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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.

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 such as emergence or feeding sites which are heavily frequented by

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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 mates 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 ± 1 °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