Carnivorous Mushrooms

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For the solutions of NaCl plus glycine (isoelectric point, *p*H 5.97) and NaCl plus lysine (*p*H 9.70), the permeation rates of the amino acids were low at their isoelectric points (at which amino acids are neutral), but the rates of positively charged amino acids were as high as those of Na^+. The permeation rate of Na^+ was also dependent on *p*H but less than the permeation rates of amino acids.

Our charge-mosaic membrane can be envisioned as a model of a biological membrane in that it has a mosaic structure composed of small cation and anion domains. Thus, the charge-mosaic membrane may be useful not only as a selective membrane for organic species of low molecular weight and inorganic salts but also for some biomedical materials.

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12. The reflection coefficient σ_m was calculated from volume flows J_v measured under osmotic pressure difference $\Delta\pi$ with aqueous solutions of sucrose and under chemical potential difference $\Delta\mu_s{}^c$ of KCl across the membrane by use of the equation

$$J_v = L_p{}^m(\Delta p - \Delta\pi) + c_s L_p{}^m(1 - \sigma_m)\Delta\mu_s{}^c$$

$L_p{}^m$ is filtration coefficient, Δp is hydrostatic pressure difference, and c_s is logarithmic average salt concentration.

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Abstract. *Ten species of gilled fungi, including the oyster mushroom (*Pleurotus ostreatus*), have been shown to attack and consume nematodes. It is suggested that these wood-decay fungi utilize the nutrients in their prey to supplement the low levels of nitrogen available in wood. This mode of nutrition is similar in principle to that of carnivorous higher plants.*

Approximately 450 species of flowering plants are known to capture and digest small animal prey (*1*). These carnivorous plants, including sundews, pitcher plants, and the venus flytrap, derive a significant proportion of their nitrogen nutrition through their carnivorous habit (*1*). This ability to utilize a unique source of nutrients has enabled such plants to thrive in nitrogen-poor environments such as acid bogs.

Fungi that prey on small animals are also well known, including some 150 species that attack nematodes (*2*). Many of these fungi produce specialized trapping devices such as adhesive knobs, nets, or constricting rings to capture their victims, which are subsequently colonized and digested. Among the predatory fungi are nine species of *Nematoctonus* (Hyphomycetes), in which adhesive secretory cells are produced on either hyphae or asexual spores (conidia). Conidial fungi are given "form names" which apply only to their conidial state. If a sexual stage of a conidial fungus is discovered, the fungus is then given the name reflecting its classification in the system devised for the sexually reproducing fungi. The form genus *Nematoctonus* Drechsler (*3*) is distinguished from all other nematode-destroying fungi by hyphae with clamp connections, a characteristic feature of the Basidiomycotina. Originally the genus could not be linked conclusively with any described fungi possessing sexually reproducing structures (*3*). However, one isolate of *Nematoctonus* from soil subsequently produced fruit bodies of a gilled mushroom (*Hohenbuehelia* sp.) in culture (*4*). Species of *Hohenbuehelia* are found on soil or plant debris but are more commonly associated with rotting wood (*5*).

Rotting wood is a nitrogen-poor environment, as are many bogs. In the early stages of decay the ratio of carbon to available nitrogen is high, and C:N ratios from 350:1 to 1250:1 have been found in sound wood (*6*). Several mechanisms have been suggested by which wood-decay fungi may overcome high C:N ratios. Some of the primary colonizers of dead wood, including *Armillariella mellea* (the honey mushroom), quickly take advantage of sites of high nitrogen concentration such as the cambium, inner bark, and ray cells (*7*). Some of the polypores which penetrate huge volumes of wood conserve the nitrogen obtained by translocating their cytoplasm into the actively growing hyphae (*6*). Shigo (*8*) and others have suggested that the succession of wood-inhabiting microorganisms, including nitrogen-fixing bacteria, may play a role in increasing the amount of nitrogen available to decay fungi. It has been shown that the nitrogen content of wood increases as decay progresses (*9*), but nitrogen may still be limiting because of intense microbial competition. The ability of a *Hohenbuehelia* to capture nematodes suggested a novel means by which wood-rotting fungi could overcome the nitrogen limitations of their primary substrate.

The purpose of our study was to determine the extent and ability of lignicolous gilled fungi to attack and digest nematodes. Twenty-seven species of gilled fungi (Agaricales) were tested for their ability to attack nematodes (Table 1). Pure cultures of the fungi were maintained on potato dextrose agar and incubated at room temperature (18° to 22°C). Methods used to culture nematodes were similar to those described earlier (*2*). To evaluate the nematode-destroying capability of the fungi, a 6-mm disk of each species was transferred to the center of a water agar petri plate and incubated for 7 to 14 days, by which time a thin weft of sparse hyphal growth had radiated from the disk through the adjacent agar. Ten to fifteen active nematodes were handpicked from a nematode culture and placed on the water agar plates in the vicinity of the hyphal growth.

Observations on interactions between nematode and fungus were made immediately and at 15-minute intervals for the first hour, then hourly for the next 12 hours, and again after 24 hours. At daily intervals additional nematodes were added to the culture. The observations were repeated over a period of 7 days.

Of the 27 species tested (Table 1), we found that five species of *Hohenbuehelia*, five species of *Pleurotus*, and one species of *Resupinatus* were capable of destroying nematodes. None of the other fungi had any adverse effects. Nematodes were attacked in one of three different ways.

1) In cultures of *Pleurotus ostreatus* (the oyster mushroom), nematodes be-

came inactivated very quickly, and some were immobilized within a few minutes of exposure to the fungus. These nematodes were not dead, however, and would move feebly if placed in a drop of water. After inactivation of the nematode, hyphae of the fungus grew rapidly toward, and eventually penetrated, one of the body orifices (Fig. 1B). Within 24 hours of exposure, the nematode's body was filled with the hyphae of the *Pleurotus* and its contents were digested. It appears that *P. ostreatus, P. cornucopiae, P. cystidiosus, P. strigosus*, and *P. subareolatus* release a potent toxin that completely inactivates nematodes prior to penetration. This method of attacking nematodes has not been reported previously.

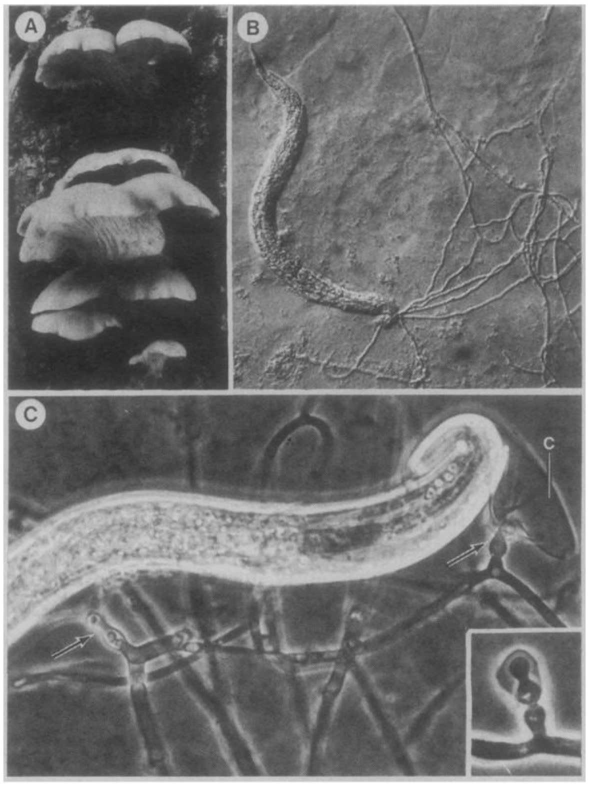

Fig. 1. (A) Fruiting bodies of *Pleurotus ostreatus* (the oyster mushroom), from which the culture in (B) was isolated, growing on a living sugar maple (*Acer saccharum*) (one-quarter life size). (B) Hyphae of *Pleurotus ostreatus* converging on the mouth of an immobilized nematode on water agar (×300). (C) A nematode caught by two adhesive trapping devices of a *Hohenbuehelia* (arrows). Note that its struggles have pulled its cuticle (*c*) away from the body at the points of capture (×800). (Inset) Detail of the trapping device of *Hohenbuehelia portegna* showing the hourglass-shaped secretory cell with its adhesive sphere (×900).

Pleurotus ostreatus (Fig. 1A) and related species are worldwide in distribution and are sought after by collectors of edible wild fungi. They are also grown commercially in parts of Europe, Israel, Asia, and more recently in North America (*10*). In nature, although occasionally found on thoroughly rotted logs, they are commonly associated with sound dead hardwood and are often seen fruiting on live trees. Species of *Pleurotus* are thus among the primary colonizers of dead wood and face the high C:N ratios that this entails. The discovery of the nematophagous habit of *Pleurotus* implies that nematodes would not be a pest in the commercial cultivation of *Pleurotus* as they are in the cultivation of *Agaricus*, the common commercial mushroom. Instead, nematodes may represent a valuable means of increasing the nitrogen available to *Pleurotus* under cultivation.

2) In *Resupinatus silvanus*, hourglass-shaped adhesive cells were produced on the hyphae. Passing nematodes became attached to these and were penetrated and colonized. The hyphae produced conidia typical of *Nematoctonus*, and the method of capture was similar to that reported by Drechsler (*11*) for *Nematoctonus campylosporus*. However, the conidial state of *R. silvanus* was distinct from any of the described species in *Nematoctonus*.

A similar method of capture was found in *Hohenbuehelia atrocaerulea, H. grisea*, and *H. portegna* (Fig. 1C). These species also produced conidia and were referrable to *Nematoctonus*. The conidial states of *H. atrocaerulea* and *H. grisea* do not match any of the described species in *Nematoctonus*, but that of *H. portegna* is close to *N. campylosporus*. These findings lend support to contemporary classification systems (*5, 12*) in which the genera *Hohenbuehelia* and *Resupinatus* are considered closely related. Kühner and Romagnesi (*12*) considered *Hohenbuehelia* and *Resupinatus* to be congeneric (as *Geopetalum*) and to belong in the Pleurotaceae. Jülich, however, has placed *Hohenbuehelia, Resupinatus*, and *Pleurotus* in three separate families (*13*). Both *Hohenbuehelia* and *Resupinatus* are found primarily on decaying wood or woody debris, but they are also found on soil or living plant stems (*5*). These two genera could be considered secondary decay organisms and face intense microbial competition for their nitrogen supply.

Table 1. Evaluation of 27 species of gilled fungi for their ability to destroy nematodes. +, Nematodes killed and consumed; −, no effect.

Fungus species	Ability
Agaricus bisporus f. albida	−
Armillariella mellea	−
Campanella subdendrophora	−
Coprinus lagopus	−
Cyptotrama asprata	−
Flammulina velutipes	−
Geopetalum carbonarium	−
Hohenbuehelia atrocaerulea	+
Hohenbuehelia grisea	+
Hohenbuehelia mastrucata	+
Hohenbuehelia niger	−
Hohenbuehelia petaloides (France)	+
Hohenbuehelia petaloides (United States)	−
Hohenbuehelia portegna	+
Hypsizygus tessulatus	−
Lentinellus ursinus	−
Oudemansiella radicata	−
Panellus serotinus	−
Phyllotopsis nidulans	−
Pleurocybella porrigens	−
Pleurotus cornucopiae	+
Pleurotus cystidiosus	+
Pleurotus ostreatus	+
Pleurotus strigosus	+
Pleurotus subareolatus	+
Resupinatus silvanus	+
Schizophyllum commune	−
Xeromphalina campanella	−

3) In *Hohenbuehelia mastrucata* and *H. petaloides*, adhesive knobs were not produced on the hyphae. In the presence of nematodes, conidia of these fungi produced adhesive knobs. These knobs attached to the cuticle of passing nematodes which were then penetrated, colonized, and digested. This method of attack is similar to that described by Drechsler (*3*) for *Nematoctonus leiosporus*. The conidial states of both *Hohenbuehelia mastrucata* and *H. petaloides* are disinct from previously described species of *Nematoctonus*.

Hohenbuehelia mastrucata is found on rotting, often thoroughly rotted, hardwood logs in the Northern Hemisphere. Although fruiting bodies are seldom found, the repeated recovery (*14*) of nematodes infected by its conidial state would suggest that this is a common fungus at least in Ontario. *Hohenbuehelia petaloides* is considered one of the most common members of its genus and is found in Europe, North America, and Japan on both thoroughly rotted wood and soil. Host specificity may explain the inability of *H. niger* and the North

American strain of *H. petaloides* to attack nematodes in this study, since only one species of nematode was used.

More species of gilled fungi capable of attacking nematodes will likely be discovered. The distribution and importance of these carnivorous fungi in nature and their ecological relation to other microbial inhabitants of their environments remain to be determined. Shigo (*8*) has reported a succession of microorganisms during the process of tree decay. In areas of advanced decay he found a variety of organisms in addition to the primary wood-rotting fungi (Hymenomycetes), including bacteria, nematodes, and other fungi. Blanchette and Shaw (*15*) reported that the presence of bacteria and yeasts significantly increased wood decay by the three Hymenomycetes tested.

Whatever their direct roles in nitrification and decay, bacteria are an important food source for free-living nematodes, and it would not be surprising if substantial populations of nematodes, as indicated by Shigo (*8*), were present in the decayed cores of standing trees. However, quantitative data on nematode populations in such habitats are scarce. We had the opportunity to analyze a sample collected from the rotting core (5 to 10 cm in diameter) of a standing maple (60 cm in diameter) within 6 hours of felling, and 912 nematodes per 100 ml of decayed wood were recovered after a 24-hour extraction with the use of a Baermann funnel.

In habitats such as rotting wood where nitrogen is limiting because of scarcity or intense microbial competition, the ability of fungi to feed on nematodes may be a significant advantage. Gilled fungi which supplement their carbohydrates obtained by the breakdown of wood with a diet of captured nematodes are analogous in principle to the carnivorous higher plants which supplement their photosynthetic energy with protein from captured insects.

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Late Triassic Naticid Drillholes: Carnivorous Gastropods Gain a Major Adaptation but Fail to Radiate

Abstract. *Infaunal and reclining bivalves of the Late Triassic Cassian Formation of northern Italy contain drillholes that closely resemble those produced by modern naticid gastropods. The oldest drillholes previously reported are from the late Early Cretaceous; this suggests that the drilling adaptation was lost soon after its appearance in the Late Triassic and originated independently in another naticid clade 120 million years later. The perceived selective value of such an adaptation may thus not always be a good predictor of its long-term survival, which is ultimately governed by factors that affect the speciation and extinction rates of the clade that carries it.*

Drillholes produced by certain carnivorous gastropods, which penetrate the shells of their prey, are among the most readily recognized traces of predation in the fossil record. Analysis of fossil faunas for drillhole frequencies, positions, and preferred prey provides a direct means of assessing the evolution of predation and its ecologic impact on marine communities (*1, 2*). Occurrence of apparent naticid drillholes in Late Triassic bivalves, 120 million years older than the oldest previously known drillholes in the late Early Cretaceous, suggests that drilling capability evolved at least twice in the Naticidae. The innovation was apparently lost at the end of the Triassic, presumably because of extinction of the clade in which it originated, and reappeared in an Early Cretaceous species.

The Late Triassic (Carnian) Cassian Formation of the Southern Alps contains several benthic associations dominated by mollusks that inhabited fine-grained substrata in protected shallow-water environments (*3*). At least two kinds of borings occur in the shells of Cassian bivalves. One is a cylindrical tube 0.2 to 1.3 mm in diameter that penetrates the shell at various angles; borings of this type are difficult to attribute to any particular predator or sessile shell-borer. The second type of boring closely resembles holes drilled by naticid gastropods. These holes occur in the infaunal deposit-feeder *Palaeonucula* and the reclining suspension-feeder *Cassianella* (exclusively in the lower, buried valve) and less commonly in other infaunal bivalves such as *Palaeocardita* and *Prosoleptus*; thus an infaunal or semi-infaunal habit is indicated for the producer of the drillholes. The borings are circular to slightly oval in plan view (outside diameter, 0.65 to 1.3 mm; mean, 1.07; $N = 39$) and exihibit the typical parabolic cross section of modern naticid drillholes (*1–6*) (Fig. 1A). Many of the borings are incomplete, which is unusual but not unknown in assemblages of naticid-drilled shells (*1, 2*), and incomplete holes exhibit the central boss typical of naticid holes—a result of the characteristic hole-center to hole-margin radular movements when drilling (*4, 6*). The drillholes are only present in articulated shells, as would be expected if the driller were an infaunal predator. Some shells contain four or five incomplete drillholes, but invariably only a single complete hole is present, further supporting the predator interpretation. Stereotypy of prey-handling behavior similar to that observed in naticids (*1, 4–7*) is suggested by the concentration of drillholes in the center of the lower valve of *Cassianella* and near the umbo in *Palaeonucula*; as observed by Kitchell *et al.* (*1*) in experiments with living naticids, multiple drilling sites on a single shell are adjacent to, or overlap, one another.

Naticid gastropods that could have been responsible for the drillholes are abundant in the Cassian Formation, no-