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Review

Natural variation in learning rate and memory dynamics in parasitoid wasps: opportunities for converging ecology and neuroscience

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Although the neural and genetic pathways underlying learning and memory formation seem strikingly similar among species of distant animal phyla, several more subtle inter- and intraspecific differences become evident from studies on model organisms. The true significance of such variation can only be understood when integrating this with information on the ecological relevance. Here, we argue that parasitoid wasps provide an excellent opportunity for multi-disciplinary studies that integrate ultimate and proximate approaches. These insects display interspecific variation in learning rate and memory dynamics that reflects natural variation in a daunting foraging task that largely determines their fitness: finding the inconspicuous hosts to which they will assign their offspring to develop. We review bioassays used for oviposition learning, the ecological factors that are considered to underlie the observed differences in learning rate and memory dynamics, and the opportunities for convergence of ecology and neuroscience that are offered by using parasitoid wasps as model species. We advocate that variation in learning and memory traits has evolved to suit an insect's lifestyle within its ecological niche.

Keywords: learning; memory; ecology; parasitoid wasp; neurobiology

1. INTRODUCTION

Parasitoid wasps lay their eggs in or onto other insects that eventually will be killed by the developing parasitoid larvae. There are parasitoids that develop in either eggs, larvae or pupae, generalists and specialists, gregarious (i.e. laying several eggs into one host) and solitary species; and there is a similar diversity in the ecology of their host insects and their respective food sources. Finding a host insect is a daunting task for all female parasitoids, because hosts are under strong selection to remain inconspicuous to their natural enemies, irrespective of whether the host is in the egg, larval or pupal stage. The use of indirect, chemical information is an important solution for parasitoid wasps to solve this detectability problem. For instance, parasitoids of dipteran hosts use odours of decaying substrates like mushrooms, fruits or carcasses to find host fly larvae, and parasitoids of herbivorous larvae use odours of the plant their host is feeding from. Alternatively, parasitoids can rely on information from other, more conspicuous host stages than the one under attack, such as egg parasitoids that use the pheromones of the adult host stage [1]. These odours are very complex blends, however, and minute differences between these blends may signal the presence of suitable hosts [2]. Dukas [3] proposed that generalist species may need to divide their attention over a wide variety of cues, which reduces searching efficiency owing to limited brain capacity for simultaneous processing of information. This may be a reason why so many insects are specialists, since these specialist species can focus on a more reduced set of stimuli compared with generalists [4]. Learning may provide a more flexible way of specialization. For instance, parasitoid wasps that experience the current presence of a certain host species, thereafter narrow their (olfactory) 'search image' by learning, as a form of temporal specialization [5]. Although learning was considered as a trait that is more important for generalists that specialists, it has become clear that learning is also important as for specialist parasitoid wasps [6]. Indeed, although female parasitoids have an innate preference for certain odours, in most investigated species associative learning optimizes their foraging efficiency [7,8].

Recent research unveiled remarkable natural variation in learning rate and in the dynamics of memory formation between closely related parasitoid wasp species [9–13]. These studies suggested that this variation reflects adaptations to species-specific ecological constraints. The

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rich behavioural diversity among parasitoid wasp species offers a wealth of possibilities for a comparative approach to address both ultimate and proximate questions on the evolution of learning rate and memory formation. As we will argue in the last part of this paper, such a comparative approach creates unique opportunities for the convergence of ecology and neuroscience.

2. LEARNING IN PARASITOID WASPS

Finding suitable hosts is a difficult task for female parasitoid wasps, but inexperienced wasps by no means search randomly. They respond innately to stimuli that are derived from their hosts or that indicate suitable hosts [7,8], comparable to innate colour preferences in pollinators [14,15]. Parasitoids can change their innate preferences for odour cues that guide them to patches with hosts after an oviposition experience. This allows female wasps to find hosts faster, thus probably increasing their lifetime foraging success [16]. This oviposition learning of long-range odour cues is the main focus of this review.

In parasitoids, learning of odour cues has been studied most extensively (e.g. [8,12]), but parasitoids are also able to learn other cues such as colours, shapes, patterns and spatial information (e.g. [7,17,18]) Parasitoid wasps also learn information about the availability of suitable hosts and use that information during subsequent visits to other patches. For parasitoids of dipteran larvae, it was found that the lower the quality of hosts in previously visited patches, the longer they search on patches with high-quality hosts [19]. Similarly, previous experience with unparasitized hosts reduces the acceptance of subsequently encountered hosts that already have been parasitized [19]. Addressing oviposition learning is highly relevant from an evolutionary ecological point of view, because the success of a female parasitoid to find and parasitize a host is directly linked to its Darwinian fitness [20,21].

(a) *Oviposition learning*

In nature, a parasitoid first has to localize the microhabitat that probably contains hosts by using long-range odours (long-distance search, in most cases flight) and subsequently it has to localize the host within this microhabitat. It is only during this local search that the parasitoid encounters host-derived cues, such as faeces, silk, saliva and plant damage. These host cues provide direct information on host presence and suitability and initiate the actual oviposition behaviour; the ovipositor is inserted in the host and eggs are laid. In laboratory set-ups, oviposition-learning bioassays are used to study parasitoid learning and memory formation in ecologically relevant simulations of the natural situation. In most oviposition learning bioassays, researchers immediately bring the parasitoid in contact with host-derived cues, thereby bypassing the parasitoid's long- and short-distance searching behaviour. In those cases, in which a conditioned stimulus (CS) (an odour) is directly followed by a reward, the conditioning procedure can be considered as a form of classical (Pavlovian) conditioning, where an association is formed between the odour and the oviposition reward. The unconditioned stimulus (US) in oviposition learning consists of two main components, i.e. contact with the host traces and the oviposition in the host, whereas complex natural odour blends are usually employed as CS (see the electronic supplementary material for further description of cues involved in oviposition learning). After oviposition, the wasp is gently removed, thus ending the conditioning trial.

Different set-ups are used for testing memory retention in parasitoids; all are olfactometers in which a wasp can fly or walk towards the learned odour [9,22,23]. In a two-choice odour preference test, wasps can choose between the learned odour and a reference odour. Memory retention is calculated for groups of wasps and considered to be present if there is a shift in preference: i.e. when the percentage of trained wasps choosing the 'learned' odour is increased compared with naive wasps. The strength of the innate responses to each of these odours has to be carefully considered to avoid masking of memory retention [24].

3. VARIATION IN LEARNING RATE AND MEMORY DYNAMICS

In parasitoid wasps, we encounter interesting variation in learning between closely related species and we argue that learning rate (defined here as the number of trials required for long-term memory (LTM)) and memory dynamics are functional traits involved in the optimization of the foraging task, and shaped by the balance between costs and benefits of these traits.

First of all, learning has several ecological costs. For instance, it would be costly for a parasitoid to change a valuable innate preference after a single oviposition experience on a plant species on which its hosts rarely occur. To prevent such maladaptive associations, animals usually require repeated learning experiences, spaced in time, before they form long-lasting memories but there are also animals that have a high learning rate, i.e. that learn instantly after only one learning experience [12,22,23,25]. Second, and as a consequence of the previous, learning takes time. During the learning process animals behave suboptimally, which infers a cost to the individual [26]. Furthermore, there are costs in terms of energy for maintenance and signalling in the nervous system [27] and for memory formation itself [28–30]. The sum of all these different costs explains why every insect does not learn instantly: the possible benefits of a high learning rate may just not outweigh the costs. Parasitoid wasps, with their range of interspecific variation in learning rate and memory dynamics, can be instrumental in elucidating which ecological factors are important in this context.

(a) *Innate and learned behaviour*

It is important to consider how learning changes innate behaviour. Vet *et al.* [24] proposed that learning affects innate responses depending on their strength or evolutionary importance: strong innate responses are less affected by experience than weak innate responses, and the ranking of the importance of cues that evoke these responses may be altered by experience. In this concept, stimuli can become more or less important after experience. This process is regarded as a shift in preference. For instance, *Cotesia glomerata*, a parasitoid of cabbage white caterpillars, has a low innate preference for odours of nasturtium, a less common food plant of its hosts [9]. One or multiple ovipositions in hosts on nasturtium will, however, increase the preference towards nasturtium, resulting in a higher percentage of wasps that choose nasturtium over the innately preferred cabbage in a two-choice bioassay. The innate preference for cabbage odours returns after 4 days, however [31]. Innate responses can also be highly persistent. *Leptopilina fimbriata*, a specialist of fly larvae in decaying plant substrates can learn to respond to other substrates, such as fermenting fruits. In the presence of its innately preferred substrate, the majority of the wasps still choose the innately preferred substrate, however. Only when testing these wasps on the learned substrate, it becomes clear that their response to this substrate has increased [32]. Thus, innate responses and learned responses should not be considered as two separate traits, but as two dynamically interacting components of insect behaviour.

(b) *Memory dynamics*

Learning rate cannot be considered separate from memory dynamics. The impact of learning is determined by the way the learned information is stored in memory, whereas the type and number of conditioning events determine what type of memory will be formed. To study this aspect, it is essential to discriminate between different forms of memory, since these forms vary in terms of energy consumption, stability and duration.

Eisenhardt [33] reviewed memory types in the honeybee, whereas a comparable classification exists for the fruitfly, *Drosophila melanogaster* [34]; both classifications are based on highly similar mechanisms [35], but the used terminology is somewhat different. First, there is early short-term memory (eSTM or working memory) and late STM (lSTM). These types of memory can be disrupted with anaesthesia, such as a brief exposure to a cold shock in insects, and are known as forms of anaesthesia-sensitive memory (ASM) [36,37]. ASM lasts, depending on the investigated species, for several minutes up to a few hours [13,36,37]. During the ASM phase, the formation of long-lasting memory types starts, a process called memory consolidation [34]. Two main forms of consolidated memory can be distinguished based on their sensitivity to protein synthesis inhibitors. LTM requires protein synthesis and can consequently be disrupted by feeding insects a protein synthesis inhibitor. The other type, called mid-term memory in honeybees, is resistant to cold shock but not dependent on protein synthesis, hence it is a form of anaesthesia-resistant memory (ARM). ARM is based on changes in existing proteins, in contrast with LTM consolidation [38]. ARM and LTM may occur in parallel, and the process of their consolidation may last for hours to days [12]. ARM is less stable and durable than LTM, but it does not require protein synthesis, and is therefore regarded as 'cheaper' memory than LTM; flies that consolidated ARM lived longer than those consolidating LTM [29]. The type of memory that is consolidated depends on both the number of conditioning trials and the intertrial interval. In aversive conditioning of the fruitfly, single trainings or 10 trainings given without intertrial interval (massed conditioning) induced only ARM formation; LTM was typically formed only after 10 spaced trainings [39]. Studies with bees have shown that conditioning procedures with an intertrial interval of 10 min are sufficient to be regarded as a spaced training protocol [40].

Menzel [41] explained the adaptive value of these different memory types by correlating them to the different use of memory during foraging decisions of a honeybee. In this view, eSTM (in the range of seconds, see [42] and references therein) is used for intra-patch decisions such as whether to stay or leave a patch of similar flowers, whereas lSTM (in the range of minutes up to 1 h [33,41]) is used to store information from different patches of flowers that can either be more or less rewarding. Memory-inhibiting genes (see §4*b*) could prevent early memories from being consolidated unless several learning trials, stored in lSTM provide the required 'spaced training'. After a return visit to the hive, memory of the previous foraging bout can be retrieved from ARM, or memory of previous days can be retrieved from LTM, and used to evaluate the quality of subsequent flower patches. Thus, the temporal dynamics of the different memory types serve a specific role in time- and event-specific behaviour of the honeybee. Likewise, the evolution of learning and memory of parasitoid wasps may also be driven by resource distribution.

The dynamics of time- and event-specific learning experience can be expected to vary immensely between species that forage for instance on solitarily versus gregariously feeding hosts, and between specialist and generalist parasitoid species. Wasps that are foraging for solitarily feeding hosts, for instance, experience many single conditioning trials spaced in time. By contrast, wasps foraging for gregariously feeding hosts may lay half of their eggs when finding a single patch with many hosts, which represents only one, massed-conditioning cycle. Hence, variation can be expected in memory dynamics between closely related species that differ in this ecological aspect. It is clear that the timing of a memory test must be carefully determined and the type of memory that is formed should be known for proper interpretation; information stored in STM has a different function than information stored in LTM.

(c) *Ecological factors determining variation in learning rate and memory dynamics*

Several factors can be proposed as causes of variation in learning rate and memory dynamics. Here, we first describe different factors in the context of memory dynamics, and then give examples of how a combination of factors can result in different learning and memory dynamics.

Stephens [43] argued that variability of the environment can determine the value of an innate response, and therefore its susceptibility to be altered by learning. In the case of a parasitoid wasp that experiences the temporal contingency of a cue with an oviposition reward, the variability determines whether that cue reliably predicts subsequent oviposition opportunities. If the variability in that cue is high within a generation, learning can be adaptive but memory must be stored in forms that can be changed easily, so in STM or ARM, not in LTM, because of the risk of learning irrelevant information. In that case, the reliability of a single experience is low; hence information can be only stored as LTM after several repeated conformational experiences. If variability changes slowly over the season, but is stable within a generation, reliability of learned information is high. This could result in a high learning rate: a single experience would be reliable and can then result in LTM formation. However, if between-generation variability is low, the value of innate responses is high, resulting in a strong innate response and a low learning rate. For instance, if hosts can always be found on the same host plants throughout the year, the value of innate preferences is higher than under more variable conditions [1,24,43]. Indeed, both in parasitoids and in bumble-bees it was found that strong innate preferences are less likely to be changed after experience [15,32].

The reward value is an important factor that influences learning; stronger rewards increase learning rate [44]. In the case of oviposition learning in parasitoid wasps, the reward value may also vary considerably. First, hosts can occur solitarily or in clusters; obviously, a cluster of host eggs or larvae will be a much larger reward for a parasitoid wasp. Second, the quality of a host influences the reward value, a larger host species may, for example, allow for the deposition of more eggs and may result in better quality offspring [45]. Some host species may have reduced immune responses compared with other species, resulting in increased survival of the parasitoid's larvae [19]. Third, if hosts have been parasitized already by another parasitoid, their value as a reward can be experienced as lower than the value of unparasitized hosts [19]. In fact, the value of a reward also depends on the reliability of the learned cue (see above), but if the reward value is high, a less reliable cue may still be valuable to remember.

Roitberg [46] proposed that the number of lifetime learning experiences may influence the value of learning. Indeed, an animal that has only few lifetime learning experiences has less opportunity to spend several experiences to optimize its behaviour, thus slow learning may not be feasible in such a case. This may result in absence of learning ability [47], but also in a high learning rate [48].

(d) *Learning and memory dynamics in parasitoid wasps*

Research on a number of species of the genera *Cotesia*, *Leptopilina* and *Trichogramma* is described here more in detail, to assess the differential effects of ecological factors on learning rate and memory dynamics.

(i) *Genus* Cotesia

Four species of the genus *Cotesia*, each with a very different ecology, have been investigated extensively with regard to learning and memory formation. *Cotesia marginiventris* is a highly generalistic species that parasitizes larvae of many lepidopteran species on a number of plant species [49]. The availability of different host species changes over time and learning may allow *C. marginiventris* to search for the currently most abundant host species [7,11]. By contrast, *Cotesia flavipes*, does not shift its preference after a host encounter [47]. This species parasitizes several species of stemborer larvae that typically occur in large fields of perennial grasses. This is an example of a highly constant and predictable environment in which innate preferences would suffice [43]. Furthermore, the average number of oviposition experiences is low, because the gregarious *C. flavipes* female attacks its host larvae by entering the stemborer tunnel, causing a high mortality rate of the parasitoid owing to the chance of being crushed between the host and the wall of the tunnel. Learning may, therefore, be of little use in this situation [47].

Cotesia glomerata and *Cotesia rubecula* are closely related parasitoid species of cabbage white caterpillars (*Pieris* spp.) that differ not only in learning rate, but also in memory dynamics [12]. *Cotesia glomerata* formed LTM after a single conditioning trial, whereas *C. rubecula* needed three trials spaced in time before it formed LTM; massed experiences did not cause such an LTM, as has also been shown in fruitflies [34]. After both single and multiple conditioning trials, *C. glomerata* consolidated LTM within 4 h, as determined from the maximum effect achieved from application of a protein synthesis inhibitor. By contrast, a single oviposition or three massed ovipositions resulted in ARM formation in *C. rubecula*, which lasted 8 h but had waned after 24 h [13]. Three spaced ovipositions did result in LTM consolidation, but this process was completed only after 3 days in *C. rubecula*, suggesting that ARM was present in parallel. These results suggest that both learning and memory formation in *C. rubecula* occur slower than in *C. glomerata* [12,13]. There are a number of reasons why this slow learning and memory consolidation may be adaptive for *C. rubecula*, but not for *C. glomerata*. *Cotesia rubecula* is a solitary parasitoid and a specialist on the solitary caterpillar *Pieris rapae*. *Cotesia glomerata*, on the other hand is a gregarious species and its preferred host, *Pieris brassicae* is a gregariously feeding caterpillar. First, the value of the reward may differ for the two species. The fact that *P. brassicae* occurs in clusters means that finding these hosts provide a larger rewarding value compared with finding a single *P. rapae* caterpillar. The second factor relates to the distribution of caterpillars over plant species. *Pieris brassicae* lays clusters of eggs on dense stands of similar plants. By contrast, *P. rapae* randomly distributes single eggs over different host plant species and travels rather long distances between two oviposition events [50]. The association between the plant odour and host presence is therefore expected to be less reliable for *C. rubecula*. The third factor that differs is the lifetime number of learning experiences, which is low in *C. glomerata* compared with *C. rubecula*. *Pieris brassicae* caterpillars occur in groups on a single leaf, allowing *C. glomerata* to deposit hundreds of eggs at once, which is a large part of its lifetime fecundity, in what is in fact one (massed) conditioning trial. In *C. rubecula*, on the other hand, each oviposition constitutes a single learning trial. The lower lifetime number of learning experiences allows *C. glomerata* to consolidate LTM after a single encounter with *P. brassicae*, whereas such instant learning would be costly for *C. rubecula*. Both the lower learning rate and slow consolidation of memory allow *C. rubecula* wasps to assess the reliability of the information over a longer time window.

(ii) *Genus* Trichogramma

Wasps of the genus *Trichogramma* are minute egg parasitoids of lepidopteran eggs. These gregarious parasitoids have a limited control over flight direction and may instead hitch-hike on female butterflies to the site where they lay their eggs. Two closely related *Trichogramma* species, *Trichogramma brassicae* and *Trichogramma evanescens*, exploit species-specific anti-aphrodisiac pheromones of two of their hosts, the gregarious *P. brassicae*, and the solitary *P. rapae*. These pheromones are transferred from male butterflies to females during mating to render them less attractive to conspecific males [51]. When *T. brassicae* detects an anti-aphrodisiac, it innately mounts on a mated (and thus egg-laying) female butterfly and hitch-hikes to a plant where the wasp parasitizes the butterfly's freshly laid eggs [52,53]. *Trichogramma evanescens* exploits the anti-aphrodisiacs in a similar way, but only after learning. A single operant conditioning trial, where approaching and mounting of a mated female *P. brassicae* butterfly upon the odour stimulus is followed by oviposition in a butterfly egg, induces LTM formation within 24 h [22]. *Trichogramma evanescens* is expected to have a wider range of host species than *T. brassicae* [22,53], and although it innately climbs onto butterflies, it does not discriminate between mated female, virgin female and male *Pieris* butterflies. LTM formation after one successful ride, especially on a gregarious-mated female *P. brassicae* butterfly, is adaptive for *T. evanescens* as a few limited opportunities to hitch-hike with such females should be enough to lay all the eggs a female wasp produces during her short lifespan [54]. This is similar to the situation for *C. glomerata*.

(iii) *Genus* Leptopilina

Species of the genus *Leptopilina* parasitize *Drosophila* larvae, which they find by probing in very different substrates such as fermenting fruits, decaying mushrooms or decaying plant material. Some species of *Leptopilina* are generalist species that attack multiple drosophilid species in several habitats, whereas other species have a more restricted host and/or substrate range. Poolman Simons *et al.* [32] compared the generalist *Leptopilina heterotoma* and the specialist *Leptopilina boulardi* parasitoids and showed that both species shifted their preference towards a learned odour after a single oviposition experience. Other studies have found that a preference shift was maintained up to 3 days in *L. heterotoma* [55] and approximately 1–2 days in *L. boulardi* [32,56]. An important difference between *L. heterotoma* and *L. boulardi* that affects their foraging behaviour is observed in innate preferences, as *L. boulardi* responded invariably strongly to innately preferred apple substrate, regardless of previous experiences, whereas the response of *L. heterotoma* depended on previous experience in all cases tested [32,55]. This result highlights the importance of addressing learning as well as innate behaviour to understand foraging behaviour of a parasitoid wasp. Interesting questions that remain are whether differences in memory dynamics exist and how different training regimes affect these memory dynamics. Species of the genus *Leptopilina* are well suited to investigate such questions using a comparative approach.

(iv) *Using a comparative approach to study learning rate and memory dynamics*

Although many studies have focused on learning and memory in parasitoid wasps, only few of these have used a comparative approach. The above mentioned studies on wasps of the genera *Cotesia*, *Leptopilina* and *Trichogramma* have provided valuable insights into the range of variation and the ecological factors that probably contribute to the observed differences. Below we describe some additional comparative studies in this context. Tamo *et al.* [11] have studied effects of single trial conditioning in three generalist parasitoid species, *Cotesia marginiventris*, *Campoletis sonorensis* and *Microplitis rufiventris*, and showed that the effect of conditioning was different for the three species. While *C. marginiventris* showed a preference shift towards the learned odour, *M. rufiventris* increased its innate preference and *C. sonorensis* did not show any change in its preference. This shows that host range by itself does not satisfactorily explain differences in learning rate in this case [6,11]. Two species of pteromalid wasps, *Nasonia vitripennis* [57] and *Lariophagus distinguendus* [23] were analysed for their memory dynamics by using either an inhibitor of ARM or LTM. In *L. distinguendus*, LTM was formed after single trial conditioning, comparable to *C. glomerata*. In *N. vitripennis*, however, memory formed after a single trial waned between 4–6 days and was therefore assumed not to be LTM. Instead, this memory could be inhibited by a blocker of intermediate memory forms (presumably ARM). Such a difference can be explained by differences in distribution patterns of their respective hosts. *Lariophagus distinguendus* parasitizes stored grain beetles, which occur in large patches, so similar to the situation for *C. glomerata*. *Nasonia vitripennis* is a parasitoid of fly pupae that can be found predominantly in bird nests in low densities (mostly between 1 and 10), which would favour ARM-like memory rather than LTM after a single experience. This is an interesting finding, also because *N. vitripennis* has become a new model species for which genomics tools have been developed (see §4*c*). Future experiments may reveal a more complete overview of memory dynamics of this species.

In order to properly compare learning rate and memory dynamics of different parasitoids wasp species, it is important to understand how different conditioning set-ups and test procedures can affect a parasitoid's response. Generally speaking, it will be most feasible to compare species that can be assayed with very similar methods, because this will reduce the likelihood that the conditioning itself, instead of the ecological factors under investigation, will influence observed differences.

(e) ***Intraspecific variation in learning and memory***

Besides species-specific differences, intraspecific variation in learning can also be expected. For instance, some bumble-bee (*Bombus terrestris*) populations from geographically different locations had a stronger and more persistent innate preference for blue flowers than others, and had a lower learning rate [15]. Colonies obtained from a commercial supplier differed in learning rate, and those with a lower learning rate were less efficient in foraging for nectar [58]. This shows that innate preferences differ profoundly between populations of the same species, and that these differences affect learning rate that may have influence on efficiency of nectar collection. Thus, at population level, specific adaptations to ecological differences are likely to be common as well as at species level. Similar studies can be performed with parasitoid wasp species that have, for instance, different hosts in geographically different populations. Two populations of *L. heterotoma*, were compared, one of which coexists with its superior competititor *L. boulardi*. This population, therefore, has a limited time window for successful parasitation, resulting in a higher innate response level than the other population. However, no differences in learning were found in this study [56].

Another way to unravel the effects of different ecological factors on learning traits is to perform artificial selection experiments. This approach can be used to test specific predictions raised by species comparisons. For instance, Smid *et al.* [12] hypothesized that *C. glomerata* does not form ARM after a single learning trial, but only LTM. An artificial selection experiment with this parasitoid species, aimed to decrease learning rate, resulted in a low learning rate line in less than 10 generations [13]. Wasps of this low learning-rate line formed normal LTM after spaced conditioning, but formed only ASM after single trial conditioning, instead of LTM, which is formed in unselected wasps. This showed that *C. glomerata* does not form ARM instead of LTM when selection pressure is used against a high learning rate. Apparently, single-trial LTM formation is 'hard-wired' in this species, when it is rewarded with *P. brassicae*. Experiments to assess costs of having a high or low learning rate are a logical next step when such selection lines are established. In *Drosophila*, it was shown that learning has both operating costs as well as constitutive costs: flies from a high learning line had a reduced larval competitive ability [28] and a reduced longevity [30].

4. OPPORTUNITIES FOR CONVERGENCE OF ECOLOGY AND NEUROSCIENCE

Ecologists have eagerly exploited parasitoid wasps to investigate the adaptive value of variation in learning rate and memory dynamics, providing insights on the ecological relevance of these differences. However, to fully understand learning and memory, a multi-disciplinary approach is needed in which ecological and evolutionary studies are combined with mechanistic studies. The vast amount of neuroscientific knowledge of a few well-established insect model species, mainly fruitflies and honeybees, provides valuable reference information and promising candidate neurons and genes to investigate mechanisms underlying learning and memory formation [33,59].

(a) *Neural pathways involved in learning and memory*

When insects learn odours, these odours are detected by olfactory receptor neurons and the resulting information is processed in the insect brain. Differences in perception or neurological processing of the odour cues may play a role in the observed differences in learning rate. An analysis of antennal responses to individual odour components emitted by cabbage plants showed that *C. glomerata* and *C. rubecula* have a comparable olfactory receptive range [60] and also the morphology of their antennal sensilla is similar [61]. Furthermore, a three-dimensional analysis of the glomeruli in the antennal lobe (AL), which is the first brain structure in the olfactory pathway, showed that the glomerular organization is similar for both species [62]. These first results suggest that the perception and processing of odours in these species are comparable but more research is necessary to compare different species and include higher integrative centres, like the mushroom bodies (MB) and the lateral horn (LH) [59].

Another possibility that may explain differences in memory dynamics of parasitoid wasps is the perception of the US. During oviposition, host traces and host haemolymph are perceived by the antennae and the ovipositor [63,64]. It is currently unknown how this information is transmitted to the brain. In honeybees and fruitflies, octopaminergic neurons were found to transmit rewarding stimuli in the brain, which is distinctly different from the dopaminergic pathway that transmits information on aversive stimuli [65]. In honeybees, a sucrose reward is detected by receptor neurons on the mouthparts, which activate the VUMmx1 neuron. This neuron innervates the AL, MB and LH, which are, therefore, all putative locations for the convergence of the US and CS [66]. It is expected that neurons with similar properties transmit the host reward signals in the parasitoid's brain, and differences in response characteristics or in the density of axonal endings, from which octopamine is released, may underlie the observed species-specific differences in learning rate and memory dynamics. Several octopaminergic neurons have already been identified in *C. glomerata* and *C. rubecula* [67] and it would be interesting to investigate which neurons transmit which reward signals and whether differences in this pathway can be correlated to differences in memory dynamics.

(b) *Genes involved in learning and memory*

The genetic pathways that are involved in memory formation are highly conserved, even for organisms ranging from insects to mammals [68]. Research on model insects has resulted in a long list of genes that are involved in memory formation (e.g. [59]). One of the most extensively studied pathways involved in learning is the cAMP-dependent signalling pathway [33,34]. The CREB (cAMP responsive element binding protein) gene is a transcription factor in this pathway, which plays a decisive role in the initiation of LTM formation [69,70]. Several different isoforms resulting from alternative splicing of CREB transcripts are known, which can act as either transcriptional activator or suppressor [71]. It was shown that high expression levels of CREB-suppressor isoforms inhibit LTM formation in a variety of species [69,72]. This resulted in the hypothesis that the balance between CREB activators and inhibitors acts as a molecular switch, determining the number of spaced training events that is required for LTM formation [71,73,74]. Nine different CREB transcripts were identified in *C. glomerata* and *C. rubecula*, predicting putative CREB activator and suppressor isoforms, which are identical in both species. A first study on CREB expression in naive wasps of these two species showed the relative abundance of each of the transcripts, which was similar for the most abundant transcripts [74].

So far, the CREB gene, which is a promising candidate gene for differences in learning rate, has been the only gene investigated in parasitoids in this context. The candidate gene approach [75] can be used to study other genes that may be involved in differences in learning and memory dynamics.

(c) *Future perspectives*

Some exciting new developments contribute to the potential of parasitoid wasps as model organisms for multi-disciplinary studies. Three species of the genus *Nasonia* have recently been sequenced and annotated [76] and molecular research on other parasitoid species can benefit greatly from this genome sequence information. Furthermore, several molecular tools and resources, such as microarrays and expression array data, are available for these species and it is possible to interbreed the different *Nasonia* species, allowing back-crossing of loci of one species into the genetic background of another species. This allows for identification of quantitative trait loci [77].

Other new tools that will probably accelerate neuroscientific research in parasitoid wasps are RNAi and next-generation sequencing. RNAi makes it possible to use a direct genetic approach in non-model organisms [78]. It can be used to reduce the expression of a specific gene and to subsequently investigate the direct effect of this gene on the behaviour of the insect or on the expression of other genes. Next-generation sequencing provides a genomics approach for non-model organisms [79]. It will provide information on expression levels of all genes and will allow a better understanding of the genetic networks that cause interspecific variation in memory dynamics.

Research on parasitoid wasps can provide a wealth of information on both the ecological relevance and the neural and genetic mechanisms underlying variation in learning and memory formation. Such multi-disciplinary research is necessary to understand the mechanisms that underlie naturally occurring variation, but it will also elucidate the true significance of neural or genetic variation. Both ecologists and neuroscientists can greatly benefit from a convergence of their fields. Considering the conserved genetic pathways that are involved in learning and memory formation [68], this integration may not only further the field of insect behaviour, but may also simultaneously enhance our understanding of learning and memory in higher animals.

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