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The complexity of learning, memory and neural processes in an evolutionary ecological context

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Abstract

The ability to learn and form memories is widespread among insects, but there exists considerable natural variation between species and populations in these traits. Variation manifests itself in the way information is stored in different memory forms. This review focuses on ecological factors such as environmental information, spatial aspects of foraging behavior and resource distribution that drive the evolution of this natural variation and discusses the role of different genes and neural networks. We conclude that at the level of individual, population or species, insect learning and memory cannot be described as good or bad. Rather, we argue that insects evolve tailor-made learning and memory types; they gate learned information into memories with high or low persistence. This way, they are prepared to learn and form memory to optimally deal with the specific ecologies of their foraging environments.

Highlights:

- **Environmental variation, spatial foraging behavior and the distribution of resources are factors that drive the evolution of differences in prepared learning**
- **Considerable heritable variation exists in numerous learning and memory traits**
- **Insects have evolved tailor-made memory types, adapted to the specific ecology of their foraging environment**
- **The *foraging* gene is an important gene underlying natural variation in learning and memory**
- **Dopaminergic signaling may drive the adaptive gating of information into different memory forms**

The use of previous experience to optimize behavior in an adaptive manner is obviously of great benefit to all animals, including insects [1,2]. However, this does not mean that insects should learn and remember information from all possible experiences they encounter. Studies on diverse insect species have revealed the daunting complexity of different forms of memory each with different stabilities, including short-, mid-, anesthesia resistant- and long term memory (STM, MTM, ARM and LTM), e.g. [3-6]. In Fig. 1 we provide a basic description of these memory forms and their abbreviations. Why does learning not always result in the formation of LTM? To ensure the reliability of learned information, most animals require multiple, spaced experiences before information is stored as LTM. Nevertheless, some animals form LTM after a single experience suggesting that they may have evolved to rely on the

value of the learned information more readily than other animals, rather than having evolved superior learning and memory abilities [7]. Several factors have been described that play a role in learning and memory including the high energetic costs of memory formation [8,9], the effects of age, physiological state, longevity, stress and the number of lifetime experiences [10-14].

This review focuses on aspects of variation in the importance and reliability of the learned information. An animal must continuously be able to adjust its behavior according to previous experiences, but only if that experience is still relevant at the time of memory retrieval. Thus, insects evolve preparedness to learn and form memories in a manner that is adaptive in the context of their specific ecology. The term prepared learning [15] is used in this review to describe preparedness to learn (for instance to learn odors more easily than colors) but also preparedness to gate the learned information into specific forms of memory that differ in stability (for instance in MTM or in LTM). Below we will describe recent advances in studies that focus on ecological factors driving natural variation in prepared learning.

Effects of environmental variation and spatial foraging behavior

Environmental variation has been proposed as one of the major factors driving the evolution of variation in learning and memory retention [7,16-18]. More variable environments promote short lasting memories, which can be updated continuously. If environmental variation is low, learned information remains relevant for a long time, which favors long lasting memories.

Spatial foraging behavior of the insect influences variation in the perceived environment. An insect that migrates after learning area-specific information does not benefit from LTM formation because the learned information becomes obsolete after migration. This could drive the evolution of differences in prepared learning, because formation of LTM would be more beneficial to insects that stay longer in a certain area than insects that tend to forage over longer distances to areas with very different types of resources. Such variation in prepared learning may have evolved within natural populations of *Drosophila melanogaster* flies (both in larvae and in adults), where so called *rover* flies tend to forage for food over longer distances, whereas *sitter* flies tend to aggregate and exploit a local food source extensively [19]. This behavior has been shown to result from a single nucleotide polymorphism on a gene encoding a cGMP dependent protein kinase (PKG), called *foraging (for)*. *Rover* flies have a higher PKG activity level [20] and experience a much more variable environment than *sitter* flies due to their explorative behavioral lifestyle. The *for* gene has been implicated in memory formation in several studies, for instance in visual orientation memory in flies [21], and there is accumulating evidence that this gene plays an important role in natural variation in prepared learning. The more explorative *rover* type flies are prepared to learn fast but remember the learned information for a shorter period than *sitter* flies, which can, due to their less explorative behavior benefit more from long lasting memories. It was found that in adult *sitter* flies, aversive memory retention was higher 24 hours after spaced conditioning, but lower 15 mins after single trial conditioning compared to *rover* flies [22]. A similar pattern was described for fly larvae after appetitive conditioning [23]. If *rover* flies were sequentially conditioned to associate two different odors with an aversive stimulus, they showed strong memory retention for the last experienced odor but poor memory for the first, whereas *sitter* flies remembered both odors equally well [24]. The foraging allele even determines whether or not flies use social learning [25,26]. Both in an olfactory and a spatial

learning task, *sitter* flies perform better when conditioned and tested in a group than alone, whereas this difference was not present for *rover* flies. Again this may be an adaptation to the more aggregated behavior of *sitter* flies, versus the more explorative, solitary behavior of *rover* flies. Using specific PKG inhibitors or activators, these differences could be correlated to PKG activity levels [25].

Other examples of specific adaptations of prepared learning to foraging behavior were described recently in locusts [27] and butterflies [17]. In the desert locust *Schistocerca gregaria*, individuals normally forage in a solitary manner and reject toxic food. They also are capable of aversive as well as appetitive taste learning when in the solitary phase. Under crowded conditions, locusts become gregarious, and in this phase they migrate over long distances. The increased risk for predation in the gregarious phase is compensated for by accepting toxic food to acquire unpalatability to deter predators. Gregarious locusts are incapable of aversive taste learning, whereas they do show normal appetitive learning [28]. Remarkably, the gregarious phase correlates to higher PKG expression in the brain [29], again linking the *foraging* gene with variation in prepared learning. In the small cabbage white butterfly, *Pieris rapae*, a correlation between explorative behavior and learning was also demonstrated. In this species, individuals with smaller wings and thoraxes perform better in optimizing their foraging efficiency through experience in complex habitats compared to individuals with larger wings and thoraxes, which can forage over larger distances [30].

Another aspect of foraging behavior that can drive the evolution of learning and memory is the level of resource specialization [31]. Generalists have been shown to change their innate preference more easily than specialists [32]. This was again supported by a direct comparison of the generalist aphid *Myzus persicae* that can feed on over 40 different plant species, and a closely related subspecies *Myzus persicae nicotianae*, which is a specialist on tobacco [33]. The generalist but not the specialist aphids change their preference for the host plant odors on which they were reared, to odors of a plant on which they subsequently had experience. Moreover, this difference in preference learning was correlated with expression levels of the *foraging* gene in winged morphs, where the specialist had lower expression levels than the generalist. Also in parasitic wasps, profound differences in learning between closely related generalist and specialist species have been described, as for example between two parasitic wasps that parasitize fly pupa, *Nasonia vitripennis* and *N. giraulti* [34]. *Nasonia vitripennis* is a generalist species that parasitizes a wide range of different fly species, occurring in manure, carcasses and bird nests, whereas *N. giraulti* is a specialist, which parasitizes on flies of the genus *Protocalliphora*, which occur in bird nests. In this comparison, the generalist formed LTM after a single host encounter, whereas the specialist formed ARM; only after multiple oviposition experiences spaced in time it formed LTM.

Effects of resource distribution patterns

The variability in the distribution patterns of resources can also drive the evolution of learning and memory as shown in the case of parasitic wasps that attack butterfly hosts [35]. The large cabbage white butterfly *P. brassicae* lays clusters of over 100 eggs, and carefully selects clusters of host plants of the same species to guarantee sufficient food resources to its offspring. In contrast, the small cabbage white butterfly (*Pieris rapae*) lays single eggs on different, isolated, plant species. This results in profound differences in host distribution for parasitic wasps. In this type of tritrophic relations,

parasitic wasps learn to remember the host plant odors on which they encounter their hosts, but the reliability of the association between host plant and host insect varies as a consequence of the oviposition behavior of the host. Finding a caterpillar of *P. brassicae* constitutes a much more significant reward than of *P. rapae*, because due to the gregarious behavior of *P. brassicae* it predicts the presence of many additional caterpillars on the same host plant species. Indeed, *C. glomerata* wasps, which can parasitize both species, show a profound difference in memory formation upon encountering a caterpillar of these two butterfly species. When *C. glomerata* wasps were given a single oviposition experience with either *P. rapae* or *P. brassicae* as a host, their 4hr memory retention level with either species was high and did not differ significantly, however, after 24 hrs memory retention was lower with *P. rapae* [35]. Using cold shock and transcription inhibitors (see Fig. 1) it was found that 4hr memory was ARM with *P. rapae* as a reward, whereas it was LTM using *P. brassicae*. Interestingly, this clear-cut effect of host species on memory formation was also found for the egg parasitoid *Trichogramma evanescens*, which can also parasitize both *P. brassicae* and *P. rapae* [36]. The formation of ARM and not LTM was described previously for a closely related parasitic wasp, *Cotesia rubecula*, which only parasitizes *P. rapae* [37]. This further strengthens the link between foraging behavior of the host and the wasp's specific learning and memory characteristics. *Cotesia rubecula* only forms LTM after three oviposition experiences spaced in time. Similarly, the associative learning of plant odors by another *Cotesia* species, *C. marginiventris*, using two different host caterpillar species as a reward, showed that these wasps were more prepared to learn the association with an optimal host species, *Spodoptera exigua* than with a suboptimal host species, *Trichoplusia ni*, in which a high percentage of mortality occurs [38]. However, in *Nasonia* parasitic wasps, which parasitize fly pupae, such an effect was absent [39], even though the different fly hosts were profoundly different in host suitability. Thus, parasitic wasps have evolved different forms of prepared learning, resulting in tailor-made memories adapted to the specific ecology or suitability of their host species.

Evolutionary aspects of prepared learning

How such a difference in prepared learning may evolve was recently demonstrated by Dunlap and Stephens [15], using experimental evolution with different selection regimes over 40 generations. Flies experienced two food dishes, of which one dish contained an aversive taste stimulus. Each dish was tagged with a different odor and color combination. Flies were allowed to oviposit on two new dishes with food tagged with a color and odor, but without the aversive taste stimulus. Eggs were collected for the subsequent generation from those food dishes where either the color or the odor matched the experienced situation (so where flies avoided the substrate that had the aversive stimulus during the conditioning phase), whereas the other modality was combined randomly. This way, only color or odor reliably predicted oviposition opportunities, and it was found that this selection regime resulted in congruent adaptation in prepared learning; a selection regime where color cues were reliable resulted in improved color learning, but decreased odor learning and vice versa. Thus, genetic diversity within a population allows for relatively fast adaptations in prepared learning.

Differences in prepared learning resulting in different gating of information into ARM and LTM could also be selected for, and again an inverse correlation in prepared learning was found. Lagasse et al., [40] selected flies bidirectionally for improved ARM

or improved LTM, and found a functional and evolutionary trade-off between these two memory forms; flies with improved ARM had reduced LTM and vice versa. Interestingly, this finding correlates well with reports that ARM and LTM are mutually exclusive in flies; the formation of ARM prevents LTM consolidation and vice versa [41].

The dependency of LTM and ARM was also investigated by bidirectional artificial selection over nine generations in *C. glomerata* [42]. As explained above, this species forms LTM for plant odors after a single oviposition experience when oviposition on *P. brassicae* is used as a reward. In this experiment, it was determined whether genetic diversity that allows selection for wasps that formed ARM instead of LTM exists in this species. Wasps were given a single experience on a plant with oviposition into *P. brassicae* as a reward and were subsequently tested for memory for the learned plant odor in a two-choice windtunnel set-up. Wasps were selected for propagation of a next generation, when they showed ARM-specific memory dynamics [37], namely memory retention at 4hr but not 24 h after conditioning. In other words, the reliability of the learned association was limited to 4hrs, whereas for the control line it was reliable also at 24 hrs. This resulted in a wasp strain where single trial learning induced STM but not ARM or LTM, whereas in the control line STM was directly followed by LTM. Both lines showed normal LTM after 3 oviposition experiences spaced in time. From these experiments, it can be concluded that in *C. glomerata* the 'switch' that gates LTM or ARM formation after a single oviposition experience is completely determined by the host species used as a reward (Fig. 2). Genetic variation that is present in the population allows for selection of strains that do not express LTM after a single oviposition experience on *P. brassicae*, but ARM is only formed when *P. rapae* is used for oviposition [35].

Interestingly, these studies on *C. glomerata* using single or spaced experiences with *P. brassicae*, resulted in consolidation of LTM within 4 hrs. However, in *C. rubecula*, spaced experiences with *P. rapae* resulted in LTM consolidation that progresses at a much slower rate [37]. First, ARM is formed which in the course of 3 days is replaced by LTM, as shown by Fig. 1c. Hence, more complex memory dynamics, such as the speed of consolidation, can be part of prepared learning.

These studies show that there exists considerable, heritable variation within a population that allows rapid adaptations in prepared learning, not only in the sense of what is learned (for instance odor or color) but also how the learning information is gated into specific forms of memory. As a consequence, results from highly inbred lines may yield strain specific results. Indeed, a comparison of four different strains of *N. vitripennis*, including homozygous as well as genetically diverse populations [43], and a panel of different isofemale lines of *D. melanogaster* [44] showed considerable differences in memory formation. Likewise, variation in learning was found between a sexual and asexual population of the parasitic wasp *Venturia canescens* [45], between individuals of different bumblebee colonies [46,47], and between genetically different worker bees [48].

Mechanisms underlying natural variation in learning and memory

Recently, considerable progress has been made to identify genes [49] and neural networks [50,51] involved in learning and memory formation. However, the specific mechanisms underlying natural variation in prepared learning are still unknown because most researchers that investigate learning and memory mechanisms do not

consider natural variation. At the gene level, natural variation in prepared learning in *Nasonia* and *Cotesia* parasitic wasps offered opportunities to identify differentially expressed genes because of the clear-cut dichotomy between ARM and transcription dependent LTM [36,37,52]. In *Cotesia*, differentially expressed genes were analysed between brains of wasps after a single oviposition experience on *P. rapae*, which induces ARM, and on *P. brassicae*, which induces LTM. Furthermore, brains of *C. glomerata* and *C. rubecula* were analysed after 3 spaced oviposition experiences, which induces LTM in both cases. In *Nasonia*, a comparison was made between *N. vitripennis*, which forms LTM and *N. giraulti*, which forms ARM, both after a single oviposition experience. As expected, given the very large number of genes involved in learning and memory [49], many genes were differentially expressed in LTM in both *Cotesia* and *Nasonia* but there were also some genes that were differentially expressed in opposing directions between the ARM and LTM memory forms. These latter genes may be interesting in the context of the natural variation in prepared learning and the observed trade-offs between ARM and LTM. One of the genes detected in *Cotesia* was the protein kinase C α (PKC α) gene; one transcript was downregulated in ARM but upregulated in LTM memory forms. The orthologous *Drosophila* gene which is involved in the regulation of LTM formation is called PKC98E and is involved in hyperphosphorylation of the transcription factor cAMP responsive element binding (CREB) protein [53]. Another gene that was detected in this comparison was the *radish* gene, a GTPase activating gene specifically required for ARM in *Drosophila* [54]. This gene was downregulated in LTM memory forms in *Cotesia*, possibly to inhibit ARM formation. Finally, the PKG gene *foraging* was upregulated in the ARM memory form in the *Nasonia* comparison. This is particularly interesting because the activity of this gene clearly correlates with many examples of natural variation described above. This gene acts within the nitric oxide (NO) - cGMP - PKG signaling pathway, which is known for its role in LTM formation [21,55]. A phosphodiesterase involved in regulation of intracellular cGMP levels was downregulated in LTM forms, both after a single and after 3 spaced oviposition experiences in *Cotesia*.

In another study, the genetic background underlying the observed difference in memory formation between *N. vitripennis* and *N. giraulti* was also investigated using introgression techniques [28]. Since crosses between these species produce fertile offspring after removing the endosymbiont *Wolbachia* by treatment with antibiotics, hybrids can be produced from isogenic strains [56]. Hybrids were tested for their memory and showed the *N. giraulti* phenotype, suggesting that the trait to form ARM after a single experience is dominant over LTM formation. Hereafter, hybrid females were backcrossed for several generations with males of *N. vitripennis* and only females with *N. giraulti*-like memory phenotypes were used for the next generation. This way, the genes underlying the *N. giraulti*-like memory phenotype were isolated in a *N. vitripennis* background. After genotyping, two chromosome regions could be identified that were linked to *N. giraulti*-like memory [28].

At the neuron level the apparent inverse correlation between ARM and LTM formation, or in more general terms between short and long lasting memory, points to a mechanism that gates information either towards short lasting memory types, or more robust, long lasting memory forms (Fig.2). In *Drosophila*, two pairs of dopaminergic neurons determine whether memory is gated into ARM or LTM, and these neurons are therefore candidates to play a role in the observed natural variation in this form of prepared learning [41]. Such a gating mechanism has so far only been found in

Drosophila, and comparative studies of this mechanism on other species are complicated by fundamental differences in reward pathways between flies, honeybees and crickets. Until recently, signaling of aversive and appetitive reinforcement in insects was thought to be mediated in a dichotomous manner by dopaminergic and octopaminergic neurons respectively. This view was and is still supported by studies in crickets [57,58], in the Honeybee [59-61] and initially also in *Drosophila* [62]. However, in *Drosophila* this view changed as octopamine signaling is only involved in appetitive learning, leading to short lasting memory formation, whereas dopamine signaling is involved in learning leading to long lasting appetitive memory [14,63,64]. It is unclear to what extent this discrepancy in reward pathways between species is caused by differences in methodology [65]. Given the supposed role of octopamine rather than dopamine in appetitive conditioning of honeybees, direct comparisons of the morphology of octopaminergic neurons have been performed between closely related species of parasitic wasps of the genera *Cotesia* [66] and *Nasonia* [67], but no differences in the number of octopaminergic neurons and their projections could be linked to the species-specific differences in learning and memory. Possibly, dopaminergic rather than octopaminergic neurons play a role in these species-specific differences.

Tailor-made memory described by learning and memory types

Studies of natural variation in learning and memory refer to individuals as good or bad learners. This generalization is misleading, because the significance of individual differences in learning and memory strongly depends on the types of learning and memory that are investigated as illustrated with the differences in memory retention between *rover* and *sitter* flies at different times after learning [22]. In fact, a 'bad' learner may have developed preparedness to gate information in short lasting memory or to high levels of active forgetting, because of its specific lifestyle or environment, and therefore learns in an adaptive, optimized manner. We conclude that this natural variation in prepared learning should not be captured by such simple terms as good or bad. We previously coined the term 'tailor-made memory', referring to the observation of species- or population-specific adaptations [68] and by including the way how the acquired information is stored in memory. The *learning and memory type* for a given population can be described by the total properties and temporal dynamics of acquisition, consolidation, waning, forgetting and retrieving of memory after a specific type of learning. Below we summarize the concept of prepared learning discussed in this paper. Variation in prepared learning occurs at different levels. Within the behavioral repertoire of individual insects, there is plasticity in the learning and memory type, for instance depending on the host species of parasitic wasp involved [35]. At the population level, there is genetic variation in prepared learning associated with foraging behavior, linked to the *foraging* gene [22,24-26], or due to genetic variation as for example in prepared learning of visual and olfactory cues [15]. Finally, between species there exists variation due to species-specific adaptations of prepared learning to ecological needs, such as described between specialist and generalist species [33]. Together, this variation allows for continuous fine-tuning of prepared learning in order to adapt to changes in the reliability and significance of information, supporting optimal decision making.

Captions:

Fig 1. Memory retention can be determined by various behavioral choice assays [69,70], such as depicted in 1a, or by measuring conditioned reflexes [48] at different intervals between conditioning and memory retention tests, resulting in a graph as shown in 1b (left panel). This memory is composed of at least three different memory types, uncovered by specific inhibitors are used. Short term memory (STM) can be erased by anesthesia such as cold shock (cooling insects on ice, b mid panel). Long term memory (LTM) requires the production of new proteins and can be inhibited by translation and/or transcription inhibitors (b, right panel). Memory that is not affected by both treatments is anesthesia-resistant memory (ARM). Note that the acronym ARM is used specifically in fly literature, and mid term memory (MTM) or intermediate term memory (ITM) is used as equivalent to ARM in other species. MTM in flies refers to a late phase of STM [71]. STM, ARM and LTM have further been subdivided in early and late forms by other specific inhibitors, see e.g. [4-6]. From the combined results of control, cold shock and protein synthesis inhibitor experiments, memory type graphs can be prepared as shown in c (left panel). Here the memory type is shown where first STM is formed (orange), followed by ARM (blue) and LTM (green), with the sum of all memories (observed without inhibiting treatments) depicted as yellow. Variations exist, for instance in the speed of consolidation of LTM and consolidation with or without intermediate ARM (c, left vs mid panel) or in the consolidation of ARM only (c, right panel) [37]. Also, the persistence of memory forms over time can vary between species.

Fig 2. Tailor-made memory can be described by the learning and memory type, which comprises the dynamics of memory formation and retention in insects induced by one or more types of conditioning. Learned information can be gated into different forms of memory, here represented as different lanes on the memory highway. Left lanes refer to short lasting memory forms, STM and ARM, whereas the right lanes refer to long lasting memory forms, such as the combination of STM, followed by ARM and LTM (slow consolidation), or LTM directly after STM (fast consolidation). Prepared learning in this example is shown as the tendency to use the left or right lanes for gating of information. Examples of factors described in this review that drive the evolution of prepared learning towards specific memory gating patterns are environmental variation, foraging behavior and host behavior (leading to specific resource distribution patterns). The upper graphs indicate four possible examples of memory dynamics.

Note that a single experience generally results in STM and ARM, whereas LTM requires multiple experiences spaced in time, but some insects already form LTM after a single experience [7]. Repeated experiences, spaced in time, increase the reliability of information and thereby also drive the gating of information towards the right lanes.

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