Host movement initiates oviposition behavior of *Meteorus pulchricornis*, a braconid parasitoid of free-living lepidopteran larvae

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Abstract

*Meteorus pulchricornis* is a solitary endoparasitoid of various lepidopteran larvae that attacks free-living hosts exposed on plant foliage. We tested the necessity of host movement for oviposition behavior in *M. pulchricornis* using dummy hosts as well as natural host larvae (*Spodoptera litura*). We quantified the occurrence of three steps in oviposition behavior, i.e., host orientation, oviposition stance, and oviposition attack, to natural hosts under light and dark conditions. We also examined the incidence of these behaviors on natural hosts, moving dummy hosts, and motionless dummy hosts under light conditions. Few successful attacks occurred on natural hosts under dark conditions and no oviposition attempts were made against motionless dummy hosts. These results indicate that visual cues from moving host larvae are necessary for successful oviposition. Furthermore, host movement seems important for female wasps to orient the ovipositor toward the host body, since the location of the oviposition attack on natural hosts changed markedly with the mode of host movement.

Key words: Dummy; exposed living; host searching; *Spodoptera litura*; visual cues

INTRODUCTION

*Meteorus pulchricornis* (Braconidae) is a solitary endoparasitoid of various free-living lepidopteran larvae that usually expose themselves on plant foliage (Maeto, 1989; Berry and Walker, 2004; Chau and Maeto, 2008). Its original distribution spanned the Palearctic ecozone (Hudleston, 1980), but biparental strains were introduced from Europe into the United States for biological control of the gypsy moth *Lymantria dispar* (Fuester et al., 1993). Uniparental strains are also important natural enemies of pest lepidopterans in East Asia, including *Helicoverpa* and *Spodoptera* (Takashino et al., 1998; Liu and Li, 2006). In New Zealand, a uniparental strain was first detected in 1996 and attacks a wide range of indigenous species of Lepidoptera (Berry and Walker, 2004).

Parasitoids that attack free-living hosts must recognize the location of moving hosts to perform oviposition. In addition to chemical cues, physical signals such as shape, size, movement, sound, sight, and color may also be important for host searching by parasitoids (Vinson, 1976; Godfray, 1994; Michaud and Mackauer, 1994, 1995; Quicke, 1997; Morehead and Feener, 2000; Oliai and King, 2000; Demas et al., 2002). Host motion has been well demonstrated to be essential for host searching and successful oviposition in polyphagous tachinid parasitoids, i.e., *Exorista* spp. (Stireman, 2002; Yamawaki et al., 2002; Yamawaki and Kainoh, 2005); however, only a few studies have suggested the necessity of host movement for oviposition behavior in hymenopteran parasitoids (Richerson and De Loach, 1972; Nakamatsu and Tanaka, 2005). Host motion seems to be less important or not necessary in many parasitoid wasps, even when attacking exposed-living hosts (Wilson et al., 1974; Sato, 1988; Michaud and Mackauer, 1994, 1995; Demas et al., 2002).

In this study, we tested the necessity of host movement for successful oviposition in *M. pulchricornis*. First, we compared oviposition behavior to natural hosts under light and dark conditions. Second, oviposition behavior to natural hosts, moving dummy hosts, and motionless dummy hosts was...
examined. Third, the location of oviposition attacks on natural hosts was compared between different modes of host movement.

MATERIALS AND METHODS

Parasitoid wasps. A uniparental strain of Meteorus pulchricornis that originated in Kagawa Prefecture, Shikoku, Japan, was maintained on Spodoptera litura (Noctuidae) in the laboratory. Adult wasps were fed a 50% honey solution absorbed on paper wipes (Nippon Paper Crecia, Kimwipe) in a small plastic culture plate (BD Biosciences, Falcon tube; 35 mm in diameter, 10 mm in height) at 20°C under a 16L8D light regimen. Female wasps 14 to 17 days old were used for all oviposition experiments. Following emergence, 9- to 12-day-old female wasps were individually housed with five second-instar host larvae and a piece of artificial diet (Nosan, Insecta LFS) in a small plastic culture plate for 48h at 25°C and 16L8D for the wasps to gain oviposition experience. Host larvae and the artificial diet were replaced once during the trial. Wasps were then kept in a 15°C incubator (16L8D) for 3d until the beginning of the experiments. Every wasp was used only once. All oviposition experiments were performed at 25°C during the light period from 9 to 13 h after the lights went on in the 16L8D photoperiod cycle.

Oviposition behavior. Oviposition behavior of M. pulchricornis was composed of three sequential steps. First, female wasps showed host orientation when they recognized a host and began to approach it (distance to host: 1.7±0.4 mm, n=20). Second, they made an oviposition stance in which the tip of the ovipositor was just below the head (Fig. 1A). They often performed the oviposition stance without demonstrating distinct orientation. In such cases, the wasp had already shown a short bout of host orientation. During both host orientation and the ovipositional stance, antennal contact with the host body was rarely observed. Third, they made an oviposition attack, which consisted of extending their abdomen and ovipositor toward the host, and then inserting the ovipositor into it (Fig. 1B).

Natural hosts. Second-instar larvae (3–5 mm in length, 0.4 mm in head width) of S. litura reared on an artificial diet (Nosan, Insecta LFS) in an incubator at 25°C and 16L8D were used for all oviposition experiments. Host larvae movement was classified into three categories: forward movement (Fig. 2A), upper shaking while turning in a different direction (Fig. 2B), and motionless. During forward movement, the larvae moved forward at 0.9±0.2 mm/s (mean±SD, n=20). For upper shaking, they shook their head at 2.2±0.6 mm/s (n=20). While they were motionless, movement was too slight to be detected by video observation.

Dummy hosts. Black rubber tubes (7.2–7.3 mm in length, 2.0 mm in diameter) were used as dummy hosts (Fig. 3). Four dummy hosts were attached to the second hand (Seiji, SH-12W) of an electric clock (Seiji, SP-350) at 5.0 mm and 14.5 mm from the pivot point (Fig. 3). The dummies moved around beneath the arena at constant velocity (0.5 mm/s and 1.5 mm/s at 5.0 mm and 14.5 mm from the pivot, respectively). As described below, the oviposition arena (O) was placed on a transparent plastic plate set with the dummies.
Oviposition Behavior of *Meteorus pulchricornis*

Fig. 2. Movement of second-instar larvae of *Spodoptera litura*. Forward movement (A) and upper shaking (B).

Fig. 3. Four dummy hosts (D) and a female wasp (P) in the oviposition arena (O).

(Fig. 5). A successful oviposition attack was defined as when the female wasp touched the bottom of the oviposition arena with its extending ovipositor.

**Observation stage.** In Experiments 1 and 2 (with natural hosts), one female wasp was placed with four natural hosts in a small transparent plastic plate (BD Biosciences, Falcon tube; 35 mm in diameter, 10 mm in height), called the oviposition arena (O). The arena was placed on the bottom of a transparent plastic plate (T) (130 mm in diameter, 10 mm in height) upon a white paper towel (W) (115 mm×115 mm) (Nippon Paper Crecia, Comfort Service Towel). These components were set on a plastic stage (P) (31 mm in height) (Fig. 4). This observation stage was placed in a top-opening rectangular parallelepiped box (Honey Ware, QBOX 20; 290 mm length, 170 mm width, 130 mm height), the inside of which was covered with a white paper towel.

In Experiment 2 (with dummy hosts), one female wasp was placed in the oviposition arena (O) on the transparent plastic plate (T) set with four dummy hosts on double-layered transparent plastic plates (D) with the white paper towel between them (Fig. 5). This observation stage with the clock (C) was placed within the same top-opening rectangular parallelepiped box.

**Experiment 1.** To examine the effects of light on wasp oviposition, we compared the oviposition behavior to natural hosts under light and dark conditions. One female wasp was placed with four host larvae in the oviposition arena (O) (Fig. 4). The observation stage was either placed under fluorescent light (NEC Lighting, FLR40SEX-D/M/36-
The proportion of wasps showing each step of oviposition behavior to natural hosts was 80.6%.

Data analyses. The proportion of wasps showing each step of oviposition behavior was compared between light and dark conditions (Experiment 1) using Fisher's exact test (two-tailed). It was also compared among natural hosts, moving dummy hosts, and motionless dummy hosts (Experiment 2) with the Chi-square test, followed by the Holms sequentially rejective Bonferroni test or Fisher's exact probability test (two-tailed).

The frequency of host orientations, oviposition stances, and oviposition attacks observed per wasp for wasps showing each behavior was compared between light and dark conditions (Experiment 1) and between natural and moving dummy hosts (Experiment 2) using the Mann-Whitney U-test (two-tailed).

The location of oviposition attacks on the body of natural hosts was measured in ten equal-length segments from the head (1) to the apex of the abdomen (10). The value was compared just after "forward movement" and "upper shaking" of the hosts using the Mann-Whitney U-test (two-tailed).

All statistical analyses were performed using SPSS Ver. 11.5 (SPSS Inc., Chicago, IL) with the Exact Tests option.

RESULTS

Experiment 1

Only two wasps (2/21 = 9.5%) exhibited host orientations, oviposition stances, and oviposition attacks under dark conditions, while 76.5% (16/21) of the wasps demonstrated host orientations and 66.7% (14/21) showed oviposition stances and attacks under light conditions (Table 1). The proportion of wasps showing each step of oviposition behavior was significantly different between light and dark conditions (Table 1).

The frequency of host orientations, oviposition stances, and oviposition attacks per wasp was not significantly different between light and dark conditions, although that of host orientations and oviposition stances per wasp was somewhat greater under dark conditions than under light conditions (Table 2).

Experiment 2

The proportion of wasps showing each step of oviposition behavior to natural hosts was 80.6%
Oviposition Behavior of *Meteorus pulchricornis*

Table 1. Proportion of wasps showing each step of oviposition behavior

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Luminous condition</th>
<th>Hosts</th>
<th>No. of wasps</th>
<th>% of wasps showing host orientation</th>
<th>% of wasps showing oviposition stance</th>
<th>% of wasps showing oviposition attack</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Light</td>
<td>Natural</td>
<td>21</td>
<td>76.2 <strong>p</strong>&lt;0.001</td>
<td>66.7 <strong>p</strong>&lt;0.001</td>
<td>66.7 <strong>p</strong>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>Natural</td>
<td>21</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>2</td>
<td>Light</td>
<td>Natural</td>
<td>31</td>
<td>80.6</td>
<td>80.6</td>
<td>80.6</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Moving dummy</td>
<td>20</td>
<td>70.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Motionless dummy</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Fisher's exact test (two-tailed) was performed between treatments for experiment 1. The Chi-square test was performed among treatments for experiment 2. The same letters in each column indicate no significant difference at *p*<0.05 by the Holms sequentially rejective Bonferroni test using Fisher's exact probability (two-tailed).

Table 2. Frequency of oviposition behaviors observed per wasp

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Luminous condition</th>
<th>Hosts</th>
<th>No. of host orientations per wasp</th>
<th>No. of oviposition stances per wasp</th>
<th>No. of oviposition attacks per wasp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Light</td>
<td>Natural</td>
<td>2.9±1.2 (16) <em>U</em>&lt;11.5</td>
<td>1.9±0.9 (14) <em>U</em>&lt;6.0</td>
<td>1.7±0.8 (14) <em>U</em>&lt;12.5</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>Natural</td>
<td>5.5±3.5 (2)  <em>p</em>&lt;0.5</td>
<td>4.0±2.0 (2)  <em>p</em>&lt;0.1</td>
<td>1.5±0.5 (2)  <em>p</em>&lt;1.0</td>
</tr>
<tr>
<td>2</td>
<td>Light</td>
<td>Natural</td>
<td>2.5±1.2 (25) <em>U</em>&lt;58.0</td>
<td>1.3±0.8 (25) <em>U</em>&lt;37.0</td>
<td>1.2±0.5 (25) <em>U</em>&lt;48.5</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Moving dummy</td>
<td>6.9±4.5 (14) <em>p</em>&lt;0.001</td>
<td>5.4±3.5 (14) <em>p</em>&lt;0.001</td>
<td>3.9±2.8 (14) <em>p</em>&lt;0.001</td>
</tr>
</tbody>
</table>

*Mean±SD (n).*

The difference of distribution between treatments was checked by the Mann-Whitney *U* test (two-tailed).

(25/31) and 70.0% (14/20) to moving dummy hosts (Fig. 6), but no oviposition behavior was observed toward motionless dummy hosts (Table 1). No significant difference was observed for the proportion of wasps showing oviposition behavior toward natural hosts and moving dummy hosts, while the proportion was significantly different to that toward motionless dummy hosts (Table 1).

The frequency of host orientations, oviposition stances, and oviposition attacks per wasp toward moving dummy hosts was more than twice as often as toward natural hosts, and the differences were significant (Table 2).

**Location of oviposition attacks**

In both experiments, oviposition attacks were concentrated in the anterior half of the host body when natural hosts had shaken their head just before oviposition (Fig. 2B), while in the posterior half when they had moved forward (Fig. 2A), and the distribution was significantly different (Fig. 7).
DISCUSSION

Few successful oviposition attempts against natural hosts were observed under dark conditions (Experiment 1), and no oviposition occurred against motionless dummy hosts under light conditions (Experiment 2). These results indicate that the sight of the host and host movement are necessary for the oviposition behavior of *Meteorus pulchricornis*. Our results show that host movement initiates host orientation and usually begins the sequential steps leading toward oviposition attack. Furthermore, movement of the host seems important for female wasps to orient the ovipositor toward the host body since the location of oviposition attacks changed with the mode of host movement.

Two possibilities exist as to why two wasps showed oviposition behavior under dark conditions. First, the wasps may have been able to perceive a very weak, red or infrared light emitted from the video camera. Second, they may have randomly contacted the host prior to oviposition. It is also unusual that the number of host orientations and oviposition stances shown per wasp under dark conditions was more than that under light conditions, although not significantly. This may suggest that a few wasps with high sensitivity to weak physical cues from host larvae can successfully oviposit even under dark conditions, but we have no further observations to support this speculation.

The more frequent oviposition behaviors per wasp toward moving dummy hosts than natural hosts may have been due to the large size of the dummy hosts compared to the natural hosts, but the continuous motion of the dummy hosts should also be considered.

Among many parasitoid wasps of free-living insects living on foliage, the necessity of host movement for oviposition behavior has been firmly demonstrated in a braconid parasitoid (*Dinocampus coccinellae*) of aphidophagous coccinellid adult beetles (Richerson and De Loach, 1972) and in an eulophid parasitoid (*Euplectrus separatae*) of *Mythimna* larvae (Nakamatsu and Tanaka, 2005). Host motion is not essential to the oviposition behavior of many braconid aphid parasitoids (Michaud and Mackauer, 1994, 1995), and is less important than chemical cues in an ichneumonid parasitoid (*Campoplexis sonorensis*) of *Heliothis* larvae (Wilson et al., 1974). Additionally, Sato (1988) reported that the braconid parasitoid (*Cotesia glomerata*) of *Pieris* larvae is able to make successful oviposition under dark conditions with only red illumination.

Note that two braconid genera *Dinocampus* and *Meteorus*, which both appear to use visual cues for oviposition, belong to the same subfamily Euphorinae (Maeto, 1990; Quicke and van Achterberg, 1990), indicating that the visual recognition of moving host insects may be a phylogenetic trait of this monophyletic group. In fact, parasitism of free-living larvae and adults of various insect orders is very common in the braconid subfamily Euphorinae (Shaw, 1988).

*Meteorus pulchricornis* is a highly polyphagous endoparasitoid, hitherto having been recorded from more than twelve lepidopteran host families (Maeto, 1990; Berry and Walker, 2004). This may be why female wasps show oviposition behavior toward artificial dummy hosts without any chemical extracts from host materials; however, our results did not exclude the use of chemical cues during
host searching by this species. Female wasps likely use some volatiles released from host plants, host excretion, or the host themselves to locate the habitat and place of host larvae.

Parasitism by parasitoids using visual cues for oviposition may be affected by numerous environmental factors that change host movement; for example, plant volatiles released from infested corn plants activate the movement of *Mythimna separata* larvae (Shiojiri et al., 2006), which may increase larval parasitism by parasitoids. Further studies are necessary to elucidate the interactions among host plants, hosts, and parasitoids using visual cues in host searching.

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