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Annual Review of Entomology

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<https://doi.org/10.1146/annurev-ento-011019-024939>

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Annual Review of Entomology
**Microbial Symbionts
of Parasitoids**

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Annu. Rev. Entomol. 2020. 65:171–90

First published as a Review in Advance on
October 7, 2019

The *Annual Review of Entomology* is online at
ento.annualreviews.org

<https://doi.org/10.1146/annurev-ento-011019-024939>

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Keywords

ecology, multitrophic interactions, parasitoid–host interactions, insect–plant interactions, viruses, bacteria, direct interactions, indirect interactions

Abstract

Parasitoids depend 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 hematophagous insects, parasitoid symbionts do not provide nutrients. Instead, they are involved in parasitoid reproduction, suppression of host immune responses, and manipulation of the behavior of herbivorous hosts. Moreover, recent research has shown that parasitoid symbionts such as polydnviruses may also influence plant-mediated interactions among members of plant-associated communities at different trophic levels, such as herbivores, parasitoids, and hyperparasitoids. 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 even 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 (96). 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 are attracting rapidly increasing attention. Parasitoid wasps have evolved various intricate symbiotic associations with viruses, most of which are mutualists (84, 114). Parasitoid-associated viruses are well known to suppress host immunity, thus promoting successful development of the parasitoid in its host (12, 32, 61, 86, 92, 94, 112). However, recent studies have shown that parasitoid symbionts may influence host phenotype more extensively (23). This influence 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 (117), thereby influencing the plant phenotype with consequences for plant immunity (98), interactions between the plant and herbivores (17), parasitoids that attack the herbivores (76), and hyperparasitoids that attack the parasitoids (76, 117). 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 use the term symbiosis in its original broad sense to mean the intimate association between two dissimilar entities living together (21). In some cases, such as for the mutualistic association between polydnviruses (PDVs) and ichneumonoid wasps, the interaction is so ancient and tight that the symbiont has become part of the host (syngiogenesis) and the viral nature of the symbiont has been questioned (34, 90).

We present the current state of research on microbial symbionts of insect parasitoids in an ecological perspective. We summarize symbiont diversity and transmission patterns. We then 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 already exist (107, 113). 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). In particular, a wide variety of viruses have been reported as symbionts of parasitoid wasps, representing double-stranded DNA viruses (Ascoviridae, Polydnviridae, Entomopoxviridae), single-stranded RNA viruses (Coronaviridae, Iflaviridae, Rhabdoviridae), and segmented double-stranded RNA viruses (Reoviridae) (5). The vast majority of viral symbionts are PDVs, which are associated with

approximately 40,000 species of the hymenopteran superfamily Ichneumonoidea. They form specific obligate mutualistic associations with parasitoids and are divided into two genera: the bracoviruses (BVs), associated with six subfamilies of braconid wasps, and the ichnoviruses (IVs), associated with two subfamilies of ichneumonid wasps (19, 24, 30, 31, 95). The life cycle of PDVs is divided into a part in the primary host (the wasp), in which the virus replicates, and a part in the 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 into 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 in 33, 107, 113). Little attention has been given to the general bacterial community of parasitoids. Next-generation sequencing and diagnostic PCR have been used to characterize the bacterial community present in *Nasonia* species (10), *Asobara tabida* (119), *Megaphragma amalphantum* (69), the *Eretmocerus* species *Eretmocerus mundus* and *Eretmocerus eremicus*, and *Encarsia formosa* (22). In *Nasonia* species, species of the bacterial genera *Providencia* and *Acinetobacter* were most frequently recorded (10). In *A. tabida*, the genera *Acetobacter*, *Acidomonas*, *Bacillus*, *Brevibacillus*, *Duganella*, *Herbaspirillum*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Rickettsia*, and *Wolbachia* were recorded (119). Interestingly, in the wasp species *M. amalphantum*, no representatives of the *Rickettsia* and *Wolbachia* genera were detected (69). The wasp species *E. mundus*, *E. eremicus*, and *E. formosa* share a group of bacteria belonging to the genera *Staphylococcus*, *Streptococcus*, *Rothia*, and *Arthrobacter*, as well as unclassified members of the Pasteurellaceae family (22). Based on these studies, the main bacteria recorded in parasitoids are members of Proteobacteria and Firmicutes.

Only a few fungi associated with parasitoids have been described. The most detailed study involved a yeast-like organism related to *Candida* species (Saccharomycotina) found in *Comperia merceti* (37, 38, 58). Additional cases of symbiotic associations between parasitoids and unicellular fungi have been reported in older literature for the braconid wasp *Dapsilarthra* (= *Alysia*) *apii* (54) and the ichneumonid wasp *Pimpla turionellae* (64), but the precise nature of these relationships has never been unveiled.

Microbial symbionts of hyperparasitoids have to the best of our knowledge not been reported.

Localization

Parasitoid symbionts have been reported mainly for their presence in ovaries and the venom gland. The ovary of insect parasitoids hosts endosymbiotic bacteria, several viruses, virus-like particles (VLPs), and a few unicellular fungi, which are nonspecific, as they have been described occurring in different tissues (**Figure 1**). PDVs are produced in specific cells localized in the calyx region of the ovary (93). 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 a source of host immune suppression factors is especially important in parasitoids not associated with PDVs (3, 4, 66). Some viruses, VLPs, and very few fungi have been reported in venom glands, whereas no bacteria have been reported so far, although 16S analyses of these organs should be carried out to confirm the lack of bacterial symbionts (65).

The microbial composition of the parasitoid gut has been poorly investigated. Sequencing approaches in insect parasitoids generally have characterized the operational taxonomic units for total individuals, possibly because dissecting the gut of parasitoids (especially from larvae) for microbial analyses is a challenge. Nonetheless, microscopic techniques have occasionally been used to study the gut bacteria of adult parasitoids. As in herbivores, most of the bacteria in three

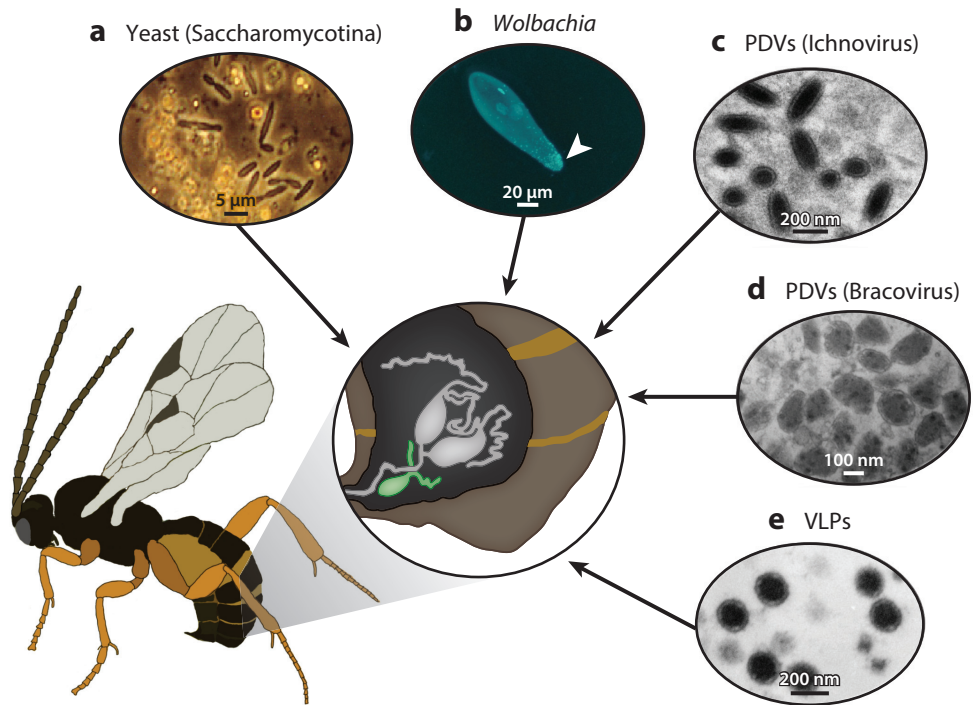


Figure 1

Diversity of parasitoid-associated symbionts localized in the reproductive tract of adult females. (*Middle*) Ovaries of the parasitoid are depicted in gray, and venom gland is depicted in green. (*a*) *Saccharomycotina* yeast associated with the encyrtid parasitoid *Comperia 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 (4',6-diamidino-2-phenylindole)-stained egg of the trichogrammatid parasitoid *Trichogramma kaykai*. (*c,d*) Polydnviruses (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*) The ichnovirus associated with the ichneumonid parasitoid *Hyposoter didymator*; (*d*) The 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. Panel *a* adapted courtesy of Cara Gibson (<http://www.caragibson.com>). Panel *b* adapted courtesy of Merijn Salverda (Wageningen University) and Richard Stouthamer (University of California at Riverside) (104). Panels *c–e* adapted courtesy of Marc Ravallec (DGIMI, INRA, France).

Nasonia species are located in the hindgut. The bacterial community of *Nasonia* parasitoids is dynamic, and it diverges as parasitoids develop from larvae to adults in a species-specific manner according to the phylogenetic distance between species (10). Gut bacterial composition might play a role in speciation of *Nasonia* (11), but studies on other parasitoids will be required in order 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 represents the best-documented case of vertical

transmission in insect parasitoids (94, 113). Horizontal transmission from infected larvae to uninfected larvae via the shared host appears quite common and has been demonstrated for *Wolbachia* (50), fungi (37, 38), and viruses (105), among others. 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, as demonstrated for whiteflies (14, 60). Acquisition of viruses from insect hosts followed by endogenization (i.e., integration into the genome of the wasp species) may be important for evolution of viral symbiotic associations in insect parasitoids (114). Vertically transmitted symbionts form stable associations with their hosts, yet they can be replaced, indicating that the symbiosis is dynamic over evolutionary time (75).

EFFECTS OF THIRD-TROPHIC-LEVEL SYMBIONTS ON PARASITOIDS

Manipulation of Parasitoid Reproduction

Because of the developmental lifestyle of parasitoids, their symbionts can affect 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 in 33). Manipulators of parasitoid reproduction may induce cytoplasmic incompatibility, feminization, male killing, and parthenogenesis in their associated parasitoids (107, 113). All of 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 double-stranded DNA virus named LbFV is capable of vertical and horizontal transmission and manipulates the oviposition behavior of the parasitoid by inducing superparasitism in infected females (105). 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 interspecific competition (73). 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 by both 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 in that it increases the adult longevity of its host wasp (109). This effect is expected to be beneficial for the virus because it may enhance virus spread in the insect population by infecting more wasps.

No Evidence of Nutritional Benefits for Parasitoids

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 a presumed mutualistic relationship in terms of nutrition was originally described for a yeast-like symbiont (a Saccharomycotina species originally described as *Candida* sp.) in *C. merceti*, an egg parasitoid of the cockroach *Supella longipalpa* (58). However, more recent investigations failed to reveal any evidence for this presumed

nutritional benefit conferred to the wasp. Instead, the fitness costs associated with the yeast were that the infected wasps attacked fewer hosts and had longer development time compared with wasps cured from the yeast (37, 38). Why nutritional mutualistic symbioses have not yet been reported in parasitoids may be due to the nature of parasitoid–host relationships in which the parasitoids are provided with sufficiently high-quality food by the host; thus, the need to establish a symbiotic relationship to supplement the regular diet is likely less important in parasitoids compared with insects feeding on plant sap or animal blood, which typically provide an unbalanced diet (e.g., 2, 26, 27, 63, 88). We note, however, that symbionts in carnivorous insects have been understudied.

Survival of Parasitoid Offspring

The best-known symbiont-mediated defense in parasitoid wasps results from PDVs that 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 develop successfully. 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, 86, 92). PDVs have been extensively documented as mutualistic viral symbionts associated with braconids and ichneumonids, protecting parasitoid eggs by preventing encapsulation (94). In addition to PDVs, other parasitoid-associated viruses (ascoviruses, reoviruses, entomopoxviruses) and VLPs provide 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 (56). An ascovirus associated with *Diadromus pulchellus* (DpAV4) contributes to immunosuppression of the lepidopteran host, *Acrolepiopsis assectella*. Complex interactions between ascoviruses and reoviruses (DpRV1) may occur in this parasitoid–host system and are described in detail below (see the section titled Dynamic Interactions Among Symbionts in Parasitoids and Their Hosts). 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 (114). In particular, in the braconid parasitoid wasp subfamily Microgastrinae (in which PDVs are associated with all species), significant diversification of species occurred after the mutualistic association with PDVs was established. This finding suggests that the success of the Microgastrinae may be due to the advantages provided by viral symbionts to exploit novel host resources (115).

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 (e.g., hyperparasitoids), have not yet been documented. Hyperparasitoids are top-level carnivores that lay their eggs in or on the body of other parasitoids (97). As common components of terrestrial trophic webs, hyperparasitoids can inflict significant mortality on their parasitoid hosts (41, 76). 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 endohyperparasitoids, and whether microbial symbionts are involved, has not yet been explored. Even if not strictly considered a form of protection, parasitoid symbionts have been suggested to mitigate the toxicity of pesticides. Bacteria of the genus *Arthrobacter* attenuate the 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

Manipulating Host Behavior

A fascinating aspect of parasitoid–symbiont ecology is that the symbiont may manipulate the behavior of the parasitoid's host (23, 103). In the model system comprising *Dinocampus coccinellae* (hymenopteran parasitoid) and *Coleomegilla maculata* (coccinellid host), the host displays zombie-like paralytic behavior, which protects the parasitoid offspring. Interestingly, the behavioral manipulation occurs after the parasitoid larva has egressed from the host. An RNA virus of the parasitoid *D. coccinellae*, *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 that correlate with the paralytic symptoms typical of the behavioral manipulation. After clearance of the virus, normal coccinellid behavior is restored, suggesting that changes in lady beetle 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, zombie-like behavior is displayed by some caterpillars attacked by braconid parasitoids (1, 43, 45, 55). *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 into the host. Whether CgBV plays a role in protecting the parasitoid pupae is unclear. 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. boucardi* (59) that was presented in the section titled Effects of Third-Trophic-Level Symbionts on Parasitoids. Transcript levels of the viral gene *ORF13* of LbFV are more abundant in the head of *L. boucardi* than in the abdomen (59). However, whether CgBV and LbFV are responsible for caterpillar manipulation and wasp manipulation, respectively, has yet to be investigated.

Modifying Competitive Abilities

Other aspects of parasitoid–host ecology that can be affected by parasitoid symbionts are intra- and interspecific 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 the 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 that naturally coexist in the field (73). Under controlled laboratory conditions, *L. boucardi* outcompeted *Leptopilina heterotoma* in the absence of LbFV, whereas the parasitoid species coexisted when *L. boucardi* was infected by LbFV. As the viral symbiont induces superparasitism and egg wastage in *L. boucardi*,

the resulting reduced host exploitation abilities allow the coexistence of the inferior competitor *L. heterotoma* (73).

Promoting Interspecific Parasitoid Facilitation

Finally, parasitoid-associated symbionts may also promote interspecific facilitation when a parasitoid species benefits from interspecific competition (16). This may occur when a parasitoid species that is a superior competitor in larval competition interacts with another species that 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 interspecific facilitation. Although no competitive experiments were carried out, Vinson & Stoltz (108) 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 *Pieris rapae* hosts (77) but whether this outcome is mediated by PDVs remains to be investigated.

DYNAMIC INTERACTIONS AMONG SYMBIONTS IN PARASITOIDS AND THEIR HOSTS

As case studies of symbiotic associations of microbes with parasitoids have so far been mostly of a viral nature, studies of the dynamic interactions among multiple microbes often involve viruses. Multiple symbionts may interact in both the adult parasitoid and the parasitized host because parasitoid-associated symbionts are commonly injected into the host by the female wasp together with 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.

Interactions Among Viruses

Complicated interactions among multiple viruses can result in host immunity suppression and allow for 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 coinjected 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). Another RNA virus packaged within DpRV1 particles has been hypothesized to play a role in this process, highlighting the complexity of these interactions (83, 89). There may be other cases of multiple interactions among 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 coinjected 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) (57). 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 (56).

Interactions Among Bacteria

Co-occurrence of bacterial symbionts may occur, too. For example, *Wolbachia* infection inducing cytoplasmic incompatibility is particularly common in *Drosophila* parasitoid species such as *L. heterotoma* and *A. tabida*, in which up to three different endosymbionts have been detected (106). Co-occurrence 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 (67, 68), although caution is needed because the cost to the host and competition among symbionts are not necessarily causally linked. A diverse set of *Wolbachia* strains is important because loss of one of the *Wolbachia* strains may result in exposure of females to cytoplasmic incompatibility, a risk that can be particularly high in species prone to *Wolbachia* infection, such as *Drosophila* parasitoids.

Interactions Among Bacteria and Viruses

Because *Wolbachia* infection is widespread in parasitoids and their hosts (113) and parasitoids often inject viruses, interactions between viruses and *Wolbachia* may occur in the parasitized host. Such interactions are interesting because *Wolbachia* strains associated with *Drosophila* larvae confer protection against viruses (48, 72, 100). 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 (62). The increase in 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) that promote hemolymph melanization, which is often required for encapsulation of the parasitoid egg (101). The costs and benefits to different *Wolbachia* strains should be investigated to determine the specificity of this tripartite interaction.

Symbiont Effects on Resident Microbiome of the Parasitoid's Host

Many parasitoid-associated viral symbionts negatively influence 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, 74, 82, 85, 99). As a consequence, the resident microbiome of the parasitized host is likely to be affected after parasitism. The parasitoid *Habrobracon bebetor* injects a paralytic venom in *Galleria mellonella* host larvae that alters the host midgut microbiota by shifting the community composition from a predominance of enterococci to enterobacteria (80). How the herbivore's microbiome changes after injection of parasitoid-associated symbionts is not yet known, but there is evidence that these 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 (111).

Effects on Secondary Pathogenic Infections

Other indirect species interactions involving microorganisms can result from parasitism events. The 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. For parasitoids that attack hosts developing in decaying fruits and vegetables (e.g., *Drosophila* larvae) in particular, contamination with opportunistic pathogens may be common. Parasitoids may enhance the pathogenic effects of bacteria and fungi. 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 the bacteria (51). When *G. mellonella* moth larvae were exposed to envenomation by the parasitoid *H. hebetor*, they were more susceptible to developing fungal infections caused by *Beauveria bassiana* (80). However, these parasitoids have evolved prophylactic strategies mediated by their venom, which can prevent naturally occurring secondary infections that result in early host mortality and hamper parasitoid development (65). For example, the venom of *Pimpla hypochondriaca* exerts antimicrobial activity against gram-negative bacteria (18), and antimicrobial peptides have also been identified in the parasitoid *Pteromalus puparum* (87). It is not known whether such antimicrobial compounds are also present in the venom of parasitoids associated with hosts known to harbor important nutritional and protective symbionts such as aphids or whiteflies.

THE EXTENDED PHENOTYPE: EFFECTS OF THIRD-TROPHIC-LEVEL SYMBIONTS ON PLANT-MEDIATED MULTITROPHIC INTERACTIONS

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, and protection against natural enemies of herbivores (36, 44, 71). Microorganisms in herbivores thus affect the strength of trophic relationships and insect community organization (118). Several recent studies have shown that parasitoid-associated symbionts may also directly or indirectly affect multitrophic interactions and community organization.

Injection of parasitoid-associated symbionts such as PDVs into the host during parasitization may alter herbivore traits as well as plant responses to herbivory and may subsequently affect the direction and strength of plant interactions with other organisms (17, 98, 117). Plants may respond differentially to attack by parasitized versus unparasitized caterpillars and aphids (70, 76, 78, 79, 102, 116). These responses result in altered interactions of the plant with herbivores, parasitoids, and hyperparasitoids (17, 53, 76, 78, 79, 98, 116, 117). Direct evidence that these interactions are caused by PDVs and not by the parasitoid larvae comes from manipulative studies in two very different plant–herbivore–parasitoid tritrophic relationships (17, 98, 117). 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 (98). Injection of *C. glomerata* PDV (CgBV) and the parasitoid's venom, which catalyzes PDV activity, into caterpillars of *P. brassicae* feeding on cabbage plants affects subsequent colonization of the plant by the diamondback moth (*Plutella xylostella*) as well as attraction of hyperparasitoid enemies of *Cotesia* (Figure 2) (17, 117). PDVs directly target the 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

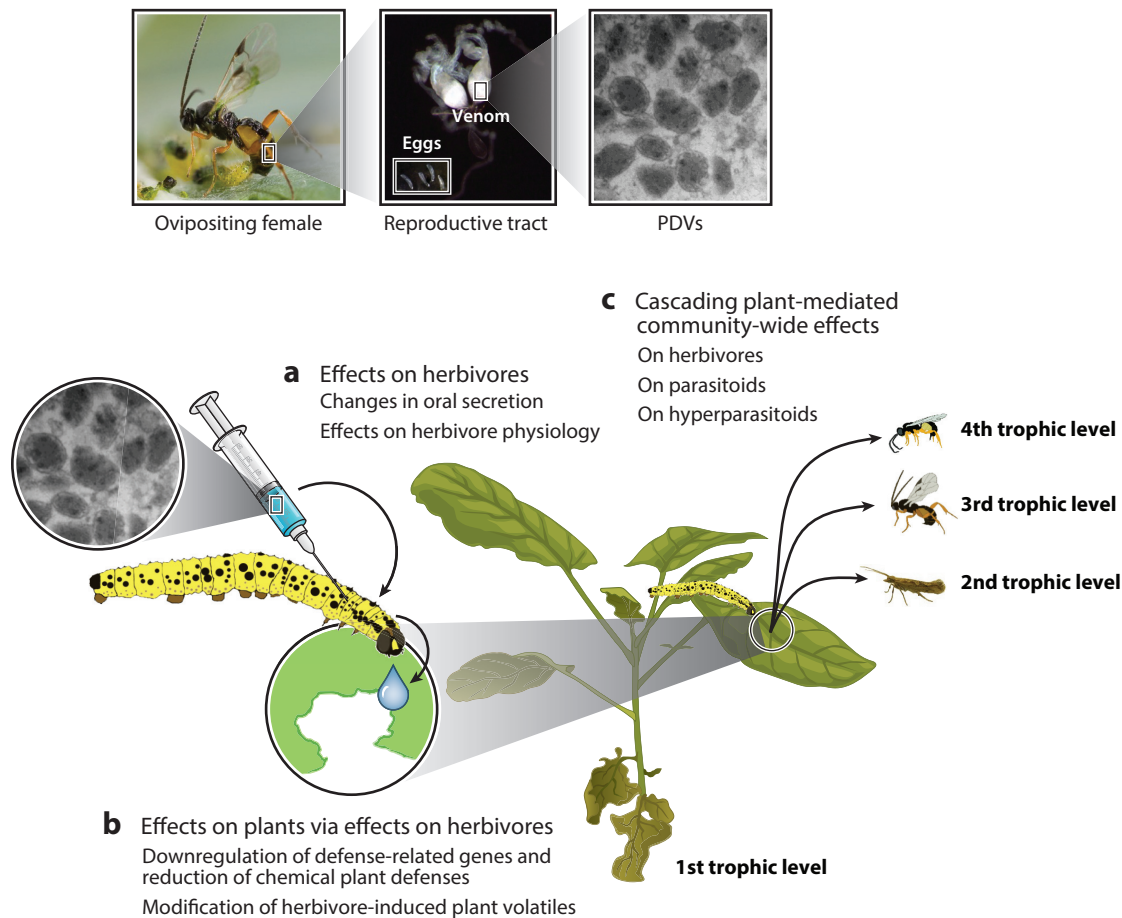


Figure 2

The extended phenotype of polydnnaviruses (PDVs) from a plant-insect perspective. *Cotesia glomerata* polydnnavirus (CgBV) is injected by *C. glomerata* into a *Pieris brassicae* caterpillar along with wasp eggs and venom during parasitism. (a) PDVs experimentally injected into a caterpillar induce changes in oral secretions (regurgitate, saliva) as well as herbivore physiology (development, feeding rate). (b) PDV-induced phenotypic changes in the caterpillar affect the subsequent interaction between the caterpillar and its food plant. In response to herbivory by PDV-injected caterpillars, plants downregulate defense-related genes, reduce chemical defenses, and alter herbivore-induced plant volatile blends. (c) In turn, phenotypic changes in the induced plant (first trophic level) affect subsequent interactions with insect community members across multiple trophic levels (second, herbivores; third, parasitoids; fourth, hyperparasitoids). Images at the top of the figure adapted courtesy of (from left to right) Hans Smid (Wageningen University, The Netherlands), Antonino Cusumano, and Marc Ravallec (DGIMI, INRA, France).

response to parasitized caterpillars (17, 98, 117). However, direct induction of plant responses by the PDVs cannot yet be excluded. A transcriptome analysis of *P. brassicae* caterpillar salivary glands revealed the expression of viral genes; thus, viral proteins may come into direct contact with damaged plant tissue through the oral secretions of the caterpillar (117). These examples are currently restricted to braconid parasitoids and their BVs. It remains to be determined whether ichneumonid parasitoids and their IVs, which have a different evolutionary origin than BVs (94), affect host saliva in similar ways.

In addition to their qualitative effects on herbivore saliva, PDVs may affect the damage patterns of herbivores quantitatively and, thereby, influence plant responses to herbivory (17). Both BVs

and IVs may regulate herbivore growth and development time. For example, the BV of the gregarious parasitoid *Cotesia congregata* extends the developmental time of its host, *M. sexta*, whereas the IV of the solitary parasitoid *Hyposoter didymator* arrests development of its host, *Spodoptera frugiperda* (6, 25). Interestingly, parasitoids that lack PDVs, such as aphid parasitoids and parasitoids from the Encyrtidae family that attack caterpillars, also affect plant responses to herbivores (70, 102). It is unknown whether these responses are caused by the parasitoid larvae, by microbial symbionts other than PDVs, or by maternally delivered virulence factors such as venom toxins.

Emerging studies of the effects of parasitized versus unparasitized herbivores on multitrophic interactions suggest that parasitoid symbionts have extensive ecological consequences. Parasitized herbivores may differentially induce plant gene expression in a parasitoid species-specific manner (79), and may affect primary and secondary plant compounds (70), including the emission of parasitoid-specific parasitized-herbivore-induced plant volatiles (76). Parasitoid symbiotic microbes are at least partly responsible for each of these plant phenotypic changes (17, 98, 117). These induced plant responses mediate a range of interactions in a plant-associated insect community. Some of the interactions directly benefit the parasitoid; for example, PDVs that increase food-plant quality benefit parasitoid development (98). However, PDVs can also cause the induction of parasitoid-specific herbivore-induced plant volatiles that attract the enemies of parasitoids, namely hyperparasitoids (117). These microbially induced interactions are costly to the survival of the parasitoid. The plant responses induced by parasitized herbivores can affect the performance of parasitoids developing in other caterpillars that feed on the same plant (78, 98). 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 affect 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 (76, 116, 117). The emerging view on the intriguing effects of parasitoid symbionts on various aspects of plant-herbivore-parasitoid interactions should lead to intensification of investigations of these interactions. To understand the complex interaction networks and ecological consequences arising from parasitoid-associated microbial symbionts, it is critical to determine whether the microorganisms are the drivers regulating these multitrophic interactions (52, 118). If so, this has extensive consequences for how we should view indirect defense of plants that involves the attraction of natural enemies of the herbivores (81). Although plants may benefit from attracting natural enemies of herbivores, 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 subsequent interactions with herbivores, parasitoids, and hyperparasitoids. Third-trophic-level symbionts should therefore be included in our frameworks of community organization as well as of trait selection of individual community members.

FUTURE PERSPECTIVES

Studies of parasitoid symbionts have 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 develop successfully (94, 110). Other symbionts, such as *Wolbachia* bacteria (113) or the virus LbFV (62), modify their wasp host's reproduction or

behavior exclusively for their own benefit. Parasitoid endosymbionts that support their host's nutrition seem to be rare, likely because of the carnivorous nature of the parasitoid larvae.

Upon injection into the host, PDVs infect most immune cells (hemocytes) (94). There is ample evidence that PDVs interfere with different components of their host's immune response, including parasitoid egg encapsulation and molecular immune pathways (94). 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 influence the emission of volatiles by *B. oleracea*, which mediate the attraction of the hyperparasitoid *Lysibia nana* (117). It will be important for future studies to investigate the mechanisms underlying PDV effects on host phenotypic traits other than those involved in the host's immune response. If suppression of the host's immune response by PDVs 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, which has 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, 98, 117), 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 suppress plant resistance to the parasitoid's host, as was shown for McBV (98), this has a positive effect on parasitoid fitness (**Figure 3**). Thus, the full range of ecological consequences needs to be investigated in order to assess the overall effect of a PDV on the fitness of the parasitoid with which it is associated, raising the question of what the limits of the extended phenotype are. Such studies are likely to fundamentally influence our understanding of multitrophic interactions within a plant-associated insect community.

Finally, generating access to parasitoids with and without functional PDVs or specific PDV genes will be required to identify the effects of PDVs on parasitoid fitness, as well as the underlying mechanisms. Genetic tools such as RNA interference may be used to knock down specific PDV genes in a parasitoid because the virus is included in the parasitoid's genome (13). For bacterial symbionts such as *Wolbachia*, antibiotic treatments have been successfully used (91).

To date, studies of symbionts' effects on parasitoid biology and ecology have focused on the effects of individual symbiont species. Although the community of parasitoid-associated symbionts seems to be limited in the number of species 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. How individual symbionts are affected by the symbiont community that they are part of remains to be investigated. Doing so will require specific manipulative tools. Such tools have been developed for transferring PDV to a parasitoid's host by, for instance, extraction of calyx tissue followed by microinjection (17). However, to manipulate symbionts in the parasitoid body, genetic tools need to be developed.

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

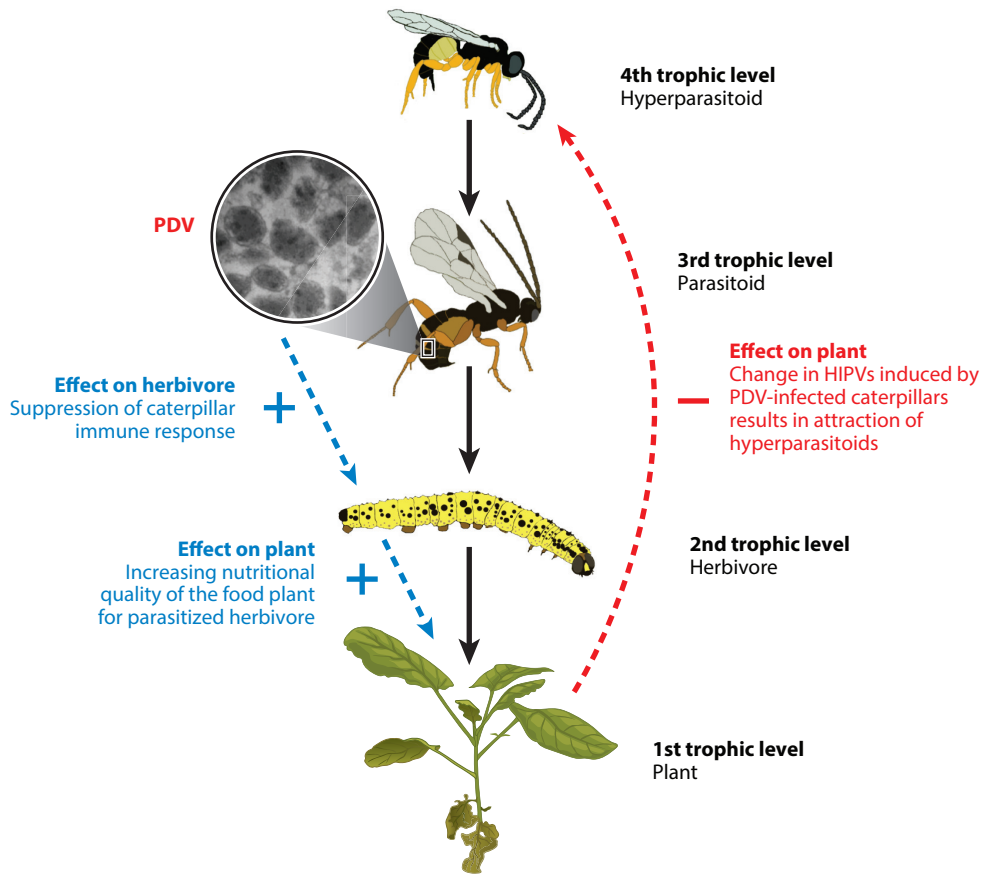


Figure 3

Benefits and costs of polydnnaviruses (PDVs) for the associated parasitoid in interactions with organisms at different trophic levels. From the 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-induced plant volatiles induced by PDV-infected caterpillars to locate their parasitoid victims, this incurs an ecological cost. Thus, the net effect of PDVs on parasitoid fitness should be evaluated in a community context. Solid lines represent trophic interactions, and dashed lines represent ecological effects of parasitoid-associated virus. Abbreviation: HIPV, herbivore-induced plant volatiles.

components of the insect’s physiology and many are even integrated in the parasitoid’s genome and considered “good” viruses (84). The extended effects of the symbionts influence a community or organisms that include insects and plants, as well as their associated symbionts. Thus, a parasitoid-associated community of macroorganisms, each carrying its own microorganisms, seems to be a meta-community. In this context, it is important to note that microbial symbionts of hyperparasitoids have not been reported, possibly because of a lack of focused studies on this. Parasitoids likely represent the most speciose group of animals (35) that are members of intricate communities (81). Parasitoid-associated symbionts further increase the complexity of interactions in such communities. Unraveling these interactions will be an exciting task for the years to come and is likely to have profound consequences for our understanding of the ecology of parasitoids.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Funding was provided by The Netherlands Organisation for Scientific Research, NWO, to M.D. (Spinoza Award) and to A.C. (ALW Open Programme ALWOP.343) and by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme to E.H.P. (grant agreement 677139). We kindly acknowledge Cara Gibson, Merijn Salverda, Richard Stouthamer, Marc Ravallec, and Hans Smid for providing pictures and drawings that were included in the figures of this review.

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