

# Host-searching responses to herbivory-associated chemical information and patch use depend on mating status of female solitary parasitoid wasps

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**Abstract.** 1. In a tritrophic interaction system consisting of plants, herbivores, and their parasitoids, chemicals released from plants after herbivory are known to play important roles for many female parasitoids to find their hosts efficiently. On the plant side, chemical information associated with herbivory can act as an indirect defence by attracting the natural enemies of the host herbivores.

2. However, mated and virgin females of haplodiploid parasitoids might not necessarily respond to such chemical cues in the same way. Since virgin females can produce only sons, they might refrain from searching for hosts to invest eggs until copulation, in order to produce both sexes.

3. Here, we investigated differential host-searching behaviours shown by mated and virgin females in the solitary parasitoid wasp, *Cotesia vestalis*, in response to herbivory-associated chemical information from cruciferous plants infested by their host larvae, *Plutella xylostella*.

4. Mated females showed a significantly higher flight preference for host-infested plants over intact plants, while no preference was observed with virgin females. Mated females also showed more intensive antennal searching and ovipositor probing behaviours to leaf squares with wounds caused by hosts than did virgin females. Furthermore, mated females stayed longer in host patches with higher parasitism rates than virgin females.

5. These results indicate that mating status of *C. vestalis* females clearly influences their host-searching behaviour in response to herbivory-associated chemical information and patch exploitation. Female parasitoids seem to forage for hosts depending on their own physiological condition in a tritrophic system.

**Key words.** *Cotesia vestalis*, herbivore-induced plant volatiles, indirect defence, sex allocation, tritrophic interaction.

## Introduction

Female parasitoids are known to use a wide variety of information to find and attack their hosts. One of the most important cues for host-searching parasitoids is chemical information produced by plants and/or by host herbivores

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(review in Godfray, 1994). For instance, female parasitoid wasps are known to efficiently locate a host patch in their habitat through the detection of volatile infochemicals emitted from plants attacked by their herbivorous hosts. Many studies have reported that volatile organic compounds produced by plants in response to herbivory (herbivore-induced plant volatiles: HIPVs) attract various parasitoid wasps (Turlings *et al.*, 1990; Takabayashi & Dicke, 1996; De Moraes *et al.*, 1998; Dicke, 1999). On a host patch, female parasitoids search

for their hosts, at close range, by means of lower volatile and/or contact infochemicals arising from hosts themselves, silk or frass ejected by the hosts, wounds on leaves caused by the hosts, etc. (Sato, 1979; Mattiacci & Dicke, 1995; Horikoshi *et al.*, 1997; Li *et al.*, 1997; Shiojiri *et al.*, 2000b). Contact infochemicals can also be produced by the plants in response to host attack (Shiojiri *et al.*, 2000b). In addition to the purpose of locating hosts, females of some parasitoid species exploit these herbivore-induced infochemicals as cues for determining how long they should stay in a host patch (Ohara *et al.*, 2003; Wajnberg, 2006; Tentelier & Fauvergue, 2007).

From the viewpoint of tritrophic interaction studies, the ecological function of such induced infochemicals has attracted much attention and has been emphasised as an indirect defence by plants against herbivorous insects, since plants suffering from herbivory express a defensive trait that affects higher trophic levels (Price *et al.*, 1980; Sabelis *et al.*, 2007), by promoting the effectiveness of host-searching in parasitoid wasps. Although such plants' indirect defence can be influenced by behavioural variability in parasitoid wasps, there have been only few studies on how plastic the information use for herbivore-induced infochemicals is in parasitoid wasps, and how their consequent behavioural outputs are affected by biotic and abiotic factors (Hilker & McNeil, 2008). Such behavioural plasticity in information use appears to be important for parasitoids themselves to behave adaptively.

Host-searching parasitoids need to make complex decisions to cope with various problems by integrating external information from the environment with their internal physiological condition. This decision-making process would allow them to adopt flexible foraging behaviour that would maximise their lifetime reproductive success under changing circumstances (van Alphen *et al.*, 2003). For example, parasitoids face the problem of whether to search for hosts to increase current reproduction or to search for food to increase future reproduction through their own survival (e.g. Sirot & Bernstein, 1996; Tenhumberg *et al.*, 2001, 2006; Lucchetta *et al.*, 2007; Bernstein & Jervis, 2008).

Another type of trade-off between current and future reproduction may arise in haplodiploid arrhenotokous parasitoid wasps. In this case, virgin females can produce progeny even without mating, but only haploid sons develop from unfertilised eggs. The enforced production of only one sex by haplodiploid females is called 'constrained sex allocation' (Godfray, 1990; Godfray & Hardy, 1993). Mated females can produce either fertilised diploid daughters or unfertilised haploid sons. Thus, virgin females may be confronted with a trade-off between searching for hosts to invest eggs in current reproduction with only sons, and refraining from doing so until copulation in order to produce both sexes. This appeared to be the case with solitary parasitoids but also with gregarious parasitoids, in which virginity and non-local mating can be prevalent and may influence sex ratio optima (Hardy *et al.*, 1998, 2000). In both solitary and gregarious parasitoids, there are several pieces of evidence that indicate that constrained sex allocation occurs in the field (Hardy & Godfray, 1990; Godfray & Hardy, 1993), suggesting that the situation and the

trade-off is ubiquitous in haplodiploid parasitoid species. However, differences in the sequence of host-searching behaviour in response to chemical information according to mating status in most parasitoids have not been demonstrated, although such a trait could be one of the bases for complex decision making by foraging female parasitoids.

*Cotesia vestalis* (Haliday) [= *C. plutellae* (Kurdjumov), Shaw, 2003] (Hymenoptera: Braconidae) is a solitary parasitoid wasp that mainly attacks the larvae of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), a specialist of cruciferous plants. To locate the hosts, female parasitoids use various infochemicals produced by plants and host herbivores. In their habitat, they can find host-infested plants (host patch) by discriminating them from intact plants using a specific blend of HIPVs in their flight orientation process (Shiojiri *et al.*, 2000a). After landing on the infested plants, female parasitoids forage for hosts in the patch. Upon encountering wounds caused to the plants by the hosts, females show characteristic behaviours that include persistent antennal drumming and ovipositor probing around the wounds, suggesting the involvement of contact infochemicals as host-finding cues (Shiojiri *et al.*, 2000b). Furthermore, foraging female parasitoids might control their residence time on host patches in the presence of infochemicals.

In this study, we tested whether mating status of females can affect each step of their host-searching behaviour in the solitary parasitoid wasp, *C. vestalis*. Females' age also can be a factor that potentially influences the host-searching behaviour, since it might be adaptive for a female approaching the end of its life to adopt behaviours that are not influenced by its mating status any more. Hence, we also tested whether the age of females can affect the behavioural steps together with their mating status in a reciprocal or independent way. To test these hypotheses, we compared patterns of flight orientation to a host patch by means of HIPVs among mated and virgin females of different ages, and measured their antennal searching and ovipositor probing responses to a wounded leaf square elicited by contact infochemicals. Flight orientation and antennal searching are expected to indicate differential responsiveness of females to these infochemicals, and ovipositor probing is indicative of their oviposition activity levels, before and after landing on host patches. Finally, we investigated quantitative differences in residence time on host patches by the female parasitoids, and evaluated the net effect of potential changes on subsequent parasitism as a function of mating status and age. This was in order to explain how females exploit host patches and decide to leave these patches according to these two biotic factors.

## Materials and methods

### *Plants and insects*

Japanese mustard spinach (Komatsuna), *Brassica rapa* var. *peruviridis* L., was cultivated in a greenhouse (25 ± 3 °C; 60 ± 10% relative humidity (RH); LD 16:8 h photoperiod). Five plants were reared in a plastic pot (90 mm i.d., 70 mm depth) for 4–5 weeks, and were used for both insect rearing and behavioural tests. *Plutella xylostella* was

originally collected from fields in Ayabe, Kyoto Prefecture, Japan, in 2001, and was mass reared on potted plants in a climate-controlled room ( $25 \pm 3^\circ\text{C}$ ;  $60 \pm 10\%$  RH; LD 16:8 h photoperiod). *Cotesia vestalis* was collected from the same fields with its host, *P. xylostella*, and was reared on *P. xylostella*-infested plants under the same conditions as their hosts. Parasitoid cocoons were collected from stock culture and individually maintained in glass tubes (25 mm i.d., 120 mm height). Newly emerged, less than 24 h old, adult females were randomly divided into the following two groups (i.e. virgin or mated), and kept individually in the same tubes with honey for food under the same condition ( $25 \pm 3^\circ\text{C}$ ,  $60 \pm 10\%$  RH and LD 16:8 h photoperiod) until experiments. In these conditions, adult females can live for a few weeks. 'Virgin females' were prepared by maintaining the females as they were, whereas 'mated females' were prepared by introducing a few males into each glass tube to allow them to mate with the females. Males were removed 1 day before the experiments. We compared host-searching behaviour of virgin and mated females at the age of 1 or 2 weeks (7 or 14 days) after emergence. Tested females had never encountered host-infested plants before the experiments (i.e. naive females). After experiments, it was confirmed that females exposed to males actually mated by checking the sex of offspring produced.

#### *Flight response of Cotesia vestalis females to host-infested plants*

The flight response of mated or virgin *C. vestalis* females was observed for 30 min in an acryl cage ( $25 \times 35 \times 30$  cm) in a climate-controlled room ( $25 \pm 3^\circ\text{C}$ ;  $60 \pm 10\%$  RH). Infested plants were prepared by allowing 15 third-instar *P. xylostella* larvae to feed on the potted plants for 24 h. At the same time, intact plants were prepared without any treatment as controls. The two different pots of infested and intact plants were placed ca 10 cm apart, inside the acryl cage. A group of 10 mated or virgin females at each age were released from a position located exactly between the two pots. After hovering over between the two pots of plants repeatedly inside the test cage, females finally landed on either of the plants in response to volatile infochemicals, with a few exceptions. The total numbers of first landings on each potted plant by the female parasitoids were counted as a result of flight preference shown by the females. After landing, females were immediately removed from the cage using an insect aspirator. A few females not landing on any potted plants within 30 min were recorded as no-choice subjects, and were excluded from the data for statistical analysis. To start the next trial, the set of potted plants was replaced with a new set, and the positions of infested and intact plants were switched. For every treatment, each trial represented by a group of 10 released females was replicated six times on three different days. Significant preferences differing from the null hypothesis of an expected ratio of 0.5:0.5 were analysed using a replicated *G*-test in view of heterogeneities suspected among replications (Sokal & Rohlf, 1995). Furthermore, effects of mating status, age of female parasitoids and the interaction between these

two main factors on the percentage of females landing on the infested plants were analysed by means of a logistic model, a generalised linear model specially designed for modelling binomial data using logistic link function (McCullagh & Nelder, 1989; Wajnberg & Haccou, 2008).

#### *Antennal searching and ovipositor probing of Cotesia vestalis females on leaf squares*

Female parasitoids frequently show typical host-searching behaviour with antennal contacts (i.e. antennal searching behaviour) and stinging behaviour with their ovipositor (i.e. ovipositor probing behaviour) on host-infested leaves. Those behaviours shown by mated and virgin females of *C. vestalis* were observed in a glass tube (20 mm diameter, 130 mm length) in a climate-controlled room ( $25 \pm 3^\circ\text{C}$ ;  $60 \pm 10\%$  RH). To prepare infested leaves, 15 third-instar larvae of *P. xylostella* were allowed to feed on the potted plants for 24 h. Just before the observation, these larvae and their visible products, such as faeces and silk, were carefully removed from the leaves with a paintbrush. A leaf square ( $1 \times 1$  cm) including a wormhole (ca 8 mm long) was cut out from the plants and placed in each glass tube containing a mated or a virgin female of *C. vestalis* of different age. Observation started on the first antennal contact with the leaf square. We recorded the total duration of antennal contacts with the leaf square and the number of stings on it by the ovipositor for each tested female. The observation was terminated after 3 min or when a female stopped antennal searching and began grooming, or when she left the leaf for more than 10 s. Each observation was replicated for 25 females per treatment. Effects of mating status, age of female parasitoids, and the interaction between these two main effects on the total duration of antennal contacts with the leaf square were analysed by means of a Cox (1972) proportional hazards model, a specific statistical method whose features seem to be particularly appropriate when analysing data on time duration (e.g. review in Wajnberg, 2006). In this case, grooming was considered as a censoring event (Bressers *et al.*, 1991; Haccou & Meelis, 1992). The same effects were tested on the number of ovipositor probing by means of a log-linear model, a generalised linear model designed to handle Poisson-distributed data using a logistic link function (McCullagh & Nelder, 1989; Wajnberg & Haccou, 2008).

#### *Patch residence time and parasitism by C. vestalis females on host-infested plants*

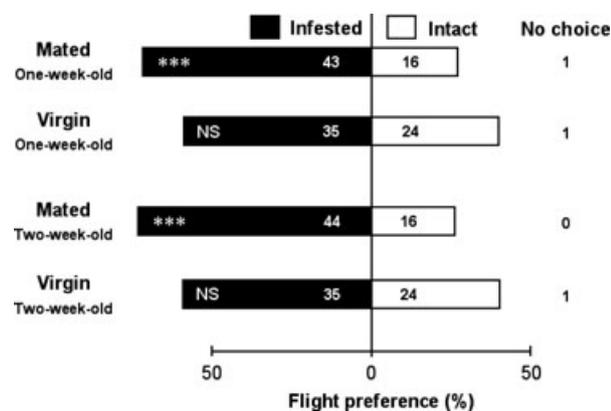
The foraging behaviour of mated and virgin *Cotesia vestalis* females of different age on potted whole plants infested by *P. xylostella* larvae was observed in a climate-controlled room ( $25 \pm 3^\circ\text{C}$ ;  $60 \pm 10\%$  RH;  $3 \times 3 \times 3$  m). Twenty third-instar larvae of *P. xylostella* were allowed to feed on the potted plants for 24 h. A pot of infested plants was considered as a 'host patch'. Each female parasitoid kept in a tube was introduced carefully, using an insect aspirator, just onto an old wound of the plants still infested by the larvae, and the total time spent

on the patch until leaving was recorded. When the female came back to the patch in a few seconds after a short departure, the observation was continued. After the female had flown away, the whole patch was caged with all the host larvae, which were then reared on the whole potted plants. The numbers of parasitoid pupae and host pupae were counted afterwards to evaluate the parasitism rate achieved during the observation. If the sum of numbers of pupae obtained was fewer than 15 (i.e. more than 25% of tested host larvae died before pupation), the data were discarded. The observation was replicated for 20 females per treatment. Effects of mating status, age of female parasitoids, and the interaction between these two main effects on patch residence time were analysed by means of a Cox proportional hazards model (see above), and the same effects were tested on the observed parasitism rates by means of a logistic model, a generalised linear model for binomial data as the above. All computations were carried out using SAS (version 9.1.3, SAS Institute Inc., Cary, North Carolina).

## Results

### Flight response of *Cotesia vestalis* females to host-infested plants

One-week-old mated females of *C. vestalis* significantly preferred host-infested plants to intact plants when offered a pair of plants in the replicated choice trials ( $G_p = 12.83$ , d.f. = 1,  $P < 0.001$ ) (Fig. 1). Two-week-old mated females also significantly preferred host-infested plants to intact plants ( $G_p = 13.59$ , d.f. = 1,  $P < 0.001$ ). However, 1-week-old virgin females showed no significant preference ( $G_p = 2.06$ , d.f. = 1,  $P = 0.15$ ), and neither did 2-week-old virgin females ( $G_p = 2.06$ , d.f. = 1,  $P = 0.15$ ). There was no significant heterogeneity among replications in each treatment (1-week-old mated females:  $G_h = 3.10$ , d.f. = 5,  $P = 0.68$ ; 1-week-old



**Fig. 1.** Flight preferences (%) to a host patch, plants infested by *Plutella xylostella* larvae (filled bars), or to intact plants (open bars), shown by mated and virgin females of *Cotesia vestalis* at the ages of 1 and 2 weeks after emergence ( $n = 60$ ). Numbers in bars indicate the total numbers of females that showed the preference by landing on each plant. Asterisks indicate significant differences within each preference test set (\*\*\*)  $P < 0.001$ ; NS: not significant, replicated  $G$ -test).

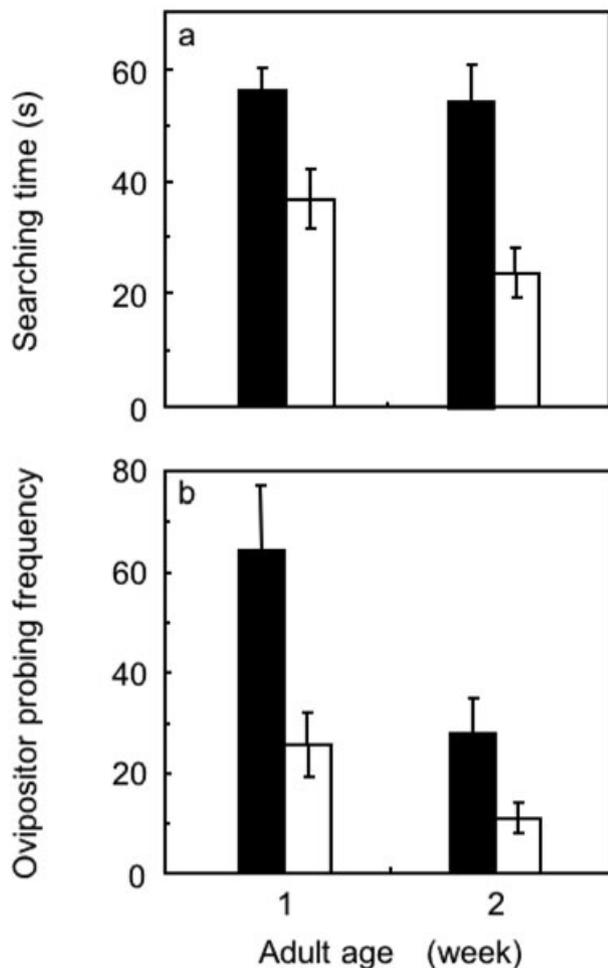
virgin females:  $G_h = 0.90$ , d.f. = 5,  $P = 0.97$ ; 2-week-old mated females:  $G_h = 3.93$ , d.f. = 5,  $P = 0.56$ ; 2-week-old virgin females:  $G_h = 0.90$ , d.f. = 5,  $P = 0.97$ ), supporting good reproducibility of the flight preference test. Furthermore, there was a strongly significant difference in the flight preference between mated and virgin females ( $\chi^2 = 11.45$ , d.f. = 1,  $P < 0.001$ ), but no significant difference between 1- and 2-week-old females ( $\chi^2 = 0.003$ , d.f. = 1,  $P = 0.95$ ). Finally, the interaction between mating status and age of female parasitoids was also not significant on their flight preference ( $\chi^2 = 0.004$ , d.f. = 1,  $P = 0.95$ ).

### Antennal searching and ovipositor probing of *Cotesia vestalis* females on leaf squares

All female parasitoids showed antennal searching and ovipositor probing behaviours on leaf squares with a wound caused by host herbivores, irrespective of their mating status or age. However, there was a strongly significant effect of mating status of female parasitoids on the duration of antennal searching behaviour on the leaf square ( $\chi^2 = 6.95$ , d.f. = 1,  $P = 0.0084$ ). Antennal searching time of mated females was longer than that of virgin females, regardless of the different ages of 1 and 2 weeks after emergence (Fig. 2a). Difference in age had no significant effect on antennal searching time ( $\chi^2 = 3.21$ , d.f. = 1,  $P = 0.073$ ), although there was a trend that older and especially virgin females searched less on the leaf square. No significant interaction between mating status and age of female parasitoids was detected in antennal searching time ( $\chi^2 = 0.32$ , d.f. = 1,  $P = 0.57$ ). Similarly, mating status of female parasitoids had a strongly significant effect on frequency of ovipositor probing ( $\chi^2 = 11.30$ , d.f. = 1,  $P < 0.001$ ). Mated females showed a higher ovipositor probing frequency than virgin females (Fig. 2b). Difference in age also had a strongly significant effect on ovipositor probing frequency ( $\chi^2 = 11.18$ , d.f. = 1,  $P < 0.001$ ), with younger females showing a higher ovipositor probing frequency. There was no significant interaction between mating status and age of female parasitoids on their ovipositor probing frequency ( $\chi^2 = 0.03$ , d.f. = 1,  $P = 0.87$ ).

### Patch residence time and parasitism by *Cotesia vestalis* females on host-infested plants

The residence time of female parasitoids on potted plants with host larvae (i.e. a host patch) was strongly affected by mating status ( $\chi^2 = 6.94$ , d.f. = 1,  $P = 0.0084$ ). Mated females stayed longer on a patch than virgin females, irrespective of their age (Fig. 3a). At the same time, there was a strongly significant effect of age on the residence time on the patch ( $\chi^2 = 9.87$ , d.f. = 1,  $P = 0.0017$ ), with older females staying longer on the patch. There was no significant interaction between mating status and age on the residence time on the patch ( $\chi^2 = 0.94$ , d.f. = 1,  $P = 0.33$ ). The observed parasitism rate during the stay on the patch was also strongly affected by mating status of females ( $\chi^2 = 15.33$ , d.f. = 1,  $P < 0.0001$ ). Mated females parasitised more

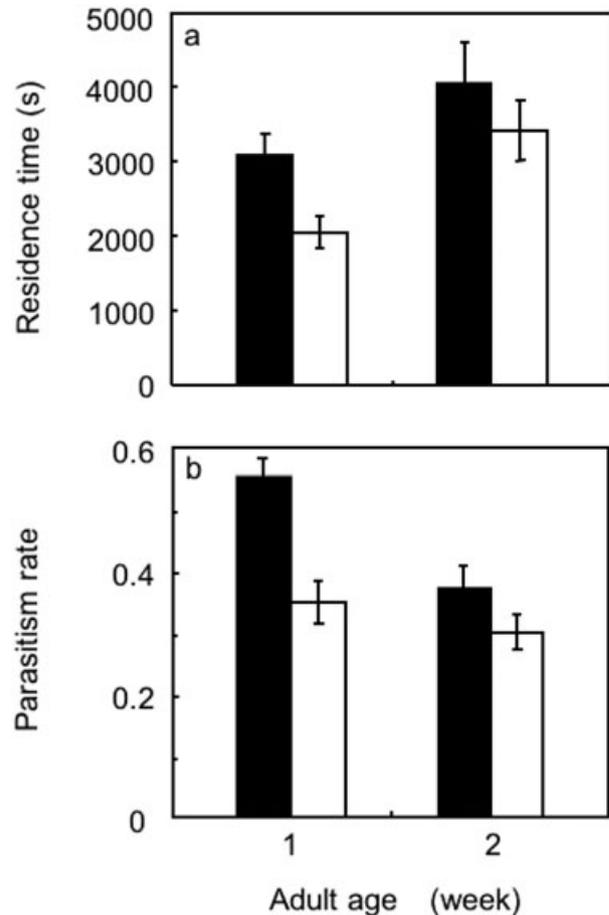


**Fig. 2.** Host-searching responses of mated females (filled bars) and virgin females (open bars) of *Cotesia vestalis* in contact with a leaf square infested by *Plutella xylostella* larvae. (a) Antennal searching time (mean  $\pm$  SE) and (b) frequency of ovipositor probing (mean  $\pm$  SE) observed in female parasitoids ( $n = 25$ ) at the ages of 1 and 2 weeks after emergence. Statistical data are presented in the text.

host larvae than virgin females, whatever their age (Fig. 3b). A significant effect of age on the parasitism rate was also detected ( $\chi^2 = 10.13$ , d.f. = 1,  $P = 0.0015$ ), with younger females parasitising more host larvae. Finally, there was no significant interaction between mating status and age of female parasitoids on their parasitism rate ( $\chi^2 = 3.46$ , d.f. = 1,  $P = 0.063$ ).

## Discussion

In a tritrophic system consisting of plants, herbivores and parasitoids, consequences of the use of chemical information by parasitoids to locate and attack their hosts can be influenced by their own physiological conditions, such as dietary status (e.g. Wäckers, 1994; Siekmann *et al.*, 2004; Desouhant *et al.*, 2005), as well as by abiotic factors supposed to affect



**Fig. 3.** Residence time and subsequent parasitism rate of mated females (filled bars) and virgin females (open bars) of *Cotesia vestalis* on a host patch, plants infested by *Plutella xylostella* larvae. (a) Patch residence time (mean  $\pm$  SE) and (b) parasitism rate in the host patch (mean  $\pm$  SE) shown by female parasitoids ( $n = 20$ ) at the ages of 1 and 2 weeks after emergence. Statistical data are presented in the text.

parasitoid behaviour (Hilker & McNeil, 2008). In this work, we demonstrated for the first time, that the whole sequence of host-searching behaviour by female parasitoids in response to chemical information from herbivore-infested plants was dependent on their mating status in the solitary parasitoid wasp, *C. vestalis*. The significant effect of age was not observed in flight preference and antennal searching response (Fig. 2a). Such host-searching responses to infochemicals seem to be influenced independently by mating status and by age, because no interaction effect between the two factors was detected in the species studied. These results suggest that responsiveness to volatile and contact infochemicals remains only in mated females late into their life, although motivation for oviposition decreased with age in both virgin and mated females.

The tendency for depressed oviposition in relation to virginity of *C. vestalis* females was also observed in their foraging behaviour once they had arrived on a host patch (Fig. 3a,b). One of the proximate mechanisms involved could be egg load in female parasitoids leading to the

observed difference in their responses to infochemicals, and to their different foraging behaviour on a host patch. In insects, it is generally known that copulation promotes oviposition behaviour and ovigenesis in females (Chapman, 1998). However, there was no significant difference observed in egg loads between the mated and virgin females of *C. vestalis*, irrespective of their age (T. Mitsunaga and Y. Kobori, unpublished).

Similar effects of mating status on foraging behaviour have been reported in a few gregarious species and solitary species that parasitise aggregated hosts: mated females of some aphid parasitoids stayed longer on a host patch and laid more eggs than virgin females (Michaud & Mackauer, 1995). In the larval parasitoid *Cotesia glomerata*, mated females laid more eggs in a host caterpillar, *Pieris rapae crucivora*, than did virgin females (Tagawa *et al.*, 1987), although the same numbers of mature eggs were stored in the oviducts of virgin and mated females of the same age (Tagawa, 1987). In the Bethyloid wasp *Laelius pedatus*, virgin females took a longer time than mated females to lay the same numbers of eggs in clutches (Mayhew & Heitmans, 2000). In *C. vestalis*, differences according to mating status were observed in the whole sequence of host-searching behaviour, including responsiveness to infochemicals mentioned above, and the differences appear to be caused by more complex factors other than egg load. In aphid parasitoid species, it has been demonstrated that HIPVs can be used as a cue for patch assessment to determine how long to stay in the patch (Tentelier & Fauvergue, 2007). In the same way, *C. vestalis* females might also adjust their patch residence time by the use of such chemical information, but in different ways according to their mating status. Further work at the physiological level is needed to better understand the underlying mechanisms of the differential behaviours caused by copulation.

Ultimately, the results presented here suggest that it might be disadvantageous for a virgin female to lay all her eggs, producing only sons before mating (i.e. constrained sex allocation). Indeed, a virgin female should take a delayed chance to make another option of having daughters if she can wait for a mate (i.e. unconstrained sex allocation). Depressed oviposition in virgin females might be also relevant to the mating system of parasitoids. In solitary parasitoids, females generally lay only one egg in each host, with less chance for the newly emerged females to mate with males emerging at different places. The situation is different from the case of most gregarious parasitoids, whose females have better opportunity to mate with sib-brothers from the same host, soon after emergence. Among the genus *Cotesia*, it has been demonstrated that females attract or arrest conspecific males by sex pheromones, unidentified as yet (Tagawa & Hidaka, 1982; Field & Keller, 1993; De Freitas *et al.*, 2004). In *C. vestalis*, females a few days after emergence are most attractive to males, although showing the host-searching responses not so clearly as other older females (S. Kugimiya and T. Yoshimura, pers. obs.). There might be a trade-off between host-searching and pheromone production by females, which needs further investigation.

On the other hand, some benefits remain for a virgin female by immediate oviposition of unfertilised eggs before mating, since it can still gain some fitness that way under the constant risks of predation, starvation or other accidental forms of death. There have been several pieces of evidence that constrained sex allocation occurs in the field among parasitoid species (Hardy & Godfray, 1990; Godfray & Hardy, 1993), suggesting the importance of virgin oviposition to their reproductive success. Actually, as we observed in *C. vestalis*, all virgin females still showed ovipositor probing behaviour to a wound on a leaf and laid eggs on a host patch, despite no flight preference for HIPVs, observed together with reduced responses to the contact infochemicals.

A dynamic programming model predicts that foragers should stay longer in host patches as they approach the end of their life, and such a prediction was verified for the parasitoid *Anaphes victus*, exploiting eggs of their hosts (Wajnberg *et al.*, 2006). The same effect of age difference on variation in patch residence time has been experimentally demonstrated in some other parasitoids but only in gregarious species (Weisser, 1994; Michaud & Mackauer, 1995; Thiel & Hoffmeister, 2004). As for *C. vestalis*, the prediction appears to be verified in the present work, with the result that older females actually stayed significantly longer on host patches (Fig. 3a). On the other hand, older females showed a significantly lower parasitism rate, despite an increase in their patch residence time (Fig. 3b), suggesting a global decrease in the efficiency of finding and parasitising hosts. The lower parasitism rate observed in older females also seems to correspond to diminished ovipositor probing in response to wounds caused by the hosts (Fig. 2b), which may imply that oviposition activity in foraging females is depressed simply as a result of ageing. In all cases, whether a female is mated or not is likely to be one of the proximate rules of patch-leaving decisions used by the solitary parasitoid species, and age is likely to be another, as suggested by the absence of interaction between the two factors. Females are most likely to integrate such internal information about their own physiological condition to adopt optimal decision-making in their foraging strategy.

Our results showing that the mating status of female parasitoids affects their response to infochemicals and their patch use, lead to two main conclusions. Firstly, as mentioned above, proximate mechanisms are regulating the host-searching behaviour of female parasitoids. Secondly, the ability of female parasitoids to locate their hosts through the use of induced plant chemicals, such as HIPVs or other infochemicals, is likely to have evolved in concert with the elaborate mechanisms involving their sex allocation strategy. At this point, tritrophic interaction studies overlap with behavioural ecology studies on sex ratio, which has long been a major focus of interest in this area. However, there are few studies on optimal allocation to host searching and mate searching (Guertin *et al.*, 1996), especially by constrained females of solitary parasitoids, although the corresponding results would give more insight into the evolutionary ecology of parasitoid behaviour. In this study, as a first step, it is clearly demonstrated that foraging females of solitary parasitoids acquire chemical information from their environment and use it according to their mating

status, which allows them to adopt flexible behaviour in a tritrophic context.

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