Notes and Comments

Explaining Dioscorides' "Double Difference": Why Are Some Mushrooms Poisonous, and Do They Signal Their Unprofitability?

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ABSTRACT: The adaptive significance of toxins in mushrooms has received very little consideration, although it is clear that poisons have appeared (and/or disappeared) many times in mushrooms' evolutionary history. One possibility is that poisons have evolved in some mushroom species to deter their consumption by would-be fungivores before spore dispersal. If this is so, then one might expect poisonous mushrooms to signal their unprofitability in some way. In this study, we have conducted the first formal analysis of the ecological and morphological traits associated with edible and poisonous mushrooms in North America and Europe. Poisonous mushrooms do not tend to be more colorful or aggregated than edible mushrooms, but they are more likely to exhibit distinctive odors even when phylogenetic relationships are accounted for. This raises the intriguing possibility that some poisonous species of mushrooms have evolved warning odors (and perhaps tastes) to enhance avoidance learning by fungivores.

Keywords: mushrooms, signaling, fungivory, odor, olfactory aposematism.

> Of fungi there is a double difference, for either they are edible, or they are poisonous. (Translation from the *Vienna Codex* of Dioscorides ca. 512 AD; cited by Ramsbottom [1953])

Dioscorides, a Greek surgeon in Nero's army during the first century AD, was describing perhaps the best-known aspect of the natural history of mushrooms. Pick up any field guide to mushrooms (e.g., Kibby 1979; Lincoff 1981; Pacioni 1985; McKnight and McKnight 1987) or a more general text on their natural history (e.g., Spooner and Roberts 2005), and it will describe which species are edible and which are poisonous. Not all mushrooms are poisonous to humans; indeed, it is the minority of species that make us seriously unwell (Ramsbottom 1953; Konno 1995). However, an important question, which none of these books raise, is why some mushrooms are poisonous.

One possibility is that poisons are not adaptive per se but arise as a by-product of other metabolic processes within the fungus. Perhaps a more probable alternative is that there has been selection in some species to deter the consumption of their fruiting bodies before spore dispersal. Indeed, both Camazine (1983, 1985; Camazine et al. 1983) and Hanski (1989) proposed that vertebrate fungivores could provide the major selective force that generates these poisons. Hanski (1989, p. 43) wrote, "It should be added that many mammals, including *Homo sapiens*, are probably much more serious enemies of fungi than insects. If the chemical compounds in fungi have an evolved defensive role, it may be against vertebrates rather than insects: the former consume entire sporophores [fruiting bodies] and are much more likely than insects to effect spore production and successful dispersal." Of course, this implies that consumption of a fruiting body reduces the mushroom's contribution to the next generation, but we consider this an entirely reasonable assumption, at least for those mushroom species that are not dependent on foragers to disperse their spores.

If mushrooms contain poisons as an antipredator adaptation, then one might also expect them to signal this fact (sensu Maynard Smith and Harper 2003) and thereby reduce the costs of animals mistakenly eating them. Folk law and popular natural history are full of heuristic rules for telling poisonous from edible mushrooms. These include boiling the mushroom with a silver spoon to see

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whether the spoon blackens, using fungi collected only from fields, and using only mushrooms that "peel." Although many books and review papers stress that none of these rules work from the perspective of being completely reliable (Ramsbottom 1953; Kibby 1979; Konno 1997), we are not aware of any formal analysis. Furthermore, it should be noted that while simple classification rules for distinguishing edible and poisonous mushrooms have been provisionally identified by computer scientists (Iba et al. 1988; Duch et al. 1997), each of these studies has been based on the same hypothetical data set created by Jeff Schlimmer (see http://www.ics.uci.edu/~mlearn/MLSummary.html), which was in turn was inspired by descriptions of only 23 species drawn from the *Audubon Society Field Guide to North American Mushrooms* (Lincoff 1981).

The unprofitability of poisonous mushrooms might in theory be signaled in a number of ways. An association between defense and conspicuousness known as "aposematism" (Poulton 1890; Cott 1940; Guilford 1990; Ruxton et al. 2004) is seen in a wide variety of animals ranging from lepidoptera (Nilsson and Forsman 2003) to dendrobatid frogs (Summers and Clough 2001). Indeed, there have been suggestions that conspicuous warning signals may have similarly evolved in plants to deter would-be herbivores (Hinton 1973; Wiens 1978; Archetti 2000; Hamilton and Brown 2001; Lev-Yadun 2001, 2003). Because several well-known poisonous mushroom species are brightly colored, then one might wonder whether the two traits are also associated in fungi. For example, the red and white poisonous fly agaric (*Amanita muscaria*) seems to have attributes consistent with this interpretation (Ramsbottom 1953). Moreover, more than 60 species of fungi are known to emit light from their mycelia and/or fruiting bodies (Spooner and Roberts 2005), and it has been postulated that luminous and white fungi have evolved conspicuous traits to signal their unpalatability to crepuscular and nocturnal fungivores (Sivinski 1981).

Other forms of defensive signal have also been postulated in the literature. In particular, Camazine (1985) has argued that some poisonous fungi (and plants) may employ odors to warn fungivores of their noxiousness, a relationship that has been termed "olfactory aposematism" (Eisner and Grant 1981). This idea is supported by the fact that opossums (*Didelphis virginiana*) develop strong aversions to toxic mushrooms (Camazine 1983) and use olfactory cues to guide them in their decision making (Camazine 1985). However, once again, no formal analysis has been made to examine the overall association between mushroom edibility and odor.

Finally, Fisher (1930) wondered how distastefulness might initially spread in a population in those cases when it confers no direct survivorship benefit to the individual that possesses it. Invoking the first formal kin selection logic, he proposed that distastefulness might be more likely to arise in those species that are clustered in family groups because consumption of one individual may allow relatives to persist. Such an argument might apply particularly well to fungi in which adjacent fruiting bodies can even be clones of the same individual.

In this note, we ask why certain species of mushroom have evolved to be poisonous and what morphological and ecological species traits are indicative of edibility. First, we make use of modern phylogenies to assess how frequently poisons have evolved. We then describe our analysis of two mushroom data sets compiled from detailed field guides to test whether poisonous mushrooms species tend to be more odorous, more colorful, or more aggregated than edible mushrooms (the analyses of two additional data sets are described in the appendix in the online edition of the *American Naturalist*). We ask whether contemporary fungivores can make use of particular traits to guide them as to whether a species is likely to be poisonous, but we also question whether these putative signaling traits are associated simply via shared ancestry or continued selection.

Data Compilation

Our data sets were separately compiled from two field guides: the *Peterson Field Guide to Mushrooms of North America* (McKnight and McKnight 1987) and *The Macdonald Encyclopedia of Mushrooms* (Pacioni 1985), which describes species occurring in Europe. Two parallel data sets, collated from *The Mushrooms of Northeastern North America* (Bessette et al. 1997) and volumes 3 and 4 of the *Fungi of Switzerland* (Breitenbach and Kränzlin 1991, 1994) are described and analyzed in our appendix with comparable results, but for parsimony they are not considered further in our main text. We chose well-recognized field guides for this analysis because they cover a range of different taxonomic groups, yet they focus on species with larger fruiting bodies, which are more likely to be of nutritive value to vertebrates. For each species, we noted the characteristics shown in table 1 (always drawn from the text descriptions rather than the pictures).

Overall, data on 245 North American species and 265 European species of mushroom with known edibility were entered. Eighty-six species of mushroom were common to the two data sets and had identical scientific names, but there were also several examples of the same species that were known by different names in the two field guides (S. Redhead, personal communication). Overall, 21.2% of mushroom species were classified as poisonous in the North American data set, while 12.1% were classified as poisonous in our European data set. Despite reduced

Table 1: Mushroom characteristics

Characteristic	Comment
1. Edibility before cooking (poisonous or edible)	The term "poisonous" covers a range of effects ranging from relatively mild illness to death; some species may not be poisonous throughout their geographical range, but if the guide stated a species was poisonous, then we classed it as such within that geographic area (North America or Europe); if the edibility of a species was not clearly stated, then we omitted the species entirely from our data set (including all those classed simply as inedible)
2. Cap color	Purple, blue, pink, red, white, gray, black, green, orange, brown, olive, yellow, yellow green, not stated
3. Stalk color	Stalk color (purple, blue, pink, red, white, gray, black, green, orange, brown, olive, yellow, yellow green, not stated [including absent]); color classes (of cap and stalk) were based on the National Bureau of Standards definitions of basic hues, which were also utilized by McKnight and McKnight 1987
4. Odor	None, not distinctive, unpleasant, raw potatoes, sweet, anise, almond, other, not stated
5. Taste	Not distinctive, anise, bitter, peppery, acidic, unpleasant, mealy, other, not stated
6. Size of fruiting body	<5 cm, 5–10 cm, >10 cm, not stated
7. Size of spores	<5 μm, 5–10 μm, >10 μm, not stated
8. Spatial structure	Solitary, scattered, grouped, clustered, various, not stated
9. Substrate	Widespread, soil, dead wood, live wood, moss, dung, leaf litter, other, not stated
10. Habitat	Coniferous woodland, deciduous woodland, both types of woodland, grassland/pasture, paths, widespread, other, not stated

power, these two nonindependent data sets were analyzed separately because of the different ways the authors had of describing traits such as odor and taste.

Mushroom Phylogeny

The majority of fungi in our two data sets were homobasidiomycetes. Historically, the anatomical simplicity of this group has created problems in understanding their evolutionary history (Hibbett et al. 1997), yet several recent molecular phylogenies (Hibbett et al. 1997; Moncalvo et al. 2000; Hibbett and Binder 2002) allow an initial analysis of the evolution of toxicity in mushrooms. The most important result from the perspective of the evolution of toxicity is that many genera contain both poisonous and edible species. The molecular phylogeny of Moncalvo et al. (2000) confirms that many of these genera are monophyletic, including the large genus *Amanita*, which contains several of the most well-known dangerous species as well as some edible ones (Jenkins 1986; Weiss et al. 1998).

When our North American data set was compared with the higher taxa molecular phylogeny of Hibbett and Binder (2002), we found that our poisonous species were restricted to three of their major homobasidiomycetes clades (Euagarics, Bolete, and Russuloid), while four other clades contained only edible species (although 59% of all our genera were in one clade, the Euagarics). These general patterns, of a mix of poisonous and edible species within the same genera and restriction of poisonous species to a few clades, were common to both our North American and European data sets. Collectively, they suggest that the poisonous condition has been gained (and/or lost) repeatedly on an evolutionary time scale, within a limited number of fungal clades.

Data Analysis

Our first-step analysis comprised an array of standard statistical tests (logistic regression and tests for association) aimed at elucidating how edible and poisonous species might be discriminated on the basis of their morphological and ecological attributes. To test whether edible and poisonous mushrooms can be distinguished on the basis of a more complex set of rules (e.g., "mushrooms with colored caps and white stalks are more likely to be poisonous"), we employed a neural network to generate a nonlinear empirical classification system (Bishop 1995; Hastie et al. 2001). The second stage of analysis involved specific tests of association that took into account the potential

Table 2: Analysis of the association between odor (four classes) and edibility (two classes) in our North American and European mushroom data sets

	Edibility		
Odor	Edible	Poisonous	Total
North American data:[a]			
None	8	1	9
Not distinct	95	14	109
Smells	28	15	43
Unpleasant	6	10	16
Total	137	40	177
European data:[b]			
None	53	2	55
Not distinct	31	3	34
Smells	128	13	141
Unpleasant	6	13	19
Total	218	31	249

Note: Note that although a relatively high proportion of species were common in the two guides, the relative frequency of classes differed considerably between the two data sets. The four classes of odor shown are none, not distinct, smells in some way (but not unpleasant), smells unpleasant.

[a] $\chi^2 = 28.882$, df = 3, $P < .001$.

[b] $\chi^2 = 60.288$, df = 3, $P < .001$.

lack of independence between attributes in different species because of their shared phylogenetic history.

Logistic Regression

Here we fitted a generalized linear model with binary response (edible/poisonous) and categorical predictor variables (2–10). For the North American data set, predictor variable 5 (taste) was omitted because there were too many unknowns (the taste of highly poisonous mushrooms was not always stated, most probably to avoid poisonings). For the European data set, predictor variables 5 (taste), 8 (spatial structure), and 9 (substrate) were omitted because this guide did not always give these details. In addition, any species with “not stated” in any of the other predictor variables could not be used. This reduced the North American data set to 128 mushroom species and the European data set (with fewer predictor variables) to 213 species (the same data were used for our neural network classification, with the exception that the analysis of cap color did not involve stalk color as a predictor).

For the reduced North American data set, a forward stepwise conditional regression (critical entry significance $P < 0.05$) ended with just one predictor variable in the model, namely, odor (Wald statistic = 5.864, df = 6, $P = .015$). A backward stepwise conditional regression (critical removal significance $P > .1$) on the same data set ended with two predictor variables in the final model, namely, odor and spore size (odor: Wald statistic = 16.152, df = 6, $P = .013$; spore size: Wald statistic = 4.165, df = 2, $P = .125$). Both forward and backward stepwise conditional logistic regression on the European data set converged to the same predictor variable for edibility, namely, odor (Wald statistic = 30.320, df = 6, $P < .001$).

Tests for Association

Here we conducted tests of association and combined classes in biologically meaningful ways to ensure that the majority of expected values were greater than 5 (Sokal and Rohlf 1995). Species in which the relevant traits were not known were omitted from the analysis. Odor was highly significantly associated with edibility in both our North American and European data sets (table 2). Not surprisingly, the odor (not distinct/none, other) of the mushroom was also associated with its taste (not distinct/none, other) in both data sets (North American data: $\chi^2 = 28.725$, df = 1, $P < .001$; European data: $\chi^2 = 140.221$, df = 1, $P < .001$). There was no significant association between cap color and edibility in either data set (table 3). Similarly, there was no significant association between degree of clustering (clustered, grouped, scattered, solitary, various) and edibility in the North American ($\chi^2 = 2.228$, df = 4, $P = .694$) and European data sets ($\chi^2 = 3.353$, df = 4, $P = .501$). Intriguingly, there was a highly significant association between cap color (brown/green, colored, white/gray) and spore size (small, medium, large) in the North

Table 3: Analysis of the association between cap color and edibility in our North American and European mushroom data sets

Cap color	Edibility Edible	 Poisonous	Total
North American data:[a]			
Brown/green	61	18	79
Colored	70	21	91
White/gray	60	13	73
Total	191	52	243
European data:[b]			
Brown/green	76	11	87
Colored	78	6	84
White/gray	71	14	85
Black	8	1	9
Total	233	32	265

Note: "Colored" means colored in some way other than described in the other classes.

[a] $\chi^2 = .802$, df = 2, $P = .670$.

[b] $\chi^2 = 3.506$, df = 3, $P = .320$.

American data set ($\chi^2 = 16.194$, df = 4, $P = .003$) and a nonsignificant trend in the European data set ($\chi^2 = 11.066$, df = 6, $P = .086$). In both cases, more of the colorful mushrooms produced medium-sized spores than would be expected when assuming no association.

Neural Network Classifier

Our neural network was a fully connected feed-forward classifier that incorporated logistic transfer functions and used the softmax function to obtain the estimated probabilities of inclusion in each class (Bishop 1995; Sarle 1997; Hastie et al. 2001). The tuning parameters for the network structure were the number of hidden layers (0, 1, or 2), the number of nodes in each applicable layer, and whether to use skip-layer connections originating at the input layer and bypassing all hidden layers. Bias nodes were added to the input layer and each hidden layer. All of the predictor variables were categorical, and each was accounted for using "1 of C − 1" encoding (Sarle 1997). The procedures for identifying optimal network structure and further explanation of the subsets method employed to identify salient predictor variables are provided in our appendix. The results reported here refer to those generated by the optimal network structure from the range of tested structures (the optimal structure was found for each set of predictor variables tested for each data set).

Two types of analysis were conducted. In the first analysis, we simply considered edibility as the response, and we used the same data sets we had used in our logistic regression analyses. For the second analysis, the response was switched to cap color. Here, cap colors were grouped into four classes (brown/green, colored, white/gray, black), edibility was used as one of the predictor variables, and stalk color was omitted from the predictor variable set because of the high correlation between stalk color and cap color.

For the North American data set, the median percent of species correctly classified over the test sets was 76% (SD 9.0), and for the European data set it was 88% (SD 5.1). In both cases, the misclassification overwhelmingly predicted that poisonous species were edible. Given that the percent edible in the data sets were 75% and 87% for the North American and European data sets, respectively, it is clear that there were no reliable patterns detected in the data, such that one would do almost as well by guessing always that the mushroom was edible. However, the subsets testing produced slightly better results. For the North American data set, the best median percent correct over the test sets was 80% (SD 8.7), and for the European data set it was 90% (SD 5.5). For the European data set, odor was clearly the most important predictor variable. For the North American data set, there was no obvious best predictor variable in terms of median test set percent correct (although in terms of median test set entropy, odor was clearly the most important predictor variable). Note that the improvement in prediction for the best subsets can be attributed to the "curse of dimensionality." As Bishop (1995, p. 8) notes, "Reducing the number of input variables can sometimes lead to improved performance for a given data set, even though information is being discarded. The fixed quantity of data is better able to specify the mapping in the lower-dimensional space, and this more than compensates for the loss of information."

For the analysis of cap color, the prediction was poor in all cases. For the North American and European data sets, 24% (SD 9.4) and 12% (SD 5.8) of neural network predictions were correct over the test sets, respectively.

Using subsets of the predictor variables improved the prediction to 48% (SD 9.8) and 37% (SD 6.7) for the North American and European data sets, respectively. For the North American data, spore size was the most important predictor variable for cap color, but for the European data, there was not a clear single most important predictor variable. The suggestion that cap color may be related in some weak way to spore size (also highlighted by our association tests) hints that cap color may play a role in mediating spore dispersal.

Phylogenetically Controlled Pairwise Comparisons

The statistical analyses we have employed assume independence of data, yet it is possible that the evident association between odor and edibility arose because certain ancestral mushroom species happened to produce both poisons and distinctive odors, and these pairs of traits have subsequently continued to be associated in related species following speciation. Given that the phylogenetic relationships of species in our data sets are not well known (a large number were not listed in the available phylogenies), we attempted to overcome the potential lack of independence by conducting sister-species comparisons (Møller and Birkhead 1992; Nunn and Barton 2001). In this way, congeneric species that differed in their edibility (poisonous/edible) were compared for their odor. The widespread taxonomic distribution of poisonous mushrooms allowed us to identify 14 pairs of congeneric species (selected at random using a computer program) from our North American data, one of which was poisonous and one of which was edible (see table A1 in the appendix). Overall, poisonous members of the pairs were much more likely to exhibit some form of smell (any odor except "none" and "not distinct") compared with their edible congenors (two-tailed sign test, $n = 14$, $P = .008$). Recent taxonomic opinion suggests it is questionable whether our two randomly selected *Clitocybe* species are truly congeneric (S. Redhead, personal communication). Even omitting this pair of species, there was a significant propensity for poisonous species to smell more than edible species (two-tailed sign test, $n = 13$, $P = .016$). No such significant difference was apparent in randomly chosen species pairs from our European data set, although a one-tailed sign test (justifiable on the basis of prior expectation) indicated that poisonous mushrooms were significantly more likely to exhibit an unpleasant odor than edible mushrooms ($n = 12$, $P = .032$). The fact that our analysis of the molecular phylogenies identified multiple gains (or loss) of the poisonous condition likewise suggests that the association between poison and odor is unlikely to be caused by shared phylogeny.

Discussion

Despite the widespread acknowledgment that some species of mushroom are poisonous, there has been very little discussion in the literature of why this might be so and what factors might facilitate its evolution. Inspection of the available phylogenies of mushrooms (e.g., Hibbett et al. 1997; Moncalvo et al. 2000; Hibbett and Binder 2002) leads us to conclude that poisons have appeared (and/or disappeared) multiple times in their evolutionary history.

The fruiting bodies of mushrooms attract a wide variety of foraging invertebrates ranging from insects to slugs (Hanski 1989; Guevara and Dirzo 1999; Wood et al. 2001). However, mushrooms contain significant quantities of fats, proteins, and vitamins (Launchbaugh and Urness 1993), such that they can also provide a valuable dietary supplement to vertebrates, particularly at times of year when other foods are scarce (Fogel and Trappe 1978; Launchbaugh and Urness 1993; Mcilwee and Johnson 1998; Pokorny et al. 2004; Vernes et al. 2004; Spooner and Roberts 2005). Indeed, in the period of austerity at the end of the Second World War, an official publication of the British government drew attention to the recent discovery of several vitamins in various species of wild fungi, recommending them as of nutritional value in times of scarcity (Ministry of Agriculture and Fisheries 1945). Intriguingly, mushroom ingestion may well account for the summer and autumn increase in the ^{137}Cs levels in roe deer *Capreolus capreolus* in Scandinavia that have been observed following the Chernobyl accident (Avila et al. 1999). Taken together, it is clear that mushrooms are consumed by a range of animals.

We are inclined to agree with Hanski (1989) and Courtney et al. (1990) that vertebrate foragers that consume whole fruiting bodies are more likely than invertebrates to adversely affect the reproductive potential of mushrooms, but we also feel that one cannot entirely dismiss invertebrates as an important selective force on this basis. While toxins may in theory help to deter vertebrate fungivores, invertebrate fungivory may be sufficiently frequent and damaging to generate selection for defensive traits in fungi including poisons, but also more specific adaptations such as sticky caps or protective skins.

If toxins have evolved in mushrooms to deter their consumption by fungivores, as Camazine (1983) and Hanski (1989) have argued, then one might expect them to evolve traits that would allow fungivores to recognize and avoid them. Contrary to expectation and despite a range of analytical methods, our study has revealed no evidence that poisonous mushrooms as a whole consistently signal their unprofitability through colorful visual traits. This interpretation is supported by results of a field study in a Mexican cloud forest that found no relationship between mushroom color and degree of damage by invertebrates (Guevara and Dirzo 1999). Of course, color does not necessarily translate to conspicuousness in the eyes of a potential fungivore, particularly in the autumn when fallen leaves provide a range of hues and colors. Similarly, our work has concentrated on searching for general patterns, and we cannot rule out that the distinctive appearance of certain poisonous species such as the fly agaric facilitates its avoidance. Intriguingly, even using a neural network we could identify no set of ecological variables that could adequately explain mushroom color.

Perhaps the lack of a visual warning signal in unpalatable mushrooms can be explained in part by the type of fungivores that the poisons have evolved to deter. Many mammalian fungivores have relatively poor color vision and forage nocturnally, and the distinctive odor of particular mushrooms (along with taste) may be the major sensory stimulus that vertebrates learn to associate with toxic effects (Camazine 1983). Experimental work on herbivory by mammals similarly suggests that odors are an important deterrent when paired with a toxic effect (Provenza et al. 2000; see also Roper and Marples 1997 for work on birds). The possibility that some poisonous mushrooms use odor as a signal is supported by our cross-species comparisons, which consistently indicated that poisonous mushrooms are more likely to smell (often unpleasantly, as judged by humans) than edible mushrooms. Whether this association has arisen largely as a cue (the odor is a result of biochemical pathways involved in production of the toxin itself) or as a signal (the odor is a consequence of an unrelated biochemical pathways) is unclear. The chemical nature of a number of mushroom odors have now been elucidated (e.g., Wood et al. 1988, 1990, 1994, 1998, 2003; Wood and Largent 1999). While potential fungivores may sometimes smell the toxins themselves (e.g., phenols; Wood et al. 1998), we see no particular reason why production of poisons should automatically involve volatile chemicals, which suggests signals rather than cues.

Of course, any examination of the odor of mushrooms may be complicated by the fact that many species of mushroom rely on invertebrates and vertebrates for spore dispersal and actively attract these animals via smell (e.g., stinkhorns [Clathraceae] attract flies, and truffles [Tuberales] attract rodents and other mammalian foragers such as boars). In addition, we cannot discount the fact that some species classed as poisonous to humans are edible to other species and vice versa. While five species of mushrooms, all of which taste pungent to humans, were also found to be unpalatable to the opossum (Camazine et al. 1983), red squirrels (*Tamiasciurus hudsonicus*) have been reported to eat "poisonous" mushroom species *Lactarius piperatus* and *Russula emetica* with impunity (Hatt 1929; cited by Camazine and Lupo 1984). Much of this literature is complicated by the possibility that some of the field observations may be of naive fungivores that have not yet learned which species to avoid (cf. Camazine 1983). There is also the possibility that mimicry evolves in certain palatable species to exploit the protection afforded to poisonous species. Collectively, these factors will almost certainly complicate any relationship between poisonousness and odor.

Our analysis did not explore the signaling role of taste because of lack of data, but it was clearly highly associated with odor in each of the data sets that we examined. The complex relationship between the taste and odor of plants (which can elicit similar but also unrelated sensory responses) has been described in botanical textbooks since Theophrastus, over two thousand years ago (Theophrastus 1926). Tooth marks typical of rodents are frequently observed in sporophores both in North America and Europe (M. L. Smith, personal communication; D.M.W., personal observation), which suggests that vertebrates do indeed engage in sample-and-reject behavior on the basis of taste.

Of course like all correlative studies, the relationships we have observed may have arisen for a number of different reasons. Reliance on guides may also give a biased impression, particularly if the authors are more likely to describe the odor of a poisonous mushroom as unpleasant. Experimental work is now clearly necessary to directly test the hypothesis, beginning with more studies to test whether foraging animals can discriminate between poisonous and edible fungi and learn to avoid the former. If our general interpretation is correct, then we propose that poisonous mushrooms may have evolved odors and taste rather than colors as the primary means of warning fungivores that they are poisonous. Defended plants may also make widespread use of odors to aid avoidance learning by herbivores, and more work on olfactory signaling is sorely needed.

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The poisonous (and hallucinogenic) fly agaric (*Amanita muscaria*). If mushrooms are poisonous to deter being eaten, then do they signal their unprofitability in some way? Photograph by Antoine Morin, University of Ottawa.