Intimate Rendezvous in a Tritrophic Context? Nothing but the Girls for Male Lysiphlebus testaceipes

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Received: June 27, 2014
Initial acceptance: August 8, 2014
Final acceptance: September 24, 2014

doi: 10.1111/eth.12332

Keywords: mating system, sex pheromones, synomones, herbivore-induced plant volatiles, mate finding

Abstract
In insects, mating often occurs after natal dispersal, and hence relies on a coevolved combination of sexual communication and movement allowing mate encounter. Volatile sex pheromones are widespread, generally emitted by females and triggering in-flight orientation of conspecific males. In parasitoid wasps, unmated females can start laying unfertilized eggs via parthenogenesis so that host patches could serve as sites of rendezvous for mating. Males could therefore use cues associated with host patches to focus their search on females that have successfully found oviposition sites. We hypothesized that in parasitoids exploiting herbivorous hosts, sex pheromones, and herbivore-induced plant volatiles (HIPV) should act in synergy, triggering male orientation toward ovipositing females. We tested this hypothesis with the aphid parasitoid Lysiphlebus testaceipes.

Results from both field and laboratory experiments show that males are strongly attracted to virgin females, but that volatiles from aphid-infested plants have no effect on male orientation, neither has a cue, nor in interaction with the female sex pheromone. The absence of synergy between sex pheromones and HIPV contrasts with results on other species and raises interesting questions on mating systems and sexual selection in parasitoid wasps.

Introduction
Mating systems are generally defined by how males and females obtain mates in a population, which depends on the spatial distribution of sexually mature individuals (Hardy 1994). In parasitoid wasps, strict local mate competition (LMC), where all matings occur on the natal patch and only mated females disperse, has long been considered as the most pervasive mating system (Godfray & Cook 1997). It is now well accepted that strict LMC is an exception rather than a rule and is limited to a few gregarious species with flightless males. Many gregarious and quasi-gregarious parasitoid species have a partial local mating structure with both local and non-local mating: a significant proportion of individuals disperse and mate away from where they emerge (Godfray 1994; Hardy 1994; Fauvergue et al. 1999; Hardy et al. 2005). Off-patch mating is possible if mates develop abilities and stratagems to find one another from a distance.

In parasitoid wasps with partial local mating or panmictic mating structures, long-range, volatile sex pheromones are widespread. These compounds can be released by either sex, but in most species, they are emitted by virgin females (i.e., females stop attracting males once mated; Fauvergue et al. 1999). Sex pheromones are often considered sufficiently effective on their own to induce male positive anemotaxis and insure successful orientation and mate encounter (Hardy 1994; Quicke 1997; Fauvergue et al. 1999; Ardeh et al. 2004). However, some evidence exists to suggest that males sometimes combine sex pheromones with environmental cues such as those provided by host insects and host plants (McAuslane et al. 1990; Metzger et al. 2010). In this paper, we test the hypothesis that other chemical information,
specifically herbivore-induced plant volatiles, are also involved in mate finding.

The use of cues from hosts and host plants by males seeking for females relies on three underlying assumptions. First, virgin females should visit oviposition sites. Most parasitoid species belong to the order Hymenoptera and are therefore haplodiploid. Virgin females are thus able to produce unfertilized, haploid eggs that develop into males and are for this reason likely to search for oviposition sites before being mated. Consequently, females can be fertilized by males while foraging for hosts (Fauvergue et al. 2008). Generally, it is recognized that host-infested plants are a good place to encounter a mate (West et al. 1997; Hardy et al. 2005). Second, the use of complementary information should confer a selective advantage. Indeed, males that combine sex pheromones with host cues focus their search on females that have found oviposition sites. Hence, males with such a behavior are more likely to sire daughters than males orienting toward sex pheromones only. In addition, if female traits that underpin host finding are heritable, these males may sire fitter daughters (Thornhill & Alcock 1983). Third, males should be capable of exploiting cues from hosts or host plants. In parasitoids, females locate hosts from a distance via kairomones (host-derived products such as pheromones, feces, exuviates; Powell, Hardie et al. 1993; Hardy 1994; Zuk and Kolluru 1998) or synomones, such as herbivore-induced plant volatiles (Dicke & Sabelis 1988; Turlings & Tumlinson 1991; Steinberg et al. 1993; Tumlinson et al. 1993; Takabayashi et al. 1994; Dicke & van Loon 2000). In species where such behaviors have evolved in females, unless they imply sex-linked traits, there should be no major impediment for their expression in males.

Phytophagous insects provide numerous examples on the influence of plant volatiles in sexual communication. Plant volatiles may either increase the production and release of sex pheromones by females or enhance the response of males (Landolt & Phillips 1997; Ruther et al. 2001). In parasitoids, only a few studies have reported the use of kairomones (Vandijken et al. 1989; Nadel & Luck 1992; Ruther & Steidle 2000) or synomones (McAuslane et al. 1990; Stelinski & Liburd 2005) by males. In particular, McAuslane et al. (1990) and Metzger et al. (2010) showed that volatiles from hosts acted synergistically with pheromones to increase male response. For instance, with both wind tunnel and field experiments on Venturia canescens, we showed that male orientation toward females was at least three times more successful when pheromones were associated with kairomones (Metzger et al. 2010).

Lysiphlebus testaceipes is an appropriate model organism to study the role of herbivore-induced plant volatiles in mate finding. It is a generalist parasitoid, which parasitizes a wide variety of aphids on many different host plants (Mitrovic et al. 2013). In this species, females use plant volatiles to find their hosts and exploit host patches (Grasswitz 1998; Lo Pinto et al. 2004; Tentelier et al. 2005; Tentelier & Fauvergue 2007). Field evidence strongly suggests that L. testaceipes has a panmictic mating structure with aphid colonies as sites of rendezvous. Although it appears quasi-gregarious as a consequence of the clumped distribution of its aphid hosts, microsatellite-based parentage analyses showed that females spread their eggs across many different host patches, probably to dilute hyperparasitism or predation. In the wild, Tentelier et al. (2008) found that despite large colonies of hundreds of aphids, the number of L. testaceipes emerging per colony was surprisingly small (4.9 adults on average), and the average clutch size was estimated at only 1.8 offspring per female and per colony. Hence, encounters between unrelated males and females on emergence patches are improbable, and sib-mating should be a rare event. Congruently, genetic analyses showed no deviation from Hardy–Weinberg, which also suggests there is little or no inbreeding. In the field, virgin females start to oviposit and are approached and mated by males while exploiting aphid colonies (Fauvergue et al. 2008). Given these characteristics, we expect male L. testaceipes to use herbivore-induced plant volatiles as well as sex pheromones for mate finding. Here, we test this prediction with both field and laboratory experiments.

Material and Methods

General Methods

Tested individuals were mass-reared on Aphis gossypii/Cucumis sativa (var. Serit) as a host insect/host plant complex and maintained in a controlled environmental chamber kept at 22°C, 45–55% relative humidity and under a L16:D8 photoperiod. To obtain inexperienced adults, parasitized aphids were isolated at the mummy stage in glass hemolysis tubes (8 × 60 mm). Insects were collected daily as they emerged, sexed, and fed with a 50% honey solution. Mated females were obtained by enclosing one male and one female until mating was observed. They were then isolated back in their emergence tubes until the beginning of the test. Before being tested, males were transferred
4 h into a rearing cage where they could train to fly. All the parasitoids used were <12-h old.

The cucumber plants were reared in a greenhouse at 20–30°C and 60–70% RH. At 16 d, these plants had grown two leaves. At that time, some plants were infested with ten parthenogenetic *A. gossypii* females that were then allowed to reproduce for 5 d. Other plants were not exposed to aphids. Both infested and healthy plants were 21 d old when tested, and infested plants were bearing 200–300 aphids.

**Evidence for a Sex Pheromone in a Wind Tunnel**

In a first experiment, we studied the response of male *L. testaceipes* to volatiles from different status of conspecifics, in combination with volatiles from host insects and host plants. For this, we quantified male in-flight orientation when exposed to 4 × 3 odor sources: virgin females, mated females, males, or no conspecific (conspecific type); healthy plants, aphid-infested plants, or no plants and no aphids (plant type). The experiment was therefore structured as a factorial design with 12 odor combinations and 38–40 males tested per combination, for a total of 475 males tested.

The experiment was conducted in a laboratory wind tunnel (150 × 70 × 60 cm) described on Fig. 1 (Lo Pinto et al. 2004; Metzger et al. 2010). Air filtered by a 5-cm-thick layer of activated charcoal was drawn through the tunnel with an electric fan. Two sheets of stainless steel and one sheet of polyester were placed after the fan to produce a constant 16 cm/s laminar airflow in the flight chamber. Four neon tubes placed 15 cm above the flight chamber produced an intensity of approximately 4600 lux at the takeoff platform. A layer of tracing paper was slipped between neon tubes and the flight chamber to light diffuse. Several pieces of colored cardboard (green, blue, and yellow) were randomly placed on the floor and walls of the flight chamber to provide visual cues to flying insects. Plants and insects used as odor sources were enclosed in a glass container (40 × 25 cm) placed under the tunnel. A pump pushed air at a flow of 1 l/min into the container via 1 l bubbler filled with 500 ml of deionized water. Air loaded with volatiles from plants and/or insects was then pushed through a pipe into the flight chamber, 15 cm downwind from the sheet of polyester and 60 cm upwind from the takeoff platform of tested males. Three factitious plants, made of cardboard, were positioned between the pipe and the takeoff platform to increase odor dispersion and allow parasitoid landing.

Bioassays were conducted between 13:00 and 18:00 h at 23–26°C and 50–70% RH. Ten minutes

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**Fig. 1:** Wind tunnel used to test insect orientation in the laboratory. Tests consisted in releasing male *Lysiphlebus testaceipes* on the takeoff platform (H) and observing takeoff and flight, possibly toward the factitious plant (I). Odors were introduced in the main airflow by pushing air via a glass cage (E) containing different types of plants and insects. Other components were A. layer of activated charcoal; B. electric fan, C. sheets of stainless steel and polyester; D. Neon tubes; F. electric pump; G. pipe; J. flow meter.
Evidence for a Sex Pheromone in the Field

Field experiments were carried out on a hedge of oleander (Nerium oleander L.) located in an urban environment in Juan-les-Pins, south of France (43°34'38" N, 7°52'25"E). The hedge was approximately 100 m long and 70 cm tall. It was heavily infested with Aphis nerii and to a lesser extent, Aphis fabae. Many of the A. nerii were parasitized by L. testaceipes and fewer by Aphidius colemani. A. fabae was parasitized by Lysiphlebus fabarum only. Hyperparasitoids and predators such as ladybirds and hoverflies were also present in this environment.

To determine whether the sex pheromone found in the laboratory allows the attraction of conspecific males by virgin females in natural conditions, we applied the same type of treatment in the field. Traps, 5-cm-diameter PVC rings closed by polyester organy, were baited with a virgin female, a mated female, or a male L. testaceipes; we also used control traps with no insect inside. In contrast with laboratory experiments, we used a single individual as emitter in each trap. Individuals used as baits were approximately 1 d old (24–30 h). As a food source, a drop of honey was applied in each trap (including control traps). Twenty traps were hung along the oleander hedge according to a randomized block design. A block corresponded to 1 m of the hedge on which the four types of traps were positioned randomly. Traps were hung 10 cm from one another within blocks and 5 m between blocks. For each block, the local abundance of parasitoids and hosts was estimated via a visual classification of the number of aphids and an exhaustive count of all mummies per leaf in a 0.25-m² quadrat (Lapchin et al. 1997). All insects found on traps were captured every 30 min during three mornings (06:00–14:00, June 3, 5, and 7, 2004) and three afternoons (14:00–21:00, June 4, 6, and 11, 2004). Captured insects were identified and sexed in the laboratory. Captured L. testaceipes males and virgin females used as baits were mounted between slides and cover slip and their hind tibia were measured under an inverted phase contrast microscope Nikon TE2000U equipped with NIS-Elements software (Nikon Instruments Europe B.V, the Netherlands). We also collected mummies directly on the edge and measured the hind tibia of the 60 emerging males.

The effects of baited female size, aphid density, and mummy density on the number of males captured were analyzed with generalized linear modeling, and the interactions was assessed with log-likelihood ratio tests. The tibia lengths of males captured on the traps and males obtained from local population were compared via a Student t-test.

Results

Evidence for a Sex Pheromone in a Wind Tunnel

Male L. testaceipes were attracted to virgin females. In the wind tunnel, more males flew upwind when exposed to volatiles from virgin females (52%; 62/119) than to volatiles from male (14%; 16/118), mated females (18%; 22/120), or pure air (14%; 16/118) (Tables 1 and 2; Fig. 2). Irrespective of male response to females, hosts and host plants had no effect on male orientation, as shown by the non-significant main effect of plant type (Table 2). Hosts and host plants did not affect male attraction to virgin females; the non-significant interaction between insect and plant type (Table 2) suggests that males were not more attracted to virgin females when females were associated to volatiles from healthy or aphid-infested host plants. These results suggest that virgin females emit a sex pheromone, which attracts conspecific males; emission (or attractiveness) is influenced by female mating status but not by key environmental variables such as plants and hosts.
Before taking off, males spent some time walking on the takeoff platform within the odor plume. This latency period can serve to estimate male motivation to respond when perceiving volatiles. However, time between odor perception and takeoff was influenced by volatiles from neither conspecifics nor infested plants (Table 2).

Body size of the bioassayed males was used as covariable in the analyses of male attraction and latency duration. Body size did not clearly influence male behavior in the wind tunnel. Although we found a tendency for bigger males to be more successful in their orientation toward virgin females ($\chi^2 = 3.64, \text{df} = 1, p = 0.0565$), we considered this trend non-significant and excluded size from the analyses mentioned above.

### Evidence for a Sex Pheromone in the Field

A total of 164 insects were captured across six sampling periods of 7–8 h. With no exception, all were male *L. testaceipes* and all were captured on traps baited with virgin females. These results confirm that virgin females emit a volatile sex pheromone that attracts males from a distance. The number of males

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**Table 1**: Number of male *Lysiphlebus testaceipes* flying upwind and landing on the factitious plants in the wind tunnel versus insect and plant types providing odor sources. Mean male body size and mean latency duration are also shown.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Plant</th>
<th>Landing</th>
<th>N</th>
<th>Body size (µm)</th>
<th>N</th>
<th>Latency duration [s]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin female</td>
<td>Healthy</td>
<td>21</td>
<td>40</td>
<td>442 ± 8</td>
<td>28</td>
<td>16 ± 3</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Infested</td>
<td>23</td>
<td>39</td>
<td>419 ± 10</td>
<td>30</td>
<td>11 ± 3</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>18</td>
<td>40</td>
<td>400 ± 8</td>
<td>34</td>
<td>29 ± 7</td>
<td>38</td>
</tr>
<tr>
<td>Mated female</td>
<td>Healthy</td>
<td>6</td>
<td>40</td>
<td>411 ± 8</td>
<td>27</td>
<td>12 ± 4</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Infested</td>
<td>8</td>
<td>40</td>
<td>420 ± 10</td>
<td>32</td>
<td>17 ± 5</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>40</td>
<td>413 ± 10</td>
<td>31</td>
<td>19 ± 5</td>
<td>37</td>
</tr>
<tr>
<td>Male</td>
<td>Healthy</td>
<td>3</td>
<td>40</td>
<td>429 ± 10</td>
<td>28</td>
<td>6 ± 2</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Infested</td>
<td>6</td>
<td>40</td>
<td>400 ± 8</td>
<td>32</td>
<td>9 ± 3</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7</td>
<td>38</td>
<td>425 ± 8</td>
<td>30</td>
<td>16 ± 4</td>
<td>36</td>
</tr>
<tr>
<td>None</td>
<td>Healthy</td>
<td>6</td>
<td>39</td>
<td>424 ± 10</td>
<td>34</td>
<td>13 ± 3</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Infested</td>
<td>7</td>
<td>39</td>
<td>427 ± 11</td>
<td>32</td>
<td>17 ± 5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
<td>40</td>
<td>411 ± 11</td>
<td>30</td>
<td>9 ± 2</td>
<td>36</td>
</tr>
</tbody>
</table>

**Table 2**: Results from logistic regression and survival (Weibull distribution) models testing the effect of odor source on attraction (proportion of males landing on the source) and takeoff latency (time before takeoff) in *Lysiphlebus testaceipes* males.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Khi 2</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect on male attraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect</td>
<td>3</td>
<td>26.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plant</td>
<td>2</td>
<td>1.55</td>
<td>0.46</td>
</tr>
<tr>
<td>Insect x Plant</td>
<td>6</td>
<td>4.52</td>
<td>0.61</td>
</tr>
<tr>
<td>Effect on latency duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect</td>
<td>3</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Plant</td>
<td>2</td>
<td>1.68</td>
<td>0.43</td>
</tr>
<tr>
<td>Insect x Plant</td>
<td>6</td>
<td>2.99</td>
<td>0.81</td>
</tr>
</tbody>
</table>

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Fig. 2: Proportion of male *Lysiphlebus testaceipes* flying upwind toward the factitious plant in the wind tunnel versus conspecific type and plant type (white bars: no plant; light-gray bars: healthy plant; dark-gray bars: aphid-infested plant).
captured varied dramatically among days and blocks, but without any constant pattern (Table 3). Among days, the arrival of males at virgin-female baited traps span from 07:30 in the morning to 20:00 in the evening with no apparent difference between morning and afternoon (Table 3). To explain the variability among days and blocks, we fitted the daily number of males captured with a log-linear model where day, aphid abundance, mummified aphid abundance, and female size were included as explanatory variables. Except for an effect of day, none of the other variables were significant (Table 4). This result is confirmed by non-significant Spearman correlations between the number of males captured and mummy abundance, host abundance, or female size. As expected in case of a positive relation between body size and fitness, males arriving at virgin-female baited traps were on average bigger than males emerging from randomly collected mummies (mean length of hind tibia and 95% CI; successful males: 551 μm [539–562], n = 148; random males: 515 μm [492–538], n = 60; t = −3.02, p < 0.01).

Discussion

Our laboratory and field experiments show that in the parasitoid wasp Lysiphlebus testaceipes, males are attracted to virgin females from a distance; but contrary to our prior expectation, attraction does not depend on volatiles from the plant-host complex. This suggests that mate finding only relies on a specific signal emitted by virgin females and eliciting male in-flight orientation. In L. testaceipes, males do not use environmental cues such as herbivore-induced plant volatiles that are associated with reproducing females. Our discussion focuses on possible explanations for these results.

The first line of discussion is that although host plants and/or hosts have been shown to influence mate finding, it may only concern a minority of species. The expectation that male L. testaceipes (Bracoinidae) could use herbivore-induced plant volatiles was derived from two previous studies on parasitoid wasps. In Campoletis sonorensis (Ichneumonidae), males were three times more successful in locating females when females were placed on host plants (McAuslane et al. 1990). In Venturia canescens (Ichneumonidae), the same increase in male mating success occurred when female and host volatiles were combined (Metzger et al. 2010). These two studies contributed to an interesting body of literature on insects suggesting that host plants can elicit changes in either the biosynthesis and release of female sex pheromones (as in moths of the genus Helicoverpa; Raina et al. 1992) or male orientation toward the pheromone source (as in the European cockchafer Melolontha melolontha; Reinecke et al. 2002). Our results suggest that volatiles emitted by healthy and aphid-infested cucumber plants do not enhance the production and release of sex pheromones by virgin female L. testaceipes nor do they act synergistically with sex pheromones to improve male orientation.

An absence of evidence is of course disputable because we could have omitted specific conditions that would have revealed the expected response. Several arguments suggest that it is probably not the case.

Table 3: Number of wild male Lysiphlebus testaceipes captured on virgin-female baited traps on five locations of an oleander hedge (blocks A–E) and across 6 d of experiment. Each number represents the cumulated number of males over 17 daily observations (every 30 min from 06:00 to 14:00 in morning sessions or from 14:00 to 21:00 in afternoon sessions). No male was captured on empty traps or on traps baited with mated females or males.

<table>
<thead>
<tr>
<th>Morning</th>
<th>Afternoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>03/06/04</td>
<td>05/06/04</td>
</tr>
<tr>
<td>Block A</td>
<td>1</td>
</tr>
<tr>
<td>Block B</td>
<td>0</td>
</tr>
<tr>
<td>Block C</td>
<td>49</td>
</tr>
<tr>
<td>Block D</td>
<td>6</td>
</tr>
<tr>
<td>Block E</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
</tr>
</tbody>
</table>

Table 4: Results from generalized linear models testing the effects of day, host density, mummy density, and female size on male attraction (number of males captured) in the field.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Khi 2</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>5</td>
<td>11.85</td>
<td>0.0037</td>
</tr>
<tr>
<td>Host density</td>
<td>1</td>
<td>1.72</td>
<td>0.19</td>
</tr>
<tr>
<td>Parasitoid density</td>
<td>1</td>
<td>0.00</td>
<td>0.97</td>
</tr>
<tr>
<td>Female size</td>
<td>1</td>
<td>2.51</td>
<td>0.11</td>
</tr>
</tbody>
</table>
In our laboratory experiments, aphid-infested plants were raised in a greenhouse according to methods that have proved successful to elicit the emission of herbivore-induced volatiles (Tentelier et al. 2005; Tentelier & Fauvergue 2007). Further, the strong attraction of males to virgin females in the wind tunnel suggests an adequate bioassay. Yet, *L. testaceipes* is a generalist parasitoid (Mitrovic et al. 2013) for which associative learning could be an important prerequisite to the exploitation of environmental cues. In this species, the role of learning has been demonstrated in the context of females searching for hosts (Grasswitz & Paine 1993; Tentelier & Fauvergue 2007), and hence learning could also intervene in male orientation. The use of naïve males in the laboratory would have yielded spurious conclusions. However, field results appear to contradict this hypothesis: wild males that did respond to virgin females had the opportunity to learn local HIPV but were not more attracted to females surrounded by high host densities. This result, obtained in a context where males could have learned environmental cues, reinforces our conclusion of an absence of synergy between pheromones and host-associated cues in *L. testaceipes*.

Assuming our results reflect a true absence of evidence, they raise the question of why do some species use environmental cues to complement sex pheromones while others do not. From a proximal viewpoint, it is possible that in some species, long-range volatile sex pheromones are not as efficient on their own to trigger successful mate finding. This could explain why for *C. sonorensis* and *V. canescens*, male efficiency to locate females suffered a threefold decrease in the absence of plants or hosts (McAuslane et al. 1990; Metzger et al. 2010). A putative underlying mechanism could be the short range of diffusion of the sex pheromone or a relatively high male detection threshold (i.e., short detection distance). For instance, in the braconid wasp *Psychita concolor*, although males are strongly attracted to synthetic fruit-mimicking blends, they orient toward virgin females only when female signals are provided at very short distances (Benelli & Canale 2013). In this case, the sex pheromone is not a long-range signal, but it complements highly attractive environmental cues associated with the presence of females. In contrast, males of *L. testaceipes* are efficient to locate females even in absence of plant and host odors, which suggest that sex pheromones are sufficient to induce successful male response.

Our finding questions the prior assumption that, ultimately, searching for females that have reached oviposition sites benefits male fitness. We have raised several arguments in the introduction: males that find and fertilize virgin females on host patches insure an immediate reproductive output (the daughters they sire), and if the ability of females to find hosts is heritable, males mating with females that have proved successful to find hosts may sire fitter daughters (Thornhill & Alcock 1983). The benefits of such a strategy, for males, may nonetheless depend on host distribution. Rare and scattered hosts represent a stronger challenge for foraging females than abundant and aggregated hosts, so that males in the former case may gain by selecting the successful females. This difference in host distribution is indeed one of the most apparent contrasts between *L. testaceipes* and *V. canescens*. In the latter species, most resource patches (dry fruits) bear no host, and for the 9% infested fruits (Schneider et al. 2003), only a single host larva generally occurs (Driessen & Bernstein 1999). In contrast, in species such as *L. testaceipes*, hosts can form colonies bearing more than 500 individuals (Borges et al. 2006) making host finding easier. Although such differences could explain our results, the number of species compared yet is too small to allow strong evolutionary hypotheses.

Males may also gain by focusing on females in the vicinity of hosts if hosts are associated with food. In aphid parasitoids, honeydew provides carbohydrates that fuel costly behaviors such as flight (Hopkinson et al. 2013). Of course, the simultaneous exploitation of two complementary resources, mates, and food, makes sense from an evolutionary perspective (Desouhant et al. 2005). However, opposite arguments also hold. In a population where males constrain their search on females that have reached host patches, a rare mutation for mate location via sex pheromones only could spread because males searching females anywhere could find females before they reach oviposition sites, that is, before they are mated by competing males. Hence, in species where females are monandrous, males relying on sex pheromones only could have a drastic advantage. Given that *L. testaceipes* females stop attracting males once mated; they certainly mate only once, which gives a selective advantage to a male strategy minimizing the time to find virgin females. To conclude, it could be that the finding in *Venturia canescens* (Metzger et al. 2010)–a synergy between sex pheromones and environmental cues–is more an exception than a rule, which opens exciting research perspective on the costs and benefits of synergisms between pheromones and kairomones.

Although our data failed to demonstrate the roles of volatiles from hosts or plants on mate searching in *Lysiphlebus testaceipes*, they strongly suggest that...
females emit a volatile sex pheromone that attracts males from a distance. Given the literature on mate finding in parasitoid wasps, this finding is not surprising. Volatile sex pheromones have been found in many species (Fauvergue et al. 1999), notwithstanding a few cases of non-volatile ‘substrate-borne’ compounds (Fauvergue et al. 1995; Pompanon et al. 1997; Kapranas et al. 2013). In most species, as was found in *L. testaceipes*, only virgin females attract males, which suggest that these semiochemicals are true signals, with emissions and responses having coevolved for the specific purpose of mate finding. The sex pheromone of *L. testaceipes* certainly provides reliable information. In field experiments where virgin females were released on host patches, half were found by a wild male within <10 min, that is, within <1% of their adult lifespan. Efficient mate finding mechanisms are particularly important in species with panmictic or partially local mating structures. Therefore, our results are consistent with our knowledge of the mating system of *L. testaceipes*. In this species, like many other species of quasi-gregarious parasitoids (Fauvergue et al. 1999) males and females have a low probability of mating at emergence from their natal patch (Tentelier et al. 2008) and have therefore evolved information and behaviors allowing mate encounter. The fact that *L. testaceipes* males do not respond to cues from hosts and host plants does not mean that mates inevitably encounter females away from host patches (we indeed showed that some females could be found by males while foraging on host patches; Fauvergue et al. 2008). Rather, our results imply that males are also capable of finding conspecific females anywhere in the environment.

**Acknowledgements**

This research was partially granted by INRA (Département Santé des Plantes et Environnement). We thank Alessandro Lo Genco for field assistance, Elodie Vercken for advices with statistical analyses, and Leslie Martin for intimate rendezvous in the wild.

**Literature Cited**


Benelli, G. & Canale, A. 2013: Do tephritid-induced fruit volatiles attract males of the fruit flies parasitoid *Psyttalia concolor* (Szepligeti) (Hymenoptera: Braconidae)? Chemoecology 23, 191—199.


Intimate Rendezvous in a Tritrophic Context

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