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Impact of herbivore symbionts on parasitoid foraging behaviour

Short title: Symbiont effects on foraging

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Abstract

Parasitoids are insects that lay eggs in other insects, but before this they have the remarkable task of locating and successfully attacking a suitable individual. Once an egg is laid, many herbivorous hosts carry defensive symbionts that prevent parasitoid development. Some symbioses can act ahead of these defences by reducing parasitoid foraging efficiency, while others may betray their hosts by producing chemical cues that attract parasitoids. In this review we provide examples of symbionts altering the different steps that adult parasitoids need to take to achieve egg laying. We also discuss how interactions between habitat complexity, plants and herbivores modulate the way symbionts affect parasitoid foraging, and parasitoid evaluation of patch quality based on risk cues derived from parasitoid antagonists like competing parasitoids and predators.

Keywords:

Symbiont costs; parasitoid antagonists; habitat complexity; induced plant defences; intraguild predation; alarm pheromone; cuticular hydrocarbon profile; honeydew; parasitoid nutritional ecology

1. Introduction

Parasitoids are insects that live at the expense of other arthropods by laying eggs on, inside, or in the vicinity of their hosts, which develop into larvae that eventually kill the host [1]. Before egg laying, parasitoids have the remarkable task of locating, and successfully attacking a suitable individual [1,2]. Once the egg is laid, many insects have evolved immune strategies to prevent successful parasitoid development, while others have outsourced defence to symbiotic microbes. Defensive symbioses can protect their hosts by improving host vigour, priming the host immune system, or by producing defensive chemical compounds [3,4]. A well-studied case of a defensive symbiont in insects is the aphid symbiont *Hamiltonella defensa* (see Glossary). This bacterium is not required for aphid survival (it is facultative, not obligate) but it increases aphid resistance to

parasitoids, likely through the production of defensive toxins [3,4]. As we will review here, increasing evidence suggests that some symbioses act ahead of these types of defences by reducing parasitoid foraging efficiency, while others betray their hosts by producing cues that attract parasitoids [5,6].

In this manuscript, we explore the multitude of ways in which herbivore symbionts alter parasitoid foraging (Figure 1). We provide examples of symbionts altering the different steps that adult parasitoids need to take to parasitize a host. Following the classic parasitoid literature, these steps can be fractioned into detection of cues from the microhabitat, the host plant, and direct and indirect host cues [1,2]. We also consider how in complex habitats detection of these cues can be affected by plant diversity, and by the diversity of cues originating from parasitoid antagonists like competing parasitoids, hyperparasitoids and predators [7,8].

2. Habitat complexity affects symbionts through changes in foraging efficiency

High plant diversity systems tend to host a higher diversity of herbivores and herbivore natural enemies [9]. In such systems, parasitoids may face the challenge of discriminating useful cues for host location from background noise [7], or they can otherwise forage more efficiently if they have access to more diverse food and prey resources that extend their longevity and activity levels [10]. A high diversity of natural enemies can impact herbivore defensive traits because prey may require a larger set of strategies to avoid predation or parasitism. As a consequence, natural enemy diversity can increase the diversity of defensive symbionts [11]. This has been particularly studied in aphids because they associate with microbial symbiotic partners that provide highly specific resistance to different natural enemies [11,12]. In agreement, Zytynska and co-authors [13] found, in a plant diversity experiment, that relative to low diversity plots, high diversity ones hosted a larger variety of symbionts across the population despite hosting fewer at the individual level. The hypothesis was that hosting multiple protective symbionts was too costly to compensate for the protection acquired against diverse natural enemy assemblages. More recent work also found a positive relationship between the abundance of different parasitoid species and symbiont communities in field plots [14]. Moving to the laboratory, Hafer-Hahmann and Vorburger [15] demonstrated that to maintain a larger diversity of symbionts in an aphid population, high genotypic variation within the parasitoid population was also necessary. Significant correlations between symbiont and natural enemy diversity likely arise through local selection for specific defences at low parasitoid diversity (both at the species and population level), which are disrupted when parasitoid diversity increases and more variable defences are required [11,12]. A recent theoretical approach points towards parasitoid foraging as a potential cause leading to selection for symbiont defences that are specific to abundant natural enemies [16]. The authors of this report suggest that specialisation may be promoted via

constraints arising from the costs of learning new cues when switching hosts. The role of parasitoids on symbiont diversity over-time was also explored in two independent field studies highlighting the potential impact of natural enemy diversity on the strength of symbiont selection. The first study detected stronger temporal effects of parasitoids on aphid symbionts in a lower diversity system with only two dominant parasitoid species [17]. The second observed a higher diversity of parasitoids, collecting six species, but only detected a limited interaction between parasitoids and aphid symbionts [18]. Although both studies found that frequency of infection by the defensive symbiont *H. defensa* was not unequivocally linked to parasitoid pressure, parasitoid foraging in the field, and ultimately on symbiont frequencies can therefore not be ruled out. Behavioural studies of parasitoid nutritional ecology and foraging that consider defensive symbionts may be useful approaches to better understand these field observations.

3. Symbiont effects on cues from the microhabitat and the host plant

Parasitoids can directly home in on volatile cues emanating from symbionts as signals of host microhabitat. This has been reported in nutritional symbionts that live outside the host's body (ectosymbioses), e.g. volatiles emitted from fungal and yeast symbionts attract parasitoids of bark beetles [19], woodwasps [20], and Drosophila flies [21], but also in beetle gut symbionts [22]. Even if enemy attraction can potentially disrupt the symbiotic relationship, evolution towards reduced enemy attraction may be constrained because the volatiles are often metabolic by-products of the nutritional service provided by the symbiont [21–23]. Thus, these symbionts may be ray their hosts by attracting parasitoids. Important cues for parasitoids may also come from plant volatiles that signal infestation by the sought herbivore host, i.e. herbivore-induced plant volatiles (HIPVs). Symbionts of insects can defend their hosts by reducing such emissions, and therefore reduce the chance of being discovered by a parasitoid; demonstrated in an experiment with the pea aphid Acyrthosiphon pisum, and several of its associated endosymbionts [24]. Other plant defences that are induced by herbivory include changes in plant physical defences like leaf pubescence, glandular trichomes and waxy surfaces that may reduce herbivory [25]. These changes, however, can act as physical obstacles that reduce the foraging efficiency of small parasitoids [26,27]. Herbivore symbionts have been increasingly linked to changes in plant physiology and the modulation of plant defences [5,6]. If these changes alter plant physical structures they can feedback to the insect host through modifications in parasitoid foraging efficiency. In a diverse environment, detection of chemical cues emitted as plumes depends on volatile density and persistence [28]. Symbionts may be selected to minimise such emissions, or to emanate blends that are difficult to distinguish from background noise.

4. Symbiont effects on direct and indirect host cues

Once parasitoids locate a plant with potential hosts on it, short-range cues come into play. Parasitoids have long been known to use volatiles emanating from frass and honeydew as indirect host cues [1], which can be of microbial origin. To locate the moth Acrolepiopsis assectella, the parasitoid *Diadromus pulchellus* homes in to dialkyl disulphides that emanate from the moth's fresh frass, and which are produced by gut microbes [29]. Similarly, microbes found in aphid and mealybug honeydew produce volatiles that attract parasitoids [30,31]. In these examples the microbe betrays the insect as it reveals host presence to the parasitoid. In a more recent example, however, Goelen and co-authors [32] cultured bacteria from different parasitoid habitats and excreted fluids, and found that out of 38 bacterial strains tested, only the volatiles emitted by a bacterium isolated from honeydew was repulsive to the parasitoid Aphidius colemani. This is an example of a honeydew microbe that may act as a defensive symbiont by repelling aphid enemies. Even if parasitoids locate their hosts mostly using indirect chemical cues, direct visual cues are also important. Pea aphids, A. pisum, have green and red morphs, the red ones being preferred by ladybirds and the green ones by parasitoids [33]. By increasing the concentration of blue-green polycyclic quinones the endosymbiont *Ricketsiella* alters aphid colour from red to green thus increasing aphid attack rate by parasitoids, but defending them from ladybirds [34]. In a diverse system, higher plant habitat complexity, herbivore and natural enemy diversity could lead to dynamic balancing selection on protective symbionts depending on the prevailing natural enemy [35].

5. Symbionts effects on host handling, host acceptance, and parasitoid learning

After a female parasitoid locates its host, it usually taps or drums on it with the antennae to assess its quality for offspring development. Parasitoids for which the host keeps growing after egg laying (koinobionts), require hosts of a specific developmental stage or size [1]. Through services that improve nutrient acquisition, symbionts may defend their hosts simply by speeding-up juvenile development, and reducing the window of vulnerability to such enemies (e.g. [36]). Similarly, nutritional symbionts of the saw-toothed grain beetle *Oryzaephilus surinamensis* speed-up cuticle melanisation, increasing handling time by spiders, and ultimately reducing attacks on the beetle [37]. Even if the host has the right size, parasitoids may adapt their foraging strategies when defensive symbionts are present. For example, aphid parasitoids have been observed to avoid laying in hosts infected by the defensive *H. defensa* symbiont [38], or to lay multiple eggs in them probably to exhaust symbiont-based defences [39]. The way by which aphid parasitoids can detect the quality or defensive status of hosts is not yet clear, but changes in the honeydew composition

[30,31] or in cuticular hydrocarbon profiles [40] are the most likely explanations. Once accepted by female parasitoids, herbivores can display defensive behaviours that discourage parasitoid oviposition. For example, *A. pisum* aphids infected with *H. defensa* exhibit more aggressive kicking in response to predator attack [41], while *Sitobion avenae* aphids infected with *Regiella insecticola* exhibited reduced aggressiveness, but tended to emit higher concentration of alarm pheromone (E- β -farnesene) [42,43]. Even if in these examples symbionts may help their hosts avoid parasitoid attacks, other studies revealed the opposite as aphids carrying symbionts showed reduced defensive behaviours [44,45]. Another way by which herbivores can avoid parasitism is by flying away in response to risk cues. Winged aphids are produced in response to such cues (e.g. alarm pheromones), and there is some evidence that aphid symbionts can enhance [46] or reduce wing production [47]. Yet, there is insufficient data to base a robust hypothesis on how this impacts parasitoid success.

Detecting the first host is a crucial step for naïve parasitoids, but being efficient in finding the next ones is also important for their reproductive success. Particularly in long-lived parasitoids, such success may depend on learning from their experiences while foraging. The role of symbionts in insect behaviour has only been recently appreciated [48,49], and whether they may provide novel behavioural strategies to avoid parasitism risk is a question that remains to be explored. So far, evidence of symbionts impacting herbivore behaviour is limited. For example, gut symbionts have been found to alter social interactions in leaf-cutting ants *Acromyrmex echinatior* [50]. In aphids, the endosymbionts *H. defensa* and *Arsenophonus* were found to alter feeding behaviours including the time insects spent sucking sap, or the number of plant cell punctures they performed [51,52]. These feeding behaviours are often used by aphids to weaken plant induced defence [53], and they could, for instance, explain the reduction in herbivore-induced plant volatiles associated with symbiont carrying that we reported in a previous example [6,24].

6. Symbionts alter risk cues associated to parasitoid antagonists

Antagonistic interactions with other species (e.g. competition, hyperparasitism and intraguild predation) must be avoided by parasitoids before oviposition [8], and risk cues used to do so may be altered by symbionts. For example, insect symbionts may alter competition between parasitoid larvae inside their host [54]. Female parasitoids thus need to take these intricate interactions into account to lay eggs in hosts where the developing young has greater chances of survival, even if the host is already parasitized. These decisions may be particularly important for egg-limited parasitoid species. Parasitoids are known to avoid patches where hyperparasitoids and intraguild predators are abundant [8], but little is known on how symbionts may modulate these behaviours. In aphids, one potential way is through aphid alarm pheromones. As we discussed above, even if the synthesis of

these pheromones is not symbiont dependent [55], symbionts may impact emission amount and frequency [42,43]. Such emissions can feedback on foraging parasitoids [44] because they may signal low quality patches where hyperparasitoids and intraguild predators are already present [56,57].

In addition to protective microbial symbionts, many phloem-feeding insects are also protected from parasitoids by ants in return for sugar-rich honeydew excreted by the host. Ant presence in colonies of honeydew producers may therefore signal danger. As a consequence, parasitoids may avoid searching in these colonies unless they can deceive ants by emulating their cuticle hydrocarbon profiles (e.g. *Lysiphlebus* parasitoids, [58]). The protection of ants may reduce the need for symbiont protection, as it was found that ant-tended *Aphis* sp and *Dysaphis* sp. hosted fewer symbionts than unattended species or colonies within species [59,60]. However, these interactions are likely host, parasitoid and symbiont dependent since no such association was observed in other systems [61,62]. In a diverse system, parasitoids may be able to forage more effectively with more diverse resources, but they also encounter more antagonists and must avoid these multitude of cues in order to seek and gain suitable hosts.

7. Conclusions

In this review we highlight the importance of symbionts in modulating parasitoid foraging behaviour. Symbionts can either be beneficial (or defensive) by undermining the ability of parasitoids to locate their hosts, or the Achilles heel if parasitoids use symbiont-derived cues to locate them [5,6]. Relative to within-host defences, preventing enemy attacks may be a beneficial strategy because it does not require a constant boost of the immune system, and it prevents the injury imposed by oviposition and auto-toxicity when bioactive molecules are involved (e.g. [63]). This strategy, however, may be less efficient in complex habitats where parasitoids forage on a multitude of cues, which include not only attractive host cues, but also risk cues (like those derived from parasitoid antagonists) to be avoided [8].

Aphids have been an important model system for the study of insect symbiosis, and of defensive symbionts in particular [3]. More research on other taxa is needed, especially in diverse groups like lepidopterans and coleopterans where symbionts are increasingly well understood [6]. Relative to aphids, symbionts in these groups are dominated by diverse gut communities, a feature that may increase the chances of finding symbionts providing previously unsuspected anti-predatory services. As shown in the study by Goelen and co-authors [32], screening for parasitoid attraction or repulsion to different members of a gut community could be a good starting point to identify their interactions with enemies. From an applied perspective, culturable microbes of insect origin that are

attractive to natural enemies could provide novel bioactive molecules to be used to manipulate enemy behaviour, and to protect crops [64].

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a. Large-scale habitat location



Figure 1. Herbivore symbiont effects on parasitoid foraging behaviour. Symbionts are represented by bacteria icons, and different colours of herbivores, flowers or symbionts are used to represent diversity. Landscape diversity is represented with a circular buffer with different colours representing different habitat types. a. Habitat complexity affects symbionts through changes in foraging efficiency. Natural enemies are more diverse, and their foraging is more efficient in complex habitats with more diverse plant communities and flower resources. This may increase the diversity of defensive symbionts a the population level (a1). Volatile chemical cues used by parasitoids to locate their hosts are modulated by herbivore symbionts, but their detection depends on the complexity of the habitat, and of the volatile blend (a2). b. Symbiont effects on cues from the microhabitat, the host plant, and the insect host. Parasitoids can detect volatile cues that emanate directly from symbionts (b1). Induced plant defences can be modulated by herbivore symbionts by reducing the emission of herbivore-induced plant volatiles (b2), or by altering plant physical structure (b3). Symbionts can alter indirect host cues emitted from frass (b4) or honeydew (b5), but also direct visual cues like insect body colour (b6). c. Symbionts effects on host handling and acceptance. By increasing growth rate or melanization, nutritional symbionts may reduce herbivores' window of vulnerability to parasitoids (c1). Parasitoids can detect insects defended by symbionts and adapt oviposition strategies (c2). Symbiont detection likely occurs through changes in honeydew composition (c3) or in cuticular hydrocarbon profiles (c4). Symbionts affect the

defensive behaviours displayed by herbivores (c5), and how they disperse to avoid parasitism (c6). d. Symbionts alter risk cues associated to parasitoid antagonists. Symbionts modulate parasitoid competition (d1), and alarm pheromones emitted in response to intraguild predators (d2). Parasitoids evaluate patch quality based on the presence of antagonists like herbivore-defensive ants, which can alter symbiont hosting frequencies. Some parasitoids can deceive ants by emulating their cuticle hydrocarbon profiles (d3).

Highlights

- Symbionts can undermine parasitoid foraging, or attract parasitoids and betray their hosts.
- Herbivore symbionts alter parasitoid foraging through changes in plant and hosts cues.
- Habitat diversity alters parasitoid foraging with consequences for symbionts.
- Patch quality for parasitoids depends on the presence of symbionts and antagonists.