

Pre-pupation behaviour of the aphid parasitoid *Aphidius ervi* (Haliday) and its consequences for pre-imaginal learning

Cristián Gutiérrez-Ibáñez · Cristian A. Villagra ·
Hermann M. Niemeyer

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Abstract Olfactory learning may occur at different stages of insect ontogeny. In parasitoid wasps, it has been mostly shown at adult emergence, whilst it remains controversial at pre-imaginal stages. We followed larval growth of the parasitoid wasp, *Aphidius ervi* Haliday, inside the host aphid, *Acyrtosiphon pisum* Harris, and characterised in detail the behaviour of third instar larvae. We found that just before cocoon spinning begins, the third instar larva bites a hole through the ventral side of the mummified aphid exoskeleton. We then evaluated whether this period of exposure to the external environment represented a sensitive stage for olfactory learning. In our first experiment, the third instar larvae were allowed to spin their cocoon on the host plant (*Vicia faba* L.) surface or on a plastic plate covering the portion of the host plant exposed to the ventral opening. Recently emerged adults of the first group showed a preference for plant volatiles in a glass Y-olfactometer, whereas no preference was found in adults of the second group. In a second experiment, during the period in which the aphid carcass remains open or is being sealed by cocoon spinning, third instar larvae were exposed for 24 h to either vanilla odours or water vapours as control. In this experiment, half of the parasitoid larvae were later excised from the mummy to avoid further exposure to vanilla. Adult parasitoids exposed to vanilla during the larval ventral opening of the mummy showed a significant preference for vanilla odours in the olfactometer, regardless of excision from the mummy. The larval behaviour described and the results of the manipulations performed

are discussed as evidences for the acquisition of olfactory memory during the larval stage and its persistence through metamorphosis.

Keywords Chemical legacy · Hopkins' host selection principle · Parasitoid larval behaviour · Host preferences · Insect learning

Introduction

Changes in behaviour derived from olfactory experiences may occur at different stages of the life of an insect and influence its preference when searching for food, mates and oviposition site (Wcislo 1987; Vet and Groenewold 1990). Numerous studies have shown that learning of olfactory cues occurs during adult emergence or in young adults (e.g. Turlings et al. 1993; Du et al. 1997; Fujiwara et al. 2000). It has also been shown that pre-imaginal environment has an important influence on adult behaviour (e.g. Thorpe 1939; Kudon and Berisford 1980; Chow et al. 2005). This latter effect has been proposed to occur in two different ways: First, as a result of larval experiences, which implies that the larva can learn from its environment and that this memory may be transferred from pre-imaginal stages to the adult—the so-called Hopkins' host selection principle (reviewed by Barron 2001); second, at eclosion of the imago, in which the larval environment is carried over to the adult stages and olfactory learning occurs during its contact with olfactory cues at emergence—the so-called “chemical legacy” hypothesis (Corbet 1985). Most studies fail to distinguish between these two phenomena (e.g. Manning 1967; Smith and Cornell 1979; Rojas and Wyatt 1999; Rietdorf and Steidle 2002); hence, there is a need for fine grain experiments to distinguish between the effect of

C. Gutiérrez-Ibáñez · C. A. Villagra · H. M. Niemeyer (✉)
Departamento de Ciencias Ecológicas, Facultad de Ciencias,
Universidad de Chile, Casilla 653, Santiago, Chile
e-mail: niemeyer@abulafia.ciencias.uchile.cl

experience during the larval stage and during eclosion (Vet et al. 1995, Gandolfi et al. 2003).

Endoparasitic wasps show obligatory development inside an arthropod host from which they emerge as adults (Quicke 1997). During this period, such parasitoids can be affected by stimuli derived from the host, the host's food and the surrounding environment (Turlings et al. 1993; Godfray 1994); however, relevant external influences may not be evident to an observer. In particular, aphid parasitoids are known to bite a hole in the mummified aphid's ventral side of the exoskeleton during development just before cocoon spinning (Van Achterberg 1984; Quicke 1997), thus briefly exposing the larva to the outside environment. Early exposure of the larva to the plant in endoparasitic wasps may correspond to a similar kind of early learning as in ectoparasitic wasps (Gandolfi et al. 2003) and may lead to host fidelity in the adult insect. In this work, we test if the chemical stimuli to which the larva of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) is exposed through the hole affect the behaviour of the recently emerged adult, and we further test whether the learning of olfactory cues is due to a chemical legacy effect or to a true pre-imaginal learning process.

Materials and methods

Parasitoids Parasitoids were maintained in the laboratory on *Acyrtosiphon pisum* Harris on broad bean plants (*Vicia faba* L.). To get parasitoids of the same age, synchronised aphids were obtained by leaving 30 to 50 fourth instar aphids on broad bean plants during 8 h. Adults were removed, and 48 h later, groups of 20 to 30 nymphs were placed on small Petri dishes with a female parasitoid during 20 to 30 min. Thereafter, the nymphs were returned to broad bean plants. All manipulations were carried out in a room at 21°C and 14L/10D photoperiod.

Characterisation of pre-pupation behaviour Aphids parasitised at the same time as described above ($N=30$) were isolated 7 days after parasitisation and observed every half hour to determine the timing of aphid death (i.e. the moment at which the aphid no longer responded to physical harassments) and ventral opening. To observe and record the detailed behaviours of the third instar parasitoid before and after the ventral opening occurred, the legs of a parasitised aphid ($N=10$) were attached with adhesive tape to a glass microscope slide, and changes in the ventral side of the aphid were recorded with a digital colour video camera (Sony SSC-DC324) attached to a stereoscopic microscope (Nikon). Recordings lasted from 24 to 48 h.

Training with plant odours during pre-pupation period To assess the effect of ventral opening and pupation behaviour on the initial response of the adult parasitoid to the host plant, two groups of parasitised aphids were assembled, which had just died but had not yet been perforated by the larva inside. One group was left to mummify over a leaf of broad bean, and the other was manipulated in such a way that a plastic plate was inserted between that parasitised aphid and the broad bean leaf. After 24 h, the mummies that had just formed were isolated in Petri dishes containing a piece of wet filter paper and kept at 21°C and 14L/10D photoperiod. Newly eclosed male and female parasitoids were fed only with water and tested in olfactometers between 12 and 36 h after eclosion. Data were analysed with two-way analysis of variance (ANOVA) and by paired *t* tests.

Training with vanilla odour during pre-pupation period Aphids that had just died but had not yet been perforated by the parasitoid larva were individually placed lying on their dorsal side on clean glass Petri dishes. Over each aphid, a plastic Petri dish with an orifice in its base not bigger than the total width of the dead aphid was placed in such way that the orifice was exactly over the ventral side of the dead aphid where the ventral opening was to occur. Two 2.5- μ l drops of vanilla extract (Marcopolo®) or two 2.5- μ l drops of distilled water were placed near the walls of the dish in such a way that the protruding larva would not reach them and would only be exposed to vanilla odours or water vapours. To assess effects at eclosion, 24 h later, half of the larvae in each treatment were excised and were individually placed in Petri dishes containing a layer of agar gel at 5%, and the other half was allowed to develop in Petri dishes containing a piece of wet filter paper. Newly eclosed male and female parasitoids were fed only with water and tested in olfactometers between 12 and 36 h after eclosion. Olfactometric data were analysed with three-way ANOVA and by independent two-way ANOVAs for sexes. Data for females were ranked.

Olfactometric studies Y-tube olfactometers were used to investigate the responses of *A. ervi* adults when offered a choice of odours. Olfactometers utilised (9-cm-long tube and 6-cm-long arms forming an angle of 80°) were designed to allow parasitoids to explore both arms and move from one arm to the other (Villagra et al. 2005). Olfactometers were changed after each test and cleaned successively with ethanol, odour-free soap and distilled water. Pure air to feed into the arms was obtained from an ultra-pure synthetic air cylinder (Indura®). The air flow during the tests was maintained at 250 ml/min, light intensity at 3,600 lx, and temperature at 20°C. Relative

humidity varied between 40 and 50%. Experimental parasitoids were exposed to air only and to air containing the odours from a commercial vanilla extract (Marcopolo®) at a concentration of 0.02 µl extract/ml air, following Villagra et al. (2005). In the case of plant conditioning experiments, air in the stimulus arm came from a chamber containing a broad bean plant. As a measure of the attraction to an olfactory stimulus, the time spent in each arm of the olfactometer was determined. The times devoted to the following behaviours were also determined: antennating, exploring, standing still, grooming the abdomen and grooming the antennae. Tests were performed between 09:00 and 11:30, and lasted 5 min. Behavioural data were collected utilising the JWatcher software 0.9® (Blumstein et al. 2000) and analysed with two-way ANOVAs.

Results

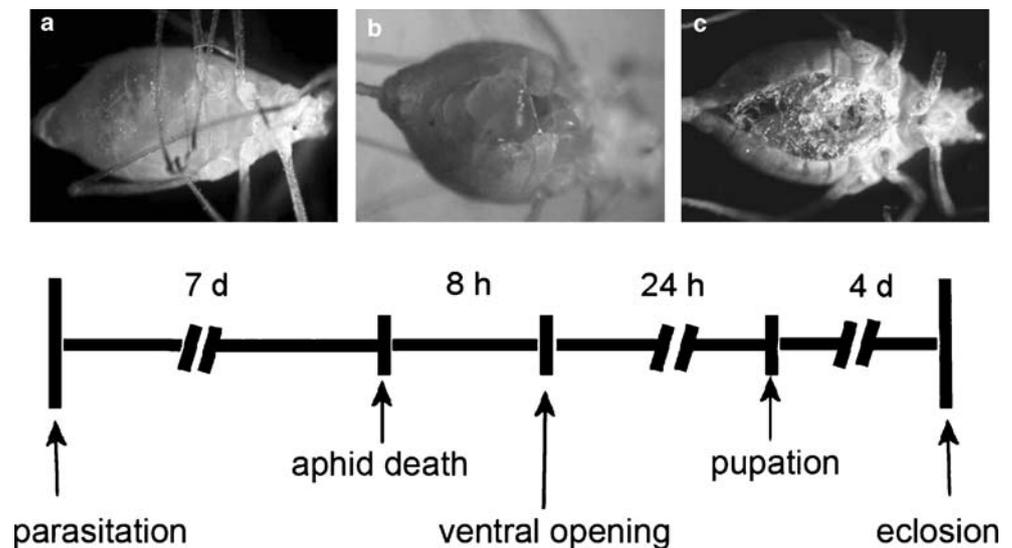
Characterisation of pre-pupation behaviour Using the protocol designed, we were able to define and characterise in detail the different stages of larval activity that culminate with ventral opening and attachment to the pupation surface. The parasitoid larva showed a series of stereotyped movements during its development inside the aphid host. Under the conditions of our observations, 168.5±6.3 h (mean±SE) after parasitisation, the aphid host died and became progressively transparent (Fig. 1a). At this time and during the next 7.4±0.5 h (mean±SE), the third instar larva of the parasitoid could be seen spinning slowly inside the host. Then the larva pushed with its head the ventral side of the host's abdomen and bit through its exoskeleton.

Further larval activity expanded the crack in the abdomen to produce a hole that occupied the abdominal and thoracic plates and exposed the larva to the external environment (Fig. 1b). Immediately thereafter, the larva began to deposit silk inside the exoskeleton of the host, first at the borders of the ventral opening and then towards the center of the hole until the latter was sealed. The larva continued to spin at an increasing speed, and after 24.2±0.5 h (mean±SE), a cocoon was produced (Fig. 1c). Whilst the hole was open, the larva protruded its head outside the host's corpse and made contact with the surface where the dead aphid was standing (Fig. 1b) and secreted silk, which glued the host's exoskeleton to that surface through the entire area of the opening (Fig. 1c). During this period, the aphid acquired the characteristic opaque colouration of a "mummy". Eclosion took place between 4 and 5 days after pupation.

Figure 1 also shows the duration of the main stages of the parasitoid life inside the host under the described laboratory conditions.

Training with plant odours during pre-pupation period Two-way ANOVA with ranked data showed no effect of sex ($F_{1,55}=0.200$, $p=0.819$), place of occurrence (host plant or plastic plate) of ventral opening ($F_{1,55}=0.715$, $p=0.401$) or their interaction ($F_{1,55}=0.182$, $p=0.671$) on the difference between times spent on the stimulus and the control arms of the olfactometer. The parasitoids (pooled females and males) that spun their cocoon directly on the host plant surface showed significant differences between times spent in the olfactometer arms (paired t test, $t=2.083$, $N=31$, $p=0.046$, Fig. 2). The individuals that spun their cocoon over plastic plates covering the plant surface showed no differences between time spent in the olfactometer arms (paired t test, $t=0.747$, $N=28$, $p=0.92$, Fig. 2).

Fig. 1 Duration of different periods of development of *A. ervi* until eclosion. **a** *A. pisum* recently killed by the activity of *A. ervi*. **b** Ventral opening through the aphid's exoskeleton, showing the protruding head of the *A. ervi* third instar larva. **c** Partial closure of the ventral opening (ca. 6 h after it was produced) by silk produced during cocoon spinning of *A. ervi*



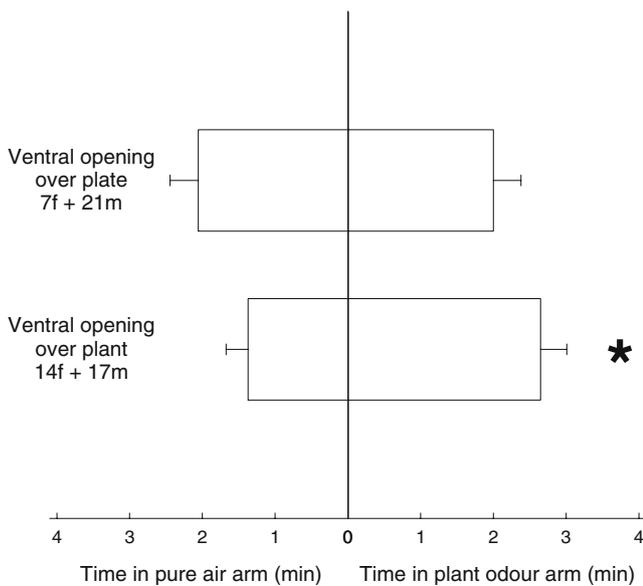


Fig. 2 Time spent (mean and standard error) by *A. ervi* adults in olfactometer arms permeated by air passing through a host plant or by pure air. At the larval stage, the parasitoids were allowed to open the ventral side of their host aphids either directly over the host plant or over a plastic plate covering the host plant. The asterisk shows a significant difference ($p=0.046$, paired t test). Number of individuals used (f , females; m , males) are indicated

Two-way ANOVA showed no effect of treatment or sex in any of the behaviours (see **Materials and methods**) performed by the parasitoids in the olfactometer (data not shown).

Training with vanilla odour during pre-pupation period - Three-way ANOVA showed significant effects of exposure (vanilla or water) and sex on the difference between times spent on the stimulus and the control arms of the olfactometer (stimulus: $F_{1,92}=11.60$, $p<0.001$; sex: $F_{1,92}=4.38$, $p=0.04$). No effect was found for manipulation of eclosion experience (mummy excision or normal eclosion; $F_{1,92}=0.370$, $p=0.544$) or for any of the interactions between treatments. Independent two-way ANOVA's were performed for males and females. Both showed a significant effect of exposure on the olfactometric response (males: $F_{1,59}=4.40$, $p=0.04$; females: $F_{1,33}=7.49$, $p=0.01$), and a non-significant effect of manipulation of eclosion experience (males: $F_{1,59}=0.008$, $p=0.92$; females: $F_{1,33}=0.72$, $p=0.40$; Fig. 3).

Two-way ANOVA showed no effect of exposure (vanilla or water) or manipulation of eclosion experience on any of the behaviours performed by the parasitoids (see **Materials and methods**), with the exception of exploring behaviour (data not shown). In this latter case, the two-way ANOVA showed a significant effect of stimulus on the difference between the times spent exploring in the vanilla arm and the control arm

of the olfactometer for both female ($F_{1,33}=4.86$, $p=0.031$) and male ($F_{1,59}=9.39$, $p=0.004$) parasitoids.

Discussion

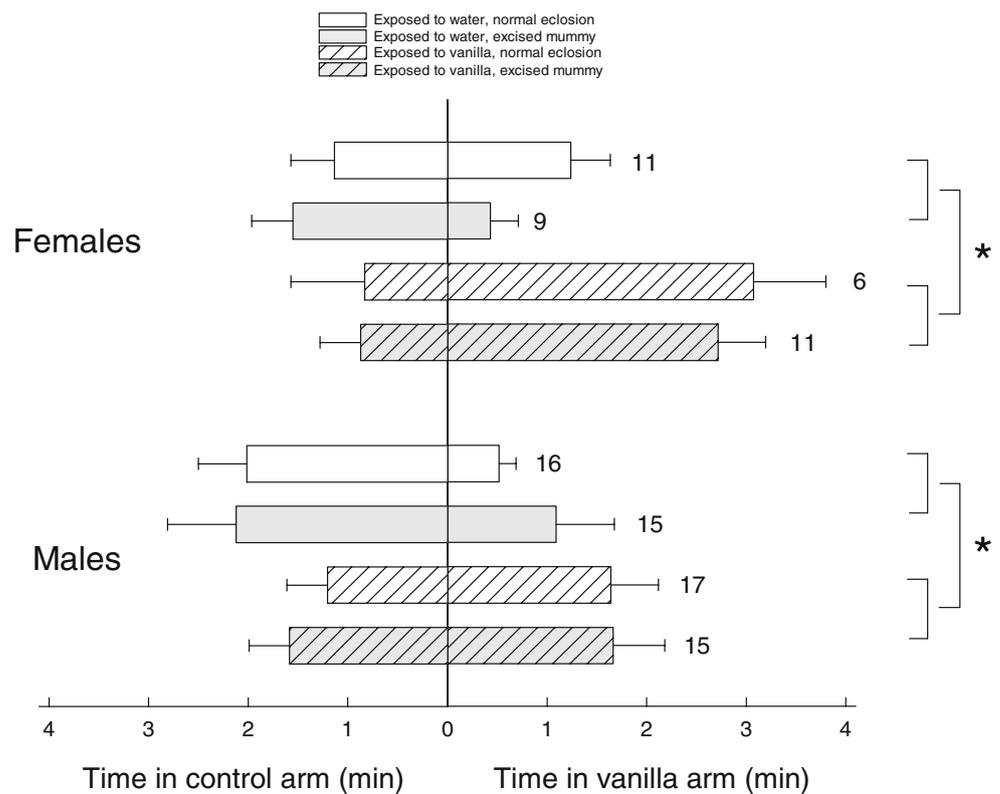
The third-instar larval stage of *A. ervi* was shown to be a behaviourally active stage during which the larva opened a hole in the ventral side of the aphid mummy. Through this hole, the larva actively glued the mummy to the surface beyond, thus confirming similar reports on other braconids (Shaw and Huddleston 1991).

Most braconid parasitoids pupate outside the host. In these wasps, the last-instar larva crawls out of the remains of the host and spins a cocoon next to it; in this way, the pupa is glued onto the surface where the aphid host was attached (Quicke 1997). Internal pupation is a derived character within the braconids, which is present only in Aphidiidae and Rogadinae (Belshaw and Quicke 1997). Hence, it seems that at least within the Aphidiidae, gluing to the pupation surface has been conserved during the transition from external to internal pupation by means of the ventral opening behaviour. This indicates the importance of this event for the survival of these parasitoids: if the ventral opening didn't occur, the mummy could easily fall from the plant surface.

During the period of ventral opening, the growing larva is exposed to the external environment (Fig. 1). The importance of this event is shown by the fact that only parasitoids that had direct contact with the plant through the ventral opening showed a preference towards the plant odours after eclosion (Fig. 2). Additionally, the larvae exposed to vanilla odour during the ventral opening period were attracted to vanilla when adults, independent of whether the larvae experienced normal eclosion or were removed from the mummy before eclosion (Fig. 3). The small amount of vanilla used, the brief exposure to it and the form in which vanilla was presented to the larva (see **Materials and methods**) make it unlikely that the response of the adult parasitoid to this compound were due to traces of vanilla present in the imago at the moment of eclosion. Collectively, these results indicate pre-imaginal learning.

In the aphid parasitoid, *Aphidius rhopalosiphii*, van Emden et al. (1996) reported that the pupation period was critical in determining the olfactory response of the adult to plants. Thus, when parasitised aphids were transferred between different plants, the adult parasitoids were attracted to the plant over which they had mummified. Moreover, in a subsequent report, van Emden et al. (2002) showed that adult parasitoids responded to odours of an alien plant only if it was present at mummification. The evidence presented herein suggests that exposure of the larvae to the external

Fig. 3 Effect of vanilla exposure during ventral opening and manipulation of the eclosion experience on the olfactometric responses of *A. ervi* male and female adults (mean and standard error). The asterisks show significant differences between groups (see Results). Number of individuals used are indicated by the bars



environment during the ventral opening of the aphid may be responsible for these reported behaviours.

Conditioning to odours present during mummification disappeared if the pupae of *A. rhopalosiphii* were excised from the mummies (van Emden et al. 1996, 2002). However, in *A. colemani* conditioning to odours present during pre-imaginal stages persisted despite the excision of the larva from the mummy (Vamvatsikos et al. 2004). Differences may be due to the different biology of the parasitoid species or/and particularities of the host-plant systems studied. On the other hand, Storeck et al. (2000) raised *A. colemani* in two different hosts and reported that when parasitoids were left in one of the hosts until pupation, extracted from their mummy and further exposed as imagoes to an empty mummy of the second host, the adults showed a preference for the odours of the second host. Thus, although a part of the evidence for the effect of eclosion environment on adult olfactory preferences is still controversial, it seems clear that odours present at eclosion affect the behaviour of the adult parasitoid. Thus, good evidence exists for two successive instances of learning: at the pre-imaginal stage and at eclosion. Furthermore, if odours present at eclosion are different from those present during ventral opening, experience at eclosion may bias the olfactory preference of the adult.

In recent years, pre-imaginal conditioning has been questioned as a mechanism to explain the influence of larval environment on adult behaviour, and “chemical

legacy” has been suggested as the sole explanation to this phenomenon (van Emden et al. 1996; Barron and Corbet 1999; Barron 2001). The present results show that pre-imaginal conditioning may also determine olfactory responses of the imago. Survival of memory through metamorphosis has been convincingly demonstrated in many insects (Alloway 1972, Isingrini et al. 1985; Carlin and Schwartz 1989; Tully et al. 1994; Ray 1999), and neurobiological evidences exist to support its occurrence (Armstrong et al. 1998). Further research is needed, which focuses on the mechanism of integration of pre-imaginal and eclosion experiences and their effect on the behaviour of the adult.

The early exposure to the host-plant complex could have important consequences for the fidelity of parasitoids to their host. Early preference towards a given host-plant complex due to pre-imaginal learning could be reinforced if adult activities such as oviposition or mating occur in the same system because females during oviposition (reviewed by Vet and Groenewold 1990) and males during mating (Villagra et al. 2005) can be conditioned to the environment where these activities are performed. Thus, if parental behaviour determines the environment where the offspring will develop, by choosing on which host-plant complex to copulate or oviposit the learned responses could transcend to the following generation as parental effects (Bernardo 1996), and fidelity towards the host-plant complex could become transgenerational and constitute an epigenetic

inheritance of host-plant complex preference (Rossiter 1996, Maturana and Mpodozis 2000). This may provide a stable ecological niche promoting the splitting and speciation of natural populations (Bush 1968, Pennacchio and Tremblay 1989). However, comparative and experimental research is needed to know how experiences during ontogeny are related to the maintenance of parasitoid host range and specificity through evolutionary time.

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References

- Alloway TM (1972) Retention of learning through metamorphosis in grain beetle (*Tenebrio molitor*). *Am Zool* 12:471–477
- Armstrong JD, de Belle JS, Wang ZS, Kaiser K (1998) Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in *Drosophila*. *Learn Memory* 5:102–114
- Barron AB (2001) The life and death of Hopkins' host-selection principle. *J Insect Behav* 14:725–737
- Barron AB, Corbet SA (1999) Preimaginal conditioning in *Drosophila* revisited. *Anim Behav* 58:621–628
- Belshaw R, Quicke DLJ (1997) A molecular phylogeny of the aphidiinae (Hymenoptera: Braconidae). *Mol Phylogenet Evol* 7:281–293
- Bernardo J (1996) Maternal effects in animal ecology. *Am Zool* 36:83–105
- Blumstein DT, Evans CS, Daniel JC (2000) JWachter 0.9. An introductory user's guide. Animal Behaviour Laboratory, Macquarie University, Australia. <http://galliform.psy.mq.edu.au/jwachter/>
- Bush G (1968) Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). *Evolution* 23:237–251
- Carlin NF, Schwartz PH (1989) Preimaginal experience and nestmate brood recognition in the carpenter ant, *Camponotus floridanus*. *Anim Behav* 38:89–95
- Chow JK, Akhtar Y, Isman MB (2005) The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusia ni* (Lepidoptera: Noctuidae). *Chemoecology* 15:129–133
- Corbet SA (1985) Insect chemosensory responses—a chemical legacy hypothesis. *Ecol Entomol* 10:143–153
- Du YJ, Poppy GM, Powell W, Wadhams LJ (1997) Chemically mediated associative learning in the host foraging behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *J Insect Behav* 10:509–522
- Fujiwara C, Takabayashi J, Yano S (2000) Oviposition experience on a host-infested plant affects flight and antennal searching behaviour of *Cotesia kariyai* toward the host-plant complex. *Entomol Exp Appl* 97:251–256
- Gandolfi M, Mattiacci L, Dorn S (2003) Preimaginal learning determines adult responses to chemical stimuli in a parasitic wasp. *Proc R Soc Lond B* 270:2623–2629
- Godfray H CJ (1994) Parasitoids, behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ
- Isingrini M, Lenoir A, Jaisson P (1985) Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proc Natl Acad Sci USA* 82:8545–8547
- Kudon LH, Berisford CW (1980) Influence of brood hosts on host preferences of bark beetle parasites. *Nature* 283:288–290
- Manning A (1967) Pre-imaginal conditioning in *Drosophila*. *Nature* 216:338–340
- Maturana H, Mpodozis J (2000) The origin of species by means of natural drift. *Rev Chil Hist Nat* 73:261–310
- Pennacchio F, Tremblay E (1989) A new species of *Aphidius* Nees from Italy (Hymenoptera, Braconidae, Aphidiinae). *Boll Lab Entomol Agr Filippo Silvestri* 45:167–169
- Quicke DLJ (1997) Parasitic wasps. Chapman & Hall, London
- Ray S (1999) Survival of olfactory memory through metamorphosis in the fly *Musca domestica*. *Neurosci Lett* 259:37–40
- Rietdorf K, Steidle JLM (2002) Was Hopkins right? Influence of larval and early adult experience on the olfactory response in the granary weevil *Sitophilus granarius* (Coleoptera, Curculionidae). *Physiol Entomol* 27:223–227
- Rojas JC, Wyatt TD (1999) The role of pre- and post-imaginal experience in the host-finding and oviposition behaviour of the cabbage moth. *Physiol Entomol* 24:83–89
- Rossiter M (1996) Incidence and consequence of inherited environmental effects. *Ann Rev Ecol Syst* 27:451–476
- Shaw SR, Huddleston T (1991) Classification and biology of braconid wasps (Hymenoptera: Braconidae). *Handbk Identif Br Insects* 7:1–126
- Smith MA, Cornell HV (1979) Hopkins host-selection in *Nasonia vitripennis* and its implications for sympatric speciation. *Anim Behav* 27:365–370
- Storeck A, Poppy GM, van Emden HF, Powell W (2000) The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomol Exp Appl* 97:41–46
- Thorpe WH (1939) Further studies on pre-imaginal olfactory conditioning in insects. *Proc R Soc Lond B* 127:424–433
- Tully T, Cambiazio V, Kruse L (1994) Memory through metamorphosis in normal and mutant *Drosophila*. *J Neurosci* 14:68–74
- Turlings TCJ, Wackers F, Vet LEM, Lewis WJ, Tumlinson JH (1993) Learning of host finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC (eds) *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York, pp 51–78
- Vamvatsikos PG, Hardie J, van Emden HF (2004) The origins of olfactory preference in an aphid parasitoid. XXII International Congress of Entomology. Brisbane, Australia
- Van Achterberg C (1984) Essay on the phylogeny of Braconidae (Hymenoptera: Ichneumonoidea). *Entomol Tidskr* 105:41–58
- van Emden HF, Sponagl B, Baker T, Ganguly S, Doulopaka S (1996) "Hopkins host selection principle", another nail in its coffin. *Physiol Entomol* 21:325–328
- van Emden HF, Eletherianos I, Rose J, Doulopakata S, Pettersson J (2002) Aphid parasitoids detect that an alien plant was present nearby during their development. *Physiol Entomol* 27:199–205
- Vet LEM, Groenewold AW (1990) Semiochemicals and learning in parasitoids. *J Chem Ecol* 11:3119–3135
- Vet LEM, Lewis WJ, Carde RT (1995) Parasitoid foraging and learning. In: Cardé RT, Bell WJ (eds) *Chemical ecology of insects 2*. Chapman and Hall, New York, pp 65–101
- Villagra CA, Vásquez RA, Niemeyer HM (2005) Associative odour learning affects mating behaviour in *Aphidius ervi* males (Hymenoptera: Braconidae). *Eur J Entomol* 102:557–559
- Wcislo WT (1987) The role of learning in the mating biology of a sweat bee *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Behav Ecol Sociobiol* 20:179–189