January 2012 » Features

Animal Mind Control

Examples of parasites that manipulate the behavior of their hosts are not hard to come by, but scientists have only recently begun to understand how they induce such dramatic changes.

By Jef Akst | January 1, 2012

Scott Youtsey/Miracle studios

A normally insatiable caterpillar suddenly stops eating. A quick look inside its body reveals the reason: dozens of little wasp larvae gnawing and secreting digestive enzymes to penetrate its body wall. They have been living inside the caterpillar for days—like little vampires, feeding on its "blood"—and are finally making their exodus to build their cocoons on its bright-green exterior.

In the caterpillar's brain, a massive immune reaction is taking place—the invertebrate equivalent of a cytokine storm—and among the factors being released is an invertebrate neurohormone called octopamine. "It's a very important compound for controlling behavior in insects," says invertebrate behavioral physiologist Shelley Adamo of Dalhousie University in Halifax, Nova Scotia. "Octopamine levels go up, and that plays a role in shutting off feeding."

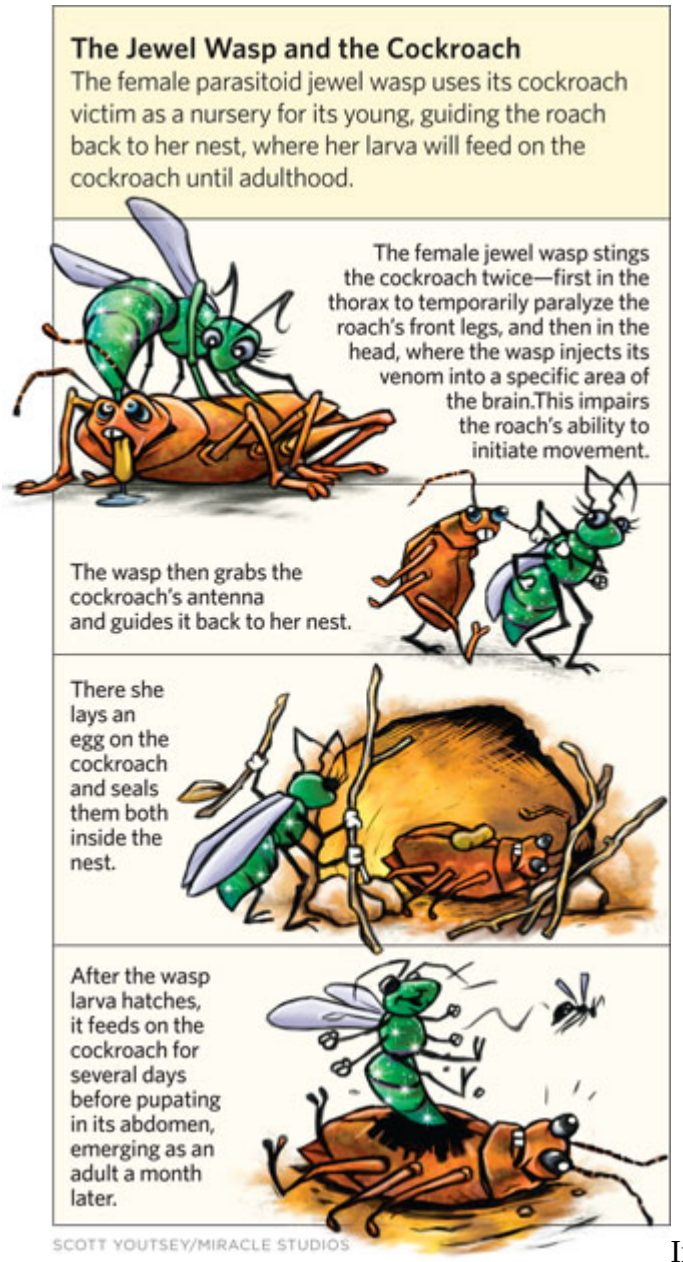
But the parasitic larvae don't stop there. They also inhibit the host's ability to break down the substance. "Octopamine levels remain high for days, and this caterpillar never really eats again," Adamo explains. "Basically, it starves to death." This plays the important role of preventing the caterpillar from picking off the cocoons, one by one, and eating the metamorphosing larvae alive. Simply killing their host isn't an option, Adamo says, because if the caterpillar dies, its body will become overrun with fungal pathogens—unwelcome visitors to a wasp nursery. Plus, non-eating caterpillars retain their defensive reflexes, which protect both them and the young wasps from

arthropod predators. “They’ve turned their host from being a meal ticket [into] their bodyguard,” Adamo says.

Although researchers have observed countless examples of parasites hijacking the autonomy of their hosts, only now are they beginning to understand how the parasites tinker with numerous systems within the host, ultimately changing the host’s behavior in grotesque and horrific ways. Taking a proteomics approach, for example, scientists have compared the proteins expressed in the brains of infected and uninfected animals to gain clues about which molecules might be involved in the manipulation. And more directed neurological approaches have flagged certain brain regions and particular neurotransmitters, such as serotonin and dopamine, as likely culprits.

“The real nuts and bolts have yet to be figured out for any system,” says Adamo. “But we have some hints—good hints.”

Pet cockroaches



Imagine a person walking his dog on a leash, only in place of the dog, substitute a cockroach, and holding the leash, picture a wasp. The female parasitoid jewel wasp doesn’t actually paralyze its cockroach victim, but impairs the roach’s ability to initiate movement of its own accord. This allows the wasp to grab the cockroach by an antenna and guide it back to her nest, where she lays an egg on the cockroach and seals them both

inside the nest. Two days later, the wasp larva hatches, drills a small hole in the bug's upper leg, and begins feeding on the cockroach. After a few days, it drills a larger hole at the base of the cockroach's leg and moves inside the abdomen, where it feeds on its host's internal organs until it pupates and emerges a month later as an adult.

"The poor zombified cockroach is sort of a living larder," says Adamo. "It gets walled up with the egg that then hatches and slowly consumes the cockroach."

One of the first thorough descriptions of this bizarre phenomenon came from Francis Williams, who was sent by the Hawaiian Sugar Planters' Association to the island of New Caledonia during World War II to identify sugar-cane pests that plantation owners feared could catch a ride on the new Pan Am Clippers and infest their crops. The jewel wasp, however, Williams regarded as a "beneficial insect," and he actually imported it to Hawaii to serve as a biological control on the cockroach population. Since that time, much has been learned about how the wasp works her magic.

To domesticate the cockroach, the wasp must sting it twice—first in the thorax to temporarily paralyze the roach's front legs, and then in the head, where the wasp injects its venom into a specific area of the brain. In 2003, behavioral neurobiologist Frederic Libersat of Ben Gurion University in Israel and his colleagues injected wasps with radiolabeled amino acids, which became incorporated into the venom proteins.[1] "Then we could trace the radioactive signal into the nervous system of the cockroach," Libersat says. They found that the venom localized primarily in the cockroach's cerebral ganglia—in particular, the supraesophageal ganglion (SupEG) and subesophageal ganglion (SEG), brain areas involved in motor control in some insects.

More recently, Libersat and his colleagues have demonstrated that by disrupting activity in the SEG, they could recreate the behaviors of stung cockroaches: treating cockroaches with a sodium channel blocker, called procaine, temporarily decreased walking behavior.[2] Injecting milked wasp venom into the SEG similarly produced the altered cockroach behaviors, suggesting that the venom was acting to decrease SEG activity—a result confirmed by recordings of spontaneous and evoked neuronal spiking activity in this brain region.

"Our current hypothesis is that neurons in the SEG send their axons to the SupEG, the central portion of the brain, and those neurons in the brain send some signals back to the thorax [to] control the initiation of movement," Libersat says. Injections of venom into the SEG essentially disrupt the first part of this pathway. Venom injections into the SupEG also had some effects, though "the results are much more complicated," says Libersat, who is studying the possibility that this may somehow be disrupting the second part of the pathway—communication from the brain to the thorax. Libersat's lab is also investigating how the wasps target their stings to these brain areas. Surgically removing the SEG region from cockroaches results in much longer stings, he notes. "The fact that the wasp spends 5 minutes stinging, instead of 30 seconds or 50 seconds, indicates that the wasp relies on sensory feedback to do the injection." His team's current research has suggested that indeed the wasps are using some sort of mechanoreceptive feedback to detect when they've hit brain tissue, but the details have yet to be published.

Fatal attraction

Cats and rodents are a classic predator/prey system, popularized in familiar cartoons and demonstrated every day by household cats around the world. Naturally, mice and rats have many defense strategies to avoid their mortal enemies, including an innate fear of the smell of cat urine. That is, until the animals become infected with a protozoan called *Toxoplasma gondii*. Then, rodents' deathly terror of cats turns into a fatal attraction.

Toxoplasma must find its way to a cat's intestines in order to reproduce sexually. The oocysts formed there are shed in the cat's feces, and use rats as a vehicle to travel to their next cat host. When the protozoa form cysts in the rat's brain, the animal not only loses its fear of cat urine, but actually seems to take a liking to it. In 2007, Robert Sapolsky of Stanford University and his colleagues showed that the cysts tended to localize in the amygdala, a brain region that responds to both predatory and sexual stimuli, and that mediates innate approach and avoidance behaviors.[3] Curious as to whether the rats really were becoming attracted to the smell of cat urine, as it appeared, or just becoming distinctly less afraid of it, Sapolsky and his PhD student Patrick House decided to take a closer look at the neural activity of infected animals. Quantifying the expression of the gene *c-Fos*, a proxy for neural activity, the researchers found that infected rats exposed to cat urine showed elevated activity in the posterodorsal medial amygdala (MEApd), a brain region involved in reproductive behaviors.[4] In fact, infected rats showed a similar level of MEApd activity in response to cat urine as uninfected rats showed upon encountering an estrous female.

"When we look in the brain, we see there's actually activity in this attraction pathway" in response to cat urine, House says.

Still, the question remains: *how* does the parasite trigger this change in neural activity? Researchers have shown that mice infected with *Toxoplasma* have about 15 percent higher dopamine levels in their brains. More recently it was learned that blocking the dopamine receptor also blocks infected animals' attraction to cat urine, suggesting a possible link between the hosts' altered behavior and the neurotransmitter, known to be key in decision making and reward. Then in 2009, a team of UK researchers identified, in the genome of *Toxoplasma*, two genes encoding a homolog of an enzyme involved in vertebrate dopamine synthesis.[5] In fact, the enzyme, called tyrosine hydroxylase, is the rate-limiting step in dopamine production, raising the possibility that the parasites are boosting dopamine levels by supplementing this enzyme in infected hosts.

"A lot of these amygdala regions are heavily dopaminergic—they have dopamine coming in, and they respond to dopamine levels," House says. "If this parasite is manipulating dopamine levels, maybe this is how it's manipulating these regions."

One of the most intriguing things about this particular system is that *Toxoplasma* is one of the few parasites that can cross the blood-brain barrier in mammals—including humans. Although *Toxoplasma* is not considered a major health problem—it mainly causes serious health consequences for severely immunocompromised patients—the parasite infects some two billion people worldwide. "If you can show these things are secreting neuroactive compounds into a rodent brain, and you know that 20 to 40 percent of the world's population have these things in their brain, you have to ask yourself, what effect is that having on human behavior?" Adamo says.

Indeed, more than three dozen studies have found a positive link between neurological disorders such as schizophrenia and *Toxoplasma* infection. "If you have schizophrenia, you're more likely to have the parasite than the average population," House says. The data, however, are merely correlational, and it could be that exposure to and infection by *Toxoplasma* is a consequence, rather than a cause, of the neurological disorder, he adds. "But it's a compelling link given the hypothesis that maybe this parasite is actually working in the rodent to increase dopamine," as the neurotransmitter has been linked to schizophrenia."

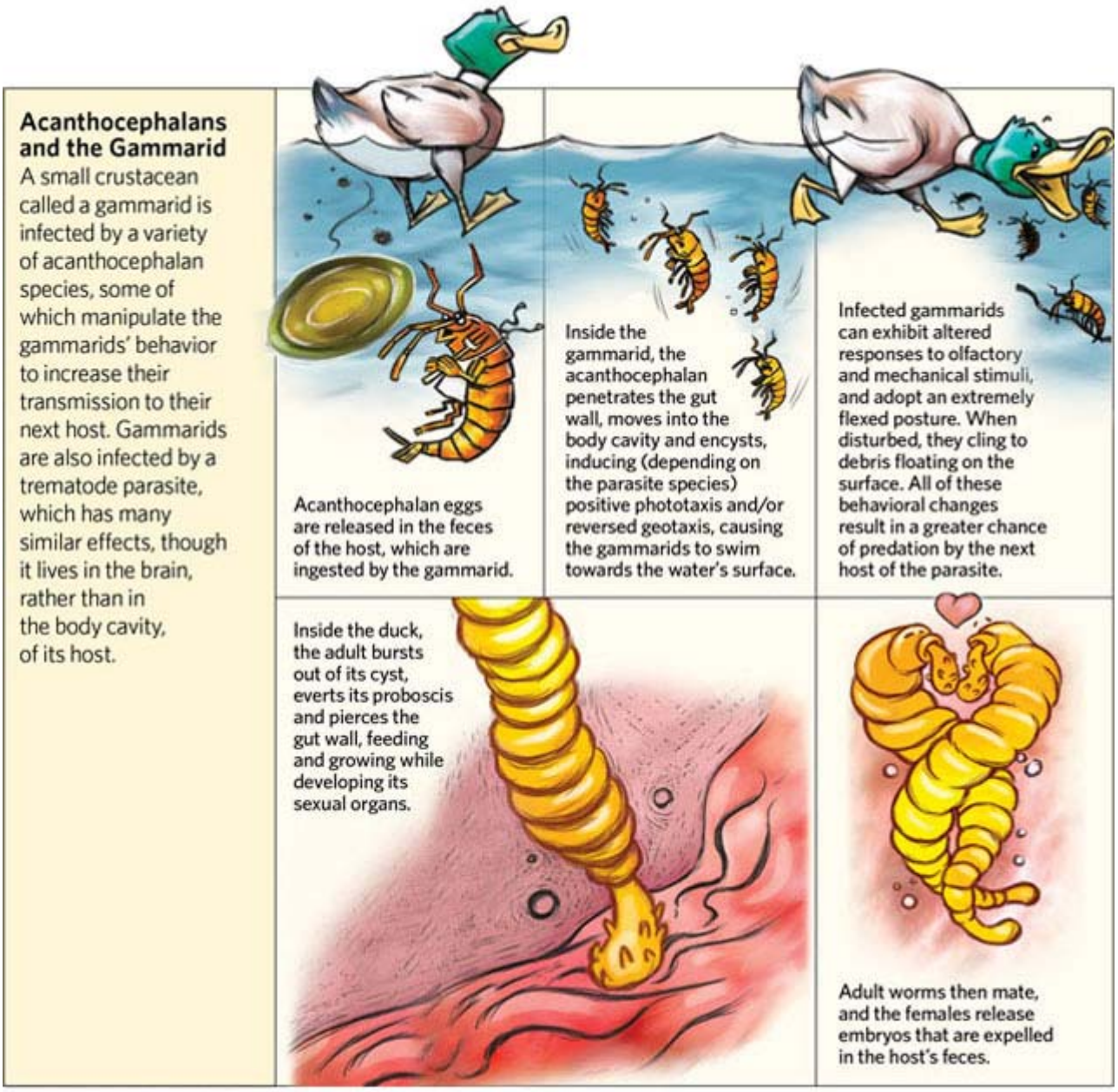
Swim towards the light

In the 1980s, Simone Helluy, a graduate student at the University of Science and Techniques of Languedoc in Montpellier, saw something bizarre in the brackish waters in the south of France: small crustaceans known as gammarids were skimming along the surface. This was unusual, she says, because normally these animals hang out at the bottom. Helluy began to investigate and soon learned that the gammarids at the surface were all infected with a trematode parasite that lodges in the brain, while those at the bottom were infection-free. She quickly recognized that this phenomenon was quite similar to one studied by Canadian researcher John Holmes, who in the early 1970s described gammarids that showed altered evasive behaviors and responses to light as a result of infection by an acanthocephalan, or parasitic worm, called *Polymorphus paradoxus*, which belongs to an entirely different phylum and lives in the host's body cavity.

"That's what's so fascinating with the parasite-gammarid system," says Helluy, now of Wellesley College in Massachusetts. "You have acanthocephalan parasites and brain trematodes that induce similar alterations of behavior in their gammarid hosts. And in both cases, it seems this altered behavior enhances predation by the definitive host of the parasites"—such as ducks, muskrats, and beavers.

More than 40 years of research on gammarids and their parasites has revealed a variety of gammarid hosts that are infected by a number of parasites with complex life histories, many of which manipulate the gammarids' behavior to perpetuate their own life cycles. Among the changes, the parasites can induce positive phototaxis, when normally the crustaceans would head

away from light; reversed geotaxis, when normally they would seek out deeper waters; and altered responses to olfactory and mechanical stimuli. Infected gammarids can also exhibit an extremely flexed posture and cling to solid materials. All these behavioral changes encourage the crustaceans to swim towards the water's surface and cling to floating debris when disturbed, rather than swim to the bottom and burrow into the mud, resulting in a greater chance of predation by the next host in the parasite's life cycle—a fish or an aquatic bird or mammal.



SCOTT YOUTSEY/MIRACLE STUDIOS

In the early 2000s, Helluy and evolutionary biologist Frédéric Thomas of the French National Center for Scientific Research (CNRS) in Montpellier used fluorescent antibodies to visualize the brains of infected gammarids, and found significantly decreased serotonergic activity in certain brain regions, including the optic neuropils, masses of tangled neurons and synapses in the optic tract.[6] They also noted that the optic tracts were deformed and the tritocerebral giant neurons—large cells known to have serotonergic activity—showed signs of degeneration in parasitized individuals. In 2006, behavioral ecologist Frank Cézilly of the University of Bourgogne also found a connection between serotonin and the altered host behaviors, but rather than a decrease, there was a 40 percent increase in overall serotonergic activity in the brains of manipulated gammarids.[7] Cézilly and others further demonstrated that injections of serotonin into certain

gammarid species can recreate the altered phototaxis and clinging behavior induced by the parasites. “This seems to indicate that serotonin is involved,” says Helluy, but how serotonin production is altered and what triggers those changes are still unclear.

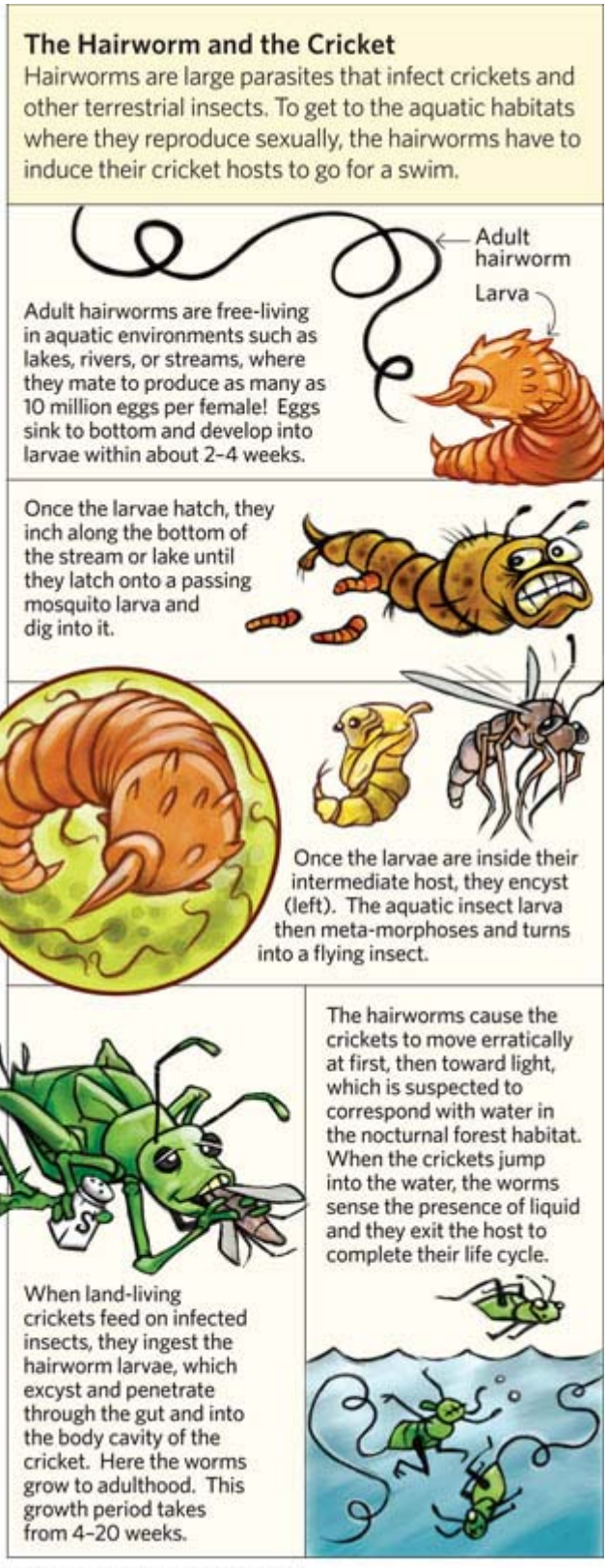
Researchers have also compared the overall protein expression patterns of uninfected and trematode-infected gammarids.[8] Among the differences were two proteins involved in the visual system, CRAL_TRIO and efhand, both of which were more highly expressed in infected individuals. Interestingly, these differences that were only seen in a gammarid species infected with the trematode M*icrophallus papillorobustus,* which induces positive phototaxis as well as negative geotaxis in the host; gammarids plagued by the acanthocephalan *Polymorphus minutus,* which induces only negative geotaxis, exhibited no increase in the levels of these proteins.

The researchers also identified higher expression of aromatic-L-amino acid decarboxylase, a protein involved in serotonin synthesis, in trematode-infected gammarids, supporting the involvement of the serotonergic system in the behavioral manipulations. And in both trematode- and acanthocephalan-infected gammarids, the proteomics analyses revealed expression changes in proteins related to the functioning of the central nervous system and immune defenses.

Changes in host immunity upon infection are to be expected, but may not always be protective. There is some evidence that many parasites can use the host immune system to their advantage. In response to infections of the brain, for example, glia, the resident immune cells of the central nervous system, release cytokines and free radicals such as nitric oxide. Last year, Helluy and Thomas examined the distribution of glial cells and nitric oxide synthase in the brains of trematode-infected and uninfected gammarids, and found key differences between the two: glia and their cellular extensions were abundant at the surface of the parasites, suggesting a proliferation of the immune cells near the site of infection, and nitric oxide synthase levels were elevated around mature parasite larvae in the brains of gammarids showing altered behavior.[9] These findings support the idea that neuroinflammation induced by the parasite is one factor that, through alterations in brain chemistry and neuronal integrity, affects the behavior of the host.

“It’s just correlation, not proof of causality,” says Helluy, but “there was a very strong immune response in the brain.” She speculates that “there could be some kind of ‘arm wrestling’ between the parasite and the host: if the host wins, then the parasite gets encapsulated and dies, but if the parasite can stay alive, then the continuous immune response to the parasite might mediate the events downstream leading to the altered behavior.”

Suicidal insects



SCOTT YOUTSEY/MIRACLE STUDIOS

Crickets don’t like water. So when researchers observe crickets seeking out forest ponds and jumping into them, they know something is amiss.

Sure enough, the water-loving crickets are infected with parasites called hairworms. Closely related to nematodes, hairworms can grow as large as 100 centimeters in length and 1–3 millimeters in diameter. With more than 300 species known, hairworms have been found to infect frogs, fish, and snails, as well as a variety of terrestrial insects, including crickets. But hairworms

that specialize in these dry-land hosts face a unique challenge—getting to water to reproduce. While the adults live on their own in aquatic environments, their larvae develop in hosts that don't remain water-bound. In the case of the crickets, researchers suspect that the worms lay their eggs in the water, where they hatch into larvae that infect water-dwelling insect larvae, such as mosquitoes. Those insect larvae then metamorphose into flying terrestrial insects and travel to dry habitats, where they die and are ingested by crickets, which acquire the worms with their meal. But then the mature hairworms must find their way back to water to complete their life cycle. Because crickets don't often venture near water on their own, this part takes a bit of mind control.

In 2002, CNRS's Thomas and his colleagues published the first formal study that confirmed the accumulating anecdotal reports: crickets infected by hairworms become "suicidal," voluntarily jumping into whatever water they can find.[10] In 2 years of watching an open-air swimming pool near a forest in southern France, Thomas's team observed nine different insect species dive into the water, at which time the hairworms emerge.

But how do the worms get landlubber insects to take the plunge? It can't be as simple as making the host like water, as the insects' normal behavior wouldn't often bring them close enough to water to express such a preference in the first place, Thomas explains. "What is very important in the cricket and hairworm system is that there are several steps in the manipulative process. In the first stage, the worm produces erratic behavior in the cricket," increasing the likelihood that it will stumble upon a source of water. Then, after the hairworm has matured, often growing to several times its host's length, it somehow induces the insect to more directly seek out water and jump in.[11]

Researchers applied a proteomics approach, analyzing snapshots of all the proteins expressed in the parasite at three different time points: before, during, and after the crickets' behavior changed.[12,13] "One of the most fascinating results was that we found proteins inside the worm that are very close to those in insects," Thomas says. Specifically, the parasites seem to make proteins of the Wnt family—involved in nervous system development—that are more closely related to insect proteins than those of the parasites' own close nematode kin, explains Thomas, suggesting this may be an example of molecular mimicry. The parasite and the host "need to speak the same language, else [they] cannot understand each other," he says. "We suspect that the worm [evolved proteins] that are similar to those produced by insects to allow the worm to manipulate the behavior."

The researchers also compared the brains of infected hosts (crickets[12] and grasshoppers[13]) with those of uninfected hosts, and identified proteins that were differentially expressed, including the visual protein CRAL_TRIO, similarly altered in infected gammarids. CRAL_TRIO expression was higher in infected hosts than uninfected individuals, with the highest expression after the hairworm had matured and the cricket was attempting to enter water. Last year, Thomas and his colleagues confirmed that hairworm-infected insects did indeed have alterations to their vision: infected hosts were attracted to light, while uninfected individuals were photophobic, just like the gammarids.[14] "It is a clever idea," Thomas says. "If you are a cricket in the middle of the forest at night [and] you want to search for water, a good option would be to go toward the light. In most cases, it will correspond to water areas."

References

1. G. Haspel et al., "Direct injection of venom by a predatory wasp into cockroach brain," *J Neurobiol*, 56: 287-92, 2003. ↩
2. R. Gal, F. Libersat, "A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in its cockroach prey," *PLoS ONE*, 5: e10019, 2010. ↩
3. A. Vyas et al., "Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors," *PNAS*, 104:6442-47, 2007. ↩

4. P.K. House et al., “Predator cat odors activate sexual arousal pathways in brains of *Toxoplasma gondii* infected rats,” *PLoS ONE*, 6: e23277, 2011.
5. E.A. Gaskell et al., “A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*,” *PLoS ONE*, 4: e4801, 2009.
6. S. Helluy, F. Thomas, “Effects of *Microphallus papillorobustus* (Platyhelminthes: Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea: Amphipoda),” *Proc R Soc B*, 270: 563-68, 2003.
7. L. Tain et al., “Altered host behaviour and brain seratonergic activity caused by acanthocephalans: evidence for specificity,” *Proc R Soc B*, 273: 3039-45, 2006.
8. F. Ponton et al., “Do distantly related parasites rely on the same proximate factors to alter the behavior of their hosts?” *Proc R Soc B*, 273: 2869-77, 2006.
9. S. Helluy, F. Thomas, “Parasitic manipulation and neuroinflammation: Evidence from the system *Microphallus papillorobustus* (Trematoda)–*Gammarus* (Crustacea),” *Parasit Vectors*, 3:38, 2010.
10. F. Thomas et al., “Do hairworms (Nematomorpha) manipulate their terrestrial host to seek water?” *J Evol Biol*, 15: 356-61, 2002.
11. M.I. Sanchez et al., “Two steps to suicide in crickets harbouring hairworms,” *Anim Behav*, 76: 1621-24, 2008.
12. D.G. Biron et al., “‘Suicide’ of crickets harbouring hairworms: a proteomics investigation,” *Insect Mol Biol*, 15: 731-42, 2006.
13. D.G. Biron et al., “Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach,” *Proc R Soc B*, 272: 2117-26, 2005.
14. F. Ponton et al., “Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation,” *Behav Ecol*, doi: 10.1093/beheco/arq215, 2011.
