

# Finding an egg in a haystack: variation in chemical cue use by egg parasitoids of herbivorous insects

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Egg parasitoids of herbivorous insects use an interplay of short- and long-range chemical cues emitted by hosts and host plants to find eggs to parasitize. Volatile compounds that attract egg parasitoids can be identified via behavioral assays and used to manipulate parasitoid behavior in the field for biological control of herbivorous pests. However, how and when a particular cue will be used varies over the life of an individual, as well as at and below species level. Future research should expand taxonomic coverage to explore variation in chemical cue use in more natural, dynamic settings. More nuanced understanding of the variability of egg parasitoid host-finding strategies will aid in disentangling the underlying genetics and further enhancing biological control.

## Addresses

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## Introduction

The challenge for egg parasitoids to find an herbivore’s inconspicuous egg in a complex environment is akin to trying to locate a needle in a very large and ever-changing haystack. Remarkably, these minuscule insects have evolved to be highly successful at host-finding. Their ability to seek out and kill an herbivore’s egg, before it has developed into a voracious larva, is highly valuable

for agriculture. The diverse host-finding strategies used by these idiobiont endoparasitoids have been thoroughly reviewed in the past [1,2], as have the ways in which these innate and learned behaviors can be exploited to improve biological control [3,4]. Thus far, research has primarily focused on species within several genera of parasitoids known to target economically important pests, namely the genera *Trissolcus* (Scelionidae), *Telenomus* (Platygastridae), and *Trichogramma* (Trichogrammatidae). As the number of species and their taxonomic coverage investigated continues to grow, so does our ability to find phylogenetic patterns in their use of chemical cues for host-finding.

Egg parasitism has evolved approximately eighteen times within Hymenoptera. Egg parasitoids have been found to predominantly parasitize herbivorous insects that lay their eggs on plants, and of these, primarily from the orders Hemiptera and Lepidoptera [5]. In this review, we focus on hymenopteran parasitoids of these herbivorous species and the chemical cues that they use to find host eggs. Egg parasitoids have antennae that can detect specific volatile organic compounds (VOCs) or non-volatile cues by contact. Many respond to chemical cues emitted directly from their hosts or indirectly induced by the host plant on which the herbivore is found and on which they then home in on their host eggs. We highlight some of the most recent research on the chemical ecology of egg parasitoid host-finding, and how the parasitoids use an interplay of chemical cues emitted by the host insect, the host plant, and potentially microbes. We then discuss how dynamic host-finding strategies can be within and between individuals, populations, and species.

## Finding hosts using chemical cues

### Using chemical cues from the host insect

At each developmental stage of an herbivorous insect, chemical volatiles are emitted that have the potential to betray the host’s location and inform parasitoids to find a suitable egg for oviposition. Egg parasitoids primarily use VOCs from the adult stage, however, there are examples of egg parasitoid attraction to larval frass volatiles, which may indicate the location of a host [6]. In short-range searching, egg-derived compounds have been shown to act as contact kairomones for several species [7]. Chemical traces left on plants by ovipositing

Figure 1



Examples of *Trichogramma* wasps using different modalities of host-finding. **(a)** Artificially colored scanning electron microscope image of butterfly scales near and on eggs (credits: Hans M. Smid), **(b)** phoresy: hitchhiking *Trichogramma* female with a female butterfly emitting antiaphrodisiac pheromones (credits: Nina E. Fatouros), **(c)** close-range plant cues such as changes in epicuticular waxes reliably indicate host eggs in close vicinity (credits: Nina E. Fatouros), **(d)** *Pieris* butterfly egg parasitized by *Trichogramma* inducing an egg-killing leaf necrosis — indirect and direct defense work in concert — double-defense line (credits: Lotte Caarls).

females can serve as a more conspicuous source of close-range cues [1]. For example, the footprints of Hemiptera have been found to be highly informative contact kairomones for egg parasitoids from several genera, and especially well studied for species of *Trissolcus* [8–10]. Kairomones from scales, i.e. cuticular hydrocarbons like tricosane, shed from the body and wings of adult Lepidoptera can also serve as close-range cues [11,12] (Figure 1a). Pheromone residues that are absorbed by these scales or deposited directly onto the egg or leaf surface may also be attractive short-range cues [13,14]. When pheromones are emitted directly by an adult insect, these relatively concentrated pheromone plumes may act as highly detectable long-range cues to which many egg parasitoids have been found to be highly sensitive [15–18]. For example, antiaphrodisiac pheromones, which are emitted by mated females, have been found to lure *Trichogramma* to *Pieris* butterflies [19]. Antiaphrodisiacs provide valuable information to an egg parasitoid because these compounds indicate that the host is likely gravid and also signal the presence of a potential ‘ride’ on the host to fresh eggs and a new patch,

known as phoresy [20] (Figure 1b). Phoresy has been found in approximately thirty species of egg parasitoids, which is far more prevalent than in any other parasitoids [21]. Further studies are needed to determine the prevalence and specificity of the combination of pheromonal espionage and phoresy across egg parasitoids and their hosts.

#### Using chemical cues from the host plant

Records of plant volatiles induced by herbivore egg deposition (Oviposition-induced plant volatiles (OIPVs)) attracting egg parasitoids are adding up across different plant species and herbivorous hosts [22,23]. Both changes in plant volatiles and epicuticular leaf wax-induced by herbivore oviposition are exploited by egg parasitoids and reliably indicate egg deposition in a habitat and/or on a host plant (Figure 1c). OIPVs have been shown to be host-specific [24,25], host egg age-specific, [26,27], and location-specific [28]. OIPVs may work in concert with direct egg-killing leaf necrosis [26,29] (Figure 1d), depend on the mating status of the egg-laying host [30], and can indicate host quality and

whether eggs are already parasitized [31]. Simultaneous caterpillar feeding by (non)hosts or egg deposition by invasive insects can alter egg parasitoid preferences to OIPVs and can even disrupt the signal. For example, the egg parasitoids *Telenomus podisi* and *Trissolcus basalisi* are only attracted to OIPVs emitted by plants when induced by eggs in combination with feeding damage of the local host. However, the wasps were neither attracted to OIPVs induced by the invasive pest nor to OIPVs of plants concurrently infested by both the local and invasive host [32,33]. The lack of response toward the invasive species might be due to the absence of a co-evolutionary history between the interacting species. Responding only to cues from suitable coevolved hosts probably reduces egg parasitoids time and energy costs [34].

Attraction of egg parasitoids by OIPVs should benefit plants and selective breeding for OIPVs could be a valuable addition to biocontrol and use of semiochemical tactics [35,36]. First attempts toward breeding for crop resistance to pests by utilizing parasitoid attraction to OIPVs have now been made by testing natural variation in commercial, transgenic, and/or landraces of maize [37–39]. Farmer-selected maize landraces were shown to emit OIPVs induced by egg deposition of the stem-borer *Chilo partellus* that are more attractive to *Trichogramma* and *Cotesia* parasitoids than commercial hybrids [39]. A genome-wide association mapping with different maize genotypes revealed > 100 SNP molecular markers associated with parasitoid attraction to OIPVs, including a receptor gene that is potentially involved in the recognition of the egg elicitor [37]. Although far from application, identification, and validation of candidate genes for parasitoid attraction and subsequent introgression into commercial hybrids, could eventually lead to higher parasitism rates and reduction in the stem-borer pest on maize.

#### Using chemical cues from, or induced by, microbes?

In recent years, there has been an increasing interest in understanding the role of microorganisms in the foraging behavior of insect parasitoids [81,82]. Extracellular microorganisms vertically transmitted from parents to offspring and inoculated onto the host eggshell [23,83] could be potentially exploited by foraging parasitoids as long-range or short-range cues. When insect host eggs are deposited on leaves, plants may perceive and respond to egg-associated microbes and recruit egg parasitoids. In this mode of action, egg-associated microbes are hypothesized to act as microbial elicitors of OIPVs that attract egg parasitoids. However, a recent study using a lepidopteran species failed to show such an eliciting role of egg-associated microbes [84]. This lack of plant response could be due to the absence of core-resident microbes in Lepidoptera [81]. It would be interesting to investigate the role of microbes in other herbivorous insects that vertically transmit symbionts via egg inoculation, such as herbivorous stink bugs (Hemiptera) that smear symbiont-containing fluids from the anus on the eggs whereby gut symbionts are transferred to the next generation [85]. As the main parasitoids of stink bugs are species that attack the egg stage — particularly members of the Scelionidae

— stink bugs and their parasitoids are ideal organisms to investigate the role of egg-associated microbes in plant-mediated parasitoid attraction. Extracellular microorganisms may also be exploited by insect parasitoids as short-range or contact cues, when wasps are in close vicinity or encounter a potential host. Many egg parasitoids extensively inspect with their antennae the surface of potential host eggs with which they recognize the host identity. However, there is no evidence yet in the literature that the presence of specific, extracellular microorganisms on host eggshells can be reliable host identity cues for egg parasitoids.

#### Variability in the use of chemical cues over time and space

With the multitude of chemical cues available to aid a parasitoid searching for eggs, the following question remains: when do parasitoids use a particular cue? The environment in which egg parasitoids live and search for host eggs is dynamic, and the availability and relative concentrations of chemical cues are ever changing along with the background odors. Recent reviews of the neuroscience and ecology of insect olfaction in complex environments have shown how multiple cues can be used simultaneously. Differences in the relative concentrations of cues against different background odors will alter a parasitoid's response to a given cue [40–42]. Often, mixtures of kairomones elicit stronger responses than kairomones presented on their own [4]. Plant and host cues may work in concert and affect the parasitoids foraging behavior in field conditions [43,44]. Which chemical cues are used likely depends on nuances of spatial and temporal context.

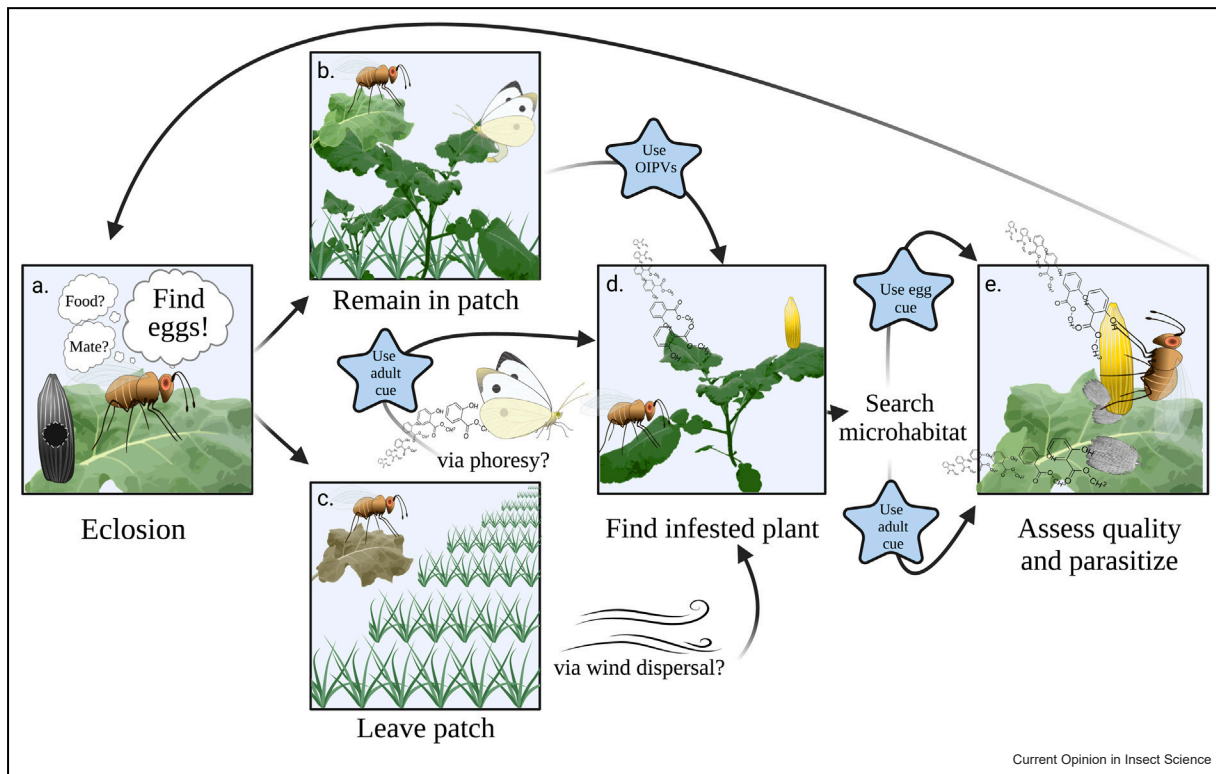
#### Intraindividual variation

Over the life of an egg parasitoid, variation in its physiological state and past experience impact how the parasitoid searches for eggs [45]. For example, transcriptional changes after mating affect parasitoid behavior [46], as do changes in egg load [47] and feeding status [48]. Learning from experiences also greatly impacts which cues parasitoids find most attractive [49–51]. Memory formation is complex, dependent on not only conditioning but also on slight differences in reward value [52]. The interplay between innate and learned cues used for host-finding is of great interest [19,53]. ‘Maladaptive learning’ can occur when a new species is introduced, and there is a mismatch between cues and preferred species for oviposition, such as *Telenomus podisi* associatively learning cues of an unsuitable host [54,55]. The learning abilities of parasitoids can be used via parasitoid olfactory conditioning to enhance the efficiency of parasitism in the field [3].

The suitability of the environment in which the emerged or released egg parasitoid finds itself will also greatly impact which cues it seeks. The variability in



Figure 2



Schematic representation of how an egg parasitoid variably uses chemical cues to find host eggs after eclosion depending on the context in which it ecloses. **(a)** Which cue is used may depend on the physiological state of the egg parasitoid (altered by, e.g. previous feeding and mating experiences), and on the quality of the patch in which it ecloses (e.g. the proximity to fresh host eggs). **(b)** If the parasitoid remains in the patch, it may use OIPVs to locate an infested plant. **(c)** If the current patch does not provide sufficient oviposition opportunities, the parasitoid may use phoresy or wind dispersal to find a more suitable patch. Adult host pheromones may act as a short-range cue for a phoront to find a host on which to ride. **(d)** Once at an infested plant, short-range cues from the egg itself or left behind by ovipositing females may guide the parasitoid to eggs. **(e)** Once host eggs are found, the parasitoid assesses their quality and decides whether to accept them for oviposition. When the offspring emerge, the process repeats as the quality of the patch and availability of different host species changes over the season. The parasitoid's response to all cues shown may be innate or learned, thus, successive host searches may be influenced by the successes or failures of earlier attempts.

landscape characteristics is often overlooked, despite several studies indicating that these factors greatly affect the biology and behavior of egg parasitoids, and thus parasitism rates [47,56,57]. Based on the quality of the patch, and which host species are readily available, an egg parasitoid must decide whether to stay and seek eggs, or whether to find a new patch. To make this assessment, entirely different chemical cues and host-finding modalities may be used (Figure 2). As the number and nuance of factors known to affect an individual's host-finding behavior continues to grow, computer modeling will likely be a valuable tool for predicting parasitoid responses and thus in designing dynamic biocontrol programs [45].

### Intrapopulation variation

Even within a single population of egg parasitoids, differences in host-finding behavior will often exist between individuals. For example, experiences before

emergence may influence host preferences, i.e. pre-maginal learning. The choice of host by the parent may alter the preferences of the offspring after only a single generation [58], possibly a result of cues gathered from antennation before the parasitoid hatches from the egg. In other cases, rearing parasitoids for multiple generations on an alternative host does not seem to create a preference for the volatiles of the alternate host over its natural host [59]. Seasonal changes, including changes in the population dynamics of hosts, may also be associated with differences in egg parasitoid cue use within or between generations. An early season parasitoid may prefer the cues associated with a host that has an egg stage early in the season, matching its phenology. Seasonal changes in host use by egg parasitoids have been found [60], as well as seasonal changes in the attraction to the host-specific chemical cues between generations [61]. For example, *Ooencyrtus pityocampae* parasitizes eggs of the pine processionary moth (PPM) but emerges after

overwintering in PPM eggs well before the next generation of these moths will fly. Instead, the parasitoids use the eggs of *Stenozygum coloratum*, the caper bug, as an alternate host in the spring and summer. These spring and summer wasps do not display attraction to the sex pheromone of the PPM, while the later generations do. DNA barcoding confirmed that these parasitoids are of the same species and are not genetically differentiated [61]. DNA barcoding is an especially useful tool for accurate species identification for many taxa of minute egg parasitoids that are difficult to distinguish morphologically and/or have suffered from inaccurate and ever-changing systematics, such as species of *Ooencyrtus* or *Trichogramma* [62]. It is clear that this species varies in host and cue use seasonally. So far, seasonal variation in egg parasitoid cue use has not been considered in designing biological control programs.

### Intraspecific variation

While it is tempting to generalize at the species level, in many cases, strains of the same species have been found to respond differently to both host- and plant-derived odors [27,29,63,64]. For application in biological control, it is essential that findings with one strain of parasitoid are not necessarily generalized to the entire species. For example, the attraction of one strain of a generalist species to particular host volatiles does not necessarily mean the same preference will be found in geographically distant population, where it may experience cues of different hosts. DNA sequencing that can identify genetic differentiation can help to discover variation in behavior below the species level, also by clarifying host associations more accurately [65]. Furthermore, discovering the genetic basis of parasitoid preferences allows for selection and breeding for these traits [66,67]. Investigating genetic variation between populations that evolved with different community interactions will give insight into the microevolutionary processes of cue use by egg parasitoids [68,69] and the coevolution of signalers and receivers [70]. It would also be interesting to investigate whether and when exploitation and natural selection on host cue use by egg parasitoids may select for changes in host (plant) chemical communication.

### Interspecific variation

On a larger timescale, we can consider how host cue use has evolved between parasitoid species. While chemical cue use is highly context dependent, some aspects of the biology of parasitoids point toward their likelihood to use a certain cue. For example, inherent differences in flight capabilities between species likely influence the propensity for an egg parasitoid to use long-range cues. For a species with strong flight capabilities, long-range host cues, such as those from calling virgin moths, may be used to locate and fly to an egg patch. On the other hand,

parasitoids that have typically been found to rely on down-wind flight for undirected dispersal [71], such as many in the families Trichogrammatidae and Mymaridae, especially benefit from phoretic behavior [21,72]. Parasitoids with weak ovipositors, such as *Telenomus*, are also often phoretic as they require freshly laid eggs of which the chorion has not yet hardened [21]. In *Trichogramma*, most known examples involve the use of long- and short-range cues derived from the host with fewer examples of cues derived from plants. Other taxa seem less reliant on phoresy and using host-derived cues for long-range host-finding. For *Trissolcus*, it seems that plant-derived compounds are the main cues utilized to find a patch, but upon contact with the plant, cues from their stinkbug hosts become increasingly important for their ultimate oviposition decision. Differences in dietary specialization between species may also impact cue use. For example, more specialist egg parasitoids respond innately to chemical cues while generalists require associative learning [73]. However, a lack of accurate host range data precludes generalization.

Divergence of cue use between egg parasitoid species may be strongly tied to divergence in their host use. Investigating the evolution of genes associated with olfaction, such as those for olfactory receptors and odorant-binding proteins (OBPs), via comparative genomics and transcriptomics coupled with electrophysiology and behavioral assays, can lead to exciting insights into the coevolution of egg parasitoids and their hosts. The rapidly growing availability of high-quality genomes, and tissue- and sex-specific transcriptomic data, allows for better annotation and more accurate comparison of olfactory genes [74–77]. For example, identification of OBPs in *Trissolcus* has revealed lineage-specific expansions as well as orthologs in other Hymenoptera [78]. The presence of large receptor families in trichogrammatids and pteromalids indicates the evolution of high complexity in olfaction in these parasitoids, while relatively simple genetics underlying chemosensation was found for a species of Mymaridae [79]. Furthermore, identification of olfactory-associated genes in parasitoids may also reveal coevolution with host genes that synthesize the compound that the egg parasitoids use as a cue. For example, two pairs of OBPs were found to be similar between *Telenomus podisi* and its preferred host *Euschistus heros*. These OBPs have no known orthologues in other Hymenoptera, suggesting that their possible independent evolution and convergence allowed *T. podisi* to use *E. heros*' semiochemicals [80]. Investigating the timing of such events can help to elucidate the coevolution of egg parasitoids and their hosts.

### Conclusions

Host finding by egg parasitoids is variable by nature, and this dynamic nature is a fundamental aspect of the

parasitoids' strategy to overcome the challenges of finding an egg under variable spatial and temporal circumstances. The oversimplification of host-finding in complex habitats can result in ill-advised release strategies leading to failure of biological control programs. While it is necessary to use laboratory assays to evaluate the physiological possibility for parasitism of a given host by a particular parasitoid, this information says little about the likelihood of the wasp to reliably seek out and parasitize the host's egg in the field. Many factors affect field parasitism rates, which cannot be directly deduced from parasitism assays in the lab. Future research efforts should elucidate a) whether the parasitoids are attracted to chemical cues emitted by a particular host population and/or cues induced from a particular host plant in the context of ever-changing background odors, and b) whether or not the parasitoid can physically travel distances to the host egg in a given landscape and under variable biotic and abiotic conditions. Investigating phenotypic and genotypic variability between individuals, populations, and species will give a more complete understanding of how egg parasitoid host finding has evolved, and how it can be exploited to enhance biological control. It is important to accept that context is highly important to how and when egg parasitoids use chemical cues. Application of egg parasitoids in biological control will require more nuanced consideration of the dynamics of a given crop and parasitoid system, and therefore a more 'personalized' approach.

#### Future aims

- Accumulate more information on population differences in chemical cue use in the field.
- Use DNA barcoding to obtain more accurate taxonomic coverage on which parasitoids use which hosts, and to compare genotypic and phenotypic variation between individuals, populations, and species.
- Elucidate the genetic basis underlying variation in cue preference between parasitoid strains and species.
- Investigate the (co)evolutionary effects of selection by eavesdroppers on host cues and signals.

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Given her role as Guest Editor, Nino Fatouros had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to David Denlinger.

#### CRedit authorship contribution statement

**LOG:** Conceptualization, Writing – original draft. **MEH:** Writing – review & editing. **ATG:** Writing – review & editing. **AC:** Conceptualization, Writing – original draft. **NEF:** Conceptualization, Writing – original draft.

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No data were used for the research described in the article.

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We declare no conflict of interests.

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#### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Fatouros NE, Dicke M, Mumm R, Meiners T, Hilker M: **Foraging behavior of egg parasitoids exploiting chemical information.** *Behav Ecol* 2008, **19**:677-689.
2. Vinson BS: **The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species.** *Biol Cont* 1998, **11**:79-96.
3. Kruidhof HM, Kostenko O, Smid HM, Vet LEM: **Integrating parasitoid olfactory conditioning in augmentative biological control: potential impact, possibilities, and challenges.** *Front Ecol Evol* 2019, **7**:1-7.
4. Ayelo PM, Pirk CWW, Yusuf AA, Chailleux A, Mohamed SA, Deletre E: **Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: a review.** *Front Ecol Evol* 2021, **9**:1-22.
5. Fatouros NE, Cusumano A, Bin F, Polaszek A, van Lenteren JC: **How to escape from insect egg parasitoids: a review of potential factors explaining parasitoid absence across the Insecta.** *Proc R Soc Lond B* 2020, **287**:20200344.

This review discusses the use of kairomones as lures for enhancing biological control. Of particular interest is the discussion on the need for an increased number of field studies that explore how to account for the dynamic background odors affecting parasitoid responses and learning.

This review screens known egg parasitoid species throughout the 32 hexapod orders, of which half seem to escape egg parasitism. Using quantitative network analysis of host-parasitoid associations, this review shows that most known egg parasitoids primarily target the eggs of insect herbivores, mainly those of Lepidoptera and Hemiptera. Patterns of specificity between the five genera with the most species of egg parasitoids and insect orders is revealed.

6. Reddy GVP, Holopainen JK, Guerrero A: **Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles.** *J Chem Ecol* 2002, **28**:131-143.
7. Hu JS, Zhang A, Cornelius ML, Vinyard BT: **Host finding behaviour of the egg parasitoid, *Gryon pennsylvanicum* (Hymenoptera: Scelionidae).** *Biocontrol Sci Technol* 2021, **31**:964-975.
8. Gomes Lagôa AC, Blassioli Moraes MC, Borges M, Laumann RA: **Selective responses of *Trissolcus basalidis* and *Telenomus podisi* to chemical footprints of preferred hosts.** *Physiol Entomol* 2020, **45**:60-71.
9. Malek R, Kaser JM, Broadley HJ, Gould J, Ciolli M, Anfora G, Hoelmer KA, Szendrei Z: **Footprints and ootheca of *Lycorma delicatula* influence host-searching and -acceptance of the**



- egg-parasitoid *Anastatus orientalis*. *Environ Entomol* 2019, **48**:1270-1276.
10. Arif MA, Alinç T, Guarino S, Colazza S, Cusumano A, Peri E: **Only females oviposit: chemical discrimination of adult stink bug sex by the egg parasitoid *Trissolcus japonicus***. *Front Ecol Evol* 2021, **9**:1-10.
  11. Vargas CC, Redaelli LR, Sant'Ana J, Blassioli-Moraes MC, Laumann RA, Borges M: **Influence of semiochemicals present in the scales of *Spodoptera frugiperda* on chemotactic behavior of *Trichogramma pretiosum***. *Entomol Exp Appl* 2021, **169**:393-402.
  12. Dong H, Zhu KH, Zhao Q, Bai XP, Zhou JC, Zhang LS: **Morphological defense of the egg mass of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) affects parasitic capacity and alters behaviors of egg parasitoid wasps**. *J Asia Pac Entomol* 2021, **24**:671-678.
  13. Noldus LPJJ, Potting RPJ, Barendregt HE: **Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies**. *Physiol Entomol* 1991, **16**:329-344.
  14. Tognon R, Sant'Ana J, Zhang QH, Millar JG, Aldrich JR, Zalom FG: **Volatiles mediating parasitism of *Euschistus conspersus* and *Halymorpha halys* eggs by *Telenomus podisi* and *Trissolcus erugatus***. *J Chem Ecol* 2016, **42**:1016-1027.
  15. Kpongbe H, van den Berg J, Khamis F, Tamò M, Torto B: **Isopentyl butanoate: aggregation pheromone of the brown spiny bug, *Clavigralla tomentosicollis* (Hemiptera: Coreidae), and kairomone for the egg parasitoid *Gryon* sp. (Hymenoptera: Scelionidae)**. *J Chem Ecol* 2019, **45**:570-578.
  16. Xu J, Huigens ME, Orr D, Groot AT: **Differential response of *Trichogramma* wasps to extreme sex pheromone types of the noctuid moth *Heliothis virescens***. *Ecol Entomol* 2014, **39**:627-636.
  17. Ahmadi S, Poorjavand N: **Behavioral and biological effects of exposure to *Tuta absoluta* (Lepidoptera: Gelechiidae) sex Pheromone on several *Trichogramma* (Hymenoptera: Trichogrammatidae) populations**. *J Econ Entomol* 2018, **111**:2667-2675.
  18. Conti E, Colazza S: **Chemical ecology of egg parasitoids associated with true bugs**. *Psyche* 2012, **2012**:651015, <https://doi.org/10.1155/2012/651015>
  19. Huigens ME, Woelke JB, Pashalidou FG, Bukovinsky T, Smid HM, Fatouros NE: **Chemical espionage on species-specific butterfly anti-aphrodisiacs by hitchhiking *Trichogramma* wasps**. *Behav Ecol* 2010, **21**:470-478.
  20. Fatouros NE, Huigens ME: **Phoresy in the field: natural occurrence of *Trichogramma* egg parasitoids on butterflies and moths**. *BioControl* 2012, **57**:493-502.
  21. Huigens ME, Fatouros NE: **A Hitch-Hiker's guide to parasitism: the chemical ecology of phoretic insect parasitoids**. In *Chemical Ecology of Insect Parasitoids*. Edited by Wajnberg E, Colazza S. John Wiley & Sons, Ltd; 2013:86-111.
  22. Fatouros NE, Cusumano A, Danchin EG, Colazza S, Nina Fatouros CE: **Prospects of herbivore egg-killing plant defenses for sustainable crop protection**. *Ecol Evol* 2016, **6**:6906-6918.
  23. Hilker M, Salem H, Fatouros NE: **Adaptive plasticity of insect eggs in response to environmental challenges**. *Ann Rev Entomol* 2023, **68**:451-469.
  24. Fatouros NE, Lucas-Barbosa D, Weldegergis BT, Pashalidou FG, van Loon JJA, Dicke M, Harvey JA, Gols R, Huigens ME: **Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels**. *PLoS One* 2012, **7**:e43607.
  25. Cusumano A, Weldegergis BT, Colazza S, Dicke M, Fatouros NE: **Attraction of egg-killing parasitoids toward induced plant volatiles in a multi-herbivore context**. *Oecologia* 2015, **179**:163-174.
  26. Fatouros NE, Pineda A, Huigens ME, Broekgaarden C, Shimwela MM, Figueroa Candia IA, Verbaarschot P, Bukovinsky T: **Synergistic effects of direct and indirect defences on herbivore egg survival in a wild crucifer**. *Proc R Soc Lond B* 2014, **281**:20141254.
  27. Milonas PG, Anastasaki E, Partsinevelos G: **Oviposition-induced volatiles affect electrophysiological and behavioral responses of egg parasitoids**. *Insects* 2019, **10**:1-13.
  28. Frati F, Cusumano A, Conti E, Colazza S, Peri E, Guarino S, Martorana L, Romani R, Salerno G: **Foraging behaviour of an egg parasitoid exploiting plant volatiles induced by pentatomids: the role of adaxial and abaxial leaf surfaces**. *PeerJ* 2017, **2017**:e3326.
  29. Afentoulis DG, Cusumano A, Greenberg LO, Caarls L, Fatouros NE: **Attraction of *Trichogramma* wasps to butterfly oviposition-induced plant volatiles depends on Brassica species, wasp strain and leaf necrosis**. *Front Ecol Evol* 2021, **9**:1-10.
  30. Salerno G, Frati F, Conti E, Peri E, Colazza S, Cusumano A: **Mating status of an herbivorous stink bug female affects the emission of oviposition-induced plant volatiles exploited by an egg parasitoid**. *Front Physiol* 2019, **10**:398.
  31. Li CZ, Sun H, Gao Q, Bian FY, Noman A, Xiao WH, Zhou GX, Lou YG: **Host plants alter their volatiles to help a solitary egg parasitoid distinguish habitats with parasitized hosts from those without**. *Plant Cell Environ* 2020, **43**:1740-1750.
  32. Martorana L, Brodeur J, Foti MC, Agrò A, Colazza S, Peri E: **Egg parasitoid exploitation of plant volatiles induced by single or concurrent attack of a zoophytophagous predator and an invasive phytophagous pest**. *Sci Rep* 2019 **9**:1 2019, **9**:1-8.
- This study finds that while oviposition by a zoophytophagous predatory bug alters plant odors in a way that makes them attractive to egg parasitoids, coinfection by an invasive pest interrupts this effect.
33. Martorana L, Foti MC, Rondoni G, Conti E, Colazza S, Peri E: **An invasive insect herbivore disrupts plant volatile-mediated tritrophic signalling**. *J Pest Sci* 2017, **90**:1079-1085 (2004).
  34. Conti E, Avila G, Barratt B, Cingolani F, Colazza S, Guarino S, Hoelmer K, Laumann RA, Maistrello L, Martel G, et al.: **Biological control of invasive stink bugs: review of global state and future prospects**. *Entomol Exp Appl* 2021, **169**:28-51.
  35. Tamiru A, Khan ZR, Bruce TJA: **New directions for improving crop resistance to insects by breeding for egg induced defence**. *Curr Opin Insect Sci* 2015, **9**:51-55.
  36. Peri E, Moujahed R, Wajnberg E, Colazza S: **Applied chemical ecology to enhance insect parasitoid efficacy in the biological control of crop pests**. In *Chemical Ecology of Insects*. Edited by Tabata J. CRC Press; 2018:234-267.
  37. Tamiru A, Paliwal R, Manthi SJ, Odeny DA, Midega CAO, Khan ZR, Pickett JA, Bruce TJA: **Genome wide association analysis of a stemborer egg induced "call-for-help" defence trait in maize**. *Sci Rep* 2020, **10**:1-12.
- Following earlier findings that certain Maize genotypes are more attractive to both egg and larval parasitoids in the field, in this paper, the genetics underlying the attraction of the larval parasitoids are investigated using GWAS techniques. The GWAS identified markers and regions of the maize genome associated with egg-induced defense that breeders can explore for selective breeding.
38. Nascimento PT, Fadini MAM, Rocha MS, Souza CSF, Barros BA, Melo JOF, von Pinho RG, Valicente FH: **Response of *Trichogramma pretiosum* females (Hymenoptera: Trichogrammatidae) to herbivore-induced Bt maize volatiles**. *Arthropod Plant Inter* 2021, **15**:107-125.
  39. Tamiru A, Bruce TJA, Woodcock CM, Caulfield JC, Midega CAO, Ogot CKPO, Mayon P, Birkett MA, Pickett JA, Khan ZR: **Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore**. *Ecol Lett* 2011, **14**:1075-1083.
  40. Renou M, Anton S: **Insect olfactory communication in a complex and changing world**. *Curr Opin Insect Sci* 2020, **42**:1-7.
- This review explores the ways in which insects process multiple complex odor cues from their environment and integrate these to respond appropriately. Recent advances in understanding the neuroscience of insect odor communication provide valuable insights into mechanisms that dictate interspecific communication, such as chemical eavesdropping by parasitoids, in complex agroecosystems.

41. Riffell JA: **The neuroecology of insect-plant interactions: the importance of physiological state and sensory integration.** *Curr Opin Insect Sci* 2020, **42**:118-124.
42. Haverkamp A, Smid HM: **A neuronal arms race: the role of learning in parasitoid-host interactions.** *Curr Opin Insect Sci* 2020, **42**:47-54.
43. Gontijo L, Cascone P, Giorgini M, Michelozzi M, Rodrigues HS, Spiezia G, Iodice L, Guerrieri E: **Relative importance of host and plant semiochemicals in the foraging behavior of *Trichogramma achaeae*, an egg parasitoid of *Tuta absoluta*.** *J Pest Sci* 2019, **92**:1479-1488.
44. Cornelius ML, Haber AI, Weber DC: **Host finding behavior of the parasitoid *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae) for egg masses of the squash bugs *Anasa tristis* and *Anasa armigera* (Hemiptera: Coreidae) in squash and cucumber fields.** *Environ Entomol* 2022, **51**:537-544.
45. Zhang S, Qian B, Ilyas A, Gong XM, Xu J, Liu PC, Hu HY: **Influence of parasitoid states on the propensity to enter and the stay in a patch.** *J Insect Behav* 2022, **35**:56-64.
46. Liu PC, Hao DJ: **Behavioural and transcriptional changes in post-mating females of an egg parasitoid wasp species.** *R Soc Open Sci* 2019, **6**:181453.
47. Paul RL, Abram PK, Lee JC: **Host patch quality increases parasitoid locomotor activity despite risk of egg limitation.** *Ecol Entomol* 2022, **47**:810-821.
48. Straser RK, Wilson H: **Food deprivation alters reproductive performance of biocontrol agent *Hadronotus pennsylvanicus*.** *Sci Rep* 2022, **12**:1-10.
49. Gonthier J, Zhang YB, Zhang GF, Romeis J, Collatz J: **Odor learning improves efficacy of egg parasitoids as biocontrol agents against *Tuta absoluta*.** *J Pest Sci* 2022, **1**:1-13.
- This study tests the effects of rearing host and of exposure to plant odors during a positive oviposition experience on the subsequent response to the same plant odors. Interestingly, the study compares learning between three species of *Trichogramma* and find that each responds differently to the treatments. *Trichogramma achaeae* was unaffected by the rearing host but showed increased parasitism rate after associative learning of the plant odor. *Trichogramma dendrolimi* response was affected by the rearing host, but not the learning experience. *Trichogramma evanescens* response to the odor was affected both by the rearing host and learning the odor, but no change in parasitism rate was observed.
50. Louâpre P, Pierre JS: **Parasitoids update the habitat profitability by adjusting the kairomone responsiveness to their oviposition experience.** *Ecol Entomol* 2014, **39**:343-346.
51. Peri E, Salerno G, Slimani T, Frati F, Conti E, Colazza S, Cusumano A: **The response of an egg parasitoid to substrate-borne semiochemicals is affected by previous experience.** *Sci Rep* 2016, **6**:1-8.
52. Kruidhof HM, Pashalidou FG, Fatouros NE, Figueroa IA, Vet LEM, Smid HM, Huigens ME: **Reward value determines memory consolidation in parasitic wasps.** *PLoS One* 2012, **7**:e39615.
53. Wilson JK, Woods HA: **Innate and learned olfactory responses in a wild population of the egg parasitoid *Trichogramma* (Hymenoptera: Trichogrammatidae).** *J Insect Sci* 2016, **16**:1-8.
54. Bertoldi V, Rondoni G, Peri E, Conti E, Brodeur J: **Learning can be detrimental for a parasitic wasp.** *PLoS One* 2021, **16**:e0238336.
- This study presents a case of 'maladaptive learning', in which *Telenomus podisi* learns the chemical cues of an unsuitable, exotic host. Following experience with the footsteps of *Halymorpha halys*, the parasitoid is subsequently arrested by these volatiles despite the hosts unsuitability. The consequences of this potential evolutionary trap are discussed in the context of biological control.
55. Konopka JK, Poinapen D, Garipey T, McNeil JN: **Understanding the mismatch between behaviour and development in a novel host-parasitoid association.** *Sci Rep* 2018, **8**:1-11.
56. Grande MLM, de Queiroz AP, Gonçalves J, Hayashida R, Ventura MU, de Freitas Bueno A: **Impact of environmental variables on parasitism and emergence of *Trichogramma pretiosum*, *Telenomus remus* and *Telenomus podisi*.** *Neotrop Entomol* 2021, **50**:605-614.
57. Romeis J, Babendreier D, Wäckers FL, Shanower TG: **Habitat and plant specificity of *Trichogramma* egg parasitoids—underlying mechanisms and implications.** *Basic Appl Ecol* 2005, **6**:215-236.
58. Boyle SM, Weber DC, Hough-Goldstein J, Hoelmer KA: **Parental host species affects behavior and parasitism by the pentatomid egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae).** *Biol Cont* 2020, **149**:104324.
59. Ali AN, Wright MG: **Behavioral response of *Trichogramma papilionis* to host eggs, host plants, and induced volatile plant cues.** *Biol Cont* 2020, **149**:104323.
60. Zhang J, Zhang F, Garipey T, Mason P, Gillespie D, Talamas E, Haye T: **Seasonal parasitism and host specificity of *Trissolcus japonicus* in northern China.** *J Pest Sci* 2017, **90**:1127-1141.
61. Samra S, Ghanim M, Protasov A, Branco M, Mendel Z: **Genetic diversity and host alternation of the egg parasitoid *Ooencyrtus pityocampae* between the pine processionary moth and the caper bug.** *PLoS One* 2015, **10**:1-21.
62. Garipey TD, Kuhlmann U, Gillott C, Erlandson M: **Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods.** *J Appl Entomol* 2007, **131**:225-240.
63. Sevarika M, Foti MC, Peri E, Colazza S, Wajnberg E: **Genetic variation in the behavioural mechanisms involved in the response of the egg parasitoid *Trissolcus brochymenae* to contact chemical cues left by the pest *Murgantia histrionica*.** *Ecol Entomol* 2021, **46**:100-105.
- This study makes the first attempts to quantify the intraspecific genetic variation of the response of a parasitoid to its hosts' chemical traces. The results show that three iso-female lines of the same species have variable walking responses when presented with the same host cues, and that this response differs between genotypes.
64. Bigler F, Suverkrupp BP, Cerutti F: **Host searching by *Trichogramma* and its implications for quality control and release techniques.** In *Ecological Interactions and Biological Control*. Edited by Andow DA. CRC Press; 1997:240-253.
65. Garipey TD, Bruin A, Konopka J, Scott-Dupree C, Fraser H, Bon MC, Talamas E: **A modified DNA barcode approach to define trophic interactions between native and exotic pentatomids and their parasitoids.** *Mol Ecol* 2019, **28**:456-470.
- This study exemplifies how DNA barcoding can be used to clarify host associations, and to discover cases of mismatched cue use where a parasitoid finds and parasitizes an egg of a host in which it cannot develop.
66. Leung K, Ras E, Ferguson KB, Ariëns S, Babendreier D, Bijma P, Bourtzis K, Brodeur J, Bruins MA, Centurión A, et al.: **Next-generation biological control: the need for integrating genomics and genomics.** *Biol Rev* 2020, **95**:1838-1854.
- This review addresses the practical challenges in selective breeding for enhanced biological control agents. An in-depth discussion of genomic techniques allowing for identification of successful strains, and identification of the genes underlying their success, points towards techniques to harness genetic variability in biocontrol agents pre- and post-release to increase biocontrol successes.
67. Lommen STE, Jong PW, de, Pannebakker BA: **It is time to bridge the gap between exploring and exploiting: prospects for utilizing intraspecific genetic variation to optimize arthropods for augmentative pest control – a review.** *Entomol Exp Appl* 2017, **162**:108-123.
68. Bertoldi V, Rondoni G, Brodeur J, Conti E: **An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host.** *Front Physiol* 2019, **10**:1-9.
69. Rondoni G, Chierici E, Giovannini L, Sabbatini-Peverieri G, Roversi PF, Conti E: **Olfactory responses of *Trissolcus mitsukurii* to plants attacked by target and non-target stink bugs suggest low risk for biological control.** *Sci Rep* 2022, **12**:1-9.
- Using Y-tube olfactometer tests, this study reveals a low likelihood of nontarget risk when releasing a non-native egg parasitoid to control an invasive stink bug. The egg parasitoid exploits the odors of its coevolved host, but not of the nontarget native Hemiptera occurring in the area it is to be released. While other studies show use of short-range cues of the



noncoevolved host and physiological capability to parasitize the non-target eggs, these results show no use of their chemical cues for long range host finding, indicating they would be unlikely to find the eggs in the field, at least innately.

70. White TE, Latty T, Umbers KDLL: **The exploitation of sexual signals by predators: a meta-analysis.** *Proc R Soc Lond B* 2022, **289**:1-10.
71. Fournier F, Boivin G: **Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions.** *Environ Entomol* 2000, **29**:55-63.
72. Pomari-Fernandes A, de Freitas Bueno A, de Bortoli SA, Favetti BM: **Dispersal capacity of the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) in maize and soybean crops.** *Biol Cont* 2018, **126**:158-168.
73. Peñaflo MFGV, Erb M, Miranda LA, Werneburg AG, Bento JMS: **Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid.** *J Chem Ecol* 2011, **37**:1304-1313.
74. Li SS, Yan ZC, Zhao JJ, Li YX: **Transcriptomic analyses of chemosensory genes in *Trichogramma japonicum* (Hymenoptera: Trichogrammatidae).** *Comp Biochem Physiol Part D Genom Proteom* 2021, **37**:100755.
75. Zhang J, Zhong Y, Tang R, Rebijith KB, Li F, Chen G, Zhang F: **Olfactory reception of host alarm pheromone component by the odorant-binding proteins in the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae).** *Front Physiol* 2020, **11**:1058.
76. Chen H, Lahey Z, Talamas EJ, Johnson NF: **Identification and expression of chemosensory receptor genes in the egg parasitoid *Trissolcus basalis*.** *Comp Biochem Physiol Part D Gen Prot* 2021, **37**:100758.
77. Lindsey ARI, Kelkar YD, Wu X, Sun D, Martinson EO, Yan Z, Rugman-Jones PF, Hughes DST, Murali SC, Qu J, et al.: **Comparative genomics of the miniature wasp and pest control agent *Trichogramma pretiosum*.** *BMC Biol* 2018, **16**:1-21.
78. King K, Meuti ME, Johnson NF: **Identification and expression of odorant binding proteins in the egg-parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera, Scelionidae, Telenominae).** *J Hymenopt Res* 2021, **87**:251-266.
79. Ma Y, Guo Z, Wang L, Wang B, Huang T, Tang B, Zhang G, Zhou Q: **The genome of the rice planthopper egg parasitoid wasp *Anagrus nilaparvatae* casts light on the chemo- and mechanosensation in parasitism.** *BMC Genom* 2022, **23**:1-14.

This study produces a high-quality genome assembly for the biocontrol agent *Anagrus nilaparvatae*, the first known genome for a member of the

Mymaridae family. Genes important for host-finding are identified and reveal relatively simple genetics underlying mechano- and chemo-sensation.

80. Farias LR, Schimmelpfeng PHC, Togawa RC, Costa MMC, Grynberg P, Martins NF, Borges M, Blassioli-Moraes MC, Laumann RA, Báo SN, et al.: **Transcriptome-based identification of highly similar odorant-binding proteins among neotropical stink bugs and their egg parasitoid.** *PLoS One* 2015, **10**:e0132286.
81. Voirol LRP, Frago E, Kaltenpoth M, Hilker M, Fatouros NE: **Bacterial symbionts in lepidoptera: their diversity, transmission, and impact on the host.** *Front Microbiol* 2018, **9**:1-14.
82. Cusumano A, Bella P, Peri E, Rostás M, Guarino S, Lievens B, Colazza S: **Nectar-inhabiting bacteria affect olfactory responses of an insect parasitoid by altering nectar odors.** *Micro Ecol* 2022, **1**:1-13.
83. Salem H, Florez L, Gerardo N, Kaltenpoth M: **An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects.** *Proc R Soc Lond B* 2015, **282**:20142957.
84. Paniagua Voirol LR, Valsamakis G, Lortzing V, Weinhold A, Johnston PR, Fatouros NE, Kunze R, Hilker M: **Plant responses to insect eggs are not induced by egg-associated microbes, but by a secretion attached to the eggs.** *Plant Cell Environ* 2020, **43**:1815-1826.
85. Tada A, Kikuchi Y, Hosokawa T, Musolin DL, Fujisaki K, Fukatsu T: **Obligate association with gut bacterial symbiont in Japanese populations of the southern green stinkbug *Nezara viridula* (Heteroptera: Pentatomidae).** *Appl Entomol Zool* 2011, **46**:483-488.

## Glossary

**VOCs:** Volatile organic compounds

**Idiobiont endo-parasitoid:** Parasitoids that prevent further host development when ovipositing into the often-sessile host

**Semiochemical:** Chemical compound or mixture of compounds emitted by one organism that affects the behavior of another

**Kairomone:** A semiochemical that is eavesdropped on by another species to the detriment of the emitter

**Pheromone:** Chemical signals are used for intraspecific communication by most insects

**Synomone:** A semiochemical that benefits both the emitter and a receiver from another species

**Phoresy:** Behavior in which one organism travels on the body of another

**OIPVs:** Oviposition-induced plant VOCs