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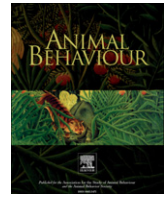
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## Animal Behaviour

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# Synergy in information use for mate finding: demonstration in a parasitoid wasp

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In many animals, mating takes place after natal dispersal. Consequently, use of reliable information is required to increase the probability of encounters between the sexes. Most of the studies on mate finding in parasitoid insects have focused on the role of a single information source: a sex pheromone. Other sources have been mostly ignored. We studied the nature of olfactory information used for mate finding by the parasitoid *Venturia canescens* both at a distance and at host patch level, and investigated how this information is used. We tested which sex attracts the other and whether mate location is improved by combining different sources of information. We found that males simultaneously used two types of olfactory cues to find their mate: information directly related to females and an environmental cue provided by hosts. Male efficiency in locating virgin females was enhanced threefold by the association of females with hosts, whereas host patches, on their own, were unattractive to males. Our results also suggest that females emit a volatile pheromone. At the host patch level, males used chemical marks left by females foraging for hosts. These results led us to consider the distinction between signals and cues and we suggest that the volatile pheromone emitted by the females, always described as a signal, could rather be a cue. Although evidence for a volatile sex pheromone is pervasive in parasitoids, our study stresses the role of other cues in mate-finding strategies.

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Mating systems depend on the reproductive biology of both sexes, the intensity of intrasexual competition and the distribution of partners in time and space (Emlen & Oring 1977; Thornhill & Alcock 1983; Godfray 1994; Shuster & Wade 2003). Mate finding is a crucial step in the mating system. Increasing both mating success and the probability of finding a mate requires the use of reliable information relative to the environmental noise.

Although animals can use multiple cues for decision making (Fawcett & Johnstone 2003; Campbell & Borden 2009), studies of mate finding have primarily focused on a single information source: sex pheromones (Quicke 1997). A sex pheromone is a chemical signal that informs the other sex or alters the sexual behaviours of conspecifics. Females can release contact or close-

range pheromones which arrest and attract nearby males and/or elicit male courtship behaviour (Danci et al. 2006; reviewed in Kainoh 1999; see also Ruther et al. 2000). Depending on the mating system, chemicals might have different roles (Godfray 1994). When just emerged individuals mate before dispersing from the emergence site, these chemical signals can increase the probability of encountering mates. Female sex pheromones can also be involved in mate location from a distance when the matings take place after dispersal, as happens in most parasitoid species (Hardy 1994). In numerous species females attract males by emitting volatile sex pheromones that are detectable from a distance (reviewed in Fauvergue et al. 1999). Finally, virgin females of some species also deposit trail pheromones on the substrate which indicate the females' presence and guide males towards them (Fauvergue et al. 1995).

Although the importance of volatile female sex pheromones in mate finding has been intensively investigated in parasitic wasps, attempts at identifying other chemical cues involved in mate location have been very limited. This may be because female volatile sex pheromones are usually considered to be sufficiently effective on their own (Quicke 1997; Fauvergue et al. 2007). None the less, as in other insect groups, male parasitoids should have a selective advantage if they concentrate their searching in areas

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such as emergence or feeding sites which are heavily frequented by females (Thornhill & Alcock 1983). In parasitic wasps, host patches exploited by females should be favourable places for mate encounters. Indeed, male parasitoids have been observed courting females in host foraging or emergence patches (Godfray & Cook 1997; Hardy et al. 2005). Male attraction to host-associated volatiles (van Dijken et al. 1989; Nadel & Luck 1992; Ruther & Steidle 2000) or plant volatiles (McAuslane et al. 1990; Stelinski & Liburd 2005) has also been reported.

Because haplodiploidy allows females to produce male offspring, virgin females may attract males and search for hosts simultaneously (Fauvergue et al. 2008). Yet, whether or not males combine information provided by host-related cues and female sex pheromones has rarely been considered in parasitic wasps (but see McAuslane et al. 1990). None the less, several host plant volatiles are known to influence communication between males in diploid herbivorous insects by being directly used by males to locate females (Ruther et al. 2001). In addition, these host plant volatiles can enhance sex pheromone production or release (McNeil & Delisle 1989) or act synergistically with female sex pheromones to increase the male response (as observed in e.g. the tobacco budworm, *Heliothis virescens*: Dickens et al. 1993; the codling moth, *Cydia pomonella*: Yang et al. 2004).

The aim of this study was to establish whether or not parasitoids combine different kinds of olfactory information to find their mates. The parasitoid *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) was chosen because our knowledge of this species' biology allows us to predict that both a volatile sex pheromone released by females and odours emanating from hosts should guide males towards females when the latter are foraging on host patches. The reasoning is as follows. In this solitary endoparasitoid of pyralid larvae (only one larva can achieve development from a single host; Salt 1976), finding a mate may pose a challenge, particularly at low population density and when ovipositions are not aggregated. This is the case in *V. canescens*. Female wasps find hosts in dried fruits such as figs, carobs, medlars and dates (Driessen & Bernstein 1999). A single host larva usually develops in each infested fruit (Driessen & Bernstein 1999) and the level of parasitism in the field can be low (e.g. in carobs less than 9% of the fruits were infested by host larvae and less than 3% were parasitized, Schneider 2003). Consequently, males and females should generally emerge in different places and times, and therefore require information to find each other. Sex pheromones and host kairomones (i.e. host mandibular gland secretions deposited in the host food medium) could be reliable information sources. Host kairomones are known to be highly attractive from a distance for mated and virgin *V. canescens* females (Corbet 1971; Metzger et al. 2008). Furthermore, mated and virgin females have the same oviposition rate once on a host patch (Metzger et al. 2008). This suggests that host patches are good locations for males to encounter females. Consequently, host kairomones may enable males to locate host patches with females. We therefore expected sex pheromones and host kairomones to guide males simultaneously towards foraging females.

To test whether *V. canescens* use these two different types of olfactory information for mate location from a distance as well as at host patch level, we defined five specific objectives corresponding to five experiments. First, a factorial experiment in a wind tunnel was performed to measure the differential attraction of virgin males and virgin females to: (1) volatiles from conspecific virgin males and virgin females; (2) volatiles from hosts; and (3) volatiles from the association between conspecifics and hosts. This factorial design enabled us to determine (1) which sex emits olfactory cues and which sex responds to these cues, (2) which cues are used for mate location from a distance and (3) whether wasps are attracted

by single or combined cues. A second experiment was aimed at testing whether males perceive and combine independent information from both females and hosts or rather change their response as a result of changes in chemical emissions of females in the presence of hosts. Mate finding might be influenced by mating status and mating system (Godfray 1994). Indeed, whereas in monandrous species females usually cease producing male attractants after mating, polyandrous species should continue to attract additional males (Fauvergue et al. 1999). We therefore conducted two experiments to test whether males are attracted to both virgin and mated females (third experiment) and whether each *V. canescens* female mates with only one male (monandry) or several males (polyandry; fourth experiment). The fifth experiment was conducted at the host patch level to investigate whether males are arrested on host patches that had been visited by females.

## METHODS

### Biological System

*Venturia canescens* wasps were reared on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), derived from a mass culture (Biotop, Valbonne, France). Hosts were fed with wheat semolina. *Venturia canescens* females attack second- to fifth-instar *E. kuehniella* larvae. Insect cultures were maintained under constant laboratory conditions ( $24 \pm 1^\circ\text{C}$ ; 60–80% relative humidity; 12:12 h photoperiod) and adult parasitoids were fed with a 50% water-diluted honey solution.

### Experiment 1: Cues at a Distance

To test whether virgin males and females were attracted by virgin conspecifics in the presence and absence of hosts, we designed a factorial experiment based on bioassays in a wind tunnel. Virgin males and virgin females were released individually downwind from a single target box in which a host patch was either present or absent (factor: Hosts, with two levels) and which contained conspecifics (factor: Conspecifics, with three levels: three virgin females, three virgin males or no conspecifics). Two measures characterizing the behavioural response of the released wasps were recorded using an event recorder (The Observer 5.0, Noldus Information Technology, Wageningen, The Netherlands): the time before take-off (take-off latency) and the success in reaching the target (target reached or not reached). The factorial design allowed us to consider several issues simultaneously. First, because both female and host-related cues represent information involved in the rendezvous mating system (i.e. males searching for females at emergence or reproduction sites), we tested male attraction to virgin females and to hosts with separated as well as associated cues. Second, females' attraction to males was also considered because males might release pheromones that are involved in male swarms which have been described in some species in the families Braconidae and Ichneumonidae (reviewed in Godfray & Cook 1997; Quicke 1997). Finally, we examined communication among individuals of the same sex which could lead to either aggregation (e.g. male aggregations in a mating swarm) or spacing behaviour (e.g. in females associated with a host).

The wind tunnel used was previously described by Lo Pinto et al. (2004). The flight chamber was 150 cm long, 50 cm high and 70 cm wide. The air speed at the release point was 22 cm/s, and the light intensity was 4600 lx. Because visual cues favour oriented flight in insects (Vickers 2000), coloured pieces of paper (diameter around 5 cm) were fixed at random on the transparent sides, and on the top and bottom of the chamber. Hosts and wasps used as odour sources

were placed in 5 × 5 cm boxes that were 2 cm deep. The target boxes were made of a cardboard frame closed with polyester organza and a rubber band, allowing air circulation through the two wider sides of the box. Host patches in the target boxes, which were the source of host kairomones, contained 30 third-instar host larvae. The larvae had been reared in a plastic dish (diameter 5 cm) with 8 g of semolina 10 days before the experiment to produce a high concentration of kairomones. The boxes were placed crosswind on a 10 × 10 cm transparent horizontal platform, 25 cm above the floor and 30 cm downwind from the air source.

All the wasps used, either in the target boxes or released in the wind tunnel, were 1 day old and virgin. After their emergence, males and females were caged separately in groups of 50–100 individuals (cages measured 50 × 30 cm and 30 cm high) and were provided with food (a 50% water-diluted honey solution). Four hours before the beginning of an experimental session, the cages were placed inside the flight chamber to acclimatize the insects to the experimental conditions (24 ± 1 °C; relative humidity 70 ± 5%; wind chamber structure). The experiments were conducted between 1200 and 1600 hours. The wasps used to test attractiveness of a given target were randomly chosen and individually transferred from the insect cage to plastic tubes (3 × 8 cm) at least 5 min before they were used (maximum 30 min). They were released individually from these tubes in the flight chamber 60 cm downwind from the target. The top of the release tube was positioned at the same height as the middle of the target box.

The target was considered to have been reached if the wasp landed on it or flew near the platform less than 5 cm away from the target but landed elsewhere in the wind tunnel. Each tested wasp was removed immediately after landing. The behaviours of the tested wasps were observed until they took off and landed or until 5 min had elapsed. After a first flight, the same wasp was immediately released for a second time (following Lewis & Tumlinson 1988; Bleeker et al. 2006) and the same variables were measured. The proportion of individuals reaching the targets at least once during two assays was regarded as a measure of attractiveness of the volatiles from the target box.

For each of the six types of target (three Conspesifics × two Hosts levels) the flight responses of 42 males and 42 females were recorded (overall 252 wasps of each sex were tested). On each day of the experiment, four to five replicates of each type of target and each sex were done. To reduce pseudoreplication, targets were changed every day (nine different targets were used for each of the six combinations of treatment). Moreover, the order of the presentation of the six different types of target was randomized within each day of the experiment and, for a given target; the sequence of males and females released was also randomly drawn. We used individuals from laboratory cultures established from a population of sexual wasps (arrhenotokous reproduction) collected in Nice, southern France, in 2005.

Male and female responses (take-off latency and number of individuals reaching the target) were analysed separately, with Conspesifics and Hosts as explanatory variables. The take-off latency was analysed via a survival model. The effect of the two factors was tested by fitting a parametric proportional hazard model with a Weibull frequency distribution (for the baseline hazard) allowing right-censored data. Take-off latency was measured and analysed for the first flight only. We excluded 23 females (15.1%) and 11 males (4.3%) from the data set because they did not leave the release tube within 5 min. Data from 28 females and 17 males were right censored because individuals stayed outside the tubes but did not take off before 5 min had elapsed. Proportions of males and females reaching the target boxes were analysed by means of logistic regressions (binomial errors and log links). Individuals that did not fly, and those that flew once during

the two trials and missed the target during this single flight, were excluded from analyses.

#### Experiment 2: Host and Female Cue Interaction

This experiment aimed at testing two alternative hypotheses arising from the first experiment: specifically, do males combine independent information from females and hosts or do volatiles from females or from hosts change when females and hosts are in contact with, or perceive, one another? In the same wind tunnel, we tested attraction of males to three virgin females which were either placed with a host patch or separated from hosts to prevent their olfactory perception of nearby hosts as well as oviposition opportunities. Because the perception of host kairomones could elicit a change in female signal emission, a third treatment level was added in which females were physically separated from the hosts but placed downwind, so that they could perceive (and potentially respond to) the hosts. A fourth group in which females were separated from hosts and placed upwind was used to complete the experimental design and to control for the effect of the females' position (front versus rear) in the experimental device (Fig. 1).

All other experimental conditions were similar to those in experiment 1. We measured only the proportion of males reaching the cardboard target at least once on two flights (81 replicates were approximately equally distributed between the four treatment groups).

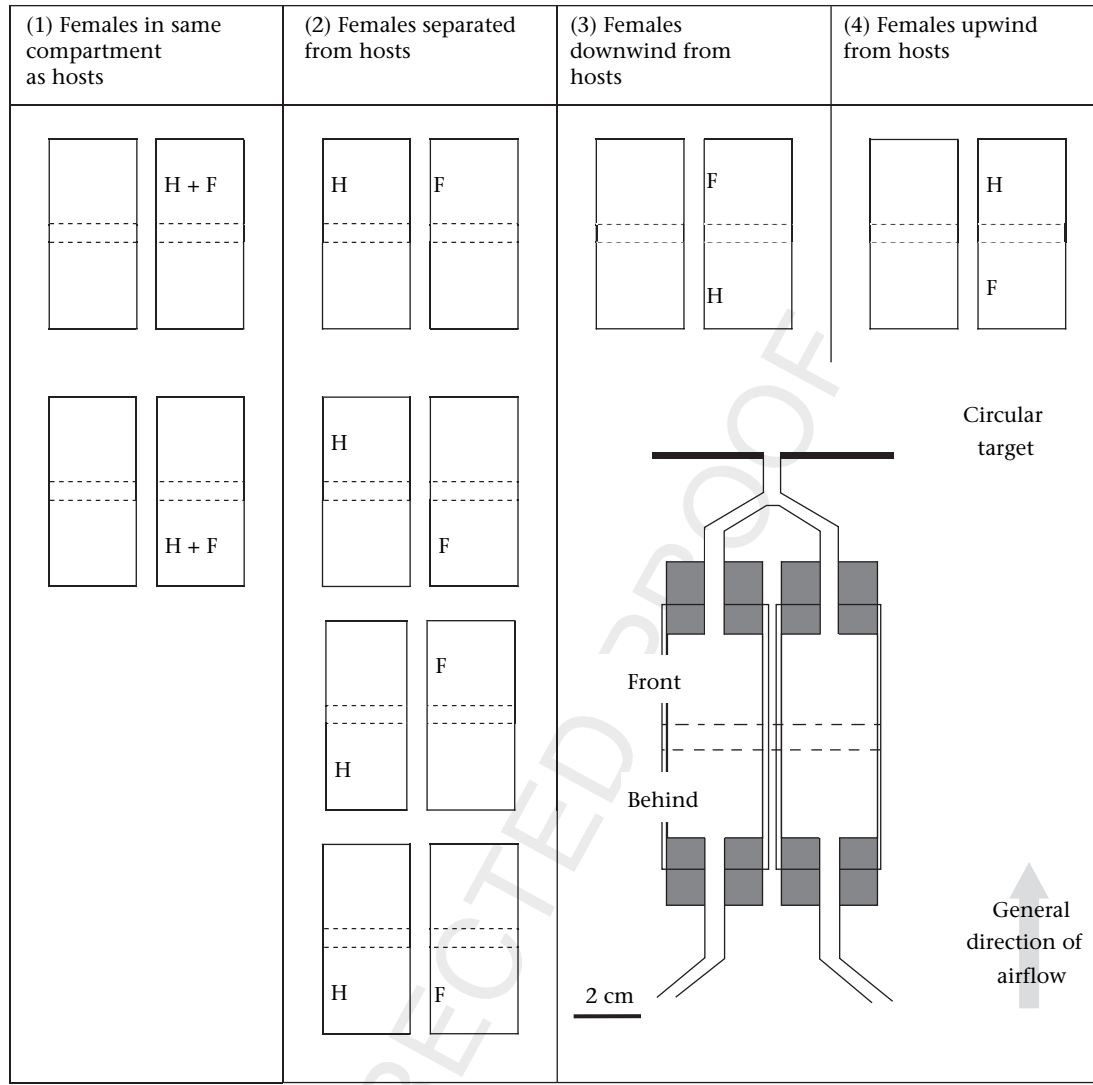
The effect of simultaneous presence of females and hosts, the effect of females' position in the tube (compartment in the front or in the back of the tube, Fig. 1), and the effect of the interaction terms between these two factors on the proportion of males reaching the target were analysed using a logistic regression with correction for underdispersion.

#### Experiment 3: Female Mating Status

The aim of this experiment was to test the influence of female mating status on male attraction. For this, males were individually released in the flight chamber as before, with the target containing either three virgin females ( $N = 35$  tested males), three mated females ( $N = 34$ ) or no conspecifics ( $N = 33$ , control). Mated females had been mated  $22 \pm 1$  h before the tests. Other experimental conditions were similar to those in experiments 1 and 2.

#### Experiment 4: *V. canescens* Mating System

To characterize the mating system of *V. canescens*, that is, monandrous or polyandrous, and better interpret the observed effect of female mating status on male attraction, we also tested whether females mate once or several times. For this, groups of three or four virgin females (0–24 h old) were exposed to 24 males for 45 min in an insect cage (14 females tested); the high operational sex ratio (OSR ≥ 6:1) is known to favour mating in this species (van Santen & Schneider 2002). Females were under constant observation. They were withdrawn from the experimental arena as soon as they mated. Two days later, to measure the rate of remating, these mated females were exposed a second time to males under the same conditions. The proportion of females accepting mates during each period of exposure was recorded. To create conditions that should favour remating, females were allowed to oviposit on large host patches (>60 hosts) between the two periods of exposure. The experiment was repeated with longer intervals between the two mating opportunities (2 days and 5 days postemergence) and with a higher number of males (OSR 8: 1,



**Figure 1.** Host (H) and female (F) positions inside the four compartments of the target box according to the experimental treatments (1–4) in the second wind tunnel experiment (the left–right reverse modalities are not shown but were also tested). The experimental device, shown from above, comprised two Plexiglas tubes, each divided into two compartments (closed at each extremity by a piece of white cloth) that were separated by a gap. Each of these tubes was closed with corks and received an airflow of 1 litre/min. After traversing each of the tubes, the airflows loaded with odours joined in a single exit, the pipe leading into the centre of a circular target made of green cardboard.

$N = 14$ ). We used wasps from laboratory cultures established from a population collected in Valence, southern France, in 2002.

#### Experiment 5: Mate Location at Host Patch Level

We tested whether males stayed longer on host patches on which females had already foraged. For this experiment, males were released on three different types of patch: (1) a patch of clean semolina without hosts (the food medium of host larvae, ‘control patch’,  $N = 18$ ); (2) a host patch that had not been visited by a female wasp (hereafter called a ‘nonvisited host patch’,  $N = 36$ ); and (3) a host patch that had been visited by five virgin females (hereafter called a ‘visited host patch’,  $N = 18$ ). Comparisons of (1) versus (2) and (2) versus (3) allowed us to discriminate between the previous presence of females and that of hosts in their effect on male patch residence time. Females are known to mark patches on which they forage: they deposit chemicals on the surface while probing the substrate and ovipositing. Such chemicals indicate to the females that the patch has already been exploited by themselves or conspecifics (Bernstein & Driessen 1996).

Host patches contained eight third-instar larvae of *E. kuehniella*, which had been placed 7 days before the trials in a petri dish plate (diameter 5 cm) filled with wheat semolina. The dish was covered with a fine cloth to prevent the escape of the host larvae, while still allowing the females to probe the semolina through the cloth and oviposit inside the hosts. ‘Visited host patches’ were obtained by exposing patches to five virgin females (1 day old) for 24 h. These females were removed 15 min before a male was released on the patch. For control patches, we used clean semolina inside a petri dish plate that was also covered with cloth. During the experiment, patches were placed in the centre of a clean semolina arena 20 cm in diameter. To release a male on a patch, we placed him in a plastic tube ( $7.5 \times 1$  cm) positioned just above the patch until he walked out of the tube and onto the patch. Male patch residence time and behaviour were recorded. Residence time was defined as the total time between entering the patch and either (1) the time the patch was left for more than 10 s or (2) the time at which he flew away, whichever happened first. The behaviours recorded were walking, antennating the patch (odour searching), resting, preening and courtship (described in van Santen & Schneider 2002). The males

tested were 1 day old and naïve; they had been maintained individually in tubes (7 × 3 cm) and fed with a drop of 50% water-diluted honey solution since their emergence. The food was removed 3 h before the trials. Each male and patch (along with the surrounding semolina) were tested only once. We used wasps from laboratory cultures established from a population collected in Valence, southeast France, in 2005.

The effects of the treatment levels ('control patch', 'nonvisited host patch' and 'visited host patch') on male patch residence time and on the time allocated to the observed behaviours by males were estimated using generalized linear models with an inverse link and a gamma distribution of errors. Contrasts were chosen to compare the 'control patch' treatment with the 'nonvisited host patch' treatment and the 'nonvisited host patch' treatment with the 'visited host patch' treatment.

For analyses using generalized linear models in each experiment, we fitted all the main effects and the interaction terms for each data set. The full model was not simplified by a stepwise procedure to avoid any bias caused by this classical procedure (Whittingham et al. 2006; Mundry & Nunn 2009). All data analyses were performed with the R freeware statistical package (R Development Core Team 2009).

## RESULTS

### Experiment 1: Cues at a Distance

#### Take-off latency period

Odours from conspecifics in target boxes influenced the time males spent waiting in the odour plume before taking off: males left the release point significantly faster when virgin females were present in the target box (Table 1, contrast F). In contrast, the take-off latency slightly increased with the presence of hosts in the target box (Table 1, contrast Presence). The males were not affected by the presence of other males (Table 1, contrast M). The interaction between Conspecifics and Hosts was not significant (Table 1, for males).

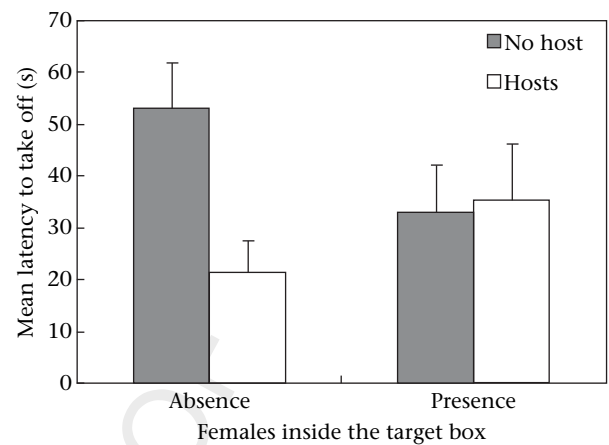
For the females, the latency period of released females was influenced by the combined odours of hosts and conspecific females in the target boxes (significant Conspecifics\*Hosts interaction; Table 1, for females). The latency period was significantly reduced by the presence of hosts in the absence of females (Fig. 2)

**Table 1**

Effect of the presence of conspecifics and hosts in the target box (experiment 1) on the time spent outside the release tube by males or females before flying away (i.e. the take-off latency)

Factors	Contrasts	Estimate	SE	P
For males				
Intercept		2.96	0.27	<0.001
Conspecifics	F	-1.09	0.37	<0.001
	M	0.12	0.37	NS
Hosts	Presence	0.78	0.38	0.04
	Conspecifics*Hosts	F*Presence	-0.74	0.52
	M*Presence	-0.80	0.52	NS
For females				
Intercept		3.879	0.332	<0.001
Conspecifics	F	-0.571	0.453	NS
	M	-0.034	0.457	NS
Hosts	Presence	-1.926	0.439	<0.001
	Conspecifics*Hosts	F*Presence	1.777	0.618
	M*Presence	1.009	0.616	NS

The estimated regression coefficient, SE and P value for each covariate in the model (parametric survival model) are also given for the chosen contrasts (F: presence of females; M: presence of males). The scale parameters are 1.55 for males and 1.75 for females.



**Figure 2.** Mean ± SE latency period (s; i.e. the time spent outside the release tube by males or females before flying away) of males in response to the presence of females, with or without hosts, in target boxes (experiment 1).

but was not affected by hosts when females were present (Fig. 2). Females did not change their time to take off in response to the presence of males in the target (Table 1).

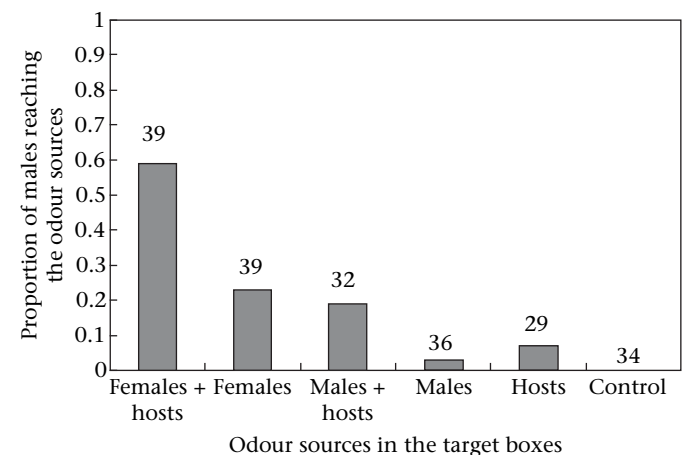
#### Proportion of wasps reaching target

As expected, males were significantly attracted by virgin females ( $\beta \pm SE = 3.25 \pm 0.81$ ,  $z = -5.62$ ,  $P < 0.0001$ ; Fig. 3), and by presence of hosts ( $\beta = 1.74 \pm 0.44$ ,  $z = 3.94$ ,  $P < 0.0001$ ). This attraction of males by virgin females was increased 2.55 times by the presence of hosts (comparison of proportions: 9/39 of males were attracted by virgin females without hosts versus 23/39 with hosts;  $\chi^2_1 = 8.95$ ,  $P = 0.003$ ; Fig. 3). Males were not attracted by the other males in the target boxes ( $\beta = 1.28 \pm 0.83$ ,  $z = 1.54$ ,  $P = 0.125$ ).

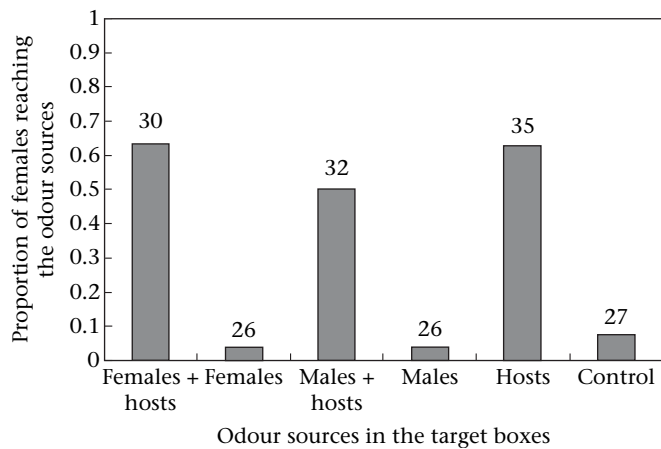
As expected, females were strongly attracted to hosts ( $\beta = 3.31 \pm 0.55$ ,  $z = 5.96$ ,  $P < 0.0001$ ; Fig. 4). They were not influenced by the presence of conspecifics in the target boxes (for males:  $\beta = -0.56 \pm 0.47$ ,  $z = -1.20$ ,  $P = 0.229$ ; for females:  $\beta = -0.09 \pm 0.47$ ,  $z = -0.19$ ,  $P = 0.851$ ).

### Experiment 2: Host and Female Cue Interaction

Males combined independent information from females and hosts to locate targets. The presence of females and hosts in the



**Figure 3.** Observed proportion of males reaching the odour source (target, see Methods, experiment 1). Numbers are sample sizes. Control: no host and no conspecific inside the target box.



**Figure 4.** Observed proportion of females reaching the odour source (target, see Methods, experiment 1). Numbers are sample sizes. Control: no host and no conspecific inside the target box.

same container (Fig. 1), and thus the opportunity for females to perceive and oviposit (and/or for hosts to respond to females' attacks), did not influence the proportion of males reaching the target (contrast: treatment 1 versus 2, 3, 4;  $\beta \pm SE = -0.068 \pm 0.25$ ,  $z = -0.27$ ,  $P = 0.834$ ). Consequently, it is not necessary to compare the males' response in treatments 3 and 4 (Fig. 1), since this comparison was initially planned to distinguish between the effects of host perception and oviposition on chemical emission by females. The position of females inside the tube had a weak influence on male efficiency to locate females ( $\beta = 2.35 \pm 0.22$ ,  $z = 10.72$ ,  $P = 0.059$ ; Fig. 5): males tended to be more efficient in reaching the females when they were situated in the front of the experimental device, which was closer to the release point. The interaction term was not significant.

#### Experiment 3: Female Mating Status

The mating status of females did not affect their attractiveness: 32% ( $N = 34$ ) of the males found the target when baited with mated

females compared to 28% ( $N = 35$ ) when baited with virgins (comparison of proportions:  $\chi^2_1 = 0.0135$ ,  $P = 0.90$ ). No male reached the empty control targets ( $N = 33$ ).

#### Experiment 4: *V. canescens* Mating System

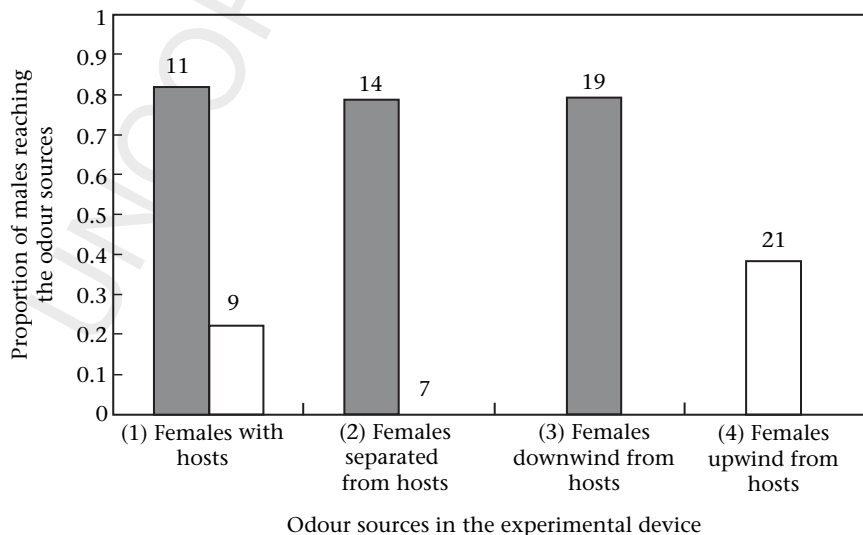
We found *V. canescens* to be a monandrous species. Of the 28 females tested, 27 accepted mates during their first exposure period to males, but no female accepted a second mating, independently of the delay (1, 2 or 5 days).

#### Experiment 5: Mate Location at Host Patch Level

The nature of patches ('control patch', 'nonvisited host patch' and 'visited host patch') affected male patch residence time ( $\chi^2_2 = 17.029$ ,  $P < 0.001$ ). Males responded to chemicals deposited by virgin females when foraging on host patches by spending more time on 'visited host patches' than 'nonvisited host patches' ( $t = 3.592$ ,  $P < 0.001$ ; Fig. 6). They spent more time exploring the surface of patches by walking on 'visited host patches' than on 'nonvisited host patches' ( $\chi^2_2 = 11.89$ ,  $P < 0.001$ ; mean walking time:  $6.11 \pm 1.5$  s versus  $2.39 \pm 0.6$  s;  $t = 3.188$ ,  $P < 0.01$ ). Males were not arrested by healthy hosts: there was no significant difference between male residence time on 'nonvisited host patches' and 'control patches' ( $t = 0.03$ ,  $P = 0.97$ ). We did not compare the time spent exhibiting behaviours other than walking because there were few or none observed.

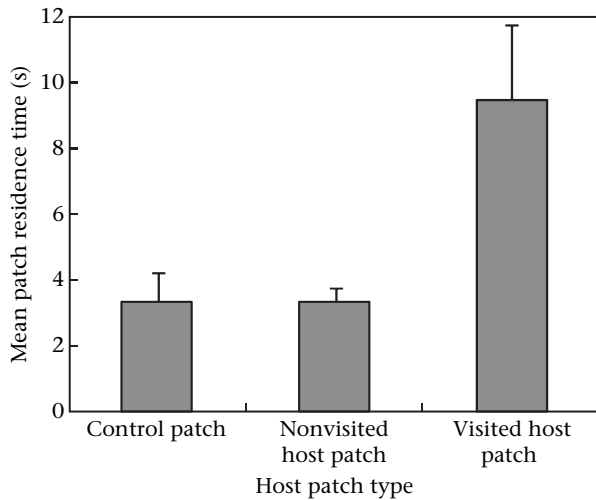
## DISCUSSION

This study shows that, to locate potential mates, *V. canescens* males responded to the co-occurrence of two types of olfactory information. Volatile cues from females, which elicit take off and guide flight orientation, were combined with volatile information from hosts. This combination of cues yielded a significant increase in mate-finding efficiency: males were three times more efficient in reaching females when the latter were foraging on host patches. In surprising contrast with the observed monandry, females did not cease their emission of volatile chemicals after mating. Because females are able to perceive each other from a distance (see also



**Figure 5.** Observed proportion of males reaching the odour sources (females and hosts) according to the treatment levels (1–4, experiment 2). Grey bars: data obtained with the females in front of the experimental device (see Table 1); white bars: data obtained with the females behind the device. Numbers are sample sizes.





**Figure 6.** Mean + SE patch residence times of males according to the experimental treatment (experiment 5).

Castelo et al. 2003), a question arising from our findings is whether mate attraction and female spacing behaviour are elicited by different signals or rely on a common compound, which would therefore be defined as a cue. Our wind tunnel experiments also revealed a common feature in parasitoid species: females were attracted to hosts, but not to males. At the patch level, males were arrested on host patches on which females had foraged, a behaviour which may increase their probability of encountering a foraging female when one is present. This begs the question whether males use a specific signal or respond to the chemical marks that females leave on host patches while foraging.

Male efficiency in locating females was enhanced by the simultaneous occurrence of females and hosts. This enhancement did not result from interactions between females and hosts, but rather from the combination of olfactory information. Hence, volatiles from hosts and from females acted synergistically on male orientation towards females. Our results therefore parallel the synergism of sex pheromones with host plant volatiles already evidenced in phytophagous insects (Landolt & Phillips 1997), including species in which males are not attracted to plant volatiles per se (Yang et al. 2004). In parasitoids, only two other studies have shown that host or host habitat cues could act synergistically with female-related cues in the context of mate finding. van Dijken et al. (1989) found that males of the mealybug parasitoid, *Epidinocarsis lopezi*, were more strongly attracted to female-baited traps when traps were placed on host-infested plants. In *Campoletis sonorensis*, volatiles and visual cues from the host plants increase the efficiency of female location by males from 13% (female on her own) to 60% (female on a plant; McAuslane et al. 1990). Such a combination of volatiles is not the rule, however. For example, males of the aphid parasitoid *Lysiphlebus testaceipes* are attracted to virgin females independently of the surrounding environment, be it healthy plants, aphid-infested plants or even no plant at all (X. Fauvergue & A. Auguste, unpublished data).

From an adaptive perspective, there are good reasons to explain why a synergistic action of volatiles produced by females and by hosts should be selected for. First, mating with females already in host patches might ensure that the male will sire some offspring. Because virgin females are attracted to host patches and oviposit at the same rate as mated females (Metzger et al. 2008), host patches represent suitable rendezvous sites to mate. Mating on host patches does not exclude the possibility that males could encounter mates outside host patches or tree stands. Second, the use, by

males, of combined information to find females on host patches could also reduce time and energy costs for finding food. Indeed, in natural conditions, fruits that harbour hosts also provide sugary food sources for adults (Desouhant et al. 2005). The ability of *V. canescens* to use different information sources simultaneously is also known in the context of host and food foraging (Desouhant et al. 2005; Lucchetta et al. 2007).

Our results contrast with the published literature for another reason. In contrast to most other parasitoids, mated *V. canescens* females, although unreceptive, are as attractive as virgins from a distance. Two nonexclusive hypotheses could explain this result: (1) emission of male attractants by females is not a controlled process and (2) these chemicals are involved in biological functions other than male attraction. The first hypothesis contrasts with the observation that in monandrous parasitoid wasps, the emission of female sex pheromones is usually a controlled process, illustrated by the fact that it ceases after mating (Quicke 1997; Fauvergue et al. 1999; Ruther et al. 2000; but see Swedenborg & Jones 1992). The second hypothesis begs the question whether these chemicals are signals or cues. In *V. canescens* the conditions for the definitions of signals and sex pheromones are not met. Sex pheromones are chemical signals that organisms use for transmitting intraspecific information and allow mate finding and mate choice (Law & Regnier 1971; Wyatt 2003). A signal can be defined as 'any structure or act that alters the behaviour of other organisms, which evolved for that effect, and which is effective because the receiver's response has also evolved' (Maynard Smith & Harper 2003). The second condition of this definition is satisfied in our case: even if signalling females are not always sexually receptive, any advantage that allows more efficient access to females should be selected for in males. However, the first condition, that is, a signal evolved in the emitter because of its effect, is less clear in our case, in particular because females continue to emit chemicals attracting males after mating. With this nuance in mind, volatiles emitted by *V. canescens* females that attract males should be referred to as a pheromonal cue (i.e. any feature of the world, animate or inanimate, that can be used by animals as a guide to future action, Hasson 1994) rather than as a pheromonal signal. In the literature, the concept of a pheromonal signal is favoured (Alexander et al. 1997; Phelan 1997; Zuk & Kolluru 1998; Wyatt 2003; but see Williams 1992) and debates have concentrated on other hypotheses explaining, for instance, the small amount of sex pheromones emitted by females, by sexual selection, particularly mate choice and asymmetrical partition of searching cost between the sexes (for a complete discussion see Zuk & Kolluru 1998). Our results resurrect the debate concerning the nature of the pheromonal information and show the need for further investigations. A similar distinction between signals and cues can be made in the context of fluctuating asymmetry (FA, i.e. random nondirectional departure from perfect bilateral symmetry). Whether FA is a cue or a signal is still the subject of debate (Maynard Smith & Harper 2003). There is mixed evidence that the degree of FA is information that could indicate developmental stability and thus 'quality' to mates (Møller & Thornhill 1998; Breuker & Brakefield 2002). Under the hypothesis that a low degree of FA is associated with high fitness, FA should be used in mate choice by females. However, even if FA is used in mate choice, it would be a cue and not a signal. To be defined as a signal, it would be necessary to show that animals performed a display whose function is to make the level of FA more apparent (Swaddle et al. 2008).

The second hypothesis explaining why females still emit volatile chemicals once they have mated is that these chemicals could be involved in other functions. This hypothesis supports the existence of a pheromonal cue and is corroborated by (1) the fact that females, while probing, deposit chemicals used by other females (Bernstein & Driessen 1996) and males (this study) and (2) the fact

that female take-off latency is higher when females and hosts are perceived, suggesting that females detect one another from a distance. The latter result is in agreement with Castelo et al. (2003), who reported that *V. canescens* females use volatile chemicals emitted by other females to avoid host patches with conspecifics. This ability was also found in the *Drosophila* parasitoid *Leptopilina heterotoma* (Janssen et al. 1995). Here, we propose that the chemicals involved in mate location in *V. canescens* are also involved in female intrasexual communication. This phenomenon, referred to as 'pheromone parsimony', occurs when the same chemical serves several functions depending on the behavioural or ecological context. It is well documented in social Hymenoptera in which the same pheromone can have both social and sexual functions (Blum 1996; Nation 2002). However, other evolutionary scenarios can be imagined. For instance, females may 'eavesdrop' on other conspecifics using a female sex pheromonal signal or cue to find a mate (Wyatt 2003). A sex pheromone that is also used by members of the same sex has also been reported in the aphid hyperparasitoid, *Alloxysta victrix*: the same pheromone attracts males and repels females (Micha et al. 1993).

In the context of intrasexual response to conspecifics, we do not have a robust hypothesis to explain why males were also slightly attracted to other males associated with hosts. Generally, male aggregation pheromones could allow males to form leks and attract females from a distance (Quicke 1997; Quimio & Walter 2000). Nevertheless, because males are not attractive to other males on their own and because females are not attracted by males, a lek mating system seems unlikely in *V. canescens*. We cannot rule out that the behavioural responses observed in intrasexual communication were due to the density at which we held the males (or females) before testing them in the wind tunnel.

### Conclusion

As a consequence of the solitary larval development and the scattered distribution of their hosts in the field, most male and female *V. canescens* emerge in different locations (Metzger et al. 2008). Virgin females emerge with a set of mature eggs, reproduce at the same rate as mated females, and have been observed to search for host patches in the field (Metzger et al. 2008). Therefore, males actively search for females that are themselves foraging for hosts. The combination of pheromonal cues from females and host-associated cues provide information required by males to locate females. Male behaviour with respect to patches with hosts but no female suggests that males do not search or guard host patches per se. Thus, the mating system in *V. canescens* should be a type of scramble competition between males for obtaining mates.

In parasitoids, when individuals disperse from their natal patches before mating, two types of mating system have been identified: 'sex rendezvous mating systems' and mating systems for which matings occur at arbitrary sites thanks to attractive female sex pheromones (Godfray 1994; Godfray & Cook 1997; Hardy et al. 2005). Mixes of different types of mating system have also been envisaged (Godfray & Cook 1997), and our study is one rare example (van Dijken et al. 1989; McAuslane et al. 1990; Fauvergue et al. 2008). Volatile sex pheromones are central components of such mixed mating systems. Here, we have shown the role of other cues which combine with pheromones to ease mate finding. Taking into account such combinations is one exciting research avenue to document novel mating systems.

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Crawley, 2007; Jervis et al., 2001; Pelosse et al., 2007; Sokal and Rohlf, 1995.

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