Not without my microbes

December 19 2012

This shows forest cockchafer larva feeding on a carrot. Cockchafer larvae (grubs) feed underground on the roots of trees during their three- to five-year larval stage. Credit: MPI for Chemical Ecology/Arias Cordero

After metamorphosis European forest cockchafers benefit from the same bacterial symbionts housed during their larval stage.

Apart from the common European cockchafer (*Melolontha melolontha*), the European forest cockchafer (*Melolontha hippocastani*) is the most common species of the Melolontha genus. These insects can damage huge areas of broadleaf trees and conifers in woodlands and on heaths. Cockchafers house microbes in their guts that help them to digest their woody food, such as lignocelluloses and xylans. Scientists of the Max Planck Institute for Chemical Ecology in Jena, Germany, have now performed comprehensive RNA analyses and identified the microbiota of cockchafer larvae feeding on roots and of the adult beetles feeding on leaves. Surprisingly, the guts of adult beetles house the same microbial species that were present in the larval midgut – despite having metamorphosized from larva to beetle. These microbes include clostridia as well as other bacterial species that are as yet unknown. Moreover, only a small percentage of the microbes living in the gut originated from the roots or leaves the larvae or beetles were feeding on. These microbes seem to be characteristic bacterial symbionts with which the forest cockchafer has long been associated.

Metamorphosis is a fascinating process: A caterpillar or larva, feeding on roots below-ground or leaves above-ground (depending on the species), turns into a butterfly or a beetle after pupation and quiescence. The cylindrical bodies of larvae are quite unspectacular in comparison to the colorful and delicate butterflies. It is usually the larvae that cause the most damage and threaten agricultural and silvicultural yields by feeding on plants. Among these herbivores is the European forest cockchafer (*Melolontha hippocastani*), a major pest of trees.

This is the European forest cockchafer (*Melolontha hippocastani*). Credit: MPI for Chemical Ecology/Arias Cordero

During the pupal stage the insects stop feeding completely. A fundamental transformation starts, a radical internal conversion that changes every single larval organ. The tissue and organs of the larva are converted into the new organs of the beetle. Yet the metamorphosis of some insect species is still not completely understood. What happens to the gut microbes that are needed for digesting plant tissues and therefore important for the insect's survival as soon as the larva is transformed? Are there any bacteria present in the gut of the new beetle and if so, which?

PhD candidate Erika Arias-Cordero from Costa Rica addressed these questions. Thanks to modern and sensitive detection methods, she was able to get an overview of the microbial species present in the guts of larvae and adult beetles. In so-called culture-independent studies, more than 300 individual RNA sequence segments were identified that were

assigned to the different taxa of known classes of microbes. Sequences of bacterial ribosomal RNA (16S rRNA) were determined that could be distinguished from insect RNA (18S rRNA). "Using this method, we could be pretty sure we had identified all classes of microbes present in the gut. A typical microbiological approach, for which bacteria from the gut would have to be cultivated first, cannot guarantee this, because we do not know the culture media, especially for microbial species we do not know yet," says the scientist.

A total of nine different classes of bacteria were found in the cockchafer gut: Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, actinobacteria, bacilli, clostridia, erysipelotrichi, negativicutes and sphingobacteria. Some are able to digest lignocelluloses and xylans, typical wood components. Interestingly, many classes of bacteria that were identified in the larval midgut were also found – after metamorphosis – in the gut of the adult cockchafer, even though the larval gut completely empties during the pupal stage. Moreover, Arias-Cordero found that the gut microbiome of the larvae overlaps only minimally with the microbiome of soil and root material. In other words, most microbes present in the larvae and beetles do not originate from the digested food. "This means that the forest cockchafer per se, that is, the larva hatching from the egg, e.g. via secretions passed from the mother, already has a basic set of bacterial symbionts which this insect species has co-evolved with over thousands of years," explains Wilhelm Boland, director at the institute.

This result confirms again the assumption that all higher organisms, such as plants, insects and animals (including humans), are equipped with microbial symbionts. Without these beneficial microbes, we could not live and survive; they must be classified as an integral part of our body.

Larvae and beetles, as well as soil, root and leaf samples, were collected in forests near Mannheim and Iffezheim. The Forstliche Versuchs- und

Forschungsanstalt Baden-Württemberg (Forest Research Center) in Freiburg and the Fritz Lipmann Institute in Jena were also involved in this research project. [JWK/AO]

More information: Arias-Cordero E, Ping L, Reichwald K, Delb H, Platzer M, Boland, W. (2012) Comparative evaluation of the gut microbiota associated with the below- and above-ground life stages (larvae and beetles) of the forest cockchafer, Melolontha hippocastani. *PLoS ONE* 7(12): e51557. DOI:10.1371/journal.pone.0051557 dx.doi.org/10.1371/journal.pone.0051557

Provided by Max Planck Society