

# Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp

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The behavioural responses of parasitic wasps to chemical cues from their hosts and host plants are known to be affected by genetic and environmental components. In a previous study of the codling moth ectoparasitoid *Hyssopus pallidus*, we found that the response of adult parasitoids to the frass of their host caterpillars depended on a learning process involving plant cues. In the present study, we investigated how and when learning takes place. A series of experiments was conducted involving exposure of parasitoids to fruit cues at different developmental stages. While parasitoids were not able to learn the fruit cues in the adult stage, exposure to fruit odour at early preimaginal stages significantly increased the adult response to frass from fruit-fed caterpillars. The olfactory memory persisted through metamorphosis, with a retention time of 14 days. Preimaginal learning was not confined to fruit cues but was also demonstrated for a host- and fruit-independent cue, menthol. Parasitoids exposed to menthol odour at the egg and larval stages no longer showed negative responses as adults. Sensitization to fruit cues and habituation to menthol are considered to be the mechanisms involved. This study provides evidence of true preimaginal learning of olfactory cues in a parasitic wasp.

**Keywords:** preimaginal learning; memory; insect; parasitoid; *Hyssopus pallidus*; *Cydia pomonella*

## 1. INTRODUCTION

Parasitoid host-searching ability depends on the response of parasitoids to chemical cues from the host and the host plant. This response may be considerably affected by genetic components (Prevost & Lewis 1990; Gu & Dorn 2000), although learning processes may also play a significant role by enabling the insects to adapt to changes throughout their life (Kaiser *et al.* 1994). The ability to learn olfactory cues related to the host–host-plant complex has been documented in several parasitoid species (e.g. Turlings *et al.* 1993 and references therein) as an essential feature of high behavioural performance (Lewis & Martin 1990). In particular, efficient host searching is required in parasitoids released in fields for biological control.

Olfactory learning may occur at different stages of insect ontogeny, though a critical phase may exist in which parasitoids are more likely to learn. Numerous studies have shown that learning of olfactory cues occurs mainly during adult emergence or in young adults (e.g. Turlings *et al.* 1993; Du *et al.* 1997; Fujiwara *et al.* 2000). Learning in preimaginal stages (Hopkins' host-selection effect, reviewed by Barron (2001)) has also been postulated (e.g. Thorpe & Jones 1937; Smith & Cornell 1979), but conclusive evidence of parasitoid learning occurring exclusively in the larval stages and affecting adult behaviour is not yet available (Caubet & Jaisson 1991; Caubet *et al.* 1992; Lecomte & Thibout 1993; Cortesero & Monge 1994; van Emden *et al.* 1996; Morris & Fellowes 2002). Often, changes in adult behaviour are induced by chemical contamination carried over from the larval to the adult environment. This phenomenon, known as 'chemical legacy' (Corbet 1985), may seem to be preimaginal learning,

when in fact learning occurs in young adults. Therefore, discrimination between larval and early-adult experience is crucial for determination of the sensitive stage for learning.

In this study, we investigated the learning mechanisms involved in maintaining good host-location capability in the parasitic wasp *Hyssopus pallidus* (Askew) (Hymenoptera: Eulophidae). *Hyssopus pallidus* is a gregarious larval ectoparasitoid and a candidate biological-control agent of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), a key pest of apple fruit, *Malus domestica* (Borkh.) (Rosaceae). The female parasitoid has the ability to enter the infested fruit, paralyse the feeding caterpillar irreversibly and deposit eggs on its body. The hatched parasitoid larvae feed from the outside on the host caterpillar (Tschudi-Rein & Dorn 2001). For successful location of its endophytic host, *H. pallidus* relies on the response to host frass traces (faeces and silk) on the surface and in the channels of the infested fruit (Mattiacci *et al.* 1999, 2000). In a previous study, we found strong deterioration of the response to frass in parasitoid females reared for more than 30 generations on caterpillars fed on an artificial diet. However, this was not the result of a selection process, since it was reversible within a single generation (Gandolfi *et al.* 2003). Rearing of parasitoids in the presence of fruit cues completely restored the original level of response to host frass, suggesting that, while the host-derived chemical components of the frass are recognized innately, recognition of the fruit-derived components may be subject to learning (Gandolfi *et al.* 2003). Here, we investigated the mechanism underlying the learning of fruit components of frass. Specifically we asked

- (i) whether parasitoids need to learn the fruit-related chemicals from fruit-fed caterpillars and their faeces, or whether exposure to fruit is sufficient, and

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- (ii) whether both chemical and physical cues from the fruit and both olfactory and gustatory stimuli are involved in learning.

Subsequently, we tested the hypothesis that there is a sensitive window in the parasitoid life history for learning the relevant stimuli for host location. The occurrence of preimaginal learning was tested both for apple-fruit cues and for a host- and plant-independent cue, menthol, which is not present in the tritrophic system of *H. pallidus*.

## 2. MATERIAL AND METHODS

### (a) *Insect rearing*

The parasitoid *H. pallidus* was reared on *C. pomonella* caterpillars from a laboratory colony (described by Mattiacci *et al.* 1999) fed on a wheat-germ-based artificial diet devoid of apple fruit cues (Huber *et al.* 1972). Newly hatched first instar caterpillars were placed individually in plastic boxes (18 mm × 18 mm × 10 mm) filled with 3.4 g of artificial diet. The boxes were kept at 26 ± 1 °C, 55 ± 10% relative humidity and an 18 L : 6 D photoperiod. After 16–21 days, fifth instar caterpillars were offered to 4- to 7-day-old mated female parasitoids in glass vials (15 cm × 2 cm diameter) at a ratio of one parasitoid per host with a droplet of undiluted honey as food. Parasitoids of the subsequent generation emerged 15 to 18 days later. Upon emergence they were transferred into Plexiglas cages (25 cm × 25 cm × 25 cm) and maintained on water and honey. During parasitization and development, the parasitoids were never exposed to apple cues.

### (b) *Bioassays*

No-choice bioassays were carried out with 4-day-old mated parasitoid females. Immediately prior to the bioassay, females were transferred individually from emergence cages into small glass dishes (2 cm in diameter, 0.8 cm in height). A half piece of circular filter paper (Whatman, 1.3 cm, grade 1, cat. no. 1001013; Merck, Switzerland) was treated with 20 µl of solvent extract or solution, and left uncovered for 3 min to allow complete evaporation of the solvent. Subsequently, it was transferred to a glass plate (5 cm in diameter). The open side of the small dish containing the wasp was positioned on top of the filter paper. Observation started when the parasitoid began to move in the arena and ended 10 min later. The behavioural parameter 'searching', defined as intensive antennal examination of the filter paper, was recorded. The total searching time was measured with a stopwatch by accumulating time periods of searching activity. The very few wasps that did not move at all (in total 2.7%) were not considered. Bioassays were conducted from 11.00 to 17.00 under 900–1800 lux, at 21 ± 1 °C and 50 ± 5% relative humidity.

### (c) *Rearing manipulations*

Parasitoids that had been reared on artificial-diet-fed caterpillars for 42–44 generations were exposed to apple fruit cues during one generation, and tested as adults for their responses to host frass cues. Eight different manipulations were carried out (table 1), and the results were compared with those from non-manipulated controls without apple cues.

To test whether a high response of adult parasitoids to host frass requires exposure to both physical and chemical stimuli from the fruit, or whether chemical stimuli are sufficient, the following manipulations were carried out.

(1) Parasitoids were reared on *C. pomonella* caterpillars fed on apple fruit (*M. domestica* cultivar Bohnäpfel, Rosaceae). Fruits were infested with first instar caterpillars and dissected carefully after 16–21 days to collect fifth instar caterpillars. Parasitization was carried out in glass vials as in standard rearing. In addition, parasitoids were exposed directly to a slice of apple fruit (*ca.* 30 mg) during development.

(2) Parasitoids were reared on caterpillars fed on an artificial diet treated with apple fruit extract. The fruit extract was prepared from a mixture of 85% grated pulp, 8.75% grated skin and 6.25% sliced seeds, soaked in diethyl ether for 18 h. The final concentration was 400 mg of apple material per ml of solvent. The composition of this mixture best characterizes the apple components of the frass (Gandolfi *et al.* 2003). Freshly prepared artificial diet at a temperature of 50 °C was treated with apple fruit extract prepared from a quantity of fruit corresponding to the quantity of diet (w : w) to which it was added. The use of diethyl ether was found to be innocuous for both caterpillars and parasitoids in preliminary experiments, on condition that the solvent was allowed to evaporate for at least 3 min. In addition, parasitoids were exposed directly to apple fruit extract during development. Filter-paper halves were treated with 20 µl extract obtained from 30 mg apple fruit. Since the apple fruit extract maintained its activity for only 7 days (data not shown), the filter papers were changed after 7 days.

Adults emerging from manipulations (1) and (2) were compared with non-manipulated control adults (0).

To test whether a high response of adult parasitoids to host frass requires exposure to both gustatory and olfactory stimuli from the fruit, or whether exposure to fruit odour is sufficient, the following manipulations were carried out.

(3) Parasitoids were reared on caterpillars fed on an artificial diet treated with apple fruit extract as in (2), and were therefore in direct contact with the apple cues.

(4) Parasitoids were reared on caterpillars fed on plain artificial diet, and were exposed to only the odour of apple fruit extracts as in (2) throughout their development.

Adults emerging from manipulations (3) and (4) were compared with non-manipulated control adults (0).

To test the hypothesis that there is a sensitive window in the parasitoids' life history for learning the relevant stimuli for host location, the following manipulations were carried out.

(5)–(8) Parasitoids were reared on caterpillars fed on plain artificial diet, and were exposed to apple fruit extracts at different developmental stages: (5) from eggs to young larvae (days 0–3 after parasitization); (6) from older larval instars until pupation (days 4–10); (7) from pupal stage until adult emergence (days 11–18); and (8) as adults (*ca.* day 21, 1 day before bioassay). For comparison, parasitoids exposed to apple fruit extracts during their entire development (4) and parasitoids without exposure to apple cues (0) were used. To avoid parasitoid larvae and pupae coming into direct contact with apple chemicals, treated filter papers were placed in the parasitization vials 4 cm away from the host caterpillar. Since the caterpillar is paralysed and so cannot move during parasitoid development, and since developing parasitoids do not leave the host, the parasitoids were never in direct contact with the filter papers during the exposure period.

The adult females that emerged from the various rearing systems were transferred together with males into Plexiglas cages (25 cm × 25 cm × 25 cm) and maintained on water and honey until bioassay. In manipulation (8) the emerging females remained for an additional day in the glass vials before being

Table 1. Schematic overview of the rearing manipulations, adding apple fruit cues to parasitoid rearing at all levels (manipulations 1 and 2), at different levels (manipulations 3 and 4) and at different times during parasitoid development (manipulations 5–8). (In control rearing (0) there was no addition of apple cues. Details are described in § 2c. A, apple fruit; AE, apple fruit extract.)

manipulation	to host diet	to parasitoids			
		egg–larva	larva–pupa	pupa–adult	adult
0	—	—	—	—	—
1	A	-----		A	-----
2	AE	-----		AE	-----
3	AE	—	—	—	—
4	—	-----		AE	-----
5	—	AE	—	—	—
6	—	—	AE	—	—
7	—	—	—	AE	—
8	—	—	—	—	AE

transferred with males into the Plexiglas cages. At the age of 4 days, mated females were tested for their responses to host frass extracts. Extracts were prepared from the frass produced by caterpillars feeding on apple fruit. The frass had been air-dried for 2 days and extracted with diethyl ether (purity of at least 99.8%) at a ratio of 0.13 g ml<sup>-1</sup> solvent. For each parasitoid group, 30 females were assessed. Differences were tested by means of one-way ANOVA, followed by pairwise multiple comparisons (Student–Newman–Keuls test) (Zar 1999).

#### (d) Preimaginal learning

In the rearing manipulations we found that exposure of parasitoids to apple fruit extract during the larval stage increased their response to the host frass as adults (see § 3). To verify that this effect was the result of preimaginal learning of fruit cues, we exposed parasitoids from egg to larval stages to fruit stimuli, and subsequently tested the responses of the emerged adults to the same stimuli. Filter-paper halves were treated with 20 µl apple fruit extract obtained from 30 mg apple fruit. Treated filter papers were added to the parasitization vials at the time of oviposition and were removed 7 days later, before parasitoid pupation. The responses of the emerged females to apple fruit extract and to diethyl ether as a solvent control were compared with the responses of females reared without preimaginal exposure to apple. To make sure that the exposure to apple stimuli occurred exclusively early in development and not after emergence, the following experiment was carried out to verify that no contamination was left on the inner surfaces of the glass vials after removing the treated filter papers. Females were introduced into untreated vials or into vials from which filter papers treated with apple fruit extract had just been removed after having been in the vial for 12 h. Their searching activity in the vials was assessed as in the bioassay described in § 2b. The responses of the females to untreated vials (1.91 ± 1.71 s; mean ± s.d.) and to the vials that previously contained treated filter paper

(1.83 ± 1.66 s; mean ± s.d.) did not differ significantly (*t*-test: *t* = 0.070, *p* = 0.94, *n* = 30).

To verify the occurrence of a general phenomenon of preimaginal learning, we exposed parasitoids from egg to larval stages to the novel cue menthol, a compound not detected in apple extracts (Gandolfi *et al.* 2003), and tested the responses of emerged adults to this substance. Filter papers were treated with 20 µl of a menthol solution prepared with menthol (Fluka, Switzerland, purity of at least 99%, racemic) and diethyl ether (purity of at least 99.8%) at a ratio of 0.15 mg ml<sup>-1</sup> solvent, corresponding to the amount of menthol present in 30 mg of the 0.01% menthol medium used by Barron & Corbet (1999), which proved to be non-toxic to insect larvae. The responses of these adults to menthol and to diethyl ether as a solvent control were compared with the responses of parasitoids reared without exposure to menthol. To find the concentration to use for bioassays, a pilot study was carried out, testing the dose-response of adults to menthol. While the menthol solution used for rearing manipulation was repellent in the bioassay (response of 0 s, *n* = 6), a 10-fold dilution was tolerated by menthol-experienced parasitoids (response of 15.8 ± 7.9 s, *n* = 6 versus response to solvent control of 11.8 ± 3.3 s). On the basis of these results, the full bioassay was carried out using the 10-fold dilution.

In both experiments, 20 wasps were tested in each exposure group and tested cue. The data were analysed separately using two-way ANOVA and pairwise multiple comparisons (Student–Newman–Keuls test) (Zar 1999).

### 3. RESULTS

#### (a) Learning mechanisms supporting parasitoid response to host frass

The response of *H. pallidus* females to the frass of their host, *C. pomonella*, was enhanced when parasitoids were reared on hosts fed on either apple fruits or artificial diet

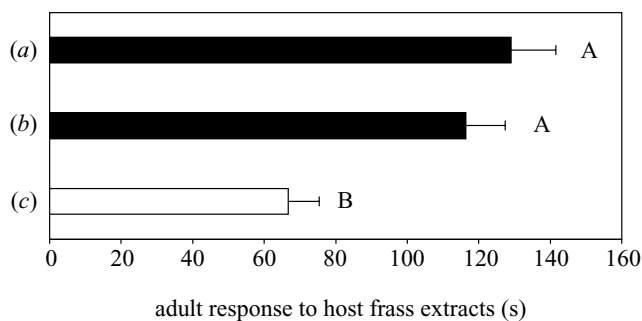


Figure 1. Effect of using either apple fruit or apple fruit extracts in rearing on the response (mean and s.e. in seconds) of adult wasps to host frass extracts. (a) Apple fruit extract (2); (b) apple fruit (1); (c) no apple cues (control) (0). Numbers in brackets refer to the type of manipulation, as shown in table 1. Different letters (A versus B) indicate significant differences between the treatments (Student–Newman–Keuls test:  $p < 0.05$ ,  $n = 30$ ).

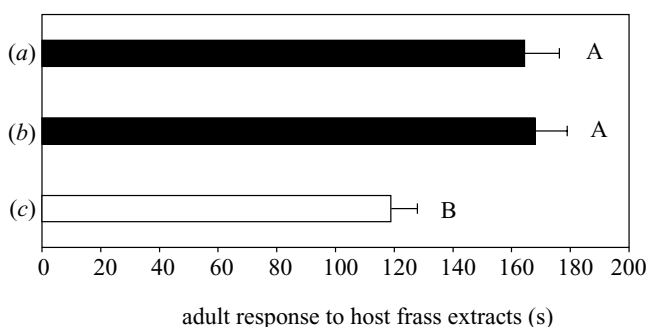


Figure 2. Effect of adding apple fruit extract either only to the host diet or only to the developing parasitoids on the response (mean and s.e. in seconds) of adult wasps to host frass extracts. (a) Developing parasitoids (4); (b) host diet (3); (c) no addition (control) (0). Numbers in brackets refer to the type of manipulation, as shown in table 1. Different letters (A versus B) indicate significant differences between the treatments (Student–Newman–Keuls test:  $p < 0.05$ ,  $n = 30$ ).

treated with apple fruit extract (one-way ANOVA:  $F_{2,87} = 6.76$ ,  $p = 0.002$ ). The use of apple fruit extract led to the same increase in response as the use of apple fruit, in comparison with the control without apple fruit cues (figure 1).

The response of females to host frass was enhanced both by adding apple fruit extract to the host diet and by exposing the parasitoids to the extract during development (one-way ANOVA:  $F_{2,87} = 5.13$ ,  $p = 0.0079$ ) (figure 2).

The exposure of parasitoids to apple fruit extract at different developmental stages affected the response of adults to host frass cues in different ways (one-way ANOVA:  $F_{6,203} = 5.87$ ,  $p = 0.0002$ ) (figure 3). Exposure at the beginning of development was the most effective. It led to the same increase in response as exposure throughout development. Exposure of parasitoids either at the pupal stage and during emergence or at the adult stage 1 day before bioassay was ineffective.

#### (b) Preimaginal learning

Parasitoids that were never exposed to apple fruit extract during development showed no significant

response to fruit extract as adults. By contrast, parasitoids exposed to apple fruit extract from the egg to larval stages showed in the adult stage a much higher response to fruit extract than did parasitoids without exposure to apple (two-way ANOVA, exposure:  $F_{1,76} = 24.4$ ,  $p < 0.0001$ ; cue:  $F_{1,76} = 52.7$ ,  $p < 0.0001$ ; interaction exposure  $\times$  cue:  $F_{1,76} = 26.9$ ,  $p < 0.0001$ ) (figure 4).

Parasitoids that were never exposed to menthol during development were repelled by the menthol solution as adults. Their response to menthol was lower than to the solvent control. Furthermore, it was observed that when they accidentally came into contact with this substance, they immediately moved away. By contrast, parasitoids exposed to the odour of menthol from egg to larval stages showed in the adult stage a much higher response to menthol than did parasitoids without menthol exposure. They were no longer repelled but showed a similar response to the menthol solution as to the solvent control (two-way ANOVA, exposure:  $F_{1,76} = 16.3$ ,  $p = 0.0001$ ; cue:  $F_{1,76} = 4.3$ ,  $p = 0.041$ ; interaction exposure  $\times$  cue:  $F_{1,76} = 23.3$ ,  $p < 0.0001$ ) (figure 5).

## 4. DISCUSSION

The parasitic wasp *H. pallidus* finds its concealed host caterpillar *C. pomonella* by means of a high behavioural response to host frass traces left on the fruit surface (Mattiacci *et al.* 1999). In a previous study, we demonstrated that a high response to frass requires the recognition of both host-derived chemical components of the frass and chemical components derived from the host's food. However, while the host components were recognized innately by female parasitoids, the fruit components needed to be learned (Gandolfi *et al.* 2003). In the present study we investigated the mechanisms underlying this process by determining how and when learning takes place.

Learning occurred in parasitoids that were reared on host caterpillars fed on an artificial diet enriched with a fruit extract. Direct contact of parasitoids with the fruit was not necessary. We conclude that chemical stimuli from the fruit are indispensable for the observed learning process, while physical stimuli such as visual or tactile cues are of minor importance. Olfactory experience during rearing yielded the same response to frass in adult wasps as a combination of olfactory and gustatory experiences. This is surprising, as the results of previous studies of the sensory background of chemical learning in insects indicate a clear role of chemical information perceived by taste receptors (Cortesero & Monge 1994), while olfaction may also be involved, but to a lesser degree (Papaj & Prokopy 1986).

The sensitive period for olfactory learning was not during emergence or in the early adult stage, in contrast to the general expectation (e.g. Kester & Barbosa 1991; Pettitt *et al.* 1992; Dutton *et al.* 2000; Fujiwara *et al.* 2000). Our experiments on exposure of parasitoids at different developmental stages to olfactory cues demonstrate that it is from the egg to the early larval stage in which learning occurs, leading to a strong adult response to host frass. Only preimaginal exposure at the beginning of development, i.e. at the greatest time interval from the adult stage (15 days), was the most behaviourally effective. Exposure at the end of preimaginal development, or even around

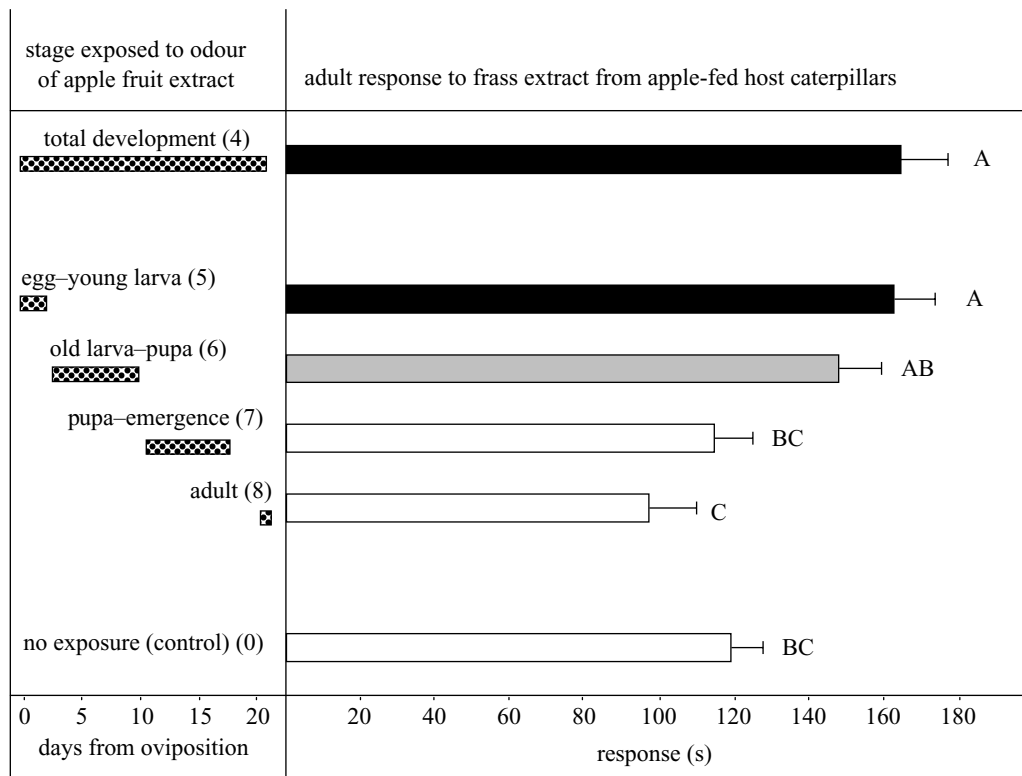


Figure 3. Effect of the development stage at which a parasitoid is exposed to odours from apple fruit extract on its response (mean and s.e. in seconds) as an adult to frass extracts from apple-fed host caterpillars. See § 2 for details. Numbers in brackets refer to the type of manipulation, as shown in table 1. Different letters (A, B, C) indicate significant differences between the treatments (Student–Newman–Keuls test:  $p < 0.05$ ,  $n = 30$ ).

the time of adult emergence, did not yield a behavioural effect in adults, even though in these two instances the interval between removal of the bioactive cues and the bioassay was much shorter. This rules out the possibility that some volatile molecules from the apple may have caused a response in adults after being first adsorbed onto the surface of the glass vials or onto the insect integument and later desorbed. The olfactory stimuli were learned during direct exposure at early preimaginal stages.

Evidence of true preimaginal learning of plant odours was provided after a further experiment, in which adult females were tested for their responses to the same fruit extract as that to which they had been given olfactory exposure as eggs and larvae. A high response at the adult stage required preimaginal exposure. This phenomenon has previously been described as ‘conditioning’ (Poppy *et al.* 1997; Barron & Corbet 1999), but empirical evidence has been missing. In our study, adult wasps recognized the stimuli to which they had been exposed during early preimaginal stages but not the pupal stage, emergence or the adult stage. The early preimaginal stages were conditioned to plant stimuli through a mechanism analogous to sensitization to chemical stimuli, which is known among adult insects. In this context, sensitization has been defined as a non-associative process in which the response to a stimulus gradually increases with repeated exposure to that stimulus (Poppy *et al.* 1997).

Preimaginal olfactory learning was not confined to plant cues but could also be demonstrated for a stimulus that is plant and host independent, i.e. menthol. Parasitoids that were never exposed to menthol during development were typically repelled by this novel cue as adults, whereas

parasitoids that had been exposed to menthol odour at early preimaginal stages no longer showed a negative response as adults. The learning mechanism involved is analogous to the phenomenon of habituation, which is known among adult insects; that is a non-associative process in which the response (positive or negative) to a stimulus gradually decreases with exposure to that stimulus (Mackintosh 1983). Here, an initially negative response to menthol waned and turned into a neutral response. Hence, preimaginal learning in *H. pallidus* is not confined to cues relevant to host searching. Learning of novel cues has previously been observed in parasitoids, but it has been demonstrated only in adults (Lewis & Tumlinson, 1988; Lewis & Takasu 1990; de Jong & Kaiser 1991; Kerguelen & Cardé 1996; Iizuka & Takasu 1998).

Our study, as far as we know, is the first in which authentic preimaginal learning of olfactory cues in a parasitoid wasp has been documented. Previous investigations of the effect of larval environment on adult behaviour have failed to demonstrate true preimaginal learning (e.g. Caubet *et al.* 1992; Barron 2001), owing to the difficulty of excluding the occurrence of a ‘chemical legacy’ (Corbet 1985). Chemical contamination from the larval to the adult environment is difficult to avoid, especially when learned cues are directly present in the insect food (Elamrani *et al.* 1991; Barron & Corbet 1999, 2000) or in the host cocoon (Wickremasinghe & van Emden 1992), even if insects are isolated from the cue source before emergence (Poppy *et al.* 1997; van Emden *et al.* 1996) and washed (Cortesero & Monge 1994). In the present study, the chemical cue was not offered as a liquid or solid substrate prone to lead to persistent contamination, but was

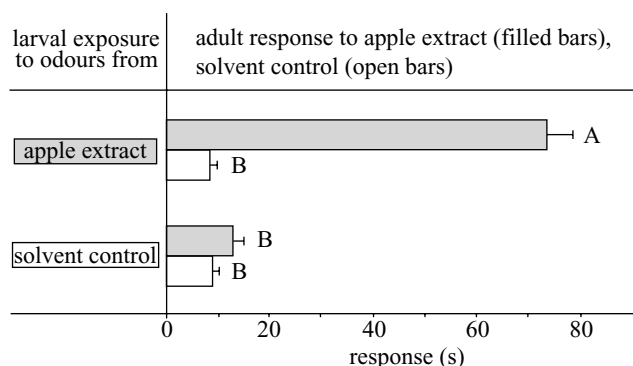


Figure 4. Preimaginal learning of apple fruit cues. Effect of preimaginal exposure of parasitoids to odours from apple fruit extract on the subsequent response (mean and s.e. in seconds) of adult females to the same extract. Different letters (A versus B) indicate significant differences both between exposure groups and between tested cues (Student–Newman–Keuls test:  $p < 0.05$ ,  $n = 20$ ).

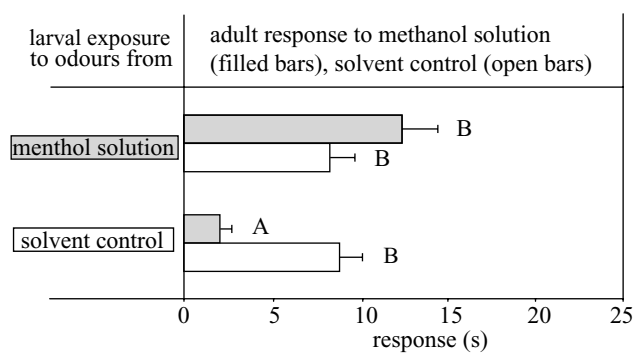


Figure 5. Preimaginal learning of a novel cue, menthol. Effect of preimaginal exposure of parasitoids to odours from a menthol solution on the subsequent response (mean and s.e. in seconds) of adult females to menthol. Different letters (A versus B) indicate significant differences both between exposure groups and between tested cues (Student–Newman–Keuls test:  $p < 0.05$ ,  $n = 20$ ).

released as a volatile substance. The source of emission could not be contacted by the developing parasitoid and was removed long before pupation and adult emergence. This renders adherence of the behaviourally effective chemicals to the cuticle of the pupa and subsequent contamination of the emerging adult's environment unlikely, in contrast to the situation in previous studies (reviewed by Barron 2001). Final evidence for preimaginal learning comes from the finding that *H. pallidus* adults failed to associate the volatile stimulus with their host when this cue was offered at their emergence. This clearly excludes the possibility that the crucial experience occurred at the young adult stage rather than during the early preimaginal period.

Olfactory memory in this parasitoid species persisted over a period of up to 14 days until the adult stage. This permanence is striking compared with the 3 to 7 days reported from studies of olfactory memory of host or host-plant cues acquired during adult emergence or oviposition (e.g. Papaj & Vet 1990; Kester & Barbosa 1991; Du *et al.* 1997). A robust memory for two weeks is even more surprising for novel cues, which are known to be even less persistent in adult insects (Barron & Corbet 2000). The

persistence of olfactory memory through metamorphosis suggests the involvement of the central nervous system (Heisenberg *et al.* 1995). Given the dramatic changes in the insect brain during metamorphosis (Barron & Corbet 1999), the finding that the adult insect retained learning acquired during the larval stage is even more impressive. Some evidence for memory persisting through metamorphosis has been reported for a few holometabolous insects (Caubet & Jaisson 1991 and references therein; Tully *et al.* 1994; Ray 1999). Though the underlying processes are largely unknown, cell survival through metamorphosis has been indicated as a possible mechanism (Ray 1999).

The ecological significance of the olfactory-learning process reported in this study remains to be investigated. Our findings emphasize the potential significance of preimaginal learning of plant stimuli for efficient host location by adults in this parasitoid species. This early determination and high permanence of memory may be of advantage for a parasitic wasp, which has to locate an endophytic herbivore within the tissue of its perennial food plant.

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