Scientists show how parasites turn marsh-dwelling brown crustaceans into neon zombies

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Orange amphipods caught the eye (and interest) of Brown University graduate students conducting field research. Credit: David Johnson

Salt marshes are home to tiny crustaceans called amphipods that keep a low profile: Their gray-brown coloring helps them blend in with their surroundings, and they spend most of their time hiding under vegetation.

But when amphipods are infected with a parasitic worm called a trematode, they turn bright orange and lose their tendency to run for cover when exposed. This bizarre behavior makes them stand out to predators—as well to scientists.

Biologists at Brown University have been studying amphipods for roughly a decade. The project started as a training exercise for students in collaboration with the Marine Biological Laboratory research institution. Over time, with advancements in molecular genetics, computational tools and biomedical technology, faculty and student scientists have made unexpected discoveries about the relationship between amphipods and the parasitic worms that prey upon them.

In a new study published in *Molecular Ecology*, Brown researchers provide a detailed analysis of the molecular mechanisms that allow the parasites to manipulate their hosts, and explain what's happening to the amphipod's biology that causes it to respond to the parasite in such distinct ways.

"Characterizing the molecular mechanisms of manipulation is important to advancing understanding of host–parasite coevolution," said study author David Rand, a professor of natural history and chair of the ecology, evolution and organismal biology department at Brown.

The relevance of the findings extends far beyond the salt marsh, Rand said, especially when considered in context of certain pathogens that infect humans.

While foodborne trematodes can make humans very sick, they don't have the same type of "zombie" effect. The amphipod system is closer to a malaria example, Rand noted, where the plasmodium parasite is carried by a mosquito that serves as an intermediate host. Studies have shown that mosquitos carrying the parasite can be more attracted to

humans than to uninflected mosquitoes.

"This may be an example of a parasite manipulating an intermediate host to ensure its own transmission between hosts," Rand said. "Rabies could be another relevant example: it drives infected individuals 'mad' so they bite others and infect the next host. Learning the molecular mechanisms of these kinds of host-parasite interactions can have important implications for how to manage pathogens generally, and in humans."

The evolution of a biological research project

The trematode worm's interaction with the amphipods makes Darwinian sense, Rand said, Parasites manipulate hosts to ensure their transmission so they can continue to reproduce. They're an example of "prudent parasites" that don't kill their hosts right away or ever, giving the parasites time to reproduce or move to another host.

Amphipods infected by a parasitic trematode change color from light grey or brown to orange and move into more exposed areas of salt marshes, which, scientists hypothesize, may increase rates of predation. Credit: David Johnson

The type of "zombie" manipulation seen in the amphipods isn't unheard of in the natural world. However, Rand said, less has been known about the precise ways that parasitic worms have been able to cause changes in the amphipods that affect behavior, appearance and immune function.

In the new study, the scientists used RNA sequencing to identify genes whose function match the three big changes in the host's traits. They discovered that trematode infection results in activation of amphipod gene transcripts associated with pigmentation and detection of external stimuli, and suppression of multiple amphipod gene transcripts implicated in immune responses.

The researchers hypothesized that suppression of immune genes and the altered expression of genes associated with coloration and behavior may allow the parasite to persist in the amphipod and engage in further biochemical manipulation that promotes transmission.

"Infected amphipods become sitting ducks for predators," Rand said. "That allows the parasites to spread into a newer, bigger, more robust host organism, and continue to reproduce and propagate their species."

In the paper, researchers concluded that the genomic tools and transcriptomic analyses they reported provide new opportunities to discover how parasites are able to alter the diverse molecular pathways that underlie or determine changes in their hosts.

The research began in 2013 as a collaborative project intended to engage

graduate students in sequencing DNA and RNA for questions in the realm of ecology, evolution and environmental science. Every year, a group of Brown Ph.D. students studying subjects including biology, applied math and computer science would take a field trip to a research site at Plum Island Estuary in Massachusetts. While several projects emerged from the site, students were perennially intrigued by the curious orange amphipods.

The *Molecular Ecology* study is a culmination of the researchers' interest and efforts, and reflects how students' skills in areas such as bioinformatics and genomic analysis increased over time.

Rand, whose lab focuses on fruit flies, said that the project was continually updated with new genomic and transcriptional data, such as the annotation of the fruit fly genome, since many of the genes and mutations in fruit flies could be correlated to changes in gene expression in the amphipods. That also helped propel the research forward, he said.

This type of study is illustrative of how far biological research has come, Rand said.

"We're not curing human diseases out there in the salt marsh," Rand said. "But compared to how ecologists of the past would be limited to looking for answers to nature's mysteries by sifting through the mud, we now have access to molecular, biomedical and computational resources to find answers to questions, and we can then apply those findings to different areas of science that previously wouldn't have seemed as connected."

More information: David M. Rand et al, Parasite manipulation of host phenotypes inferred from transcriptional analyses in a trematode-amphipod system, *Molecular Ecology* (2023). DOI: 10.1111/mec.17093

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