A SEX PHEROMONE IN MALES OF MELITTOBIA AUSTRALICA AND M. FEMORATA (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

A 4-choice arena was used to test for evidence of a volatile male-produced attractant in macropterous and brachypterous forms of 2 closely related parasitic wasps, *Melittobia australica* Girault and *M. femorata* Dahms. Virgin females of both species and forms were strongly attracted to freshly killed mashed and intact males and to living males. They were unresponsive toward males which had been dead for at least 5 days and toward empty controls. Mated females were indifferent to males, regardless of male condition. Bioassays implicated the abdomen as the source of the male sex pheromone in both species.

RESUMEN

Un escenario donde se le daba 4 oportunidades para escoger, fue usado para probar la presencia de un compuesto volátil producido por los machos, el cual atrae a hembras macropterás y brachypterás de dos especies de avispas parasíticas cercanamente relacionadas, *Melittobia australica* Girault y *M. femorata* Dahms. Hembras virgenes de ambas especies y formas fueron fuertemente atraídas hacia machos recientemente muertos, tanto machacados como intactos, y hacia machos vivos. Ellas no respondieron hacia machos que tenían al menos 5 días de muertos o hacia el testigo. Hembras previamente apareadas no fueron atraídas hacia los machos, indiferentemente de la condición de los mismos. Ensayos biológicos con ambas especies indicaron que el lugar de origen de dicha feromona masculina está en el abdomen.

*Melittobia* is a genus of small parasitic wasps whose extreme sexual dimorphism and skewed sex ratios have attracted the attention of many scientists (Dahms 1984a). Herrmann (1971) kept virgin *Melittobia* females in gelatin capsules separate from capsules with males. Surprisingly, these females chewed through their own capsules and went to the capsules with males; there, they chewed their way in or became trapped between top and bottom layers of the males’ capsules. Based on these observations, Herrmann et al. (1974) suggested the presence of a “male calling pheromone” in *Melittobia chalybii* (= *austrailica*). An apparent male pheromonal role in courtship of 2 *Melittobia* species was also noted by Evans and Matthews (1976). The present study was undertaken to further determine the nature of the presumed attractant.

MATERIALS AND METHODS

Wasps. The 2 species in this study, *Melittobia australica* Girault and *M. femorata* Dahms, were obtained from cultures maintained in our laboratory.
at the University of Georgia. Originally collected in 1983 as parasites of the mud-dauber, Trypoxylon politum Say, from the vicinity of Athens, Georgia, the wasps were identified according to Dahms (1984b).

Virgin females were isolated as late pupae and used within 24 h of eclosion. Mated females were of unknown ages and were collected as they crawled from the open top of culture vials containing numerous males. Males were of unknown ages and experience and were collected directly from the cultures.

Melittobia females are polymorphic (Schmieder 1933, Freeman and Ittyeipe 1982). Because the forms differ in some aspects of their behavior (Freeman and Ittyeipe 1982), each was used separately, and forms were never intermixed. The morph used (macropterous = type form, brachypterous = second form) is indicated for each experiment.

Experimental Apparatus. Initial experiments used an airflow olfactometer described by Vet et al. (1983) for studies of other small parasitoid wasps. However, absence of air movement and the relatively small space characteristic of the natural habitat of Melittobia (e.g. the host cocoon), suggested that another design might provide a more realistic bioassay. Therefore, for the bioassay experiments, a small plastic container was modified to serve as an experimental arena (Fig. 1). Gelatin capsules (choices) were inserted into holes drilled at 4 equidistant points around the perimeter of the container. One half of each capsule, with an end snipped off to allow entry into the plastic container, was permanently affixed to it with paraffin wax. The other half was removable. A fine mesh screen (4 sq/mm), through which females could pass, was attached to the inside

![Experimental apparatus used in experiments on Melittobia attractants.](image-url)

Fig. 1. Experimental apparatus used in experiments on Melittobia attractants.
of the chamber wall by melting the plastic and embedding the screen. A tube pushed through a hole drilled into the center of the container lid allowed introduction of experimental subjects into the container's main chamber.

General Methodology. At the start of each trial, various combinations of *Melittobia* wasps (see below) were placed in the capsules. Groups of 14-25 females were introduced into the main chamber. Once each hour for the next 4 h, the number of females present in each choice capsule was recorded. Counts were terminated after 4 h because preliminary observations indicated that by this time all females could be presumed to have had the opportunity to mate.

To control for the possibility of odor contamination, new capsule halves were used to hold the choices for each trial. To avoid possible effects of light, all trials were run in total darkness and observations were made using a flashlight with a red filter. To remove possible bias due to positional effects, the apparatus was rotated each time data were collected. In replicate trials, the choices were randomly arranged in the capsules. Experiments were run at room temperature and set at different hours between December 1983 and July 1984.

Statistical Analysis. To test the assumption that experimental replicates came from populations having identical behaviors, a heterogeneity Chi-square analysis (Zar 1974) was performed on the replicated trials of each experiment. In all cases, the null hypothesis was not rejected ($\alpha = 0.05$); therefore the data were considered to be homogeneous and subsequent statistical analyses were performed on the pooled results for each experiment. Contingency and goodness-of-fit Chi-square tests were used in the data analyses.

Experimental Protocol. The following questions were experimentally addressed:

1. Are virgin females attracted to other *Melittobia* or are they dispersing at random? Experiment 1 was run using only empty capsules as the 4 choices to determine whether females might end up in the capsules simply as a result of their efforts to disperse from the chamber and whether any features of the apparatus itself might influence wasp dispersion.

2. Are virgin females attracted more to newly eclosed males than to dead or living females or dead males? If so, is this attraction chemically cued? Experiment 2 offered virgin females choices between living males, living mated females of the same morph, and males and mated females which had been freshly killed by mashing them with the blunt end of a camel's hair brush handle.

3. Is the presumed attractant a rapidly decaying volatile chemical? In experiment 3, virgin females were offered live males, freshly mushed males, and "old" and "new" dead but intact males. "Old" dead males, taken from defunct cultures, had been dead for at least 5 days. "New" dead males were taken alive from their culture and quickly killed by close range (<2 cm) exposure to heat from a 40 watt incandescent bulb for 1 min. This treatment presumably left the body juices intact, in contrast to the mashed males whose body juices were exposed.

4. Is the attractive substance a true sex pheromone, differentially attractive to virgin females? The same choices offered in experiment 3
were repeated in experiment 4, but the test females were known to have been previously mated. Females become refractory after mating (Matthews 1975). Therefore, if the attractive substance were truly a sex pheromone rather than a generalized aggregant/arrestant, then mated females should be much less responsive to it than virgin females would be.

5. Finally, what body region is the source of the attractive substance? For experiment 5, we carefully dismembered live males and placed each body portion (head, thorax, and abdomen) into different choice capsules, with the 4th capsule left empty as a control.

RESULTS

From observations under red light, it was apparent that wasp distribution in the chamber and choice capsules had reached an equilibrium by the end of the first hour. Either females that had made their choices remained mostly stationary, or movements in and out of the choice capsules canceled out each other. Although data were recorded over a 4 h period, there were no discernable changes in distributions and census data in hours 2, 3, and 4, and the data were considered equivalent. Because over time the females’ behavior appeared increasingly to switch to a dispersal mode, thereby possibly confounding the results, subsequent analysis generally used only counts made at the end of the first hour. However, analyses using means of the 4 hourly counts produced the same levels of statistical significance as did those based on the first hour counts, so in some cases data are presented as means of the 4 hourly censuses.

Experiment 1. Virgin *Melittobia* females were not attracted to empty choice capsules. Only once was a female recorded in a choice capsule at the hourly census. This distribution was in strong contrast to the experimental results discussed below, where in every case except experiments 4 and 5, more than half of the introduced females were recorded from 1 or more of the 4 choice capsules. Therefore we conclude that females arrive in the capsules as a result of active choice, not random dispersal.

Experiment 2. From Table 1, it is apparent that virgin females offered a

<table>
<thead>
<tr>
<th>Female Species and Form</th>
<th>Wasp in Capsule:</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>Alive</td>
</tr>
<tr>
<td><em>australis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brachypterous</td>
<td>25.75 ± 0.96</td>
<td>22.00 ± 2.16</td>
</tr>
<tr>
<td>macropterous</td>
<td>25.25 ± 5.25</td>
<td>6.50 ± 1.29</td>
</tr>
<tr>
<td><em>femorata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brachypterous</td>
<td>21.25 ± 0.96</td>
<td>8.50 ± 1.73</td>
</tr>
<tr>
<td>macropterous</td>
<td>20.50 ± 5.97</td>
<td>4.75 ± 1.26</td>
</tr>
</tbody>
</table>

1Mean (± SD) of 4 counts taken 1, 2, 3, and 4 h after trial initiation.

2Includes females remaining in central chamber. All female wasp distributions were significantly different (P < 0.001) from random.
choice between conspecific males and females, both alive and dead, clearly preferred those capsules containing males (P<.001). Both morphs behaved in the same manner in this regard (australic P<.25; femorata P<.75). Similarly, there was no difference between the 2 species. Finally, although virgin females taken as a whole appeared to prefer dead to living males, the difference was not statistically significant.

Experiment 3. The attraction of virgin females to males in various conditions is shown in Fig. 2 and 3. “Old” dead intact males were not attractive to virgin females of either species. However, somewhat surprisingly, living males were the most preferred choice only for brachypterous australica females. Significantly more brachypterous females than macropterous females of M. australica preferred living males (P<.001), the only experiment for which the 2 morphs of a species did not behave in a consistent fashion. Both femorata morphs, like the macropterous australica form, showed the strongest preference for dead males (either intact “new” or mashed).

Experiment 4. Mated females of both species were almost indifferent to males offered in choice capsules, regardless of male condition. The majority of the released females remained in the chamber, either wandering or resting motionless, especially at the top.

Experiment 5. Responses of virgin females of both species to various body regions of a freshly trisected male revealed a decided preference for the abdominal section (Table 2). The thorax was approximately one-third as attractive as the abdomen; the head was totally non-attractive. The difference in attractiveness between the thorax and abdomen was statistically significant (P<.001). Neither the morphs nor the species showed any statistically significant differences in body region choice.

Fig. 2. Attraction of virgin M. australica females to conspecific males in various conditions.
CONDITION OF MALE

Fig. 3. Attraction of virgin *M. femorata* females to conspecific males in various conditions.

DISCUSSION

Both the nearly complete absence of female attraction to “old” intact dead males and females’ decided preferences for males in the other 3 conditions (experiment 3) provide substantial evidence that volatile chemical cues mediate this response. The strength of the response to the mashed male (particularly for macropterous *australia* females) suggests that the process of mashing allows the attractant odor to be released in greater quantity.

The pheromone probably originates from the abdominal region. In experiment 5, abdomens were 3x as attractive as thoraces, and heads were

**TABLE 2. VIRGIN FEMALE *Melittobia* ATTRACTION TO BODY REGIONS OF CONSPECIFIC MALES.**

<table>
<thead>
<tr>
<th>Female Species and Form</th>
<th>Male Body Part&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thorax</td>
<td>Abdomen</td>
</tr>
<tr>
<td><em>australia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brachypterous</td>
<td>4.00 ± 1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.25 ± 1.50</td>
</tr>
<tr>
<td>macropterous</td>
<td>4.75 ± 0.50</td>
<td>20.00 ± 1.63</td>
</tr>
<tr>
<td><em>femorata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brachypterous</td>
<td>9.00 ± 1.41</td>
<td>24.75 ± 4.03</td>
</tr>
<tr>
<td>macropterous</td>
<td>7.00 ± 1.41</td>
<td>21.00 ± 3.37</td>
</tr>
</tbody>
</table>

<sup>1</sup>Four choices were given (male head, thorax, and abdomen, and an empty capsule—“control”). No females responded to capsules containing either male heads, or the control.

<sup>2</sup>Mean (±SD) of 4 counts taken 1, 2, 3, and 4 h after trial initiation.
totally non-attractive. We suspect that the presumed gland may be situated near the base of the abdomen; our relatively crude dissection techniques could have resulted in some contamination of the thorax portions. Histological studies are needed to resolve the question. However, courting males typically adopt a raised-wing "calling" posture (van den Assem et al. 1982), which may behaviorally corroborate the gland position.

Results of experiment 4 seem to justify calling the attractive substance a true sex pheromone, since mated females were quite unresponsive to males, regardless of condition. These results contrast strikingly to those of experiment 2, in which virgin females were used.

Because mating occurs before dispersal, and generally does not differ for brachypterous and macropterous forms of a Melittobia species, we would have predicted that the behavioral response of the two morphs of each species to the choices offered would be consistent. Only the brachypterous morph of M. australica in experiment 3 differed from the others. Although M. australica's different responses may reflect differing underlying behavioral propensities, in the absence of hard data we prefer to label them "anomalous".

Male-produced olfactory cues are rare throughout the parasitic Hymenoptera (Gordh and DeBach 1978). Among the Melittobia, however, blind, flightless males appear to use a volatile pheromone to attract newly eclosed virgin females. Such a system appears to be highly adaptive. Melittobia live in the closed confines of the host cocoon, from which the females disperse immediately after mating and within which the poorly mobile males must compete with their brothers for copulations. Sex ratios are characteristically strongly female-biased (ca. 95:5), and the relatively uncommon males are often aggressive and cannibalistic toward other males (Dahms 1984a, Matthews 1975). The manner in which the blind males recognizes others of their own sex has not been investigated, but the recognition is probably chemically cued and could involve the sex pheromone.

Females of both Melittobia utilize the same host and displayed a similar response to male odor in our experiments. However, they belong to quite different species groups in the genus (Dahms 1984b), and their courtship patterns differ in several respects (Gonzalez and Matthews, unpublished observations). Since sexual communication systems should be expected to maintain the integrity of each species' own communication channel, the question of the existence or extent of cross-attractancy is an interesting one which will be considered elsewhere (Matthews et al. 1985).

References Cited


EFFECT OF GAMMA-RADIATED MALES ON EGG PRODUCTION IN ACARUS SIRO

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ABSTRACT

Mating of unirradiated Acarus siro L. females with normal and gamma irradiated males was studied. Females usually laid eggs throughout their life span if they were mated at least once a week with normal males. Females paired with treated males contained spermatophores but did not lay any eggs. Previously mated fertile females of A. siro subsequently mated with irradiated, sterilized males ceased egg-laying during the next 1-2 weeks. There was no significant difference when compared to the untreated control in the number of eggs laid when females were mated for one week with irradiated males and then with normal males. Weekly alternation of