The Ecology of Hyperparasitoids

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Abstract

Hyperparasitoids are some of the most diverse members of insect food webs. True hyperparasitoids parasitize the larvae of other parasitoids, reaching these larvae with their ovipositor through the herbivore that hosts the parasitoid larva. During pupation, primary parasitoids also may be attacked by pseudo-hyperparasitoids that lay their eggs on the parasitoid (pre)pupae. By attacking primary parasitoids, hyperparasitoids may affect herbivore population dynamics and they have been identified as a major challenge in biological control. Over the past decades, research on especially aphid- and caterpillar-associated hyperparasitoids revealed that hyperparasitoids challenge rules on nutrient use efficiency in trophic chains, account for herbivore outbreaks, or stabilize competitive interactions in lower trophic levels, and may use cues derived from complex interaction networks to locate their hosts. This review focuses on the fascinating ecology of hyperparasitoids in how they exploit and locate their often inconspicuous hosts, and the insect community processes in which hyperparasitoids are prominent players.

INTRODUCTION

Hyperparasitoids are insects in the fourth trophic level, parasitizing the larvae or pupae of primary parasitoids (108, 109). In insect communities, hyperparasitoids are ubiquitous and found in virtually all trophic chains of insect food webs in a wide range of environments (36, 108, 109). Hyperparasitoid larvae develop at the expense of their parasitoid hosts, which are themselves parasitizing aphids, mealybugs, scale insects, psyllids, whiteflies, beetles, caterpillars, fly larvae including leaf miners and galling insects, sawflies, wasps, bees, spiders (18, 109), and even insect eggs (77, 137). The hyperparasitic lifestyle evolved from primary parasitoids, potentially by frequent encounters with parasitized hosts and facultative utilization of the secondary host (36). Hyperparasitoids are

predominantly found in Hymenoptera (at least 17 families), but a few hyperparasitoid species can be found in Diptera and Coleoptera (93). The facultative nature of primary or secondary parasitism by some hyperparasitoids results in a single species being able to exploit different food web positions and have a wide host range (6, 8, 51, 107). They may facultatively parasitize predators, or function as fifth or higher trophic level hyperparasitoids by parasitizing conspecifics or other hyperparasitoid species (18, 51, 107). In insect communities, species richness of hyperparasitoids is large, since not infrequently, parasitoid species may serve as hosts for as many as sixteen different hyperparasitoid species (11, 29, 89).

True species richness of hyperparasitoids is likely to be highly underestimated. Hyperparasitoid species are commonly identified using morphological characters (e.g. 32, 33, 40). Species diversity in the Hymenoptera is extreme and taxonomy of some groups is difficult, requiring highly specialized and skilled taxonomists to identify the often very small hyperparasitoid species. Moreover, morphological identification can only be done on adult specimens, requiring rearing of hosts, and often precludes interpretation of trophic relationships between species present in a certain habitat. Molecular methods can be a partial solution to overcome these disadvantages. Species-specific molecular markers have been developed for hyperparasitoids (5, 16, 113) and DNA barcoding can be used to identify hyperparasitoid species and their parasitoid hosts (23, 67). These molecular techniques have revealed cryptic species and cryptic interactions between species. Thus, molecular techniques are becoming valuable tools in helping to construct and understand food webs, including the roles of hyperparasitoids (35, 60, 116).

In their seminal review about hyperparasitoids two decades ago, Daniel Sullivan and Wolfgang Völkl (109) highlighted that we knew little about hyperparasitoid ecology. Most ecological knowledge on hyperparasitoids at that time had been discovered for aphid and whitefly associated hyperparasitoids (36, 108). These study systems revealed the various life histories, host exploitation patterns, and foraging strategies of hyperparasitoids. Over the past two decades, new discoveries on aphid-associated hyperparasitoids were made on host location (10, 24), the cues exploited during host

location (9, 38), and the potential of using this knowledge in decreasing the negative impact of hyperparasitoids in biological control (20, 65, 112). Moreover, the first detailed studies on host location by caterpillar-associated hyperparasitoids have yielded new insights into the cues that hyperparasitoids may exploit in host searching (21, 89, 139, 140, 142). Ecologists have also elucidated the significance of hyperparasitoids in community processes caused by top-down control of parasitoids by hyperparasitoids (94, 95, 129).

Here, we provide a comprehensive review with emphasis on recent findings about the ecology of hyperparasitoids. We first outline the large diversity of hyperparasitoid life histories and the challenges they face in acquiring resources at the apex of insect food chains. We then summarize their sensory-based host location strategies by making use of plant, herbivore, and parasitoid cues in hierarchical foraging steps to locate their often inconspicuous or even concealed parasitoid hosts. The top-down effect of hyperparasitoids on parasitoids is then discussed as a major component of insect community processes with implications for species co-existence, apparent competition, secondary extinctions, and herbivore outbreaks. We identify how community processes may also limit hyperparasitoid prevalence, we examine the role of hyperparasitoids in communities under climate change, and we discuss challenges that hyperparasitoids present to the efficacy of biological control programs.

HYPERPARASITOID LIFE-HISTORIES

Terminology and classification of hyperparasitoids

Hyperparasitoids can be classified according to trophic relationships and different life-history characteristics (8, 109). They can be divided into *obligate* hyperparasitoids that develop exclusively at the expense of other primary parasitoids and *facultative* hyperparasitoids if they can develop either as primary or secondary parasitoids. Facultative hyperparasitism can occur through auto-parasitism (heteronomous hyperparasitism), where female offspring develop as primary parasitoids but male offspring develop at the expense of conspecifics as hyperparasitoids of the fourth trophic level. This

peculiar phenomenon is unique to parasitoids in the family Aphelinidae that attack scale insects and whiteflies (125). Based on the host developmental stage they attack, we can distinguish *true* hyperparasitoids that oviposit into parasitoid larvae through the arthropod hosting a larva (sometimes called primary hyperparasitoids) or *pseudo*-hyperparasitoids that oviposit into host pupae or pre-pupae (sometimes called secondary hyperparasitoids). Hyperparasitoids share developmental strategies and evolutionary origin with parasitoids so terminology and classification used for parasitoids can also be extended to hyperparasitoids. For example, *endo*-hyperparasitoids develop inside the body of their hosts whereas *ecto*-hyperparasitoids develop externally. They are *idiobionts* if they kill their hosts during oviposition or *koinobionts* if they allow their hosts to continue feeding and growing (6). If we consider non-adjacent trophic relationships with herbivores, then we can distinguish, for example, caterpillar-associated hyperparasitoids and aphid-associated hyperparasitoids. Pseudo-hyperparasitoids associated with aphids are often called 'mummy hyperparasitoids' because they attack parasitized aphids once they become mummified (109) (**Figure 1**).

Larval development and nutrient use efficiency

Hyperparasitoids are under selection pressure to efficiently exploit the limited resources stored in a parasitoid host, which is often not much larger than the hyperparasitoid itself (103, 104). Hyperparasitoids have served as excellent models to study optimal use of nutrients such as carbon and nitrogen (53), the latter often being a major limiting element in the diet of organisms in plant-based food chains (31). In caterpillar-associated hyperparasitoids, stoichiometric analyses have shown that the percentage of nitrogen was higher and carbon was lower in *Gelis agilis* and *Lysibia nana* compared with their primary parasitoid host *Cotesia glomerata* (51). Development of *G. agilis* as a fourth trophic level hyperparasitoid resulted in adults that were about 90% of the size of their host *C. glomerata*. When the same hyperparasitoid developed as a fifth tropic level hyperparasitoid of *L. nana*, it still was 75% as large as the primary parasitoid *C. glomerata* (51). In another example,

the true hyperparasitoid *Mesochorus gemellus* reaches 84% of its host's size and can reduce development time by parasitizing older and larger hosts (44). Similarly, stable isotope analyses have shown an increase in ^{15}N along aphid-associated food chains, with remarkably higher levels of enrichment found when mummy hyperparasitoids developed as members of the fifth trophic level (96). These examples illustrate how resource assimilation is highly efficient at the top of these food chains (51, 96). Hyperparasitoids clearly challenge the assumption that trophic chains do not extend beyond four or five trophic levels due to constraints in energy demands for higher order consumers (88, 106). Nutrient assimilation in hyperparasitoids may be very efficient because there is a close match between the developmental needs of hyperparasitoids and the resource quality represented by their parasitoid hosts, given a shared evolutionary origin and developmental lifestyle (51, 96). Due to this extraordinary efficiency in nutrient utilization, hyperparasitoids may be less constrained by availability of limited nutrients in their food than other consumers at lower trophic levels.

Effects of lower trophic levels on hyperparasitoid development

The high nutrient assimilation efficiency of hyperparasitoid larvae also suggests that hyperparasitoid size is strongly determined by bottom-up processes in trophic chains. For example, food plant quality determines aphid body size as host for its primary parasitoids and thus the quality of hosts for hyperparasitoids. As a consequence, a more diverse and abundant hyperparasitoid community was found on larger aphids compared with the diversity of the hyperparasitoid community on smaller aphids feeding from poorer quality plants (11). Interestingly, the plant-mediated effects on hyperparasitoids were stronger for generalist mummy hyperparasitoids compared with specialized true hyperparasitoids. It is possible that because koinobiont true hyperparasitoids such as *Alloxysta* spp. have established a more intimate relationship with their parasitoid hosts, they are less affected by changes in plant quality compared with more generalist mummy hyperparasitoids, such as members of the genera *Asaphes* and *Dendrocerus* (109).

In caterpillar-associated hyperparasitoids, presence of secondary plant compounds such as glucosinolates in Brassicaceae and nicotine in tobacco has been shown to negatively affect the performance of the hyperparasitoid *L. nana* (49, 50). In both plant-based food chains, it was found that the detrimental effects of plant defense compounds on the hyperparasitoid were stronger than for the primary parasitoids (*Cotesia glomerata* and *C. congregrata*) (49, 50). The primary parasitoid species are well adapted to exploit their respective herbivores (*Pieris brassicae*, a *Brassica* specialist, and *Manduca sexta*, a tobacco specialist) and consequently likely evolved strategies to deal with plant allelochemicals specific for their herbivore host's diets. On the contrary, because *L. nana* can attack both parasitoid species, the wasp can be associated with both *Brassica*-based and tobacco-based food chains and may thus be more constrained by the specific alteration in host quality associated with the herbivore diet. Little is known about the way hyperparasitoids deal with toxic compounds found in parasitoid host tissues (but see 121), although the concentration of plant allelochemicals encountered may strongly depend on the developmental strategy of their hosts. Parasitoid species such as *Cotesia* spp. develop as hemolymph feeders, so parasitoid larvae feeding inside the herbivore mainly encounter plant allelochemicals present in the herbivore hemolymph. Other parasitoids develop as tissue-feeders meaning that parasitoid larvae must consume all host resources in order to pupate, including the unmetabolized secondary compounds present in herbivore tissues. For hyperparasitoids attacking tissue-feeding parasitoids, the effects of lower trophic levels may pose more severe developmental constraints. Possibly, toxic compounds may protect parasitoids by affecting immune responses to hyperparasitoid eggs or by enhancing hyperparasitoid developmental mortality (26). It remains to be explored whether parasitoids sequester plant toxins for their own defense against hyperparasitoids.

Adult food sources and longevity

Adult hyperparasitoids are free-living and can forage for food to support somatic maintenance and reproduction. Laboratory studies have shown that carbohydrates can extend life expectancy of

hyperparasitoids (e.g. 25, 37) and can influence their host exploitation efficiency (4). In nature, carbohydrates are primarily found in floral and extrafloral nectar and honeydew, excreted by phloem-feeding insects such as aphids, whiteflies, and mealybugs (64, 130). Exploitation of these food sources by hyperparasitoids can influence their fitness and is consequently expected to impact their foraging strategies (3). Effects of honeydew on extending hyperparasitoid life span depend on the species of aphid that produces the honeydew and the plant on which it feeds, due to variation in the quantity of honeydew produced and the composition of dietary sugars (118). Different types of honeydew were found to have similar effects on longevity of four species of hyperparasitoids, irrespective of whether they were associated with honeydew-producing aphids or not (118). Honeydew may thus present an important generic food source to a wide range of hyperparasitoids and may reduce the time spent and associated risks of foraging for carbohydrates, particularly in environments where flowers are scarce. Host-feeding provides another source of adult nutrition for some hyperparasitoid taxa, but this behavior is phylogenetically constrained to several hymenopteran families (55, 63). Host-feeding on hemolymph can increase longevity and reproduction of hyperparasitoids (e.g. 66, 87), although for some hyperparasitoid species the benefit of host feeding is only found in combination with access to sugars (42, 118). The facultative generalist hyperparasitoid *Gelis agilis* must host-feed on hemolymph to produce mature eggs and it produces only few large eggs per day over its long life time. These traits are reversed in more specialized hyperparasitoids such as *Lysibia nana* and *Acrolyta nens* that do not host-feed (e.g. 42), and which produce more eggs per day but for a shorter duration.

Oviposition strategies and resource allocation

The differences in egg maturation or egg load across hyperparasitoids illustrates the diversity in life histories in terms of the trade-off between fecundity and longevity. Even closely related hyperparasitoids associated with the same parasitoid host may have remarkably divergent reproductive strategies (42, 43). Cocoons of the parasitoid *Cotesia glomerata* are attacked by

ichneumonid parasitoids in the subfamily Cryptinae, such as *Lysibia nana*, *Acrolyta nens,* and *Gelis agilis*. While *L. nana* and *A. nens* are relatively short-lived, allocating most of their eggs early in their life, *G. agilis* lives longer and produces only a few eggs per day, which are allocated evenly across an individual's lifespan. Such divergence in life-history traits probably evolved in response to hyperparasitoid host range, allowing specialized parasitoids of *Cotesia* cocoons such as *L. nana* and *A. nens* (100) to tailor their reproductive potential to their preferred host resources, whereas generalist hyperparasitoid species such as *G. agilis* (18, 99) have adopted an opportunistic reproductive strategy depending on host availability (44). In addition, host feeding by *G. agilis* to enhance its longevity is destructive and contributes to a high rate of host mortality (52), causing a trade-off between opportunities for current and future reproduction (55). In the genus *Gelis*, species differ markedly in host feeding, sex ratios of offspring, fecundity, and reproductive strategy ranging from asexual to sexual reproduction (43). Loss of wings, host feeding and sexual reproduction may be key traits in promoting divergence of reproductive strategies within this genus (128). Variation in host availability can reflect hyperparasitoid reproductive traits because true hyperparasitoids attacking more abundant host stages such as parasitoid larvae are expected to possess higher egg loads and produce smaller eggs compared with pseudo-hyperparasitoid species (62, 63). Spatial partitioning of host resources, which can even occur at the plant scale, can also be linked to hyperparasitoid traits related to dispersal and reproduction. Wingless pseudo-hyperparasitoid species, which possess limited dispersal capacities and small egg loads compared with winged species, have been shown to be more abundant at the bottom of the canopy than at the top of the canopy (47, 56). Due to large variations in life history traits and host exploitation patterns, it is not surprising that several hyperparasitoid species can be associated with a single primary parasitoid host (29, 39).

HYPERPARASITOID HOST LOCATION

Sensory systems and perception of chemical stimuli

Like their parasitoid hosts, hyperparasitoids are thought to use (volatile) chemical information to locate their hosts, although visual cues may also play a role. Here, we focus on the use of infochemicals in host location by hyperparasitoids, because substantial progress has been made in the chemical ecology of hyperparasitoids over the last two decades (20). Nevertheless, fundamental research on the sensory systems of hyperparasitoids remains scarce. Scanning electron microscopy was used to describe the sensory cells on antennae for *Cheiloneurus noxius* (131), for male and female *Alloxysta consobrina* and *A. victrix* (92), and different types of external sensilla across the entire bodies of male and female *Pachyneuron aphidis* (101). Eight different types of sensilla have been discovered on hyperparasitoid antennae, including porous sensilla that are thought to play a role in olfaction (85), such as the abundant multiporous placoidea sensilla on the antennae of *Alloxysta* spp. (92). Unfortunately, fundamental studies on sensory systems are often descriptive and rarely coupled to behavioral studies revealing the functions of sensilla. An early study on *C. noxius* is an exception in this respect (131). *Cheiloneurus noxius* is a hyperparasitoid of scale insect parasitoids. Before oviposition, female *C. noxius* tap parasitized scale insects with their antennae and it was demonstrated that the antennal tips, where porous sensilla placoidea are located, are essential in this process. These sensilla are most likely involved in close range perception of chemical stimuli that guide host acceptance in this hyperparasitoid. Overall, these studies suggest that the types of sensilla found on the antennae of hyperparasitoids are similar to those of other parasitic Hymenoptera. However, similarity in sensory 'hardware' does not necessarily mean that information use in hyperparasitoids resembles that of their parasitoid hosts.

Plant volatiles as foraging cues

Many studies on foraging behavior of parasitoid wasps revolve around the reliability-detectability trade-off: information derived from host insects reliably predicts the presence of the host, but is usually hard to detect because these insects are small and inconspicuous (123, 124). Host plant volatiles that are specifically induced upon herbivory are therefore used by many parasitoids of

herbivorous insects. These so-called herbivore induced plant volatiles (HIPVs) are thought to benefit foraging parasitoids because they are detectable from a longer distance yet reliably predict the presence of host insects (114). HIPVs may also influence the behavior of other organisms in the environment, including hyperparasitoids (91), although the presence of herbivores on plants does not necessarily mean that parasitoids are also present. *Lysibia nana*, a specialized pseudo-hyperparasitoid of *Cotesia* cocoons, discriminates between volatiles from cabbage infested with parasitized and unparasitized caterpillars (89). Injection of venom and polydnavirus by the primary parasitoid *Cotesia glomerata* affects herbivore physiology and the composition of its oral secretions, and thereby indirectly changes the profile of volatiles emitted by the plant, making these infochemicals reliable indicators of host presence to foraging *L. nana* (21, 139, 140). *Pteromalus semotus,* another hyperparasitoid of *Cotesia,* is also attracted by HIPVs induced by parasitized caterpillars (90). The role of (induced) plant volatiles in host location by other groups of hyperparasitoids is less clear, although a diversity of aphid-associated hyperparasitoid species has been studied. Some species were shown to respond to volatiles from plants, either or not in combination with aphids, but these studies used intact plants and were not aimed at investigating HIPVs (see 20 and refs therein). A comparative study on four species of aphid-associated hyperparasitoids concluded that contact cues of mummies and honeydew were more important than olfactory cues in hyperparasitoid host location (10). In contrast, a laboratory study recently showed that female *Dendrocerus aphidum* are attracted to volatiles from the plant-host complex, but this attraction was likely due to the presence of mummies and not to (induced) plant volatiles (24). Moreover, when attractive mummy hosts were presented in combination with plants, *D. aphidum* no longer preferred the smell of mummies, suggesting that plant volatiles may confuse foraging hyperparasitoids. Hence, the primary cues which guide aphid-associated hyperparasitoids to locate host-infested plants still remain to be identified.

Close range foraging cues

Besides information from (infested) host plants, hyperparasitoids may also use information from their parasitoid host, the host insect of the parasitoid, or associated products such as frass or honeydew. Infochemicals from these sources may more reliably predict the presence of a suitable host than infochemicals from plants, in particular when the primary parasitoid and its host interact. The wasp *Baryscapus galactopus* responds to odors of parasitized caterpillars and parasitism by *C. glomerata* indeed alters the composition of the odor profile of *Pieris rapae* caterpillars (142). Hyperparasitoids may also respond to cuticular hydrocarbons of the host insect of the primary parasitoid, as shown for *Alloxysta victrix* females that have a longer residence time on patches with extracts of parasitized aphids compared with unparasitized aphids (41). Such specific interactions may be expected for true hyperparasitoids that 'actively' interact with their own hosts, and are possibly mediated by the immune system of the primary parasitoid's host. Pseudo-hyperparasitoids that oviposit on the (pre)pupal stage of their host may use information from the pupal skin or cocoon. *Dendrocerus carpenteri* and *D. aphidum* are attracted to aphid mummies, and Siri (102) showed that long-chain compounds may mediate this response, at least at short-range, while de Boer et al. (24) showed that mummies can attract *D. aphidum* over a longer distance as well, suggesting that volatile compounds may be involved too (but see above). After arrival on a plant, aphid-associated hyperparasitoids may be arrested by the presence of honeydew (9), possibly because it is an important food source for them (25). Interestingly, parasitism of aphids can change the composition of the honeydew they excrete (17), suggesting that honeydew could reliably indicate host presence to foraging hyperparasitoids as well, but no discrimination was shown between honeydew from parasitized versus unparasitized aphids (9). Finally, *D. aphidum* responds to microbial volatiles, which elicit attraction or repellence, depending on the specific bacterium (38). However, it is unclear what the role of these microbial volatiles may be in natural environments, how this finding extends to other (groups of) hyperparasitoids, and why hyperparasitoids would respond to microbial volatiles.

Strategies in exploiting hosts

The large majority of described hyperparasitoids are known as solitary, perhaps because they are phylogenetically closely related and similar in size to their hosts. Examples of gregarious species include *Baryscapus galactopus*, *Tachinobia repanda*, *Pediobius bruchicida* (all Eulophidae) (111, 136, 141). Among aphid-associated hyperparasitoids, *Dendrocerus liebscheri* is an exception with up to eight individuals recorded from a single host mummy (32), while the congeneric *D. carpenteri* is solitary but may sometimes produce two or three offspring per host (70). This phenomenon of facultative gregarious development and the observation of successful development of two individuals of different hyperparasitoid species on/in the same host individual (12) may be important components of reproductive strategies in hyperparasitoids. As in primary parasitoids, facultative gregarious development in hyperparasitoids likely depends on the relative density of hosts to hyperparasitoids (and hence on the rate of superparasitism) and on host quality, particularly host size (available resources). Superparasitism and multiparasitism may be prevented or reduced when the first female that visits a patch or oviposits in a host leaves a chemical mark (an oviposition deterring pheromone). The aphid-associated hyperparasitoid *D. carpenteri* indeed uses such a mechanism that reduces the time that females spend on a previously explored patch (58, 59), but similar mechanisms have not yet been studied in other (groups of) hyperparasitoids. To secure access to hosts, pseudo-hyperparasitoids have been observed guarding parasitized caterpillars awaiting the egression of parasitoid larvae to parasitize their pupae shortly after their silk cocoons have been spun (89). Similar to some primary parasitoids known to use landmarks for spatial memory, hyperparasitoids may periodically return to plants with parasitized caterpillars to monitor if hosts are turning into the optimal stage for hyperparasitism (120).

Foraging challenges of aphid- and caterpillar-associated hyperparasitoids

Sullivan & Völkl (109) suggested that specialized endohyperparasitoids use specific cues to locate their hosts, while ectohyperparasitoids with a broad host range search at random, basing these hypotheses on aphid-associated hyperparasitoids. More recent studies on caterpillar-associated

hyperparasitoids show that at least some species of ectohyperparasitoids do respond to very specific infochemicals. Clearly, a wider range of study systems is needed in order to draw general conclusions on how hyperparasitoids use information in host location. Nevertheless, these new findings contribute to developing predictions and further hypotheses on hyperparasitoid foraging behavior that can be evaluated in future studies. As for primary parasitoids, host range breadth is likely an important trait in determining information use in hyperparasitoids. Moreover for hyperparasitoids, host range extends to two levels: the primary parasitoid and their hosts. For example, some aphid ectohyperparasitoids (mummy hyperparasitoids) are extreme generalists at the plant level, and some hyperparasitoid species (including *Gelis* species and *Tachinobia repanda*) have such a wide host range that some are but others are not associated with plants (e.g., species exploiting spider egg sacs (136)). Exploitation of (induced) plant volatiles is predicted to be most likely to occur in specialized true hyperparasitoids of parasitoids. The parasitized herbivores elicit changes in plant volatile emission and are a reliable cue of host presence to hyperparasitoids. However, the use of plant volatiles has only been found for pseudo-hyperparasitoids (*L. nana, P. semotus)* attacking parasitoid hosts in caterpillars (24, 89). An important step is to test this prediction in other hyperparasitoids such as those associated with aphid-parasitoids. The large difference in regulation of defense responses to leaf-chewing caterpillars versus phloem-sucking aphids in plants suggests that aphid- and caterpillar-associated hyperparasitoids likely exploit different plant-derived cues. Specific to aphid-associated hyperparasitoids may be the use of aphid alarm pheromones. The release of alarm pheromones by aphids in response to disturbance of a colony by parasitoids 'warns' other aphids in the same colony, which are often closely related due to clonal reproduction, but alarm pheromones may also attract hyperparasitoids, as shown for *Alloxysta victrix* (102). In contrast, caterpillars do not produce alarm pheromones, so there are no parallels with caterpillar-associated hyperparasitoids, but we may predict that caterpillar-associated hyperparasitoids exploit changes in herbivore cues resulting from interactions with primary parasitoids (**Figure 2**).

HYPERPARASITOIDS IN COMMUNITY PROCESSES

Community structure

Hyperparasitoid communities typically consist of species with wide host ranges, parasitizing primary parasitoid species on a number of herbivores that may be found on different food plant species (6, 19, 57). At the same time, host specialization as found for many primary parasitoid species extends to hyperparasitoids (98). Thus, food webs across four trophic levels consist of highly specialized compartments as well as broader connectivity among food chains through generalist hyperparasitoids. The structure of these food webs, the abundance of hyperparasitoids as well as its species richness, is determined by both temporal and spatial dynamics (30). The temporal structure is characterized by bottom-up effects of host availability through dynamics of herbivore-parasitoid relationships. Hyperparasitoid communities follow abundance of parasitoids and become more species-rich during a plant growth season (133). Fluctuations in herbivore populations cascade to fluctuations in hyperparasitoid abundance through herbivore-parasitoid population dynamics (80). Spatial composition of the environment causes local scale meta-population dynamics in herbivore-parasitoid-hyperparasitoid communities (105) and typically stabilizes herbivore-parasitoid-hyperparasitoid relationships at a larger spatial scale (138). Across meta-populations, hyperparasitoid genetic diversity was found to be lower than that of their primary parasitoid host, suggesting that hyperparasitoids effectively disperse (79). The dispersal capacity of hyperparasitoids and host searching efficiency may also result in selection on dispersal probability of lower trophic level organisms (13).

Specific landscape elements such as woodlands or cropland may affect local parasitoid-hyperparasitoid communities by affecting the vulnerability of parasitoids to hyperparasitoids and the degree of specialization in food web structure (27). However, the effects of landscape elements may be subordinate to effects caused by herbivore-parasitoid population dynamics (115), or may be unrelated to host availability and landscape composition (83). Even on smaller micro-habitat scales, hyperparasitoid communities associated with the same parasitoid host may differ widely. For

example, parasitoid cocoons positioned on plant stems closer to the ground were parasitized by different hyperparasitoids than cocoons positioned in the canopy of the same plant (47, 56). Intraspecific variation in plant traits, such as in herbivore induced plant volatiles, may result in plant genotype specific hyperparasitoid community composition (90) and induced responses to herbivory may cascade to variation in hyperparasitoid pressure on parasitoid cocoons associated with individual plants (86).

Hyperparasitoid competition, apparent competition and insect outbreaks

The species richness of hyperparasitoids associated with a single parasitoid host results in frequent inter- and intraspecific competition among hyperparasitoids. Parasitoid competition in itself is an important driver of the frequency of hyperparasitism in parasitoid communities. In primary parasitoids, enhanced levels of competition increase facultative hyperparasitism as a strategy to win competitions between primary parasitoids (68, 74). The primary parasitoid that is able to parasitize late developmental stages of its competitor gains an advantage by shifting its trophic position when unparasitized herbivores become scarce. Similar shifts in trophic position also occur in competition between true and pseudo hyperparasitoids. The endohyperparasitoids that parasitize parasitoids when they are still developing in their herbivore host can be facultatively attacked by ectohyperparasitoids. In both aphid- and caterpillar-associated hyperparasitoids, the ectohyperparasitoids attacking aphid mummies or parasitoid larvae that have egressed from their caterpillar host develop facultatively on primary endohyperparasitoids and thus may develop as fifth trophic level hyperparasitoids (45, 81, 141). The outcome of competition between hyperparasitoid species follows intrinsic competition patterns observed in primary parasitoids (22, 46). Superiority as competitor is determined by the breadth of host stages that can be hyperparasitized (15), egg hatching and development time of the larvae (141), and a head start in competition (127). However, ovicidal and host feeding behaviors by hyperparasitoids also may reduce the reproductive success of competing hyperparasitoids (81).

Scarcity of resources through competition with con- or heterospecific hyperparasitoids may also affect sex allocation, with fewer (127) or more female offspring (71) being allocated.

The top-down control of parasitoids by hyperparasitoids plays a major role in dampening competition between primary parasitoids. In a food web compartment where multiple parasitoids compete for the same hosts and are attacked by a shared hyperparasitoid, hyperparasitism promotes parasitoid co-existence by attacking the most prevalent parasitoid species (129, 132). When hyperparasitoids attack parasitoids associated with herbivores in different compartments of the food web, hyperparasitoids affect apparent competition (78, 119) and their absence in the community may result in secondary extinctions of herbivores and their parasitoids (94, 95). When hyperparasitoids function as higher order parasitoids that parasitize predators, they may affect food web structure through intraguild predation (34, 110). By reducing the population of third trophic level parasitoids, hyperparasitoids may cause herbivore outbreaks by releasing herbivores from the pressure of their natural enemies (82, 84).

Community processes affecting hyperparasitism

In turn, community members or species interactions may affect the presence of hyperparasitoids. Primary parasitoids are not defenseless against hyperparasitism. Indeed, a number of primary parasitoids have been found to usurp the behavior of their host herbivore. This may include the manipulation of the movement of the herbivore by the primary parasitoid, causing the herbivore to seek shelter, or to usurp the host's behavior until days after the parasitoid has left its host to spin a cocoon (48, 75). Usurpation of the herbivore's behavior can include spinning silk over the parasitoid cocoons, making them less accessible to hyperparasitoids (48). In addition, upon arrival of hyperparasitoids, guarding behaviors or aggressive movements of the herbivore that is still near or on the cocoons of the primary parasitoid reduce the likelihood of hyperparasitism further (48, 76).

Microbial symbionts associated with primary parasitoids may also reduce the success of hyperparasitoids (26). *Wolbachia* strains in the primary parasitoid have been found to defend the

parasitoid larva against hyperparasitism (28). Endosymbionts in herbivores such as aphids have cascading effects on hyperparasitoids primarily by reducing developmental success of primary parasitoids, and thus host availability for hyperparasitoids (72). The presence of facultative endosymbionts in aphids has been shown to increase the level of specialization in parasitoid-hyperparasitoid communities. Only those primary and secondary parasitoids that are adapted to the symbionts can persist in these communities (135).

Interactions among higher predators and hyperparasitoids may affect the hyperparasitoid community. The clearest examples come from ant-mediated effects, where ant-aphid mutualisms cause changes in functional composition of parasitoid communities. By tending aphids and guarding the aphids against predators, the ants may also attack primary parasitoids and hyperparasitoids decreasing the probability that aphids get parasitized by primary parasitoids and subsequently by hyperparasitoids. At the same time primary parasitoids may also escape the attention of ants and benefit from the ant-tending by reduced risk of intraguild predation on the parasitized aphids by predatory insects. In these situations, hyperparasitoids may benefit from ant-tending (97).

Multi-trophic interactions in a changing world and consequences for biological control

Host-parasitoid-hyperparasitoid associations are found in the most extreme ecological conditions of temperature ranges from cold to warm. In cold regions, aphid-parasitoid associations may build before temperatures rise that allow hyperparasitoid activity. Southern warmer winds may not only cause the influx of aphids, but also the arrival of already parasitized aphids that may be founders for the primary parasitoid population (117). High temperatures and heat waves that are expected to increase under climate change scenarios will strongly affect herbivore-parasitoid-hyperparasitoid associations (112). High temperatures have been found to negatively affect hyperparasitoid longevity (25), although the thermal tolerance of hyperparasitoids may be higher than that of their primary parasitoid host (2). However, heat waves affect the functional responses of hyperparasitism by different hyperparasitoid species in different ways (15), and affect the outcome of competition among

hyperparasitoids (14), making it difficult to predict how heat waves will affect herbivore-parasitoid-hyperparasitoid communities in different ecosystems. How hyperparasitoid fecundity or fitness is affected by temperature is a critical knowledge gap.

How host-parasitoid-hyperparasitoid associations respond to climate and habitat change is particularly challenging for hyperparasitoids in agro-ecosystems where they may negatively affect the effectiveness of biological control of crop pests (20, 112). In open field agro-ecosystems, hyperparasitoids may benefit from habitat management strategies that aim to enhance effectiveness of conservation biological control. For example, nectar provisioning by flower strips not only enhances primary parasitoids, but may also attract and nourish hyperparasitoids (54, 73). Native hyperparasitoids may expand their host range to include biocontrol agents introduced by classical biocontrol strategies and may thereby hamper control of new pests (7). In closed greenhouse systems, hyperparasitoids are considered pests because they release the pest herbivores from their biocontrol agents. Management strategies in greenhouse and open field cropping systems should thus include strategies to deal with negative effects caused by hyperparasitoids (20).

CONCLUSIONS AND FUTURE PERSPECTIVES

Over the past two decades, significant advances have been made in understanding the ecology of hyperparasitoids. To further understand ecological communities and the role of hyperparasitoids, we will strongly benefit from the availability of molecular tools. Presence and species identity of (hyper)parasitoid DNA can be recovered from empty aphid mummies up to three weeks after emergence, using single-stranded conformation polymorphism PCR (122). Using DNA metabarcoding, single aphid mummies may reveal not only the aphid and its parasitoid but also the suite of hyperparasitoids that attacked the mummy (67), allowing the reconstruction of food webs in great detail. Although these techniques have been applied to aphid-associated parasitoid communities (16, 23, 35, 39, 67, 113, 134), the application of molecular techniques to caterpillar-associated parasitoid communities still needs to be developed. Initiatives such as online databases of host-

parasitoid-hyperparasitoid associations will significantly contribute to our understanding of taxonomic, host range, and food web relationships for hyperparasitoids (61).

Challenges in the fundamental ecology of hyperparasitoids are to further elucidate their foraging strategies in complex habitats of host and non-host complexes. Virtually all of our understanding of hyperparasitoid host location is based on studies in which plants are infested with single herbivore species. How presence of non-hosts affects host location by hyperparasitoids, and which cues hyperparasitoids may reliably exploit in these settings remain to be determined (1). Moreover, in host location, microorganisms associated with herbivores or parasitoids have emerged as important mediators of interactions (26), providing us with opportunities to use these specific insights in developing lures to monitor and trap hyperparasitoids in greenhouse biological control systems.

Finally, the ecological significance of hyperparasitoids for plant defense evolution remains to be elucidated. A major standing question is whether hyperparasitoids, as the enemy of the enemy's enemy of plants, should be considered an enemy of plants (65, 89). The negative effects of hyperparasitoids on efficiency of biological control suggest that hyperparasitoids may be considered as balancing agents of selection on plant traits that enhance effectiveness of primary parasitoids. These effects may be most apparent in systems where reproductive organs of plants are under herbivore attack, such as relationships between gall midges, their parasitoids, and hyperparasitoids (126). However, identification of hyperparasitoid enemies of parasitoids that parasitize the brood of pollinators (69) suggests that in some ecological settings, hyperparasitoids may in fact be beneficial to plants.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

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Figure captions

Figure 1

In both aphid- and caterpillar-associated hyperparasitoids, true and pseudo-hyperparasitoids are discriminated. True hyperparasitoids parasitize the larvae of primary parasitoids through the aphid or caterpillar hosting the larvae. They are typically koinobiont endoparasitoids, allowing their parasitoid host to grow and acquire nutrients from their own herbivore host. Pseudo-hyperparasitoids parasitize the (pre)pupae of primary parasitoids. They are typically idiobiont ectoparasitoids that arrest the development of their parasitoid host. Aphid-associated pseudo-hyperparasitoids lay their eggs on parasitoid (pre)pupae that occupy the mummified aphid and are therefore also called mummy hyperparasitoids. Caterpillar-associated hyperparasitoids parasitize the (pre)pupae of their parasitoid host often developing inside the silk cocoons spun by the primary parasitoid after leaving their caterpillar host. Eggs of hyperparasitoids are indicated in red. We thank Francine van Neerbos for the drawing of the aphid-associated pseudo-hyperparasitoid.

Figure 2

Hyperparasitoids use a wide range of cues in host location. For aphid-associated hyperparasitoids (top panel), the cues used in long range host location have not yet been identified. At closer range, aphid-associated hyperparasitoids exploit cues coming from the herbivore host, such as honeydew or cuticular hydrocarbons. Mummy odors and odors produced by micro-organisms associated with aphids (mummies) are used as volatile source of information. In contrast, for caterpillar-associated hyperparasitoids (bottom panel) herbivore induced plant volatiles (HIPVs) have been identified as long range cue in host location. Parasitized caterpillars induce volatile blends that are distinctly different from those induced by unparasitized caterpillars, providing the hyperparasitoid with a reliable cue in long range host location. When searching for parasitized caterpillars or parasitoid cocoons, the caterpillar-associated hyperparasitoids use odors of parasitized caterpillars. Which other cues these caterpillar-associated hyperparasitoids use in close range foraging are not yet known.
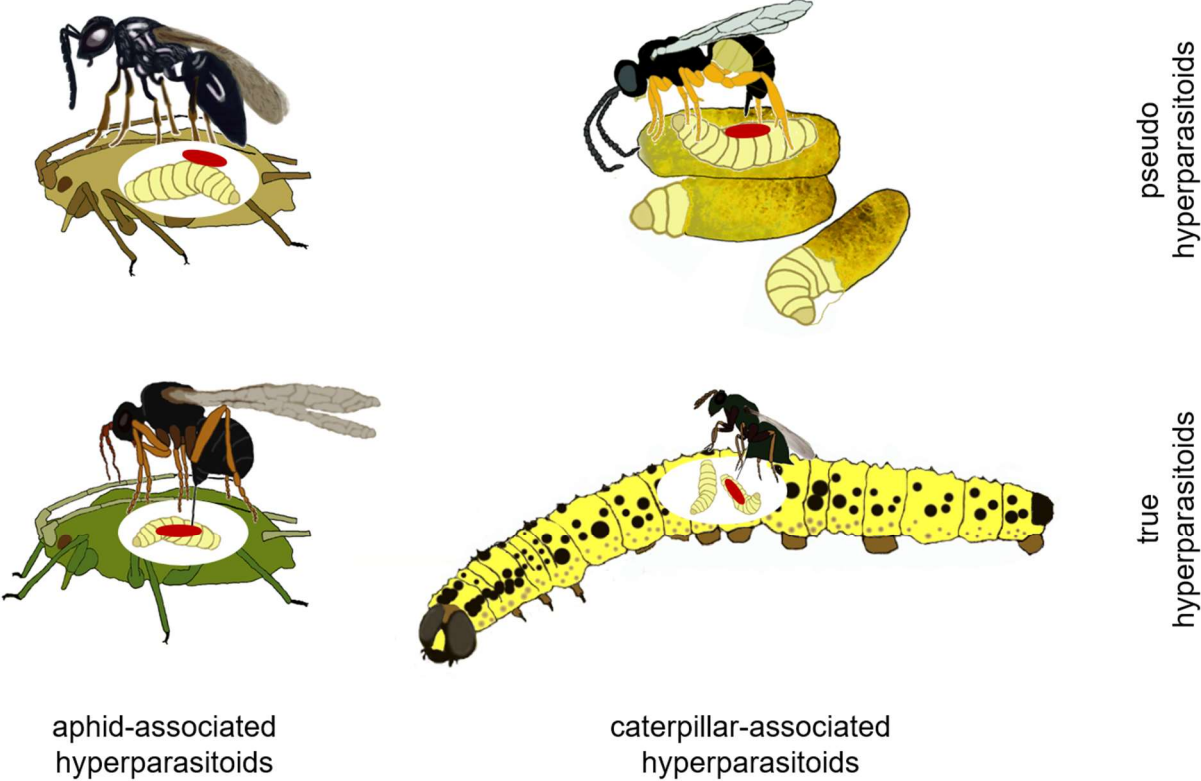
Figure 1

pseudo hyperparasitoids

true hyperparasitoids

aphid-associated
hyperparasitoids

caterpillar-associated
hyperparasitoids

Figure 2