Substance that guides ant trail is produced by symbiotic bacteria

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Atta sexdens rubropilosa worker Credit: Eduardo Afonso da Silva Junior

Researchers working on the Ribeirão Preto campus of the University of São Paulo (USP) in Brazil have discovered that a bacterium found in the microbiota associated with leafcutter ant species Atta sexdens rubropilosa produces so-called "trail pheromones," the aromatic chemical compounds used by the ants to lay a trail to their nest. An article on their findings has been published in *Scientific Reports*.

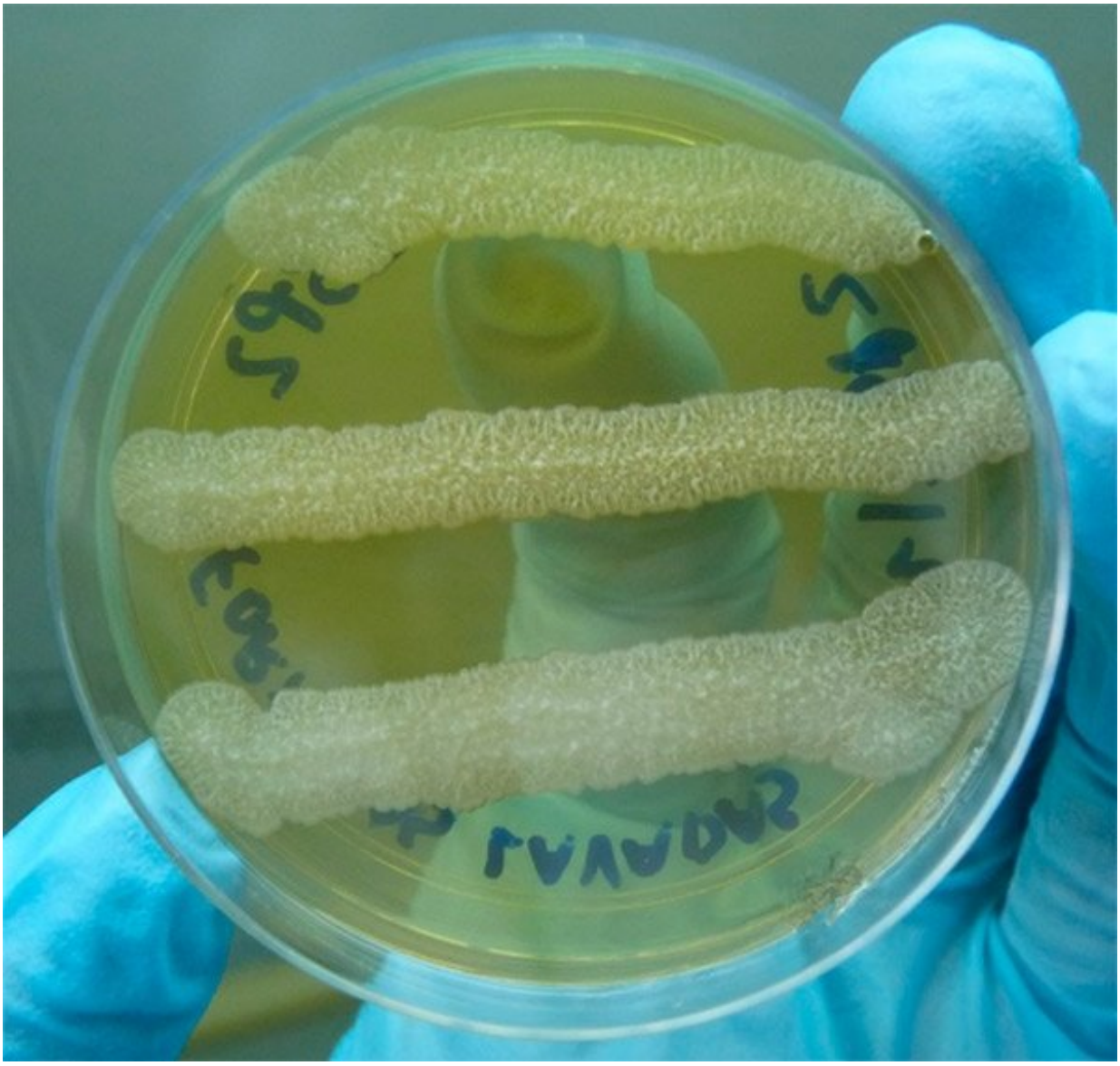
In the case of Atta sexdens rubropilosa, substances belonging to the class of heterocyclic compounds known as pyrazines guide the ants without deviation to the anthill. Researcher Mônica Tallarico Pupo, a professor in the University of São Paulo's Ribeirão Preto School of Pharmaceutical Sciences (FCFRP-USP) and the principal investigator for the project, says that pyrazine production by the ants' microbiome was observed in more than one colony.

"Are the trail pheromones produced by bacterium Serratia marcescens or does S. marcescens only help by somehow adding to the whole process?" she said.

The study was part of Eduardo Afonso da Silva Junior's PhD research and was conducted in partnership with scientists at Harvard University in the United States.

The pyrazine-producing bacterium was discovered by chance when the scientists were looking for microorganisms capable of protecting ant colonies from parasitic fungi. "The leaves carried to their nests by these leafcutters actually serve as a substrate for the cultivation of Leucoagaricus gongylophorus, the fungal species on which they feed. However, this system is susceptible to infections," Pupo explained.

"In some cases, another pathogenic species that may impair the ant colony's viability grows on the fungus they eat. The symbiotic bacteria produce compounds that can kill the parasitic fungi without damaging the food source. We set out to identify these compounds."



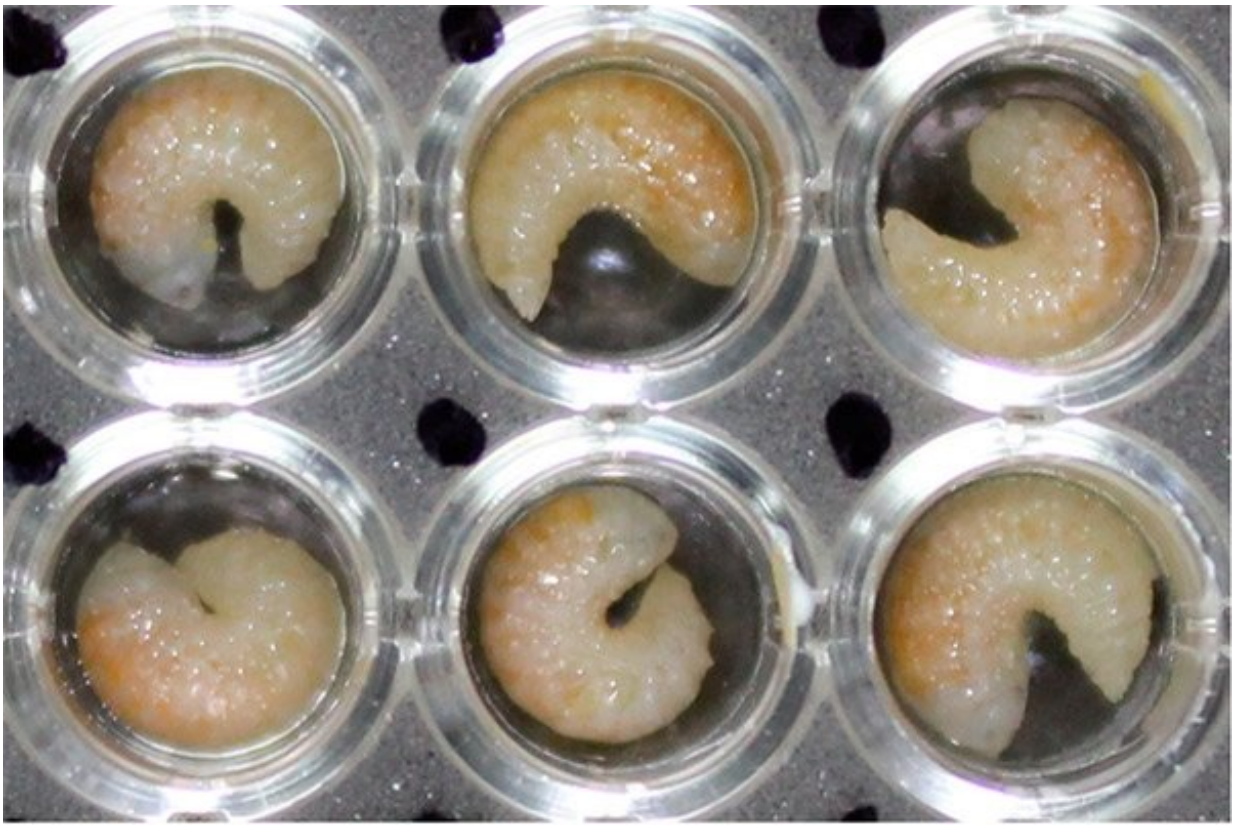
Serratia marcescens bacteria on culture plate. Credit: Eduardo Afonso da Silva Junior

The experiments described in the article involved colonies collected on USP's Ribeirão Preto campus. When the scientists succeeded in collecting the queen, part of the colony was transported to the laboratory, and all the bacteria found on the insects' surfaces and inside their bodies were isolated, characterized and placed in culture medium.

During this process, Silva Junior realized that when S. marcescens was grown in the laboratory, it released a strong aroma that closely resembled the smell of the ants kept in the same lab. "We decided to investigate the volatile compounds produced by this bacterium and discovered the pyrazines, among which there was a molecule not described in the scientific literature," Pupo said.

The researchers used a type of fiber specifically indicated for absorbing the aromatic compounds from the culture plates. Later, the material was analyzed by gas chromatography-mass spectrometry (GC-MS). "We found both pyrazines and bacteria in the ants' poison glands," Pupo said. "We don't know for sure if their synthesis is shared. Maybe the microorganism produces the aromatic compounds and the ants store them in their glands. In future studies, we plan to test techniques to remove the bacteria from the ants and observe whether the compounds continue to be produced." The group also wants to investigate whether similar phenomena can be observed in other ant species. Nothing of the kind has been described in the literature.

The cultivation of fungi in the nest for food or defense seems to be a widespread practice among social insects. According to an article by Brazilian researchers published in 2015 in the journal *Current Biology*, the newborn larvae of Scaptotrigona depilis, a species of stingless bee native to Brazil, feed on filaments of a fungus cultivated in the brood cells. Without this food, few larvae survive to become adults.



Bee larvae reared in the laboratory. Credit: Camila Paludo

This symbiosis was recently studied in greater depth by Pupo's group during Camila Paludo's PhD research. The results were published in *Scientific Reports* in January 2018.

"We know insects can't synthesize hormones, so they must obtain precursor substances from their food," Pupo said. "Our hypothesis was that the fungus supplied a precursor for the molting and pupating hormone required for larvae to complete the metamorphosis into adult bees."

The first step of the investigation consisted of isolating the fungus from

brood cells and characterizing it in the laboratory. The group found it to be a fungus belonging to the genus Zygosaccharomyces. "We aren't sure how this fungus gets into brood cells. The bees lay eggs and then fill the cells with a liquid called larval food. Some three days later, the fungus begins to grow inside the cells," Pupo said.

Using fluorescence microscopy, the researchers found an accumulation of lipids in the fungal cytoplasm from samples grown in vitro as well as samples extracted directly from bee colonies. "Steroids, the precursors of molting hormones, are lipids. Using GC-MS, we found that the predominant compound among the lipids in this fungus was ergosterol," Pupo said.

Via in vitro experiments, the researchers proved that most larvae completed pupal morphogenesis when the larval food was inoculated with the fungus and when only ergosterol was added. "The results were statistically equivalent for these two situations," Pupo explained. "When the larvae received only larval food without the fungus, they failed to reach the adult stage. We, therefore, concluded that ergosterol was in fact being used by the larvae to produce molting hormone, which reinforces the dependency between these bees and the fungus."

The group now plan to investigate whether similar phenomena occur in other species of stingless and stinging bees.

More information: Eduardo A. Silva-Junior et al, Pyrazines from bacteria and ants: convergent chemistry within an ecological niche, *Scientific Reports* (2018). DOI: 10.1038/s41598-018-20953-6

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