

# Surprising Symbiosis: Glassy-Winged Sharpshooter Eats With Friends

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Like a celebrity living on mineral water, the glassy-winged sharpshooter consumes only the dilute sap of woody plants—including grapevines in California, which is feverishly working to prevent the insect's flight into prized vineyards. Now, in a surprising study published in the June 6 issue of *Public Library of Science Biology*, researchers at The Institute for Genomic Research (TIGR), the University of Arizona, and their colleagues have discovered that the sharpshooter's deprivation diet is sneakily supplemented by not one, but two co-dependent bacteria living inside its cells.

Although insect-bacteria symbiosis is common, this is the first genomic analysis of three partners. In the study, a team of scientists led by TIGR microbiologist Jonathan Eisen, now at the University of California, Davis, uncovered an intimate metabolic co-dependency among the glassy-winged sharpshooter (*Homalodisca coagulata*) and two bacteria, *Baumannia cicadellinicola* and *Sulcia muelleri*. The sharpshooter channels the sweets from sap to the bacteria, which in turn feed the insect vitamins, cofactors, and essential amino acids.

“Much as mosquitoes transmit malaria, the sharpshooter transmits plant disease, including Pierce's disease, which threatens vineyards,” Eisen says. “In order to design methods to fight the insect, we've got to understand how it works and its weaknesses. We knew symbionts were doing something for this insect--but until this study, we had no clue what that was.”

In particular, in this case, the threesome came as a surprise. Many insects, such as aphids and cicadas, feed on the sap from a plant’s xylem or phloem, pipes that transport water and food within a plant. These sap-feeders are often known to rely on resident bacteria for a balanced diet – especially the synthesis of the “essential” amino-acids that all animals, including humans, cannot make for themselves. But researchers had assumed that the sharpshooter needed just one bacterial symbiont (in this case, *B. cicadellinicola*), as does the biologically similar aphid.

University of Arizona evolutionary biologist Nancy Moran, who has extensively studied the co-evolution of insects and their resident bacteria, recruited Eisen to the current project. “My initial interest in sharpshooter symbiosis was in the hope that we could find out exactly how xylem can be used as food,” Moran explains. “It’s terribly poor in nutrients.”

In the study, the team first carried out a painstaking forensic type of DNA analysis known as “metagenomics,” in which they sequenced the *B. cicadellinicola*’s genome from material gathered via dissections of hundreds of insects. The scientists were dumbstruck to find no evidence of the biochemical pathways needed to synthesize amino acids. Could the plant be somehow providing amino acids to its insect predator? Unlikely. Was the sharpshooter somehow cranking out its own amino acids? Doubtful. Could there be something else, some other bacteria, adding these essential ingredients?

Yes. Realizing the amino acid pathways might be carried out by other bacteria living inside the insect, the team began picking through their forensic samples of DNA sequences, removing all the sequence reads that matched neither the insect nor its known symbiotic *B. cicadellinicola* bacteria. A large amount of the leftover DNA mapped to another bacterium, *S. muelleri*. Sure enough, when they pooled the bits of sample DNA that came from *S. muelleri*, the team found all the

essential amino acid synthesis pathways.

“When doing this type of forensic metagenomics, some scientists suggest you can just analyze the whole system as one unit—a so-called ‘black-box’ approach--without knowing which piece of DNA came from which organism,” Eisen says. “But this black-box ecology just does not work well. To really understand the system, you’ve got to assign the different bits of DNA to organisms. This study shows why.”

For this particular insect-bacteria trio, genome-based reconstructions of metabolic activity suggest that the two resident bacteria are close neighbors, residing next door within host tissues and feeding each other chemical precursors needed to make nutrients. In the future, Eisen says, the bacteria will likely evolve into organelles of the insect, losing their distinction as bacteria altogether.

Looking ahead, Eisen is continuing to explore the genomes of other animal-bacterial symbioses to understand how such systems originate and work. “Symbiosis is a pervasive strategy in biological systems and we still do not understand the rules of how it works.” “This is why this sharpshooter symbiosis is so important – it has given us a good model system with two co-dependent bacteria rather than just one.”

In addition to TIGR, teams at the University of Arizona’s Department of Ecology and Evolutionary Biology and at the J. Craig Venter Institute’s Joint Technology Center contributed to this work. It was funded by a National Science Foundation grant to Nancy Moran.

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