



# A neuronal arms race: the role of learning in parasitoid–host interactions

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Parasitic wasps and their larval hosts are intimately connected by an array of behavioral adaptations and counter-adaptations. This co-evolution has led to highly specific, natural variation in learning rates and memory consolidation in parasitoid wasps. Similarly, the hosts of the parasitoids show specific sensory adaptations as well as non-associative learning strategies for parasitoid avoidance. However, these neuronal and behavioral adaptations of both hosts and wasps have so far been studied largely apart from each other. Here we argue that a parallel investigation of the nervous system in wasps and their hosts might lead to novel insights into the evolution of insect behavior and the neurobiology of learning and memory.

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## Introduction

Primary parasitoids and their larval hosts are intertwined in a constant arms race, which extends from immune responses to different behavioral strategies [1,2]. Within this evolutionary conflict, the ability to learn is of paramount importance for both sides [3], especially when the behavior of the parasitoid or host changes due to climatic or other man-made influences on natural and agro-ecosystems [4].

Here, we will review recent studies on learning and memory formation in parasitoid wasps and explore the potential behavioral and neural plasticity in enemy-avoidance strategies in their larval insect hosts. The different steps in host–parasitoid interactions will be discussed along four steps from approaching a resource to updating

memory, each time from the perspective of the host and the parasitoid, as depicted in [Figure 1](#).

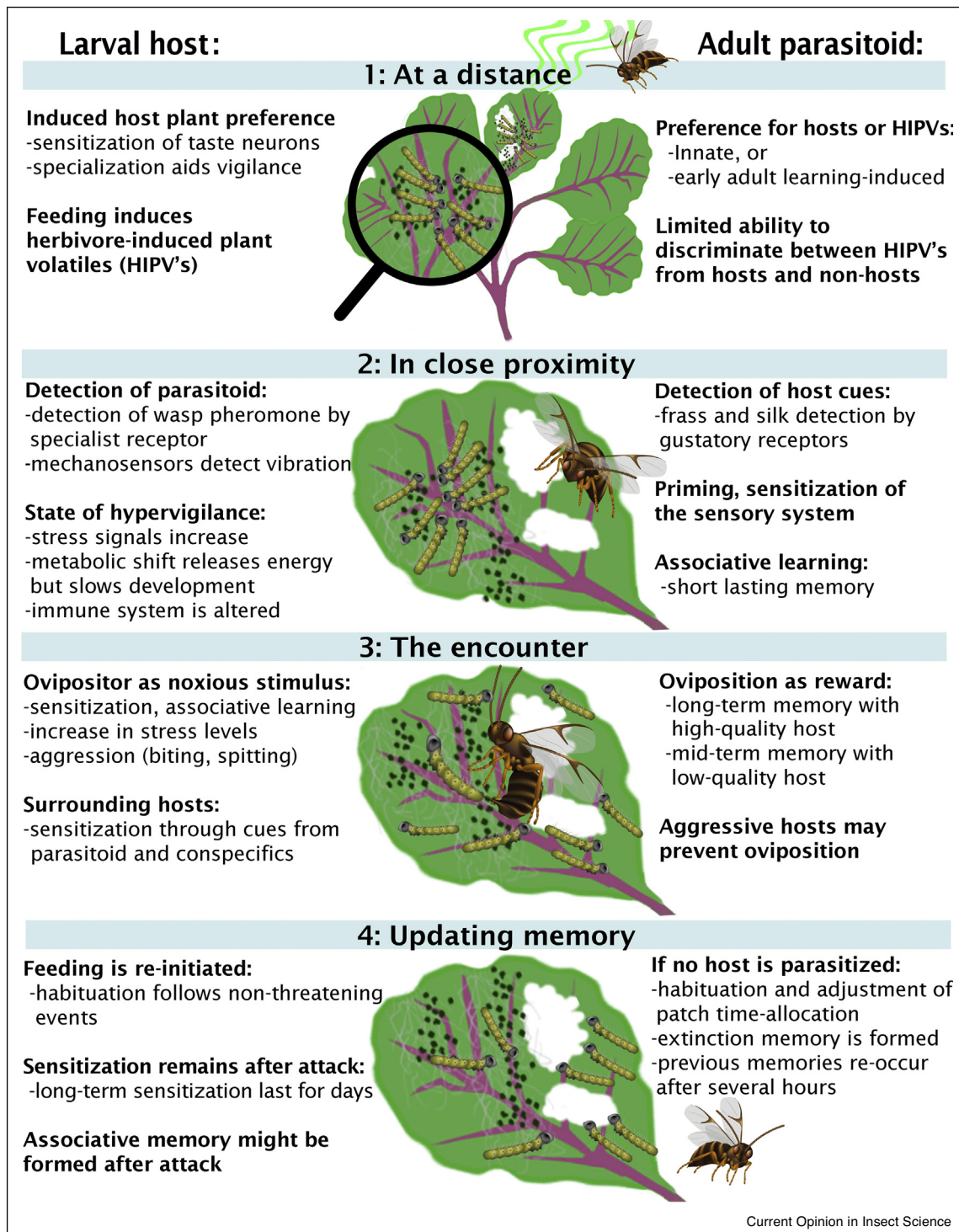
## A first approach

When herbivorous insect larvae initiate feeding, the chance of being attacked by a parasitoid or predator increases up to a hundred times as they have to give up their hiding places and because feeding triggers the release of volatiles, which are used by their natural enemy to locate their victims [5,6] ([Figure 1.1](#)). Because of this constant threat it has been argued that vigilance, that is, responsiveness to cues predicting the presence of parasitoids or predators is a major reason for host-plant specialization and host-plant learning in insect herbivores [7]. A strong plant preference, either innate or learned, will reduce the time required for choosing and ingesting food and would, therefore, provide more time and neuronal capacity for parasitoid avoidance and defensive behaviors [7].

Many herbivorous insect larvae have a short time window after emergence, in which their innate feeding preference can be either further strengthened or changed toward the acceptance of suitable, but novel host plants [8,9]. For instance, *Manduca sexta* caterpillars have an innate preference for solanaceous plants, which is further strengthened when larvae feed initially on these plants to such a degree that they will no longer accept other suitable but non-solanaceous plants. However, if the caterpillars feed initially on the suitable but non-solanaceous cowpea *Vigna sinensis*, these plants will also later on be accepted, although solanaceous plants will still be preferred [8]. This increased host-plant preference is at least partly due to sensitization of specific taste neurons, which increase their responses to indioside D, an important feeding stimulus present in many solanaceous plants, but absent in cowpea [10]. Such a sensitization of the taste neurons could be mediated by serotonin, similar to what has been shown in the blowfly *Phormia regina* [11]. Sensitization as a non-associative form of learning ([Box 1](#)) might not only help the host to avoid parasitoid enemies, but could also play a role in the oviposition choice of the adult insect in a phenomenon known as the Hopkins host selection principle [12]; a learning mechanism in which the adult oviposition choice is influenced by larval experiences.

Most parasitoids are highly specialized on a certain insect host species [13] ([Figure 1.1](#)), and consequently also have an innate preference for plant odors induced by the feeding of their hosts, so-called herbivore-induced plant

Figure 1



Parasitoids and their hosts exploit a diverse array of associative and non-associative memory traits before, during and after their encounter.

volatiles (HIPVs). The composition of this volatile blend is, however, influenced by multiple biotic factors such as other herbivores [14] or abiotic factors such as drought [15]. These variable circumstances make it particularly

challenging for naive parasitoids to find their host, especially as inexperienced wasps are often unable to discriminate plants infested by hosts from plants infested by non-hosts and even the presence of undamaged plants in the

**Box 1 Overview of basic learning and memory types**

Learning can be divided into two basic forms, **associative** and **non-associative** learning [42]. In non-associative learning, **habituation** occurs when a stimulus has no consequences after repeated exposure and the animal, therefore, learns to reduce its response in order to conserve resources. **Sensitization** occurs after a strong, meaningful stimulus, such as noxious stimuli, host traces, food and so on; the animal will increase its response level to that stimulus, but also to other stimuli; therefore, sensitization has a broader effect than habituation. From sensitization the animal learns that potential harmful events may occur or that potential resources are available. If such a stimulus is repeated without consequence, habituation can occur; a stimulus can, therefore, induce both habituation as well as sensitization.

In **associative learning**, an animal learns that a neutral, so-called **conditioned stimulus, CS**, precedes another, innately meaningful stimulus (the **unconditioned stimulus, US**), for instance an odor stimulus as CS and a rewarding food stimulus as US. The US induces a reflex, such as in proboscis extension reflex (PER) conditioning. After conditioning, memory can be measured through the occurrence of the **Conditioned Reflex (CR)** such as a PER, in response to the CS. This is a form of **Pavlovian**, or **classical conditioning** [43].

Another form of associative learning is **operant learning**, where an animal learns about the consequences of its behavior, for instance a wasp responding to plant volatiles induced by its host. When the wasp is rewarded by an oviposition experience, it learns that its foraging behavior in response to that odor leads to finding a suitable host. Thus, in **operant learning**; a behavior, in response to a stimulus, leads to a **reinforcement** (reward) or a punishment, resulting either in an increase or decrease in the probability of that behavioral response.

Learned information, either associative or non-associative, is stored as a so-called memory trace in the brain [44]. Memories can be divided in several different forms of short, mid and long-lasting memory, which can exist simultaneously and together add up to the observed memory retention levels in behavioral bioassays. The different memory forms can be discriminated from each other by various specific inhibitors or treatments, such as protein-synthesis inhibitors for long-term memory, and cold shock for short term memory [45]. Existing memory can be forgotten, but can also be temporarily inhibited by an extinction memory trace [46], formed after experiences where the expected reinforcement did not occur, such as when an expected host was no longer present on a plant that still emits attractive volatiles and contains traces of the host.

surroundings of a host-infested plant may reduce the foraging efficiency of these inexperienced wasps [16]. Early learning through contact with host cues such as frass or silk during or right after emergence from their pupae may help these wasps by inducing a more specialist foraging modus [17,18].

**In close proximity**

Foraging is a risky and stressful activity for any parasitoid female, costing not only valuable resources [19], but also exposing the parasitoid to intraguild predators and aggressively defending hosts, probably causing a state of stress similar to the one they themselves induce in their hosts [20–22].

After landing on a leaf the parasitoid will start searching for cues produced by the host, such as caterpillar frass or silk, indicating the potential presence of a suitable host, by drumming its antennae on the leaf surface and probing with the ovipositor (Figure 1.2). This first contact with host-derived cues causes a general arousal in the wasp, sometimes also called priming, which leads to an increased sensitivity of the sensory systems [23,24] and causes an intensive searching behavior of the parasitoid in the close vicinity of the host traces [25]. Interestingly, some parasitoids will already form associative memories linking their searching responses to certain plant odors after just a brief encounter with host cues, such as caterpillar frass or silk, without actually encountering the host itself [26]. These cues are likely detected by gustatory neurons on the antenna and the ovipositor [27–30]. The excitation of these neurons sufficiently serves as an innate reward leading to associative memory for the HIPVs (Box 1) lasting for 24 hours, but being less stable as memories induced by a full oviposition experience [26,31].

When approaching a potential host patch by flying or walking, even the stealthiest parasitoid will cause fine vibrations through its wings or feet and many hosts have evolved specific mechanosensory neurons in their chordotonal organs or sensory hairs on their body to detect these slight disturbances [32\*,33] (Table 1). These mechanosensors provide a major alarm system for the host, being precisely tuned to the wing-beat frequency of their air-borne predators of about 150 Hz [34,35]. Thereby they trigger a general increase in arousal and serve as an aversive stimulus during learning, as it has also been shown that hosts can learn to avoid odors associated with specific sound frequencies [36]. In addition, hosts might be able to detect specific olfactory cues emitted by their parasitoids. Larvae of different *Drosophila* species for example, which are under strong selection pressure by wasps of the genus *Leptopilina*, can detect the main pheromone compound of these wasps and quickly migrate away from the site of the odor encounter. This pheromone compound is detected through a single, highly specialized olfactory receptor in the antenna of the larvae (Or49a), which is conserved across different *Drosophila* species that are attacked by parasitoids of this genus [37]. Potentially such parasitoid infochemicals, but also alarm pheromones released by conspecifics, could also cause a sensitization of the host's sensory system, preparing the host for future attacks (Figure 1.2). In addition to sensitizing the host's peripheral receptors, the detection of parasitoid cues also increases levels of the stress neuromodulator octopamine in the hemolymph of *M. sexta*, causing a state of 'hypervigilance' [38]. In this state the caterpillars devote more resources to growth early in life, while later on more energy will be devoted to fight-or-flight responses. This will, however, delay the time to pupation and thereby extend the time a caterpillar

Table 1

## Proximate factors involved in a neuronal arms-race between parasitoids and their larval hosts

		Parasitoid	Ref.	Host	Ref.
Before encounter	Genes	225 ORs, 10 IRs ( <i>N. vitripennis</i> )	[66]	23 ORs, specifically Or49a ( <i>D. melanogaster</i> )	[37]
		190 glomeruli ( <i>Cotesia</i> sp.)		21 ORs, 10 IRs ( <i>M. sexta</i> )	[67]
	Neurons	220 glomeruli ( <i>M. croceipes</i> ) 100 glomeruli ( <i>T. evanescens</i> )	[29,68,69]	20 ORNs ( <i>M. sexta</i> ) 21 ORNs ( <i>D. melanogaster</i> )	[70] [71]
Initial detection	Genes	GRs?	?	PAINLESS, NOMPC, NANCHUNG, INACTIVE ( <i>D. melanogaster</i> )	[33,72]
	Neurons	24 gustatory neurons ( <i>L. heterotoma</i> )	[30]	24–32 md neurons/segment ( <i>M. sexta</i> ) 30 md neurons/segment ( <i>D. melanogaster</i> )	[47] [73]
During the encounter (in addition to those involved in initial detection)	Genes	<i>dunce</i> , <i>Octβ2R</i> , <i>aPKC</i> , <i>dDA1</i> , <i>GCH-1</i>	[58,74]	<i>Ddc</i>	[75]
		35–36 octopaminergic neurons ( <i>N. giraulti</i> and <i>N. vitripennis</i> ) 9 octopaminergic ( <i>T. evanescens</i> )	[60,76]	39 octopamine neurons ( <i>D. melanogaster</i> )	[77]
	Neurons (per hemisphere)	114–129 dopaminergic neurons ( <i>N. giraulti</i> and <i>N. vitripennis</i> ) 15 dopaminergic neurons ( <i>T. evanescens</i> )	[59*,76]	80 dopaminergic neurons ( <i>D. melanogaster</i> )	[78]

Abbreviations: OR = Olfactory receptor; IR = Ionotropic receptor; ORN = Olfactory receptor neuron; mdN = multidendritic neuron.

is exposed to its enemies [38–40]. Non-lethal predator attacks will also alter the immune system of the host in preparation of a potential infection [38]. Notably, changes in the immune system of the host also alter the sensitivity of nociceptive neurons, even though the exact mechanisms of this crosstalk between the immune system and the central nervous system are still unknown [41\*].

### The encounter

When being attacked by a parasitoid, caterpillars defend themselves by rapid and powerful head-strikes and oral secretion, resulting in repelling or even killing of the parasitoid (Figure 1.3) [21,22]. Similarly, *Drosophila* larvae can escape from their parasitoid enemies by fast crawling and vigorous rolling [34]. Both fly larvae and caterpillars detect nociceptive stimuli such as the sting of a parasitoid ovipositor with multidendritic neurons in their body wall, which are crucial for eliciting nocifensive behaviors [34,47]. In the caterpillar *M. sexta*, these neurons are also modified by both sensitization and habituation depending on the present threat to the animal [47–49]. This sensitization is not due to changes in the sensory neuron directly, but occurs centrally in the local ganglia [50\*] and is even generalized along the body of the caterpillar, so that an attack to one body segment still sensitizes the response at least two segments further away [51\*]. This type of non-associative learning lasts for at least 19 hours in *M. sexta* caterpillars and involves muscarinic acetylcholine receptors (mAChRs) during acquisition as well as cyclic nucleotide-gated channels (HCN), which are activated by cAMP or cGMP during memory consolidation [50\*,51\*]. These mechanisms are highly similar to the

processes involved in long-term memory (LTM) formation in other invertebrates and also in mammals [52,53]. Although this long-term sensitization is obviously adaptive in the acute presence of a predator, it also comes with both a physiological cost for protein synthesis as well as with ecological costs due to a prolonged developmental time [37,52]. It will, therefore, be interesting to assess the fitness of animals with a different preparedness for the formation of long-term sensitization in different ecological settings similarly to what has been done in parasitic wasps [52,54].

Having overcome the host's behavioral defenses, the female parasitoid inserts her ovipositor into the host and quickly injects her eggs (Figure 1.3). This oviposition experience provides a strong reward to the wasp and induces an associative memory between the reward and the odor-guided foraging behavior preceding this event [26]. Some parasitoid species readily form LTM after a single oviposition experience, while other, closely related species form LTM only after several repeated learning trials [5]. This variation in learning may correlate to the variation in distribution or quality of the host as both the larval parasitoid *Cotesia glomerata* as well as the egg parasitoid *Trichogramma brassicae* formed LTM after a single oviposition on a gregarious host, but not on a solitary host [52].

The difference in memory strength between frass-induced and oviposition-induced memories parallels the reward value of sweet taste versus caloric value in *Drosophila* [55]. In the fly, the short lasting memories of a



sweet sugar reward are reinforced through octopaminergic neurons acting upon the dopaminergic system of the mushroom bodies, while the caloric value of nutritional sugar rewards, that result in an increase in hemolymph sugar levels, is mediated by dopaminergic neurons alone [56]. It is conceivable that detection of frass and the internal signals of egg laying, are similarly mediated by octopamine and dopamine in the wasp. Indeed, dopamine and octopamine receptor antagonists impaired 24 hour olfactory memory with oviposition reward in female *Nasonia vitripennis* wasps, and injections of dopamine but not octopamine could substitute for the oviposition reward [57<sup>•</sup>]. Furthermore, a study on learning-induced gene expression comparing heads of the fast learning *C. glomerata* and slow learning *Cotesia rubecula* showed a downregulation of dopamine biosynthesis genes in *C. rubecula* as well as changes in the expression of octopamine receptors [59<sup>•</sup>]. A similar comparison of gene expression between an inbred strains of the parasitoid wasp *N. vitripennis* that learns fast, and the closely related species *Nasonia giraulti*, which learns slow, showed a downregulation of dopamine receptors as well as changes in the expression of genes involved in the dopamine biosynthesis [58]. On a morphological level, it has been shown that *N. vitripennis* has more dopaminergic neurons in clusters, known to be involved in memory formation in *Drosophila* [59<sup>•</sup>], than *N. giraulti*. In contrast to this, no such differences in the octopaminergic neurons were detected in these two *Nasonia* species [60]. One reason for this difference might be that octopamine is more involved in the formation of short term memories, as it has been shown in *Drosophila*, whereas the inter-specific and intra-specific differences in memory formation in parasitoid wasps were found rather in LTM formation [45,61–63]. Another reason might be that the octopamine-system has a broad effect on insect physiology and adaptations to oviposition learning might, therefore, be more prone to correlated, nonspecific responses [64,65<sup>•</sup>]. For instance, artificial selection on visual oviposition learning ability resulted in correlated responses to olfactory learning and male sexual learning [65<sup>•</sup>]. Thus, the specificity of the selection pressure has to be high, in order to result in specific adaptive changes in memory formation according to differences in host distribution.

#### Memory updating: habituation and extinction

Because of the variability and complexity of their environment, parasitoids need to constantly update their memories on whether certain innate or learned odor-guided behaviors are still reliably predicting suitable hosts (Figure 1.4) [79<sup>•</sup>]. When the parasitoid *Trissolcus basalis* was repeatedly exposed to traces of its host, the stink bug *Nezara viridula*, without providing an oviposition reward, the wasp showed progressively shorter search times for the next 48 hours [80<sup>•</sup>]. Interesting, this habituation effect could not be blocked using cold shock anesthesia, ATPase inactivators (ethacrynic acid), and

protein synthesis inhibitors (anisomycin), indicating that this form of non-associative memory might involve less known memory pathways [53] or might be due to direct modulations of the olfactory pathway. Findings in *Drosophila* demonstrated that olfactory habituation is mainly driven by inhibitory local interneurons in the antennal lobe [81]. It has been suggested that these inhibitory neurons are themselves modulated by different biogenic amines [82], which might also be important in the habituation of the wasp olfactory system. Independent of the physiological mechanism, this example highlights the important role which non-associative memories also play in the foraging behavior of the parasitoid. In addition to this habituation learning, the encounter of host traces in a parasitoid that already had an oviposition experience on a certain plant, will lead to a temporary extinction of memories that have previously been formed, if the encounter is not followed by an oviposition reward on the same plant [83]. However, two to four hours after such a negative experience, the original reward memory recovers. This recovery indicates that underlying memories were not erased, but rather that an additional memory trace called ‘extinction memory’ is formed that exists in parallel to the original association, rendering this association more ambiguous [46,84]. In addition, secondary negative experiences can also help to sharpen the original rewarding association, if this negative experience takes place on a different plant. If the wasp *Aphidius gifuensis*, for example, first encountered a host on wheat and was thereafter placed on a wheat plant without a host, an extinction memory was formed. However, if the negative experience took place on a bean plant, the preference of the wasp even for unfested wheat was enhanced in comparison to the unrewarding plant [85]. Hence, the combination of habituation and extinction memory forms a crucial evaluation system that helps to maintain the foraging efficiency of parasitoids also in complex environments. Further investigations into the underlying mechanisms involved in habituation and memory extinction in the interaction between host and parasitoids could benefit from combining these ecological findings with neurobiological results from *Drosophila* [82,84].

Having successfully repelled or escaped a parasitoid attacker, the host will soon need to resume feeding and switch its metabolism back from defense to growth by readjusting its neurohormonal levels, in order to minimize any costs on its development (Figure 1.4) [38]. Resources are further preserved by a quick habituation to weaker stimuli that are perceived as non-threatening by the host, such as other herbivorous insects or moving plant parts. Habituation has been demonstrated for the nociceptive neurons of *M. sexta* caterpillars [47], but has been most intensely studied in the caterpillar proleg-withdrawal-reflex. This reflex, which is triggered by the deflection of a sensory hair on the prolegs, habituates after repeated stimulation of the sensory hair without

further noxious events. Interestingly, habituation is not due to a decreasing activity of the involved sensory or motor neurons, but likely due to modulation by local interneurons, similar to those involved in the processing of noxious stimuli [34,86]. This tight interplay between sensitization and habituation within the same local neuronal network will help the host to strike the optimal balance between growth and defense, in addition to the more decentralized effects caused by biogenic amines and other neuromodulators [87].

## Conclusions

Throughout this review, we have presented various adaptations in the learning and memory system of parasitoids as well as their hosts. In parasitoids, specific adaptations in memory formation to their host's behavior are very apparent and have been studied in much detail [54]. In contrast to this, parasitoid avoidance learning in the host has been studied to a far lower degree, even though recent studies and reviews highlight the potential importance of non-associative forms of learning for the defensive behavior of the host [51,52]. To this extent, the application of molecular tools to ecological model-systems [88] will enable a direct investigation of how memory formation in parasitoids and hosts have shaped each other. Investigating these mechanisms in an ecological setting will, therefore, not only allow an exploitation of these specific adaptations for a sustainable crop-protection [42,89], but will also help to better understand the function of the nervous system in different animals based on its evolutionary context.

## Conflict of interest statement

Nothing declared.

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