

How stick insects handle indigestive food

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Young Australian stick insect (*Extatosoma tiaratum*). Credit: MPI f. Chemical Ecology/ M. Shelomi

Plant cell walls are comprised of many complex polymers that require multiple different enzymes to fully break down, such as cellulase to digest cellulose and xylanase to digest xylan. For decades scientists thought only microbes could produce cellulase, until cellulase genes were found in wood-feeding insects. Now, new research from the Max Planck Institute for Chemical Ecology in Jena, Germany, overturns another old

theory. The scientists discovered that stick insects (Phasmatodea) produce cellulases that can handle several types of cell wall polymers equally.

Cellulose as well as xylan and xyloglucan are important components of [plant cell walls](#). All walking sticks (Phasmatodea) inherited multiple copies of cellulase genes, whose enzymes can attack the glucose backbone of cellulose. However, some of these enzymes can also break down the xylose-backbone of xylan, and others the xylose-glucose backbone of xyloglucan. This discovery marks the first known xyloglucanase of any kind to be found in multicellular animals. Such enzymes in animals were previously not thought to exist.

One enzyme, many substrates

Researchers in the Department of Entomology isolated the cellulase genes from seven species of stick insect, including the Australian *Extatosoma tiaratum*, the Vietnamese *Ramulus artemis*, and the Bornean *Aretaon asperrimus*. All express multiple different cellulase enzymes from the glycoside hydrolase family 9 (GH9). Maintaining redundant enzymes does not make sense if all have the same function, so the researchers hypothesized some had lost their function or evolved to do something new.

To test what these enzymes were capable of, the genes were expressed in a stable insect cell line, and the activities of the isolated proteins tested against different plant [cell wall](#) polymers. The results showed that one groups of enzymes were active against cellulose and xylan, and another cellulose and xyloglucan, and several in each group could also degrade glucomannan. These abilities held in all families of stick insects, present in the Vietnamese *Medauroidea extradentata* (Family Phasmatidae), the Madagascan *Sipyloidea sipyilus* (Diapheromeridae), and the Peruvian *Peruphasma schultei* (Peruphasmatidae). The researchers even got

samples of the Californian *Timema cristinae* (Timematidae), considered the sister group to all other Phasmatodea, and found the same enzymes with the same new abilities.

Such multifunctionality is almost unheard of from glycoside hydrolases 9, and xyloglucanases of any family were never discovered in animals before. "If we hadn't tested these enzymes on other substrates besides cellulose, there was no way we could have discovered these functions," said Dr. Matan Shelomi, a postdoctoral fellow at the Max Planck Institute for Chemical Ecology and lead author of the study. "It was good that we did: nobody found these kind of powerful enzymes in an animal before."

A new twist on an old gene family

Most importantly, the [enzyme](#) functions matched the evolutionary relationships between the insects. Xylanase-cellulases from different species were closely related, and the xyloglucanase-cellulases also formed a monophyletic group. Because *T. cristinae* also had these activities, this means an ancestral, insect cellulase gene duplicated into several genes, some of which were then able to evolve new abilities. This happened before the Phasmatodea evolved. Next the researchers are testing other insects related to the stick insects, to see if they have multifunctional cellulases too.

The ability to break down different polymers with the same enzymes means the Phasmatodea gut is unusually efficient. Along with other enzymes such as cellobiases and xylobiases, their guts can fully degrade nearly all the [plant cell](#) wall into its component sugars, using them for nutrition as well as having more access to the easily digested cytoplasm within the cells. This means they can derive more nutrition from the same leafy diet than other herbivores. Theoretically, they could even digest wood. "There is a big community in Germany of people with stick

insects as pets," says Shelomi, "and they report them nibbling on sticks, moss, bark, and even Styrofoam and electric cables... but leaves are still their main food. Maybe their gut can break down wood, but their jaws are better suited for leaves, which probably taste better too."

More information: Matan Shelomi et al. Ancestral gene duplication enabled the evolution of multifunctional cellulases in stick insects (Phasmatodea), *Insect Biochemistry and Molecular Biology* (2016). [DOI: 10.1016/j.ibmb.2016.02.003](https://doi.org/10.1016/j.ibmb.2016.02.003)

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