

Fungus that controls zombie-ants has own fungal stalker

A specialized parasite fungus can control ants' behavior. But that fungus also faces its own deadly, specialized parasites.

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An unsuspecting worker ant in Brazil's rainforest leaves its nest one morning. But instead of following the well-worn treetop paths of its nest mates, this ant stumbles along clumsily, walking in aimless circles, convulsing from time to time.

At high noon, as if programmed, the ant plunges its mandibles into the juicy main vein of a leaf and soon dies. Within days the stem of a fungus sprouts from the dead ant's head. After growing a stalk, the fungus casts spores to the ground below, where they can be picked up by other passing ants.

This strange cycle of undead life and death has been well documented and has earned the culprit the moniker: "zombie-ant" fungus—even in the scientific literature. But scientists are just learning the intricacies of this interplay between the *Ophiocordyceps* parasitic fungus and the Camponotini carpenter ants that it infects. Fossil evidence implies that this zombifying infection might have been happening for at least 48 million years. Recent research also suggests that different species of the fungus might specialize to infect different groups of ants across the globe. And close examination of the infected ant corpses has revealed an even newer level of spooky savagery—other fungi often parasitize the zombie-ant fungus parasite itself.

"We have advanced a great deal in understanding how the fungus controls ant behavior," David Hughes, an assistant professor of entomology and biology at The Pennsylvania State University, says. Every few months scientists are discovering yet another peculiar trait that, added together, make this parasite one of the most insidious infections—or perhaps that honor goes to the parasite that ultimately kills the killer parasite.

Deadly infection

This clever *Ophiocordyceps* fungus depends on ants to reproduce and spread, but it has found an abundant host animal. As Hughes notes, ants have been incredibly successful, currently comprising an estimated half of all insect biomass worldwide.

One of the first clues that a tropical carpenter ant has become infected with *Ophiocordyceps* is that it will leave the dry tree canopy and descend to the humid forest floor, staggering over debris and plants. "Infected ants behave as zombies," Hughes and his colleagues wrote in a 2011 *BMC Ecology* paper describing some of the latest findings. The ant will walk randomly, displaying "convulsions that make them fall down and thus preclude them from returning to the canopy," they noted, comparing the stumbling gait with a "drunkard's walk."

The clumsiness cannot, however, be blamed on the ant. "While the manipulated individual may look like an ant, it represents a fungal genome expressing fungal behavior through the body of an ant," the researchers noted in the paper. Hence the zombie designation.

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Evans suggests that a nerve toxin spurred on by the fungus is at least partly to blame, "judging from the uncoordinated movements and hyperactivity of the ants infected," he says. Ants that have been dissected at this stage of infection reveal heads already full of fungal cells.

Eventually, an affected ant will stop on the underside of one leaf, roughly 25 centimeters from the forest floor, and clamp down on the leaf's main vein. (This position appears to be optimal for the fungus's later stage in which it ejects spores onto the soil directly below.) Biting leaves is not normal ant behavior. The zombies' bites are synchronized near noon (possibly cued by clock genes in the fungus)



Wikimedia Commons/David Hughes/Maj-Britt Pontoppidan/PLoS ONE

New research is uncovering how zombie-ant fungus might control its hosts. But this parasite also has its own fungal threats.

and usually occur in a north-northwestern orientation.

Scientists have found that the fungus also triggers atrophy in its victim's muscles—specifically those around its mandibles. This atrophy is prompted by metabolites that purge the muscle cells of mitochondria and sarcoplasmic reticulum (which provide energy and signals), according to the *BMC Ecology* research. Perhaps counterintuitively, when the infected ant bites onto the leaf vein in its so-called "death grip" this atrophy causes it to have lockjaw, leaving it there to die. This seemingly small detail is crucial to the fungus's success. "Without the death grip," Hughes explains, "the ant would fall to the ground," destroying the launching point for the fungus's spores.

By that stage, cells from the fungus have grown even more numerous in the ant's body. They have proliferated around the ant's brain and between surrounding muscle fibers but have not entered the brain, glands or muscles, making the manipulation all the more amazing.

Ants appear to die within six hours after their final bite. About two to three days later a fungal stalk will start to emerge from the back of the ant's head. After maturing over the course of weeks the stalk's head will shoot spores onto the soil below. Researchers have also discovered that this relatively slow-growing fungus can have its main stem broken off and regrow it later. Foraging worker ants can unwittingly pick up spores as they pass by.

The death of an ant outside of its colony and subsequent growth of the fungal stalk might be key adaptations of the fungus, researchers have hazarded. "Ants quickly remove dead nest mates so that dying in the nest would not allow sufficient time for stalk development and spore release" before the dead host ant was ejected, Hughes and his colleagues noted in their *BMC Ecology* paper.

The doomed ants do not wander too far afield, often ending up within meters of their familiar territory. And large groups of dead ants are commonly found near colonies. These graveyards can contain anywhere from 50 to hundreds of corpses, says Sandra Andersen, a researcher at the University of Copenhagen, who has studied many of these sites. If a nearby colony is new, there is not likely to be as large a graveyard—yet.

Ants' complex and large social groups are thought to be one of the keys to their global abundance. The fungus has capitalized on ants' social behavior. "Sociality can be thought of as evolution's winning lottery ticket," Hughes says. But "this zombie fungus is natural selection's tax man."

The zombie fungus, however, cannot live without the winning ants' continued success. It appears to be an obligate parasite, requiring a specific, local species of ant for it to inhabit, grow and propagate its spores.

A specialized but global threat

The ants best known for getting zombified by the *Ophiocordyceps* fungus are tree-dwelling carpenter ants found in Brazil and Thailand, but the fungus is thought to be broadly distributed in tropical areas around the globe. In fact, the full range of strange behavior—observed in Sulawesi, Indonesia—was first described in the scientific literature by Alfred Russell Wallace in the 19th century.

Although many ants in different areas are similarly infected and dispatched in this strange way, the species of fungus infecting them is not at all the same. "Instead of one variable species, there may be tens or even hundreds," says Harry Evans, a principal scientific officer at the Center for Agricultural Bioscience International. In a 2011 paper published in *PLoS ONE*, Evans, Hughes and Simon Elliot (of the Department of Animal Biology at the Federal University of Vicosa in Brazil) described four new species of the *Ophiocordyceps* fungus that were found in just a small section of rainforest in Brazil's southeastern state of Minas Gerais. Each of these species was associated with a different *Camponotus* ant species, denoting a high degree of specialization.

This hint at such vast diversity and specialization also contains broader implications for assumptions about fungus numbers in general. "We may need to increase fungal diversity estimates by these sorts of factors," Evans notes.

Ancient scourge

The zombifying fungus's vast geographic distribution also hints at the possibility that it has been possessing ants at least since before many of the continents split apart. But these delicate organisms have not been discovered in fossils. Other examples of parasitism have been found preserved in amber dating back 150 million years, and they reveal the parasite and host as a common arrangement—but not any evidence of actual behavior manipulation.

Research published in *Biology Letters* in 2010 describes a 48-million-year-old fossilized leaf from Germany that bears the distinctive

scars of a bite from an ant's mandible on its main vein. The researchers, led by Hughes, describe the find as perhaps "the first example of behavioral manipulation in the fossil record." During that time period the region of Germany would have been similar in climate to the areas of Thailand where contemporary zombie-ant fungus has been documented.

A parasite's parasite

The zombie-ant fungus is not the end of the parasitizing line, however—it meets its own death at the work of yet another parasite.

Andersen and her colleagues have found that a different breed of fungi grow over the ant corpse and the emerging fungus stalk. By covering the original fungus and its stalk, this secondary fungus—or hyperparasite—effectively prevents the zombie-ant fungus from ejecting its spores. "It looks like they completely sterilize it," Andersen says of the second-level parasite.

Even these hyperparasites seem to be specialized for growing on specific parasitizing fungi. "They're not really growing on anything else" in the area, Andersen says. This makes the hyperparasite another obligate parasite, which depends on the zombie-ant fungus, which depends, in turn, on the carpenter ant colony. "Once you're very successful, something else will take advantage of it," she notes. "It's really a little ecosystem in its own [right]."

The zombie-ant fungus's doom, of course, is little consolation for the infected ant. But the castration of the ant-killing fungus means that it will not go on to turn other local ants into zombies. This hobble might, in fact, be one of the reasons the zombie-ant fungus has been so successful over the long term. As a deadly infection, it could severely damage an ant colony. But, if another parasite renders more than half of its mature spores infertile (and more still failing to reproduce due to other interferences), that creates a sort of equilibrium with a colony. According to the research by Andersen and her colleagues (published in May 2012 in *PLoS ONE*), the actual reproduction rate for each mature zombie-ant fungus organism is a little more than one new mature organism, thus allowing the species and local population to sustain itself as long as there are ants nearby to infect.

In addition to the fungicidal fungi, scientists have also seen small bugs laying their eggs in the infected ant corpse, where their larvae can then eat the growing fungus. These bugs include specialized gall midges (in the Cecidomyiidae family) and a species that appears to be new to science, Hughes says. "It seems their entire nutrition comes from eating the fungus that manipulates ant behavior."

Are such hyper-specialized hyperparasites a freak occurrence? Apparently not: "We have found it all over the world, and most [ant] cadavers have hyperparasites exploiting the zombie-ant fungus at some stage," Hughes notes. And Anderson suspects "the more people study parasites, the more examples we'll find." Even if they are hiding in darker corners.

Indeed, Evans, Hughes and others continue to hunt for more bizarre, opportunistic organisms. Evans is collecting more zombie ants in Brazil, as part of what he and Hughes have unofficially dubbed the "World Ant Tour." The hunt may be a race against time, however. When Evans returned to a field site in Ghana where he had found different genera of possessed ants in the 1970s, "we could not find any of the forest sites," he says. They were "all cleared, gone and largely invaded by exotic weeds. In fact, the same thing occurs every time I revisit the places that I have worked over the past 40-plus years," he notes.

Learning about zombie-ant funguses is not simply an exercise in outré science. As Hughes notes, discovering more about both the fungus and the ant behavior and signaling dynamics could add to research about pest control for agriculture. "Lots of the pure discoveries we make have great import for food security and the challenges farmers in tropical countries face from insects and fungi that infect their crops," he notes. But garnering such knowledge will require more zombie corpses to collect and study.

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