

Minireview

Immune function keeps endosymbionts under control

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Abstract

How does an animal host prevent intracellular symbionts getting out of hand? A new paper in *BMC Biology* provides evidence that the mutualism between a beetle and its bacterial endosymbiont could be mediated through the expression of host immune genes.

Endosymbionts, insects and the evolution of mutualism

Mutualistic interactions between symbiotic microbes and animals are common in nature. Most such relationships are based on nutrient cycling [1], although there can be other benefits (for example, symbionts can protect hosts from parasites or pathogens [2]). Such symbionts are particularly common in insects, perhaps because most insects are specialist herbivores and plants are frequently poor-quality food for animals; the essential amino acids and biosynthetic cofactors that the animals lack can be provided by mutualistic microbes. In many cases, the relationship has become so close that the microbial partner (usually a bacterium) lives within cells in the host's body (it is then said to be an endosymbiont), is vertically transmitted from one host generation to another, and is never found in the free-living condition.

The evolution of such mutualistic relationships is, however, a challenge to evolutionary theory (for example [3]). For mutualism to evolve in the first place, both partners must share the interest of a net gain in fitness from their association and, once symbiosis is established, both partners must lose by defecting from cooperation. Interactions such

as those between legumes and rhizobia are a good example of plant hosts that have evolved mechanisms to impose sanctions on defecting bacteria [4]. At a functional level the legume-*Rhizobium* interaction is very similar to the situation of insect-symbiont interactions. The need for insects to keep their endosymbionts under control can be inferred from the observation that (as for rhizobia) in almost all cases an insect's endosymbionts are confined in a special symbiotic tissue. But what happens if the bacteria grow too much, threatening to escape, and how do the bacteria know where they should be?

Mutualists can apply sanctions on defectors by withdrawing cooperation [3]. However, the cost of sanctions is unlikely to be borne equally. In a traditional symbiotic partnership, the insect host would seem to be in a much better position to apply sanctions to the bacteria than vice versa. But we need to be careful about such conclusions, because it is widely supposed that there is a continuum of partnerships between mutualism and parasitism. Endosymbionts such as *Wolbachia*, which are at the parasitic end of this spectrum, can evidently impose fitness costs on their macroscopic partners [5]. In any case, it would be useful to know more about the sanctions that can be applied by insect hosts on their endosymbionts.



Figure 1
Sitophilus zeamais on seeds of corn. Image courtesy of Abdelaziz Heddi.

Grain weevils and their endosymbionts

A new study by Anselme *et al.* in *BMC Biology* [6] now casts light on the way in which the host insect can both monitor bacterial cooperation and apply sanctions to non-cooperators. The paper [6] focuses on the well studied [7] symbiosis between the maize weevil *Sitophilus zeamais* (Figure 1) and its obligate primary endosymbiont (Sitophilus zeamais primary endosymbiont or SZPE), a γ -proteobacterium. *S. zeamais* lives exclusively within the seeds of corn (*Zea mays*). Grain weevils grow poorly in the absence of the vertically transmitted endosymbiont and are known to gain diverse nutritional benefits from their symbiotic bacteria. As is usually the case with insect-endosymbionts, SZPE is found within specialized host cells called bacteriocytes. In larval insects these cells are located in an organ called the bacteriome, an outgrowth of the insect's gut, but during embryogenesis the bacteria must migrate through the hemocele (body cavity) to reach the bacteriome, and in the pupal ('nymphal') and adult stages, the bacteriocytes disperse to distributed locations [8].

In a recent paper about the evolution of mutualism, West *et al.* [3] have emphasized the importance of studying a variety of systems other than the 'usual suspects'. In this respect, the weevil-endosymbiont system has significant potential. Heddi and Nardon [7] have suggested that this interaction could provide a window on the early stages of host-endosymbiont co-evolution. SZPE has a relatively unreduced genome, in which large numbers of transposons have accumulated, indicating that the bacterium has been associated with the weevil host for only a relatively short period of evolutionary time (less than 25 million years) [9]. Nevertheless, the endosymbiont cannot be cultured, consistent with the hypothesis that the process of genome degradation has already begun.

Host recognition of endosymbionts

S. zeamais seems to be an ideal subject in which to investigate how host insects recognize and react to their endosymbionts. In previous studies the authors of [6] had shown that weevils react to experimental injections of non-symbiotic bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by massively increasing whole-body mRNA levels of the weevil homolog of a well-known immune-related gene, peptidoglycan recognition protein 1 (*wPGRP-1*).

Does this same system detect the endosymbiont? The previous work of the group had also shown that high levels of *wPGRP-1* mRNA are continuously present within the bacteriome of normal (symbiotic) weevils. This shows that the insect 'knows' that the symbiont is present in the bacteriome. Because the level of *wPGRP-1* mRNA is low elsewhere in the body during the larval stage, we can infer that only bacteriocytes recognize the endosymbiont's presence, whereas other tissues, not normally in intimate contact with SZPE at this time, do not 'see' these bacteria.

A problem with the work just described, however, was that only one gene related to microbial recognition was examined. Insect immune systems are complex and multiple genes mediate both recognition and antimicrobial responses; in order to understand the mutualism better, it is necessary also to study immune-effector mechanisms that have the potential to act as sanctions, thus contributing to the maintenance of the symbiosis.

Immune reactions to endosymbionts

In their new paper, Anselme *et al.* [6] have now gone much further and studied an extensive suite of immune genes. They have confirmed that larval weevils can recognize the presence of SZPE in the body cavity, and that this leads to the wide expression of an extensive set of typical genes, including several encoding typical antimicrobial peptides (AMPs) and immune-related proteins (Figure 2). This constitutes a sanction on bacteria that 'escape' from the bacteriome.

To identify immune-related genes, the authors [6] used suppressive subtractive hybridization (SSH) to generate an extensive set of expressed sequence tags (ESTs) specific to insects challenged with *E. coli*. Among this set of weevil immune genes, they identified sequences with sequence similarity to known insect AMPs (such as genes encoding peptides similar to coleoptericin, dipterin, acaloletin, cecropin, sarcotoxin, tenecin, and luxuriosin in other insects). These mRNAs are all highly (30-300-fold) up-regulated in whole insect extracts 6 hours after the immune challenge, but are not upregulated in injected controls. Other immune-related genes, including two lysozyme genes

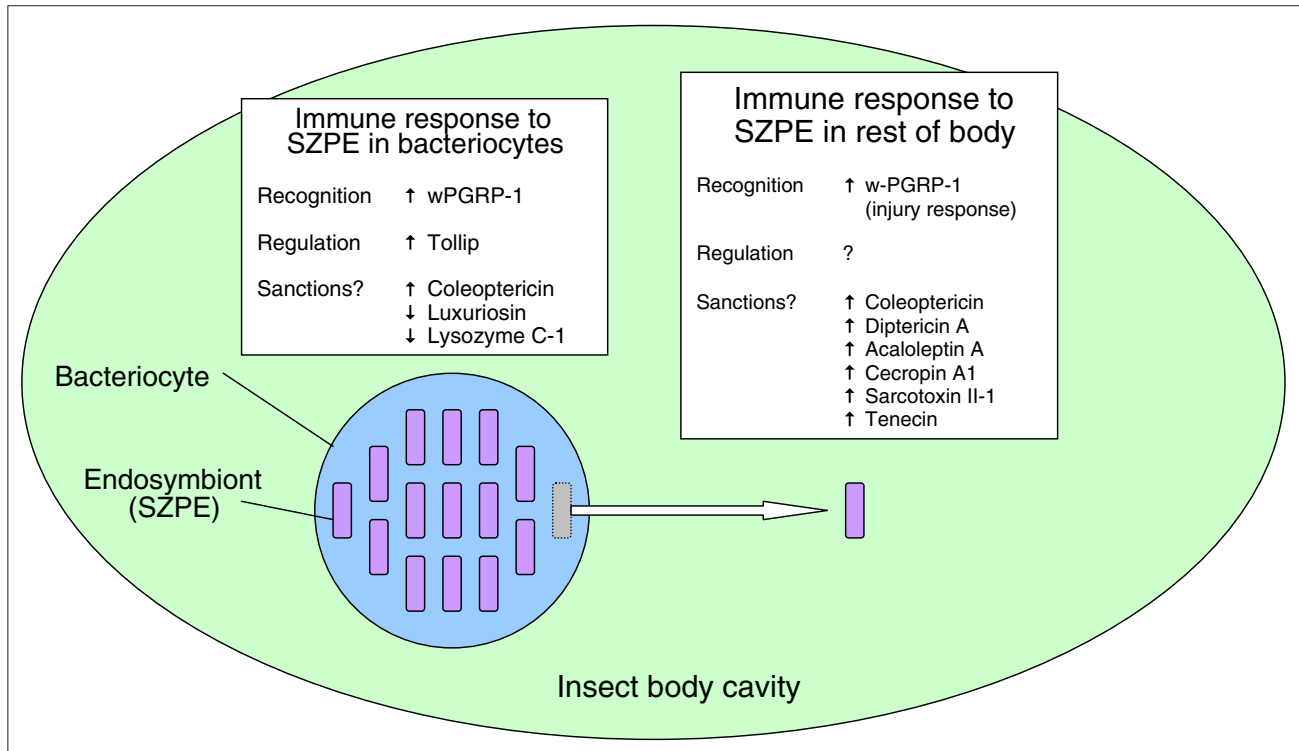


Figure 2
Summary of *Sitophilus* immune responses to its primary endosymbiont SZPE based on [6]. The left box indicates the constitutive response of bacteriocytes to the dense population of endosymbionts within it; the right box indicates the reaction of the rest of the body when bacteria ‘escape’ from the bacteriome into the insect’s hemocele (body cavity). Not all of the genes studied in [6] are listed here.

and two PGRP genes, were upregulated in the body after the immune challenge to a much smaller extent (mostly 10-fold or less), and these were also upregulated by a sterile pinprick, implying that these genes respond to injury rather than to bacteria. Injection of SZPE also caused upregulation of AMPs in much the same way as induced by the same number of *E. coli* cells. The response was similar even when the bacterial cells were heat-killed before injection, showing that the response was not due to microbial proliferation; this indicates that the weevils can recognize some heat-stable component (probably the cell wall) of the endosymbiont, just as in a non-symbiotic bacterium.

Fascinatingly, however, the transcriptional pattern in the bacteriome was quite different. Most of the mRNAs strongly upregulated in response to SZPE in the rest of the body were expressed at only low levels in this tissue. Of the mRNAs examined, only those encoding one AMP (inf-18a, one of two coleopteracin-like peptides), one presumed recognition protein (wPGRP-1), and the presumed immune signaling protein Tollip (homologous to a regulator of the Toll-like immune-signaling pathways of mammals) were expressed

more strongly in bacteriocytes than in the rest of the body of symbiont-free weevils. Other immune-related genes, including the AMP luxuriosin, a different recognition protein (wPGRP-2), and one lysozyme-like gene, were expressed significantly less intensely in the bacteriome. The failure to express most AMPs indicates that sanctions are relaxed inside the bacteriome.

Maintenance of mutualism

What is the functional significance of the insect genes that are expressed at high level in the bacteriome? First, we can infer that the weevil coleopteracin-like AMP has a special role in maintaining symbiosis. Anselme *et al.* [6] point out that coleopteracin’s mRNA includes a signal sequence, indicating probable secretion into extracellular space. It will be interesting to learn whether biologically relevant concentrations of this AMP have adverse effects on the viability of SZPE. In this case it might be hypothesized that the secreted coleopteracin is used as a constitutive local precaution against escape of the endosymbiont from bacteriocytes (in other words, it is a threatened sanction).

Alternatively, coleopteracin might be used as a signal of cooperation rather than a sanction. Nitric oxide is used in just this way in the symbiosis between *Vibrio* bacteria and bioluminescent squid; the cephalopod uses this toxic messenger to indicate the correct location for bacterial colonization [10]. In *Sitophilus*, it is even possible that coleopteracin is used in both ways, depending on the amount secreted. In their natural environments, the antibiotics secreted by free-living microbes can be used as either toxins or signals according to concentration [11].

wPGRP-1, which is highly expressed in bacteriocytes in response to the endosymbiont, seems to be a peptidoglycan recognition protein, but the subsequent response of the weevil to such recognition is unclear. PGRP family proteins in other insects have differing roles that can result in either up- or downregulation of antimicrobial responses (for example [12]). It is possible that bacteriocytes are pre-programmed to tolerate the presence of the symbiont, because w-PGRP-1 is similar to long, intracellular forms of PGRP in other insects (such as PGRP-LB of *Drosophila*), which are enzymatically active in degrading peptidoglycan and which probably serve to limit the extent and duration of immune responses by getting rid of the microbial pattern that triggers them. This may be important in preventing potentially damaging effects of persistent immune activation [13].

The weevil's *tollip* gene, which is also highly expressed in bacteriocytes in the presence of endosymbionts, is particularly interesting. The Tollip protein is a negative regulator of mammalian immune responses mediated by Toll-like receptors [14]. Although there is no convincing *tollip* homolog in *Drosophila*, BLAST searching discovers similar genes to mouse *tollip* in the genomes of several other insects. No functional data are yet available for any insect Tollip-like protein, but it is a testable hypothesis that the function of Tollip in *Sitophilus* is to regulate the immune responses of bacteriocyte cells so as to allow endosymbionts to persist there. It is an indication of such a moderating effect that most AMPs are not expressed in the bacteriome.

It is worth noting, as did Anselme *et al.* [6], that two of the three genes observed to be upregulated in symbiotic weevil bacteriocytes are known to be involved in regulating immune responses in gut tissue. PGRP-LB is expressed in *Drosophila* gut epithelium and has the function of preventing systemic immune activation in response to bacteria in the gut lumen [15]. Tollip is expressed in mammalian gut and may be responsible for the unresponsiveness of these cells to bacteria [16]. The involvement of gut-related immune modulators in the host's response to endosymbionts is consistent with the

derivation of the endosymbiont from a gut bacterium that was already associated with the host.

Mutualism and the evolution of the immune system

Finally, an important speculative implication from this work is worth highlighting. The bacteriome is derived from the gut. The results reported by Anselme *et al.* [6] are consistent with the notion that gut immunity evolved as a means of dealing with saprophytic bacteria (bacteria that live on dead material), as suggested by Hultmark [17], and, by extending this argument, with symbionts. In short, some immune responses might have evolved not as responses to pathogens but to mutualists. The ability to 'manage' symbionts in the gut has recently been invoked to explain the evolution of the vertebrate acquired immune system [18]. The gut flora, and the specialized microbes found in bacteriomes, might well also have played a role in shaping insect immunity. This context makes studies such as the one by Anselme *et al.* [6] very exciting not only as a new important example for understanding the evolution of cooperation (in the sense of West *et al.* [3]) but also as a study system to shed more light on the evolution of immunity.

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