

Microbial Symbionts of Parasitoids

Marcel Dicke, Antonino Cusumano and Erik H. Poelman

Laboratory of Entomology, Wageningen University, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Keywords

Ecology, multitrophic interactions, parasitoid-host interactions, insect-plant interactions, viruses, bacteria, direct interactions, indirect interactions

Abstract

Parasitoids are dependent on other insects for the development of their offspring. Their eggs are laid in or on a host insect that is consumed during juvenile development. Parasitoids harbor a diversity of microbial symbionts including viruses, bacteria and fungi. In contrast to symbionts of herbivorous and haematophagous insects, parasitoid symbionts are not known to provide nutrients. Instead, they are known to be involved in parasitoid reproduction, suppression of host immune responses and manipulation of the behavior of herbivorous hosts. Moreover, recent work shows that parasitoid symbionts such as polydnviruses may also influence plant-mediated interactions among members of plant-associated communities. This implies that these symbionts have a much more extended phenotype than previously thought. This review focuses on the effects of parasitoid symbionts on direct and indirect species interactions and the consequences for community ecology.

Introduction

Insect parasitoids are quantitatively and qualitatively important components of terrestrial ecosystems in terms of biodiversity and ecological impact (35, 49). Most parasitoids are hymenopterans with smaller numbers of dipteran and coleopteran species (40). They lay their eggs on or in other insects that serve as hosts for their offspring. Parasitoids are well-known as members of the third trophic level, but many are members of yet higher trophic levels, exploiting other parasitoids as hosts for their progeny (40, 47). Juvenile endoparasitoids develop in intimate association with their host. They are exposed to their host's physiology and immune system (93). Just like any other animal (39), insect parasitoids host a community of symbiotic microbes (28), including viruses, bacteria and fungi. These symbionts and their effects on parasitoid ecology receive rapidly increasing attention. Parasitoid wasps have evolved various intricate symbiotic associations with viruses, most of which are mutualists (81, 110). Parasitoid-associated viruses are well known to suppress host immunity, thus promoting successful development of the parasitoid in its host (12, 32, 60, 83, 89, 91, 108). However, recent studies have shown that parasitoid symbionts may influence host phenotype more extensively (23). This results in far-reaching ecological effects that extend well beyond interactions between the parasitoid and its host. For instance, upon injection of parasitoid-associated symbionts into their hosts, the microbes may influence interactions between the host and its food plant (113), thus influencing plant phenotype with consequences for plant

immunity (95), interactions of the plant with herbivores (17), their parasitoids (74) as well as hyperparasitoids (74, 113). Thus, parasitoid-associated symbionts influence direct interactions as well as indirect, plant-mediated interactions between organisms associated with the food plant of the parasitoid's host at different trophic levels. This means that microbial symbionts of parasitoids may influence the phenotype of the parasitoid in unprecedented ways, making them an impressive example of the extended phenotype (20).

In this review, we consider “symbiosis” in its original broad sense indicating the intimate association of two dissimilar entities living together (21). In some cases, as for the mutualistic association between polydnaviruses and ichneumonoid wasps, the interaction is so ancient and tight that the symbiont has become part of the host (symploysis) and the viral nature of the symbiont has been questioned (34, 87).

Here, we present the state of the art on microbial symbionts of insect parasitoids in an ecological perspective. We summarize symbiont diversity and transmission patterns. Subsequently, we focus on functions of parasitoid-associated symbionts and the dynamic interplay between parasitoid symbionts and other microbes. Finally, we review the effects of parasitoid-associated symbionts in plant-insect interactions in a multitrophic perspective. Reproductive manipulators such as *Wolbachia* are not extensively covered in this article, because excellent reviews exist already (103, 109). We focus on the effects of parasitoid symbionts on direct and indirect species interactions and the consequences for community ecology.

Symbiont diversity and transmission in parasitoids

Diversity

Symbionts reported in insect parasitoids include viruses, bacteria and a few fungi (5, 37). Especially a great variety of viruses has been reported as symbionts of parasitoid wasps, representing double-stranded DNA viruses (Ascoviridae, Polydnaviridae, Entomopoxviridae), single-stranded RNA viruses (Coronaviridae, Iflaviridae, Rhabdoviridae), and segmented double-stranded RNA viruses (Reoviridae) (5). The vast majority of viral symbionts are polydnaviruses (PDVs) which are associated with about 40,000 species of the hymenopteran superfamily Ichneumonoidea. They form specific obligatory mutualistic associations with parasitoids and are divided into two genera: Bracoviruses (BV) associated with six subfamilies of braconid wasps and Ichnoviruses (IV) associated with two subfamilies of ichneumonoid wasps (19, 24, 30, 31, 92). The life cycle of PDVs is divided over the primary host (the wasp) in which the virus replicates and a secondary host (usually a caterpillar) in which the virus expresses its virulence genes, suppressing the host's immune response to the benefit of the wasp's offspring. The genes responsible for viral replication are integrated in the wasp genome, but they are not packaged in the virion itself. As a consequence, the viral particle cannot replicate when injected into the caterpillar host.

Bacterial symbionts include reproductive manipulators such as *Wolbachia*, *Cardinium*, *Rickettsia*, and *Arsenophonus* (reviewed by 33, 103, 109). Little attention has been given to the general bacterial community of parasitoids. Next-generation sequencing has been used to characterize the microbial community of bacteria present in *Nasonia* species (10), *Asobara tabida* (115) *Megaphragma amalphantanum* (67) and *Eretmocerus mundus* (22). The main bacteria recorded are members of the Proteobacteria and Firmicutes.

Only few fungi associated with parasitoids have been described. The most detailed study relates to a yeast-like organism related to *Candida* species (Saccharomycotina) found in *Comperia merceti* (37, 38, 57).

Localization

Parasitoid symbionts have mainly been described for their presence in ovaries and the venom gland. The ovary of insect parasitoids is well known to host endosymbiotic bacteria, several viruses, Virus-Like Particles (VLPs) and few non-specific unicellular fungi (Figure 1). PDVs are produced in specific cells localized in the calyx region of the ovary (90). The venom gland of hymenopteran parasitoids is involved in regulation of host immune response, host paralysis, host castration, developmental alteration and antimicrobial activity. Venom as source of host immune suppression factors is especially important in parasitoids not associated with PDVs (3, 4, 64). Some viruses, VLPs and very few fungi have been described to be present in venom glands, whereas no bacteria have been reported so far (63).

The microbial composition of the parasitoid gut has been poorly investigated. Metagenomic approaches in insect parasitoids generally have characterized the Operational Taxonomic Units (OTUs) for total individuals, possibly because dissecting the gut of parasitoids (especially from larvae) for microbial analyses is a challenge. Nonetheless, microscopic techniques have been used occasionally to study the bacteria in the gut of adult parasitoids. Like in herbivores, the majority of bacteria in three *Nasonia* species are located in the hindgut. In these parasitoids, γ -proteobacteria are most abundant (10). The bacterial community of *Nasonia* parasitoids is dynamic and diverges as parasitoids develop from larvae to adults in a species-specific manner according to phylogenetic distance between species (10). Gut bacterial composition might play a role in speciation of *Nasonia* (11), but studies on other parasitoids are required to understand how widespread this phenomenon is. Whether gut microbes of adult parasitoids are involved in nutrient acquisition is not known.

Transmission

Parasitoid-associated symbionts can be transmitted vertically and/or horizontally. Transmission of endosymbiotic bacteria (*Wolbachia*) and PDVs represent the best documented cases of vertical transmission in insect parasitoids (91, 109). Horizontal transmission from infected larvae to uninfected larvae via the shared host appears quite common and has been demonstrated for e.g. *Wolbachia* (50), yeast-fungi (37, 38) and viruses (101). Parasitoids may also acquire symbionts from their hosts (15, 42),

which may be enhanced by horizontal transmission of symbionts by herbivores through their food plant (14, 59). Acquisition of viruses from insect hosts followed by endogenization in the wasps may be important for evolution of viral symbiotic associations in insect parasitoids (110). Vertically transmitted symbionts form stable associations with their hosts. Yet, they can be replaced indicating that the symbiosis is dynamic over evolutionary time (73).

Effects of third-trophic level symbionts on parasitoids

Because of the developmental lifestyle of parasitoids, their symbionts can impact not only the parasitoid itself but also the host in which the parasitoid develops as a juvenile. Among microbial symbionts associated with parasitoids, bacteria inducing reproductive manipulations (*Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophonus*) have been intensively investigated (reviewed by 33). Manipulators of parasitoid reproduction may induce cytoplasmic incompatibility, feminization, male killing and parthenogenesis in their associated parasitoids (103, 109). All these manipulations result in an increased number of infected females in the parasitoid population and maximize bacterial transmission.

Interestingly, not only bacteria, but also viruses associated with parasitoids can manipulate wasp reproduction. In *Leptopilina boulardi*, a dsDNA virus called LbFV is capable of vertical and horizontal transmission and manipulates the oviposition behavior of the parasitoid by inducing superparasitism in infected females (101). Superparasitism favors horizontal transmission when uninfected and infected females lay eggs in the same host. As *L. boulardi* is a solitary parasitoid, implying that a host can sustain the development of only a single parasitoid, this behavior is not adaptive for the wasp and can also have negative consequences for population dynamics and inter-specific competition (71). Viruses may also manipulate parasitoid reproduction by inducing sex-ratio distortion. The vertically transmitted RNA virus PpNSRV-1 infects 17-37% of *Pteromalus puparum* populations and can be transmitted to the offspring both by males and females. In females, PpNSRV-1 alters the offspring sex ratio by decreasing the number of daughters without affecting parasitism success. Whether sex-ratio alterations are due to increased female mortality or alteration of the primary sex ratio is not clear. In addition to these ecological costs for the wasp, the virus has positive effects as well because it increases adult longevity of its host wasp (105). This is expected to be beneficial for the virus as it may enhance virus spread in the insect population by infecting more wasps.

Although nutrition has been a selective driving force in the evolution of symbiotic relationships in insect herbivores (26, 27), there are no clear cases of symbiotic relationships that provide nutritional benefits for insect parasitoids. A case of presumed mutualistic relationship in terms of nutrition was originally described for a yeast-like symbiont (a Saccharomycotina species originally described as *Candida* sp.) in *Comperia merceti*, an egg parasitoid of the cockroach *Supella longipalpa* (57). However, more recent investigations failed to reveal any evidence for this presumed nutritional benefit conferred to the wasp.

Instead, fitness costs associated with the yeast were found as infected wasps attacked fewer hosts and had longer development times compared to wasps cured from the yeast (37, 38). Why nutritional mutualistic symbioses have not been reported yet in parasitoids may be the result of the high food quality of parasitoid hosts. The resources used during parasitoid development certainly represent a nutrient-rich diet. Thus, the need for establishing a symbiotic relationship to supplement the regular diet is likely less important in parasitoids compared with insects feeding on plant sap or animal blood, that compensate for the unbalanced diet by establishing mutualistic symbiotic interactions with bacteria (e.g. 2, 26, 27, 62, 85). Yet, it is important to acknowledge that symbionts in carnivorous insects have been understudied.

The best known symbiont-mediated defense in parasitoid wasps results from PDVs which suppress the immune response of the parasitoid's host (usually a caterpillar). Parasitoids that lay eggs in the body of living hosts need to suppress their immune response to successfully develop. The most common host immune response is the encapsulation of parasitoid eggs, a process in which the parasitoid egg is enveloped by a layer of hemocytes leading to its death (32, 83, 89). PDVs have been extensively documented as mutualistic viral symbionts associated with braconids and ichneumonids, protecting parasitoid eggs by preventing encapsulation (91). In addition to PDVs, other parasitoid-associated viruses (ascoviruses, reoviruses, entomopoxviruses and virus-like particles) are known to provide a similar protection. For example, the *Diachasmimorpha longicaudata* entomopoxvirus (DIEPV) occurs in the venom apparatus of female *D. longicaudata* wasps and is introduced into *Anastrepha suspensa* fly larvae during parasitism. The virus replicates both in the wasp and in the fruit fly host where it inhibits encapsulation, thus allowing the successful development of parasitoid offspring (55). An ascovirus (DpAV4) associated with *Diadromus pulchellus* contributes to immunosuppression of the lepidopteran host *Acrolepiopsis assectella*. Complex interactions between ascoviruses and reoviruses (DpRV1) have been suggested to occur in this parasitoid-host system, which are described in detail below (see section on 'Dynamic interactions between parasitoid symbionts and other symbionts'). It is not known why symbiosis between viruses and parasitoid wasps is so widespread but it has been suggested that the antagonistic nature of the interaction between wasps and their insect hosts may have selected for acquisition of insect viruses that were subsequently domesticated to benefit the wasp (110). Especially in the braconid parasitoid wasp subfamily Microgastrinae (in which PDVs are associated with all species), a large diversification of species has occurred after the mutualistic association with PDVs was established. This suggests that the success of the Microgastrinae may be due to the advantages provided by viral symbionts to exploit novel host resources (111).

Whereas protection against host immunity seems a major driving force for establishing mutualistic symbiosis in parasitoids, other forms of symbiont-conferred protection such as defense against natural enemies of parasitoids, such as hyperparasitoids, has never been documented. Hyperparasitoids are top-level carnivores which lay their eggs in or on the body of other parasitoids (94). As common components of terrestrial trophic webs, hyperparasitoids can inflict significant mortality to their parasitoid hosts (41,

74). These fourth-trophic level organisms may exert selective pressure for defenses to evolve in their parasitoid host. However whether parasitoids mount defenses against oviposition by endo-hyperparasitoids, and whether microbial symbionts are involved, has not been explored so far. Even if not strictly considered a form of protection, parasitoid symbionts have also been suggested to mitigate toxicity of pesticides. Bacteria of the genus *Arthrobacter* attenuate susceptibility of whitefly parasitoids to pesticides but further investigations are required to confirm whether these microorganisms are truly mutualistic symbionts (22).

Effects of third trophic level symbionts in parasitized hosts

A fascinating aspect of parasitoid-symbiont ecology is that the symbiont may manipulate the behavior of its parasitoid host (23, 100). In the model system *Dinocampus coccinellae* (hymenopteran parasitoid) and *Coleomegilla maculata* (coccinellid host), the host protects the parasitoid offspring displaying a “zombie-like” paralytic behavior. Interestingly, the behavioral manipulation occurs after the parasitoid larva has egressed from the host. An RNA virus of the parasitoid (*D. coccinellae* paralysis virus, DcPV) that has remained in the host after parasitoid egression, is most likely involved in this process. DcPV particles are located in the oviduct of *D. coccinellae* females and replicate within the parasitoid larvae as well as in their coccinellid hosts. In particular, DcPV replication in the coccinellid’s brain induces neuropile alterations which correlate with the paralytic symptoms typical of the behavioral manipulation. After clearance of the virus, normal coccinellid behavior is restored suggesting that changes in ladybeetle behavior are the result of manipulation by the parasitoid-associated virus rather than by the activity of the parasitoid itself (23).

Other parasitoid-associated symbionts may also manipulate their insect host by infecting the host’s brain. For example, a zombie-like behavior is also displayed by some caterpillars attacked by braconid parasitoids (1, 43, 45, 54). *Pieris brassicae* caterpillars protect their parasitoids (*Cotesia glomerata*) after the parasitoid larvae have egressed from their caterpillar host by spinning a layer of silk over the parasitoid brood and wriggling intensively when enemies of the parasitoids approach the brood (45). Interestingly, *C. glomerata* is also associated with a viral symbiont (CgBV) that is injected in the host. It remains unclear whether CgBV plays a role in protecting the parasitoid pupae. Viral manipulation of insect behavior can even occur in the parasitoid itself, and might be responsible for superparasitism behavior induced by LbFV in infected *L. bouhardi* (58). Transcript levels of the viral gene *ORF13* of LbFV are more abundant in the head of *L. bouhardi* than in the abdomen (58). However, it remains to be investigated whether CgBV and LbFV are responsible for caterpillar manipulation and wasp manipulation, respectively.

Other aspects of parasitoid-host ecology that can be affected by parasitoid symbionts are intra- and inter-specific competitive abilities (46). *Wolbachia* bacteria may negatively influence intraspecific larval

competition in the egg parasitoid *Trichogramma kaykai* when the larvae feed in a host egg, possibly due to longer developmental time and higher mortality of infected wasps (50). In contrast, infection of a Saccharomycotina yeast in the egg parasitoid *C. merceti* does not appear to affect intraspecific competition although the yeast also induces a cost in terms of longer developmental time in infected parasitoids (38). An interesting case of symbiont-mediated interspecific competition has been documented for two congeneric *Leptopilina* parasitoids which naturally coexist in the field (71). Under controlled laboratory conditions, *L. boulardi* outcompeted *L. heterotoma* in the absence of LbFV whereas the parasitoid species coexisted when *L. boulardi* was infected by LbFV. As the viral symbiont induces superparasitism and egg wastage in *L. boulardi*, the resulting reduced host exploitation abilities allow the coexistence of the inferior competitor *L. heterotoma* (71).

Finally, parasitoid-associated symbionts may also promote inter-specific facilitation when a parasitoid species benefits from inter-specific competition (16). This may occur when a parasitoid species that is a superior competitor in larval competition interacts with another species which is better at suppressing host defenses with the aid of a symbiont. Because PDVs play a major role in disrupting host immunity, these parasitoid-associated symbionts may mediate inter-specific facilitation. Although no competitive experiments were carried out, Vinson and Stoltz (104) showed that *Campoletis sonorensis* eggs developed better in the host *Trichoplusia ni* when injected together with *Hyposoter exiguae* PDVs than with *C. sonorensis* PDVs. Interspecific facilitation by *C. glomerata* that benefits the superior competitor *Hyposoter ebeninus* has been demonstrated in multiparasitized *P. brassicae* and *P. rapae* hosts (75) but whether this outcome is mediated by PDVs remains to be investigated.

Dynamic Interactions between parasitoid symbionts and other symbionts

As most of the reported symbiotic associations of microbes with parasitoids are of viral nature, it not surprising that dynamic interactions among multiple microbes often involve viruses. Multiple symbionts may interact both in the adult parasitoid as well as in the parasitized host because parasitoid-associated symbionts are commonly injected into the host by the female wasp together with the eggs. Some of these interactions can be highly complex and obligate for the successful development of the parasitoid larva. Other symbiotic interplays are facultative and may depend on the presence of specific combinations of microbes associated with the parasitoid or with the parasitoid's host.

Complicated interactions among multiple viruses can result in host immunity suppression and allow the development of the parasitoid offspring. The reovirus DpRV1 replicates in the ichneumonid wasp *D. pulchellus*, but it has no apparent impact on the wasp's fitness. This virus is transmitted to pupae of the lepidopteran host *A. assectella* where it does not replicate but still has a subtle effect. In the lepidopteran host, DpRV1 interacts with the associated ascovirus DpAV4 which is naturally co-injected during oviposition by *D. pulchellus* (8). When DpAV4 was experimentally injected into the lepidopteran host,

infection occurred very rapidly leading to early death of the host (7). However, replication of DpAV4 is much slower in natural parasitism events suggesting that DpRV1 may contribute to the development of *D. pulchellus* by regulating the replication of DpAV4 (8). In this process, another RNA virus packaged within DpRV1 particles has been hypothesized to play a role highlighting the complexity of these interactions (80, 86). There may be other cases of multiple interactions between viruses, parasitoids and the parasitoids' hosts, but the complexity of these systems has limited our understanding so far.

Multiple viruses may be present in the same venom gland and co-injected in the parasitoid host without any apparent interaction effect. A rhabdovirus (DIRhV) is commonly detected in the braconid wasp *D. longicaudata* in association with an entomopoxvirus (DIEPV) (56). The viruses are localized in different regions of the venom gland. In the parasitized fruit fly host, the effect of the rhabdovirus is not known whereas the presence of the entomopoxvirus alone is sufficient to induce apoptosis of the host's hemocytes, a component of the host's defense against the parasitoid egg (55).

Co-occurrence of bacterial symbionts may occur too. For example, *Wolbachia* infection inducing cytoplasmic incompatibility (CI) is particularly common in *Drosophila* parasitoid species such as *L. heterotoma* and *Asobara tabida* in which up to three different endosymbionts have been detected (102). This may lead to competition among *Wolbachia* strains with consequences for total bacterial abundance as well as relative abundance of each strain. However, competition among *Wolbachia* strains was not recorded in either parasitoid species suggesting that the cost of infection for the parasitoid is low (65, 66). Having a diverse set of *Wolbachia* strains is important, because losing one of the *Wolbachia* strains may result in exposure of females to CI, a risk that can be particularly high in species prone to *Wolbachia* infection such as *Drosophila* parasitoids.

Because *Wolbachia* infection is widespread in parasitoids and their hosts (109), and parasitoids often inject viruses, interactions between viruses and *Wolbachia* may occur in the parasitized host. This is interesting because *Wolbachia* strains associated with *Drosophila* larvae confer protection against viruses (48, 70, 97). Protection against viral pathogens of *Drosophila* by *Wolbachia* can also be extended to protection against parasitoid symbionts, as recorded for the virus LbFV associated with *L. boulandi*. Interestingly, the results were dependent on the *Wolbachia* strain tested (61). The increase in the encapsulation rate in response to oviposition by LbFV-infected parasitoids suggests that *Wolbachia*-mediated protection conferred by *Wolbachia* strain wAu is induced in the presence of the virus. This effect was not observed for other strains (wMel and wMelPop) known to promote hemolymph melanization which is often required for encapsulation of parasitoid egg (98). Costs and benefits to different *Wolbachia* strains should be investigated to disentangle the specificity of this tripartite interaction.

Many parasitoid-associated viral symbionts negatively impact the parasitoid's host in several ways, including suppression of immune defenses, developmental alterations, disruption of hormone balance, prevention of metamorphosis and inhibition of growth (e.g. 29, 72, 79, 82, 96). As a consequence, the resident microbiome of the parasitized host is likely to be affected after parasitism. How the herbivore's

microbiome changes after parasitism is not known yet, but there is evidence that parasitoid-associated symbionts indirectly affect the way herbivores deal with other microorganisms, including viruses. For example, injection of PDVs from *Cotesia congregata* into *Manduca sexta* caterpillars impairs the immune system, which resulted in an increased susceptibility to *Autographa californica* M nucleopolyhedrovirus (107).

Other indirect species interactions involving microorganisms can result from parasitism events. Stinging behavior of parasitoids may result in secondary pathogenic infections. Opportunistic pathogens may be present on the ovipositor of parasitoids, on the body of parasitoid hosts or in the environment. Especially for parasitoids that attack hosts developing in decaying fruits and vegetables (e.g. *Drosophila* larvae), contamination with opportunistic pathogens may be common. Parasitoids may enhance pathogenic effects of bacteria. When *Listronotus bonariensis* weevils were exposed to *Microctonus hyperodae* parasitoids that were experimentally contaminated with *Serratia marcescens* bacteria, the weevils suffered significantly higher mortality compared with weevils exposed to parasitoids not contaminated with bacteria (51). However, to prevent naturally occurring secondary infections that result in early host mortality and hamper parasitoid development, parasitoids have evolved prophylactic strategies mediated by their venom (63). For example, the venom of *Pimpla hypochondriaca* displays antimicrobial activity against Gram-negative bacteria (18) and antimicrobial peptides have also been identified in the parasitoid *Pteromalus puparum* (84).

The extended phenotype: effects of third-trophic level symbionts on plant-mediated species interactions across multiple trophic levels

The ecological importance of microbial symbiosis in insects is well recognized for herbivore-associated microorganisms in, for example, expansion of herbivore food-plant range, detoxification of plant defensive chemicals by herbivores or protection against natural enemies of herbivores (36, 44, 69). Microorganisms in herbivores thereby affect the strength of trophic relationships and insect community organization (114). Several recent studies have shown that also parasitoid-associated symbionts may directly or indirectly affect multitrophic interactions and community organization.

Injection of parasitoid-associated symbionts such as PDVs into the host during parasitisation may alter herbivore traits as well as plant responses to herbivory and subsequently affect the direction and strength of plant interactions with other organisms (17, 95, 113). Plants may respond differentially to attack by parasitized compared to unparasitized caterpillars and aphids (68, 74, 76, 77, 99, 112). These responses result in altered interactions of the plant with herbivores, parasitoids and hyperparasitoids (17, 53, 74, 76, 77, 95, 112, 113). Direct evidence that these interactions are driven by PDVs and not by the parasitoid larvae comes from manipulative studies in two very different plant-herbivore-parasitoid tritrophic relationships (17, 95, 113). The injection of PDVs of *Microplitis croceipes* (McBV) into *Helicoverpa zea* caterpillars affects tomato plant quality and benefits the performance of parasitoid

larvae growing in caterpillars that feed on the induced plant (95). Injection of *C. glomerata* PDV (CgBV) and the parasitoid's venom, which catalyzes PDV activity, into caterpillars of *P. brassicae*, feeding on cabbage plants affected subsequent colonization of the plant by the Diamondback moth (*Plutella xylostella*) as well as attraction of hyperparasitoid enemies of *Cotesia* (17, 113) (Figure 2). PDVs directly target salivary glands of the caterpillars (9) and in both study systems the PDVs influenced the activity of enzymes in the caterpillar salivary glands. PDV-altered activity of the enzymes glucose oxidase and β -glucosidase may have elicited the plant response to parasitized caterpillars (17, 95, 113). However, a direct induction of plant responses by the PDVs cannot be excluded yet. Transcriptome analysis of *P. brassicae* caterpillar salivary glands revealed the expression of viral genes and thus virus proteins may directly come into contact with damaged plant tissue through the oral secretion of the caterpillar (113). These examples are currently restricted to braconid parasitoids and their bracoviruses. It remains to be explored whether ichneumonid parasitoids and their ichnoviruses, that have a different evolutionary origin than bracoviruses (91), affect host saliva in similar ways.

In addition to qualitative differences in herbivore saliva, PDVs may also affect the damage patterns of herbivores quantitatively and, thereby, affect plant responses to herbivory (17). Both braco- and ichnoviruses may regulate herbivore growth and development time. For example, the bracovirus of the gregarious parasitoid *Cotesia congregata* extends the developmental time of its host *Manduca sexta*, whereas the ichnovirus of the solitary parasitoid *Hyposoter didymator* arrests development of its host *Spodoptera frugiperda* (6, 25). Interestingly, also parasitoids that lack PDVs such as aphid parasitoids and parasitoids from the Encyrtidae family that attack caterpillars are known to affect plant responses to herbivores (68, 99). It is unknown whether these responses are caused by the parasitoid larvae, by microbial symbionts other than PDVs or by virulence factors maternally delivered such as venom toxins. Parasitized herbivores may differentially induce plant gene expression depending on the parasitoid species developing inside the herbivore (77), and to affect primary and secondary plant compounds (68), including the emission of parasitoid-specific parasitized-herbivore-induced plant volatiles (74). Parasitoid symbiotic microbes have been identified to be at least partially responsible for each of these plant phenotypic changes (17, 95, 113). These induced plant responses may mediate a range of interactions in a plant-associated insect community. Some of the interactions may directly benefit the parasitoid, for example PDVs that increase food plant quality benefit parasitoid development (95). However, PDVs may also cause the induction of parasitoid-specific herbivore-induced plant volatiles that attract the enemies of parasitoids, hyperparasitoids (113). These microbially induced interactions are costly to survival of the parasitoid. The plant responses induced by parasitized herbivores may also affect the performance of parasitoids developing in other caterpillars that feed on the same plant (76, 95). The plant responses induced by microbes of members of the third trophic level may also influence plant performance. PDVs of parasitoids associated with one herbivore species may induce plant responses that alter the colonization of the plant by other herbivores (17) that may impact on plant fitness. Moreover, the attraction of hyperparasitoids to plants induced by parasitized caterpillars may

reduce the parasitoid population that the plant recruits to defend itself against herbivores (74, 112, 113). Thus, to understand the complex interaction networks and ecological consequences arising from parasitoid-associated microbial symbionts, it is critical to identify whether the microorganisms are in the driver's seat or are interacting passengers in regulating multitrophic interactions (52, 114). When parasitoid-associated organisms prove to be strong direct drivers of multitrophic interactions, this may have extensive consequences for how we should view indirect defense of plants that involves the attraction of natural enemies of the herbivores (78). Although plants may benefit from attracting natural enemies of herbivores, the third-trophic-level symbionts may incur costs to plants when they redirect the interaction. By manipulating the herbivorous host and food plant responses, the net plant fitness benefit of attracting natural enemies may be reduced by microorganism-induced plant-mediated effects on the subsequent interactions with herbivores, parasitoids and hyperparasitoids. Third-trophic-level symbionts should, thus, be included in our frameworks of community organization as well as trait selection of individual community members.

Future perspectives

Studies of parasitoid symbionts have especially focused on PDVs that appear to be highly specific as obligatory mutualists whose DNA has been incorporated into the wasp genome. These studies have centered around the suppression of host immune response and manipulation of host development, which allow parasitoid offspring to successfully develop (91, 106). Other symbionts, such as *Wolbachia* bacteria (109) or the *Leptopilina boulardi* Filamentous Virus (61), modify their wasp host's reproduction or behavior for their own benefit exclusively. Parasitoid endosymbionts that support their host's nutrition seem to be rare, which is likely due to the carnivorous nature of the parasitoid larvae.

Upon injection into the host, PDVs infect most immune cells (hemocytes) (91). There is ample information that PDVs interfere with different components of their host's immune response, including parasitoid-egg encapsulation and molecular immune pathways (91). Moreover, PDV infections have been recorded in cells of other tissues as well, including gut, nervous system and salivary gland (9). However, most effects of these infections on host phenotype remain to be elucidated. Interestingly, CgBV influences the transcription of genes in the salivary glands of the caterpillar *P. brassicae* and the transcription of defense-related genes in the caterpillar's host plant *Brassica oleracea*, which is mediated by salivary gland secretion (17). Moreover, the caterpillar's salivary glands also influence the emission of volatiles by *B. oleracea* which mediate the attraction of the hyperparasitoid *Lysibia nana* (113). It will be important to investigate the mechanisms underlying PDV effects on host phenotypic traits other than those involved in the host's immune response. Moreover, if suppression of the host's immune response is not successful, the parasitoid egg is encapsulated and dies. However, it remains unclear whether the host still carries PDVs and whether these continue to influence the adult host's phenotype.

PDVs appear to influence not only the phenotype of the parasitoid and its host, but also the phenotype of the host's food plant with consequences for herbivorous insects, parasitoids developing in other herbivores feeding on the same plant and even hyperparasitoids; some of these effects appear to be systemically expressed in the plant (17, 95, 113), thus tremendously extending the phenotype of the virus. The plant-mediated effect of PDVs on hyperparasitoid attraction shows that the PDV-parasitoid association also bears costs because the attracted hyperparasitoids kill the parasitoid offspring. However, when PDVs suppresses plant resistance to the parasitoid's host, as was shown for *M. croceipes* Bracovirus (95), this effect has a positive effect on parasitoid fitness (Figure 3). Thus, to assess the overall effect of a PDV on the fitness of the parasitoid it is associated with, the full range of ecological consequences needs to be investigated. This raises the question what the limits are of the extended phenotype.

Finally, to conclude on the effects of PDV on parasitoid fitness, access to parasitoids with and without functional PDVs or specific PDV genes would be required. To knock down specific PDV genes in a parasitoid, genetic tools such as RNAi may be used because of the inclusion of the virus in the parasitoid's genome (13). For bacterial symbionts such as *Wolbachia*, antibiotic treatment has been successfully used (88).

To date effects of symbionts on parasitoid biology and ecology have focused on effects of individual symbiont species. Although the community of parasitoid-associated symbionts seems to be limited in species numbers and may be spread over different tissues, interaction effects of different symbiont species on parasitoid ecology should be anticipated. Moreover, upon transfer to the parasitoid's host, the symbionts are exposed to a community of host-associated symbionts. It remains to be investigated how individual symbionts are affected by the symbiont community that they are part of. This requires specific manipulative tools. Such tools have been developed for transferring PDV to a parasitoid's host by e.g. extraction of calyx tissue followed by microinjection (17). However, for manipulating symbionts in the parasitoid body, genetic tools may be developed.

In conclusion, parasitoid-associated symbionts may be present in both the parasitoid and its host. The symbionts may influence a diverse array of traits in parasitoids and their hosts, as well as in organisms that parasitoids or their hosts interact with. Although viruses are commonly considered as pathogens interfering with their host's physiology, in parasitoids they are often integrated components of the insect's physiology and many are even integrated in the parasitoid's genome and considered 'good viruses' (81). The extended effects of the symbionts affect a community or organisms that include insects and plants, and their associated symbionts. Thus, a parasitoid-associated community of macroorganisms, each carrying their own microorganisms, seems to be a meta-community. Parasitoids likely represent the most speciose group of animals (35) that are members of intricate communities (78). Parasitoid-associated symbionts further add to the complexity of interactions in such communities. Unravelling these interactions will be an exciting task for the years to come.

References

1. Adamo SA. 2019. Turning your victim into a collaborator: Exploitation of insect behavioural control systems by parasitic manipulators. *Curr. Opin. Insect Sci.*:<https://doi.org/10.1016/j.cois.2019.01.004>
2. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, et al. 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genet.* 32:402-7
3. Asgari S. 2012. Venoms from Endoparasitoids. In *Parasitoid Viruses*, ed. NE Beckage, JM Drezen:217-31. San Diego: Academic Press. Number of 217-31 pp.
4. Asgari S, Rivers DB. 2011. Venom proteins from endoparasitoid wasps and their role in host-parasite interactions. *Annu. Rev. Entomol.* 56:313-35
5. Beckage NE, Drezen JM, eds. 2012. *Parasitoid Viruses: Symbionts and Pathogens*. Amsterdam: Elsevier. 287 pp.
6. Beckage NE, Tan FF, Schleifer KW, Lane RD, Cherubin LL. 1994. Characterization and biological effects of *Cotesia congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. *Arch. Insect Biochem. Physiol.* 26:165-95
7. Bigot Y, Drezen JM, Sizaret PY, Rabouille A, Hamelin MH, Periquet G. 1995. The genome segments of DpRV, a commensal reovirus of the wasp *Diadromus pulchellus* (Hymenoptera). *Virology* 210:109-19
8. Bigot Y, Rabouille A, Doury G, Sizaret PY, Delbost F, et al. 1997. Biological and molecular features of the relationships between *Diadromus pulchellus* ascovirus, a parasitoid hymenopteran wasp (*Diadromus pulchellus*) and its lepidopteran host, *Acrolepiopsis assectella*. *J. Gen. Virol.* 78:1149-63
9. Bitra K, Zhang S, Strand MR. 2011. Transcriptomic profiling of *Microplitis demolitor* bracovirus reveals host, tissue and stage-specific patterns of activity. *J. Gen. Virol.* 92:2060-71
10. Brucker RM, Bordenstein SR. 2012. The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. *Evolution* 66:349-62
11. Brucker RM, Bordenstein SR. 2013. The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science* 341:667-9
12. Burke GR, Strand MR. 2014. Systematic analysis of a wasp parasitism arsenal. *Mol. Ecol.* 23:890-+
13. Burke GR, Thomas SA, Eum JH, Strand MR. 2013. Mutualistic polydnaviruses share essential replication gene functions with pathogenic ancestors. *PLoS Pathog.* 9:e1003348
14. Caspi-Fluger A, Inbar M, Mozes-Daube N, Katzir N, Portnoy V, et al. 2012. Horizontal transmission of the insect symbiont *Rickettsia* is plant-mediated. *Proc. R. Soc. B-Biol. Sci.* 279:1791-6
15. Chiel E, Zchori-Fein E, Inbar M, Gottlieb Y, Adachi-Hagimori T, et al. 2009. Almost there: Transmission routes of bacterial symbionts between trophic levels. *PLoS One* 4:e4767
16. Cusumano A, Peri E, Colazza S. 2016. Interspecific competition/facilitation among insect parasitoids. *Curr. Opin. Insect Sci.* 14:12-6
17. Cusumano A, Zhu F, Volkoff AN, Verbaarschot P, Bloem J, et al. 2018. Parasitic wasp-associated symbiont affects plant-mediated species interactions between herbivores. *Ecol. Lett.* 21:957-67
18. Dani MP, Richards EH, Isaac RE, Edwards JP. 2003. Antibacterial and proteolytic activity in venom from the endoparasitic wasp *Pimpla hypochondriaca* (Hymenoptera : Ichneumonidae). *J. Insect Physiol.* 49:945-54
19. Darboux I, Cusson M, Volkoff A-N. 2019. The dual life of ichnoviruses. *Curr. Opin. Insect Sci.* 32:47-53
20. Dawkins R. 1982. *The Extended Phenotype*. Oxford: Oxford University Press. 307 pp.
21. De Bary A. 1879. *Die Erscheinung der Symbiose*. Strassburg: Verlag K.J. Trubner. 30 pp.
22. del Mar Fernandez M, Meeus I, Billiet A, Van Nieuwerburgh F, Deforce D, et al. 2019. Influence of microbiota in the susceptibility of parasitic wasps to abamectin insecticide: deep sequencing, esterase and toxicity tests. *Pest Manag. Sci.* 75:79-86

23. Dheilly NM, Maure F, Ravallec M, Galinier R, Doyon J, et al. 2015. Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behaviour manipulation. *Proc. R. Soc. B-Biol. Sci.* 282:20142773
24. Doremus T, Darboux I, Cusson M, Ravallec M, Jouan V, et al. 2014. Specificities of ichnoviruses associated with campoplegine wasps: genome, genes and role in host-parasitoid interaction. *Curr. Opin. Insect Sci.* 6:44-51
25. Doremus T, Urbach S, Jouan V, Cousserans F, Ravallec M, et al. 2013. Venom gland extract is not required for successful parasitism in the polydnavirus-associated endoparasitoid *Hyposoter didymator* (Hym. Ichneumonidae) despite the presence of numerous novel and conserved venom proteins. *Insect Biochem. Mol. Biol.* 43:292-307
26. Douglas AE. 2006. Phloem-sap feeding by animals: problems and solutions. *J. Exp. Bot.* 57:747-54
27. Douglas AE. 2009. The microbial dimension in insect nutritional ecology. *Funct. Ecol.* 23:38-47
28. Douglas AE. 2015. Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol.* 60:17-34
29. Dover BA, Davies DH, Strand MR, Gray RS, Keeley LL, Vinson SB. 1987. Ecdysteroid-titer reduction and developmental arrest of last-instar *Heliothis virescens* larvae by calyx fluid from the parasitoid *Campoletis sonorensis*. *J. Insect Physiol.* 33:333-8
30. Drezen JM, Chevignon G, Louis F, Huguet E. 2014. Origin and evolution of symbiotic viruses associated with parasitoid wasps. *Curr. Opin. Insect Sci.* 6:35-43
31. Drezen JM, Leobold M, Bezier A, Huguet E, Volkof AN, Herniou EA. 2017. Endogenous viruses of parasitic wasps: variations on a common theme. *Curr. Opin. Virol.* 25:41-8
32. Edson KM, Vinson SB, Stoltz DB, Summers MD. 1981. Virus in a parasitoid wasp - suppression of the cellular immune-response in the parasitoids host. *Science* 211:582-3
33. Engelstadter J, Hurst GDD. 2009. The ecology and evolution of microbes that manipulate host reproduction. *Annu. Rev. Ecol. Evol. Syst.* 40:127-49
34. Federici BA, Bigot Y. 2003. Origin and evolution of polydnaviruses by symbiogenesis of insect DNA viruses in endoparasitic wasps. *J. Insect Physiol.* 49:419-32
35. Forbes AA, Bagley RK, Beer MA, Hippee AC, Widmayer HA. 2018. Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecol.* 18:21
36. Frago E, Dicke M, Godfray HCJ. 2012. Insect symbionts as hidden players in insect-plant interactions. *Trends Ecol. Evol.* 27:705-11
37. Gibson CM, Hunter MS. 2009. Inherited fungal and bacterial endosymbionts of a parasitic wasp and its cockroach host. *Microb. Ecol.* 57:542-9
38. Gibson CM, Hunter MS. 2009. Negative fitness consequences and transmission dynamics of a heritable fungal symbiont of a parasitic wasp. *Appl. Environ. Microbiol.* 75:3115-9
39. Gilbert SF, Sapp J, Tauber AI. 2012. A symbiotic view of life: we have never been individuals. *Q. Rev. Biol.* 87:325-41
40. Godfray HCJ. 1994. *Parasitoids - Behavioral and evolutionary ecology*. Princeton: Princeton University Press
41. Gomez-Marco F, Urbaneja A, Jaques JA, Rugman-Jones PF, Stouthamer R, Tena A. 2015. Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control? *Biol. Control* 81:111-21
42. Gothama AAA, Sikorowski PP, McLaughlin MR. 1998. Replication of nonoccluded Baculovirus associated with the parasitoid *Microplitis croceipes* (Hymenoptera : Braconidae) in *Heliothis virescens* (Lepidoptera : Noctuidae). *Biol. Control* 12:103-10
43. Grosman AH, Janssen A, de Brito EF, Cordeiro EG, Colares F, et al. 2008. Parasitoid increases survival of its pupae by inducing hosts to fight predators. *PLoS One* 3:e2276
44. Hansen AK, Vorburger C, Moran NA. 2012. Genomic basis of endosymbiont-conferred protection against an insect parasitoid. *Genome Res.* 22:106-14
45. Harvey JA, Kos M, Nakamatsu Y, Tanaka T, Dicke M, et al. 2008. Do parasitized caterpillars protect their parasitoids from hyperparasitoids? A test of the 'usurpation hypothesis'. *Anim. Behav.* 76:701-8

46. Harvey JA, Poelman EH, Tanaka T. 2013. Intrinsic Inter- and Intraspecific Competition in Parasitoid Wasps. *Annu. Rev. Entomol.* 58:333-51
47. Harvey JA, Wagenaar R, Bezemer TM. 2009. Interactions to the fifth trophic level: secondary and tertiary parasitoid wasps show extraordinary efficiency in utilizing host resources. *J. Anim. Ecol.* 78:686-92
48. Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. 2008. Wolbachia and virus protection in insects. *Science* 322:702
49. Heimpel GE, Mills NJ. 2017. *Biological Control: Ecology and Applications*. Cambridge: Cambridge University Press
50. Huigens ME, de Almeida RP, Boons PAH, Luck RF, Stouthamer R. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc. R. Soc. B-Biol. Sci.* 271:509-15
51. Jackson TA, McNeill MR. 1998. Premature death in parasitized *Listronotus bonariensis* adults can be caused by bacteria transmitted by the parasitoid *Microctonus hyperodae*. *Biocontrol Sci. Technol.* 8:389-96
52. Kaplan I. 2012. Trophic complexity and the adaptive value of damage-induced plant volatiles. *PLoS Biol.* 10:e1001437
53. Kaplan I, Carrillo J, Garvey M, Ode PJ. 2016. Indirect plant-parasitoid interactions mediated by changes in herbivore physiology. *Curr. Opin. Insect Sci.* 14:112-9
54. Kester KM, Jackson DM. 1996. When good bugs go bad: Intraguild predation by *Jalysus wickhami* on the parasitoid, *Cotesia congregata*. *Entomol. Exp. Appl.* 81:271-6
55. Lawrence PO. 2005. Morphogenesis and cytopathic effects of the *Diachasmimorpha longicaudata* entomopoxvirus in host haemocytes. *J. Insect Physiol.* 51:221-33
56. Lawrence PO, Akin D. 1990. Virus-like particles from the poison glands of the parasitic wasp *Biosteres longicaudatus* (Hymenoptera, Braconidae). *Can. J. Zool.* 68:539-46
57. Lebeck LM. 1989. Extracellular symbiosis of a yeast-like microorganism within *Comperia merceti* (Hymenoptera, Encyrtidae). *Symbiosis* 7:51-66
58. Lepetit D, Gillet B, Hughes S, Kraaijeveld K, Varaldi J. 2016. Genome sequencing of the behavior manipulating virus LbFV reveals a possible new virus family. *Genome Biol. Evol.* 8:3718-39
59. Li SJ, Ahmed MZ, Lv N, Shi PQ, Wang XM, et al. 2017. Plant-mediated horizontal transmission of *Wolbachia* between whiteflies. *Isme J.* 11:1019-28
60. Lu ZQ, Beck MH, Strand MR. 2010. Egf1.5 is a second phenoloxidase cascade inhibitor encoded by *Microplitis demolitor* bracovirus. *Insect Biochem. Mol. Biol.* 40:497-505
61. Martinez J, Duploux A, Woolfit M, Vavre F, O'Neill SL, Varaldi J. 2012. Influence of the virus LbFV and of *Wolbachia* in a host-parasitoid interaction. *PLoS One* 7:e35081
62. McCutcheon JP, McDonald BR, Moran NA. 2009. Convergent evolution of metabolic roles in bacterial co-symbionts of insects. *Proc. Natl. Acad. Sci. U. S. A.* 106:15394-9
63. Moreau SJM. 2013. "It stings a bit but it cleans well": Venoms of Hymenoptera and their antimicrobial potential. *J. Insect Physiol.* 59:186-204
64. Moreau SJM, Asgari S. 2015. Venom proteins from parasitoid wasps and their biological functions. *Toxins* 7:2385-412
65. Mouton L, Dedeine F, Henri H, Bouletreau M, Profizi N, Vavre F. 2004. Virulence multiple infections and regulation of symbiotic population in the *Wolbachia-Asobara tabida* symbiosis. *Genetics* 168:181-9
66. Mouton L, Henri H, Bouletreau M, Vavre F. 2003. Strain-specific regulation of intracellular *Wolbachia* density in multiply infected insects. *Mol. Ecol.* 12:3459-65
67. Nedoluzhko AV, Sharko FS, Tsygankova SV, Boulygina ES, Sokolov AS, et al. 2017. Metagenomic analysis of microbial community of a parasitoid wasp *Megaphragma amalphantanum*. *Genom. Data* 11:87-8
68. Ode PJ, Harvey JA, Reichelt M, Gershenson J, Gols R. 2016. Differential induction of plant chemical defenses by parasitized and unparasitized herbivores: consequences for reciprocal, multitrophic interactions. *Oikos* 125:1398-407

69. Oliver KM, Smith AH, Russell JA. 2014. Defensive symbiosis in the real world -advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Funct. Ecol.* 28:341-55
70. Osborne SE, Leong YS, O'Neill SL, Johnson KN. 2009. Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog.* 5: e1000656
71. Patot S, Allemand R, Fleury F, Varaldi J. 2012. An inherited virus influences the coexistence of parasitoid species through behaviour manipulation. *Ecol. Lett.* 15:603-10
72. Pennacchio F, Falabella P, Vinson SB. 1998. Regulation of *Heliothis virescens* prothoracic glands by *Cardiochiles nigriceps* polydnavirus. *Arch. Insect Biochem. Physiol.* 38:1-10
73. Pichon A, Bezier A, Urbach S, Aury JM, Jouan V, et al. 2015. Recurrent DNA virus domestication leading to different parasite virulence strategies. *Sci. Adv.* 1:e1501150
74. Poelman EH, Bruinsma M, Zhu F, Weldegergis BT, Boursault AE, et al. 2012. Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. *PLoS Biol.* 10:e1001435
75. Poelman EH, Gols R, Gumovsky AV, Cortesero AM, Dicke M, Harvey JA. 2014. Food plant and herbivore host species affect the outcome of intrinsic competition among parasitoid larvae. *Ecol. Entomol.* 39:693-702
76. Poelman EH, Gols R, Snoeren TAL, Muru D, Smid HM, Dicke M. 2011. Indirect plant-mediated interactions among parasitoid larvae. *Ecol. Lett.* 14:670-6
77. Poelman EH, Zheng SJ, Zhang Z, Heemskerk NM, Cortesero AM, Dicke M. 2011. Parasitoid-specific induction of plant responses to parasitized herbivores affects colonization by subsequent herbivores. *Proc. Natl. Acad. Sci. U. S. A.* 108:19647-52
78. Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE. 1980. Interactions among three trophic levels - influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11:41-65
79. Pruijssers AJ, Falabella P, Eum JH, Pennacchio F, Brown MR, Strand MR. 2009. Infection by a symbiotic polydnavirus induces wasting and inhibits metamorphosis of the moth *Pseudoplusia includens*. *J. Exp. Biol.* 212:2998-3006
80. Renault S, Stasiak K, Federici B, Bigot Y. 2005. Commensal and mutualistic relationships of reoviruses with their parasitoid wasp hosts. *J. Insect Physiol.* 51:137-48
81. Roossinck MJ. 2011. The good viruses: viral mutualistic symbioses. *Nat. Rev. Microbiol.* 9:99-108
82. Shelby KS, Webb BA. 1994. Polydnavirus infection inhibits synthesis of an insect plasma-protein, arylphorin. *J. Gen. Virol.* 75:2285-92
83. Shelby KS, Webb BA. 1999. Polydnavirus-mediated suppression of insect immunity. *J. Insect Physiol.* 45:507-14
84. Shen XJ, Ye GY, Cheng XY, Yu CY, Yao HW, Hu C. 2010. Novel antimicrobial peptides identified from an endoparasitic wasp cDNA library. *J. Pept. Sci.* 16:58-64
85. Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H. 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp APS. *Nature* 407:81-6
86. Stasiak K, Renault S, Federici BA, Bigot Y. 2005. Characteristics of pathogenic and mutualistic relationships of ascoviruses in field populations of parasitoid wasps. *J. Insect Physiol.* 51:103-15
87. Stoltz DB, Whitfield JB. 2009. VIROLOGY Making Nice with Viruses. *Science* 323:884-5
88. Stouthamer R, Luck RF, Hamilton WD. 1990. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc. Natl. Acad. Sci. USA* 87:2424-7
89. Strand MR, Beck MH, Lavine MD, Clark KD. 2006. *Microplitis demolitor* bracovirus inhibits phagocytosis by hemocytes from *Pseudoplusia includens*. *Arch. Insect Biochem. Physiol.* 61:134-45
90. Strand MR, Burke GR. 2013. Polydnavirus-wasp associations: evolution, genome organization, and function. *Curr. Opin. Virol.* 3:587-94
91. Strand MR, Burke GR. 2014. Polydnaviruses: Nature's genetic engineers. *Annual Review of Virology* 1:333-54
92. Strand MR, Burke GR. 2015. Polydnaviruses: From discovery to current insights. *Virology* 479:393-402

93. Strand MR, Pech LL. 1995. Immunological basis for compatibility in parasitoid-host relationships. *Annu. Rev. Entomol.* 40:31-56
94. Sullivan DJ, Volkl W. 1999. Hyperparasitism: Multitrophic ecology and behavior. *Annu. Rev. Entomol.* 44:291-315
95. Tan CW, Peiffer M, Hoover K, Rosa C, Acevedo FE, Felton GW. 2018. Symbiotic polydnavirus of a parasite manipulates caterpillar and plant immunity. *Proc. Natl. Acad. Sci. U. S. A.* 115:5199-204
96. Tanaka T, Agui N, Hiruma K. 1987. The parasitoid *Apanteles kariyai* inhibits pupation of its host, *Pseudaletia separata*, via disruption of prothoracicotropic hormone-release. *Gen. Comp. Endocrinol.* 67:364-74
97. Teixeira L, Ferreira A, Ashburner M. 2008. The bacterial symbiont *Wolbachia* Induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS. Biol.* 6:2753-63
98. Thomas P, Kenny N, Eyles D, Moreira LA, O'Neill SL, Asgari S. 2011. Infection with the wMel and wMelPop strains of *Wolbachia* leads to higher levels of melanization in the hemolymph of *Drosophila melanogaster*, *Drosophila simulans* and *Aedes aegypti*. *Dev. Comp. Immunol.* 35:360-5
99. Vaello T, Sarde SJ, Marcos-Garcia MA, de Boer JG, Pineda A. 2018. Modulation of plant-mediated interactions between herbivores of different feeding guilds: Effects of parasitism and belowground interactions. *Sci Rep* 8:14424
100. van Houte S, Ros VID, van Oers MM. 2013. Walking with insects: molecular mechanisms behind parasitic manipulation of host behaviour. *Mol. Ecol.* 22:3458-75
101. Varaldi J, Fouillet P, Ravallec M, Lopez-Ferber M, Bouletreau M, Fleury F. 2003. Infectious behavior in a parasitoid. *Science* 302:1930
102. Vavre F, Fleury F, Lepetit D, Fouillet P, Bouletreau M. 1999. Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Mol. Biol. Evol.* 16:1711-23
103. Vavre F, Mouton L, Pannebakker BA. 2009. *Drosophila*-parasitoid communities as model systems for host-wolbachia interactions. *Adv. Parasitol.* 70:299-331
104. Vinson SB, Stoltz DB. 1986. Cross-protection experiments with two parasitoid (Hymenoptera, Ichneumonidae) viruses. *Ann. Entomol. Soc. Am.* 79:216-8
105. Wang F, Fang Q, Wang BB, Yan ZC, Hong J, et al. 2017. A novel negative-stranded RNA virus mediates sex ratio in its parasitoid host. *PLoS Pathog.* 13
106. Wang ZZ, Ye XQ, Shi M, Li F, Wang ZH, et al. 2018. Parasitic insect-derived miRNAs modulate host development. *Nat. Commun.* 9:2205
107. Washburn JO, Haas-Stapleton EJ, Tan FF, Beckage NE, Volkman LE. 2000. Co-infection of *Manduca sexta* larvae with polydnavirus from *Cotesia congregata* increases susceptibility to fatal infection by *Autographa californica* M Nucleopolyhedrovirus. *J. Insect Physiol.* 46:179-90
108. Webb BA, Strand MR, Dickey SE, Beck MH, Hilgarth RS, et al. 2006. Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347:160-74
109. Werren JH, Baldo L, Clark ME. 2008. Wolbachia: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6:741-51
110. Whitfield JB, Asgari S. 2003. Virus or not? Phylogenetics of polydnaviruses and their wasp carriers. *J. Insect Physiol.* 49:397-405
111. Whitfield JB, Austin AD, Fernandez-Triana JL. 2018. Systematics, biology, and evolution of microgastrine parasitoid wasps. *Annu. Rev. Entomol.* 63:389-406
112. Zhu F, Broekgaarden C, Weldegergis BT, Harvey JA, Vosman B, et al. 2015. Parasitism overrides herbivore identity allowing hyperparasitoids to locate their parasitoid host using herbivore-induced plant volatiles. *Mol. Ecol.* 24:2886-99
113. Zhu F, Cusumano A, Bloem J, Weldegergis BT, Villela A, et al. 2018. Symbiotic polydnavirus and venom reveal parasitoid to its hyperparasitoids. *Proc. Natl. Acad. Sci. U. S. A.* 115:5205-10
114. Zhu F, Poelman EH, Dicke M. 2014. Insect herbivore- associated organisms affect plant responses to herbivory. *New Phytol.* 204:315-21

115. Zouache K, Voronin D, Tran-Van V, Mavingui P. 2009. Composition of bacterial communities associated with natural and laboratory populations of *Asobara tabida* infected with *Wolbachia*. *Appl. Environ. Microbiol.* 75:3755-64

Figure legends

Figure 1. Diversity of parasitoid-associated symbionts localized in the reproductive tract of adult females. Ovaries of the parasitoid are depicted in grey and venom gland is depicted in green. **(a)** Saccharomycotina yeast associated with the encyrtid parasitoid *Camperia merceti*. This yeast is nonspecific and infects several tissues of the insect including venom gland and eggs. **(b)** *Wolbachia* bacteria depicted as light dots in a DAPI stained egg of the trichogrammatid parasitoid *Trichogramma kaykai*. **(c, d)** Polydnnaviruses (PDVs) are divided into the genera Bracovirus and Ichnovirus. The genome of the virus is integrated in the genome of the wasp and viral particles are produced in calyx cells localized in the ovary. **(c)** Ichnovirus associated with the ichneumonid parasitoid *Hyposoter didymator*. **(d)** Bracovirus associated with the braconid parasitoid *Cotesia glomerata*. **(e)** Virus-like particles (VLPs) are localized either in the ovary or in the venom gland. Unlike PDVs, which deliver virulence genes, VLPs are devoid of DNA and enclose virulence proteins. In the ichneumonid wasp *Venturia canescens*, VLPs are produced in calyx cells. Photo credits - (a): Cara Gibson, (b): Merijn Salverda and Richard Stouthamer, (c-e): Marc Ravallec.

Figure 2. The extended phenotype of PDVs in a plant-insect perspective. **(a):** *Cotesia glomerata* polydnnavirus (CgBV) which is injected by *Cotesia glomerata* into a *Pieris brassicae* caterpillar along with wasp eggs and venom during parasitism. **(b)** PDVs experimentally injected into a caterpillar induce changes in oral secretions (regurgitate, saliva) as well as herbivore physiology (development, feeding rate). **(c)** PDV-induced phenotypic changes in caterpillar affect the subsequent interaction of the caterpillar with its food plant. In response to herbivory by PDV-injected caterpillars, plants downregulate defense-related genes, reduce chemical defenses and alter herbivore-induce plant volatile blends. **(d)** In turn, phenotypic changes in the induced plant (1st trophic level) affect subsequent interactions with insect community members across multiple trophic levels (2nd: herbivores, 3rd: parasitoids and 4th: hyperparasitoids). Photo credits - (ovipositing female: Hans Smid, Reproductive tract: Antonino Cusumano, Polydnnaviruses: Marc Ravallec).

Figure 3. Benefits and Costs of PDVs for the associated parasitoid in interactions with organisms at different trophic levels. In a host-parasitoid perspective, PDVs have a positive effect on parasitoid fitness by suppressing the host immune response. PDVs can also benefit their symbiotic partner by increasing the nutritional quality of the food plant for the parasitized herbivore. Nonetheless, when natural enemies of parasitoids (i.e. hyperparasitoids) exploit changes in herbivore-induce plant volatiles induced by PDV-infected caterpillars to locate their parasitoid victims, this incurs an ecological cost. Thus, the overall net effect of PDVs on parasitoid fitness should be evaluated in a community context.

Figure 1

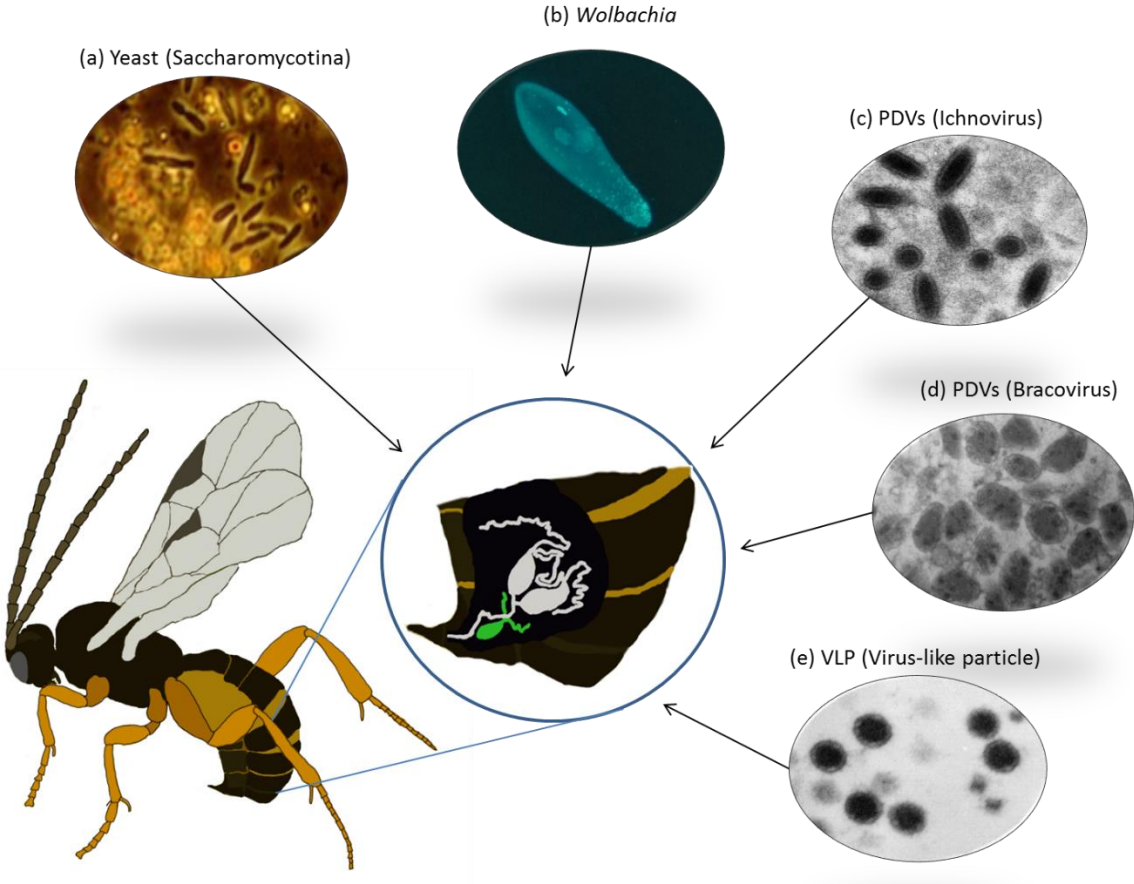


Figure 2

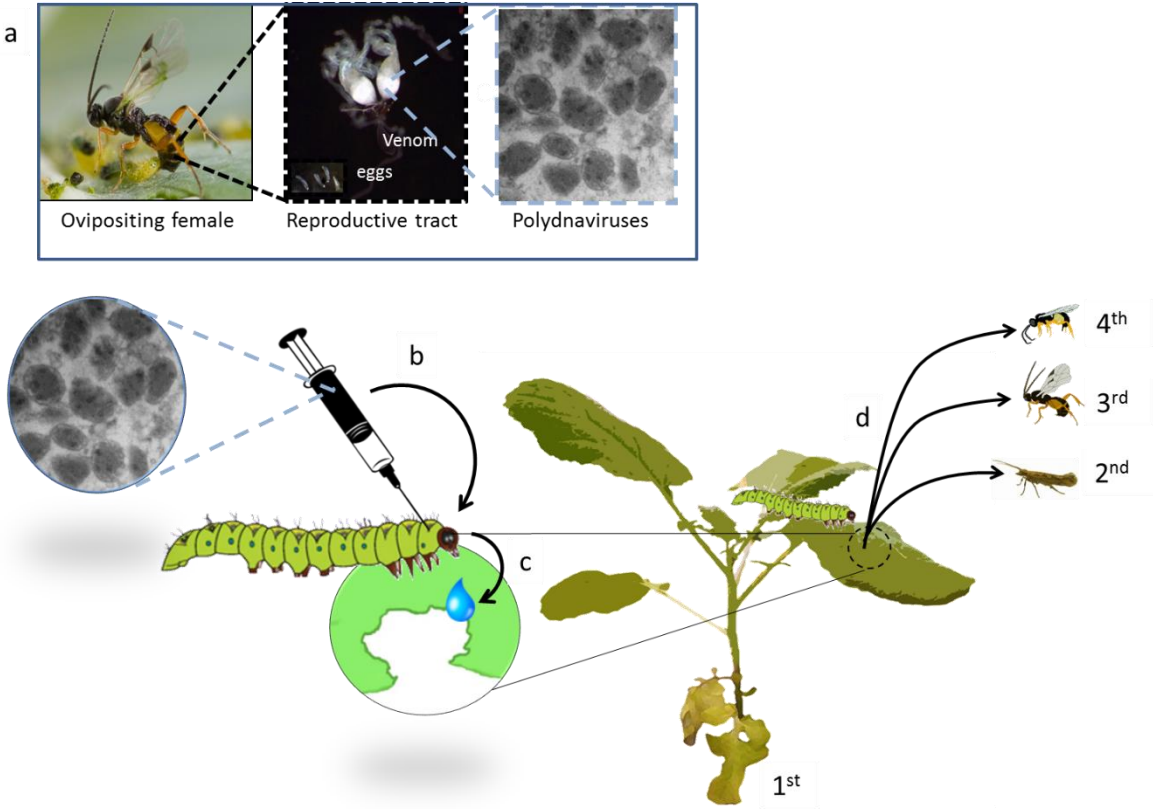


Figure 3

