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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.

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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 herbivores may then serve as cues for egg parasitoids in the next developmental stage [8].

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.
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 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, antiphrodisiac pheromones, which are emitted by mated females, have been found to lure *Trichogramma* to *Pieris* butterflies [19]. Antiphrodisiacs 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 basalis* 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 coevolutionary 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.  

### 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 [5].

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
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. preimaginal 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

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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 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.
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 prionomalids 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 independant 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.

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

Conflict of interest statement
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


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.


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.

Editorial disclosure statement

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. AC: Conceptualization, Writing – original draft.
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This study presents a case of ‘maladaptive learning’, in which the parasitoid *Telenomus* redirects its feeding behavior away from a suitable host because of a volatile cue from an unsuitable exotic host. This behavior is not influenced by the rearing host, but is affected by the volatile cues associated with it. The results suggest that the parasitoid uses a volatile cue to avoid an unsuitable host species, which may have implications for the effectiveness of the parasitoid as a biocontrol agent.

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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.


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.


Glossary

VOCs: Volatile organic compounds

Idiohost endo-parasitoid: Parasitoids that prevent further host development when ovi-positioning 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 used for intraspecific communication by most insects

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

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

OIPVs: Oviposition-induced plant VOCs