COMMENTARY

Mechanism of murderous mushrooms paves path for parasitic helminth halt

Tess Renahan[a] and Ralf J. Sommer[a,1]

"In the animal kingdom, the rule is, eat or be eaten" (ref. 1, p. 20). We will expand academic Thomas Szasz's statement to include the fungi, because in the multifaceted interactions of fungi and nematodes, this is often a steadfast rule. Indeed, soil systems that appear inert or lifeless to the naked human eye in reality teem with microscopic life dominated by interactions between nematodes and fungi. Numerous free-living *Bursaphelenchus* nematodes are mycophagous (2), and beetle-associated *Pristionchus pacificus* has expanded its dietary range to include consumption of fungi in addition to bacteria and other nematodes (3). Parasitic *Deladenus* nematodes are even commercially used for their mycophagous phase to keep fungus *Amylostereum* in check; however, the fungus has responded with hyphal invasion through the worms' vulvae (4). Indeed, *P. pacificus* has also been on the receiving end of predation: Myriad mushrooms have evolved various capabilities to capture worms, often when times are dire and nitrogen contents are low. These predation techniques include adhesive and ring traps that target different nematode developmental stages, such as in the case of *Arthrobotrys*, a well-studied nematode-trapping fungus (5). Other fungi use infective spores that either bind to the nematode cuticle or are ingested (6). As explored in PNAS by Lee et al. (7), the eatable oyster mushroom *Pleurotus ostreatus* of the Basidiomycota uses yet another mechanism by employing toxin-induced paralysis to kill its prey. While the predatory capacities of fungi are well established, the execution mechanisms are gradually being elucidated. Lee et al. delve into a cilia-dependent killing mechanism that instigates an inundation of calcium and hypercontraction of head and pharyngeal muscle cells. The study by Lee et al. not only reveals the mechanism behind an imperative ecological advantage but also provides an opportunity for anthelmintic drug development, an indispensable opportunity given the rise of resistance to current treatment and high rates of infected humans and agricultural destruction, roughly 1.5 billion people worldwide (8) and $80–120 billion in crop damages (9), respectively.

Intact Cilia of Head Sensory Neurons Needed for Instantaneous Paralysis

Using carnivorous *Pleurotus ostreatus* and free-living *Caenorhabditis elegans*, Lee et al. (7) uncover a striking mechanism of a predation tactic that differs from other well-described nematophageous fungi and successfully acts against a diversity of nematodes. Fungi relying on trapping mechanisms have co-opted the use of nematode ascaroside pheromones to lure them into their snares. Among the worms, ascarosides are used for various behaviors and communication, including mating and sensing population size (10). While these wedged worms wiggle for hours, *P. ostreatus* immobilizes *C. elegans* almost immediately upon contact with fungal hyphae, as Lee et al. observe in their study in PNAS. The authors found a substantial influx of calcium levels in the pharynx and head muscles using calcium indicator GCaMP6 under two promoters, one expressing in neurons and the pharynx and the other in various muscles. They traced this calcium source to the endoplasmic reticulum but also recognized that downstream factors participating are different from the currently known ones involved in channel-mediated neurotoxicity. Specifically, a ryanodine receptor r mutant displayed decreased calcium levels in the pharynx compared to wild type yet experienced the same ciliated sensory neuronal necrosis. This leaves the door open for further investigation.

With the power of unbiased genetic screens, Lee et al. found 12 *C. elegans* mutants displaying normal locomotion on *P. ostreatus* hyphae. Single-nucleotide polymorphism mapping and whole-genome sequencing revealed nine independent, loss-of-function mutations in genes, all of which are essential for the development of ciliated sensory neurons or amphid channel morphogenesis, the latter representing the nose of the worm, the most crucial sensory organ of these blind creatures. Using these mutants along with previously established lines with known defects in either the function of ciliated sensory neurons or impaired signaling, Lee et al. (7) surprisingly found that the intact cilia structure, but not sensory neural function,

[a]Department for Integrative Evolutionary Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany
Author contributions: T.R. and R.J.S. wrote the paper.
The authors declare no competing interest.

See companion article, "Sensory cilia as the Achilles heel of nematodes when attacked by carnivorous mushrooms," 10.1073/pnas.1918473117.
[1]To whom correspondence may be addressed. Email: ralf.sommer@tuebingen.mpg.de.
First published March 9, 2020.

is needed to elicit paralysis. However, mutants with defects in ciliated sensory neuron development displayed a range of paralysis upon hyphae contact, suggesting that several factors are at play. Together, these findings reveal that the oyster mushroom successfully evolved a hitchhiking mechanism of the essential communication systems of their nematode prey, a strategy commonly used by predators throughout all domains of life.

However, what really does happen in the victim after the attack has been initiated? Using the powerful list of genetically engineered reporter lines available in *C. elegans*, the authors found neuronal degeneration across the nervous system within 10 min after being in contact with *P. ostreatus* hyphae. Similarly, muscle cells exhibit rapid necrotic cell death. Is that effect different from those elicited by anthelmintic drugs, such as ivermectin, levamisole, and aldicarb, which are known to act at the neuromuscular junctions and to also paralyze their worm victims (11)? Lee et al. found only *Pleurotus* to cause neuronal necrosis; thus, this mechanism is different from that of current anthelmintic drugs. The authors also ascertained that a *P. ostreatus* toxin known to induce paralysis in nematodes (12) is not a response for executing the reaction they observed; therefore, this system can also be utilized to unearth the chemical compounds employed by the predator.

Findings Suggest Capacity for Innovative Anthelmintic Drugs

Potential application, however, would require a conserved mode of action of *Pleurotus* across nematodes. Indeed, the general neuroanatomy of nematodes is largely conserved even though variation is observed across species (13, 14). Reassuring therefore, Lee et al. found that *Pleurotus* has a common effect across nematodes tested, although none of them was parasitic. Two main factors contribute to the feasibility of these findings shedding light on anthelmintic drugs: the conserved effects across the nematode phylum and sensitivity of all stages. The authors maintain that all developmental stages are equally susceptible to paralysis, including the arrested dauer larvae. Dauers are a premediated response to stressful environmental conditions, have an occluded opening, and display remodeled neurons, including, in *C. elegans*, cilia retraction of a neuron in the amphid pore (14). While neuronal restructuring is common among nematode dauers, dauer entry and exit rely on chemosensation, and thus ciliated sensory neurons (15), suggesting this mechanism will be effective across nematode dauers. In terms of developing anthelmintic drugs, this is especially critical given that the analogous stage in parasitic nematodes is often the infective stage.

Lee et al. uncover a beautiful example of an intricate predator–prey relationship using a widespread fungus and ubiquitous nematodes indicating the sophisticated interactions within soil ecosystems. Of course, in our Red Queen world, these nematodes may develop an antagonistic response to the paralysis-inducing mushroom, ceasing to be the eaten and becoming the eater. Thus, soil systems reveal ever-increasing multifaceted interactions among bacteria, fungi, protozoans, and nematodes, and likely others. The time is ripe for more researchers to study them in greater detail, and not only because of rewarding application prospects.

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