Discrimination between Parasitized and Unparasitized Hosts
in an Egg Parasitoid, Trichogramma chilonis ISHII
(Hymenoptera: Trichogrammatidae)

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The effect of ovipositional experience on host discrimination by Trichogramma chilonis
females was studied using Plutella xylostella eggs as hosts. Females of T. chilonis which had
never oviposited in any host eggs after emergence was supposed not to distinguish between
parasitized and unparasitized host eggs. However, experienced females distinctly dis-
criminated parasitized host eggs from the unparasitized ones. Acceptability of unparasitized
host eggs was 90% or more irrespective of host type which females had experienced. On the
contrary, acceptance of parasitized host eggs decreased to a lower level; 15% in females
which had experienced once with an unparasitized host egg and 60% in females which
had experienced once with a parasitized host egg. Females experienced oviposition on an
unparasitized host egg became to accept parasitized host eggs more frequently 24 h after
the experience.

Key words: Trichogramma chilonis, Plutella xylostella, ovipositional experience, host dis-
crimination, superparasitism

INTRODUCTION

The diamondback moth, Plutella xylostella (L.), is a serious pest of cruciferous crops
in many countries. Recent development of insecticide resistance in P. xylostella (CHUA
and Ooi, 1986) demands a development of alternative control methods, including
biological control. An egg parasitoid, Trichogramma chilonis ISHII, is an important
solitary egg parasitoid of P. xylostella (IGA, 1985; OKADA, 1989). To establish a bio-
logical method to control the pest, various basic and applied studies on T. chilonis have
been carried out (MIURA, 1992).

Superparasitism is known to occur frequently in both the laboratory and the field
(e.g., VAN ALPHEN and NELL, 1982; HUBBARD et al., 1987) and influences the host-
parasitoid system (IYATOMI, 1943; VAN ALPHEN and VET, 1986). IYATOMI (1943)
reported that failure in biological control of using Trichogramma japonicum was due to the
superparasitism phenomenon. Understanding of oviposition behavior of female para-
sitoids on parasitized hosts is, thus, necessary for using the parasitoid in biological
control programs.

SUZUKI et al. (1984) reported the occurrence of superparasitism in T. chilonis, but
it has been unknown whether *T. chilonis* has the ability to discriminate between parasitized and unparasitized host eggs. The present study deals with two aspects related to the host discrimination: 1) Do females of *T. chilonis* discriminate parasitized host eggs from unparasitized ones? 2) If they do, how does a previous ovipositional experience influence the host discrimination?

**MATERIALS AND METHODS**

The egg parasitoid, *T. chilonis* was taken from a culture maintained with eggs of the Mediterranean flour moth, *E噗hestia kuehniella* Zellar as hosts in Laboratory of Insect Management, Shimane University. The original culture was introduced from Taiwan in 1987 (Hirashima et al., 1990). The host, *P. xylostella* was collected from cabbage fields near Matsue, Shimane Prefecture in 1990, and reared on cabbage leaves in a cabinet controlled at 24°C. Moths were introduced into a rearing cage (34 × 34 × 26 cm) containing a piece of Sealon film® (ca. 10 × 10 cm) (Fuji Photo Film Co., Ltd.) as a substrate for laying eggs, and reared on honey solution (20%).

Two experiments were carried out in the laboratory. The first was carried out in combinations of two host types and three parasitoid types as follows. Hosts: unparasitized eggs within 24 h old (UE) or same aged eggs parasitized within 10 min before the test (PE). Parasitoids: mated females within 24 h after emergence which had never oviposited before the test (IW), the same aged mated females which experienced the oviposition once within 10 min before the test on UE host (EWU), or the same aged mated females which experienced once within 10 min before the test on PE host (EWP). The wasps used in these experiments were reared under 24°C with 16L–8D and fed on sugar and water absorbed in a small piece of filter paper. We carried out this experiment with all the 6 combinations. One host egg on a piece of Sealon film and one female parasitoid were introduced into a Petri dish (9 cm in diameter, 2 cm in high), and the behavior and the ovipositional sequence of the female parasitoid were observed under a binocular microscope equipped with a video apparatus. The host egg was regarded as "rejected" when the female did not oviposit for three times of contact with the egg. Twelve of each the UE and PE eggs parasitized by IW parasitoids were dissected to count the number of parasitoid eggs laid. The rest of the parasitized eggs were reared at 24°C, 16L–8D photophase for 30 days, to determine the sex ratio and number of emerging parasitoids per host.

Next, we tested acceptability of PE hosts using EWU parasitoids, in detail. The EWU parasitoids were further divided into three types with respect to the interval between the first oviposition and the start of the test; i.e., less than 10 min (EWU-0), 24 h (EWU-1), or 48 h (EWU-2). The other procedures were the same as in the first experiment.

**RESULTS**

A result of the first experiment was summarized in Table 1. There was no difference between the percentage acceptance of unparasitized and parasitized eggs by inexperienced females (IW). No difference was observed in their pattern of ovipositional behavior and duration of each step in ovipositional sequence between different host types (Table 2).
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Table 1. Acceptance of unparasitized eggs (UE) and parasitized eggs (PE) of *P. xylostella* by *T. chilonis* females. Females were divided into three types by their oviposition experience: inexperienced (IW), experienced to oviposit once on UE hosts (EWU), and experienced once on PE hosts (EWP).

<table>
<thead>
<tr>
<th>Type of parasitoids</th>
<th>Type of host eggs</th>
<th>No. of females tested</th>
<th>% acceptance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>UE</td>
<td>35</td>
<td>82.9 a</td>
</tr>
<tr>
<td>IW</td>
<td>PE</td>
<td>32</td>
<td>81.3 a</td>
</tr>
<tr>
<td>EWU</td>
<td>UE</td>
<td>24</td>
<td>91.7 a</td>
</tr>
<tr>
<td>EWU</td>
<td>PE</td>
<td>20</td>
<td>15.0 b</td>
</tr>
<tr>
<td>EWP</td>
<td>UE</td>
<td>20</td>
<td>90.0 a</td>
</tr>
<tr>
<td>EWP</td>
<td>PE</td>
<td>20</td>
<td>60.0 b</td>
</tr>
</tbody>
</table>

* Values followed by different letters in the same column differed significantly at 5% level (Fisher's exact probability test).

Table 2. Durations in each step of ovipositional sequence by inexperienced females (IW) to unparasitized (UE) and parasitized (PE) host eggs.

<table>
<thead>
<tr>
<th>Type of host eggs</th>
<th>No. of females observed</th>
<th>Duration (Mean±S.D., s)</th>
<th>Total time on host</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Drumming</td>
<td>Tapping &amp; drilling</td>
</tr>
<tr>
<td>UE</td>
<td>12</td>
<td>14±11</td>
<td>75±40</td>
</tr>
<tr>
<td>PE</td>
<td>11</td>
<td>16±7*</td>
<td>50±40*</td>
</tr>
</tbody>
</table>

* Not significantly different from mean values for unparasitized hosts at 5% level (Mann-Whitney's *U*-test).

Table 3. Percentage of adult emergence and female ratio of *T. chilonis* from *P. xylostella* eggs attacked once or twice.

<table>
<thead>
<tr>
<th>No. of times host attacked</th>
<th>No. of host eggs tested</th>
<th>No. of host eggs from which the following no. of progeny emerged</th>
<th>Female ratio of emerging progeny (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

* Not significantly different from the ratio for hosts attacked once (5%; Fisher's exact probability test).

On the other hand, the percentage acceptance by the experienced females (both EWU and EWP) was significantly higher in unparasitized host (UE). Acceptability of UE hosts was 90% or more irrespective of the host type which females had experienced. On the contrary, acceptance of PE hosts decreased to a lower level; 15.0% in EWU and 60.0% in EWP females (Table 1).

The result of dissections of the UE and PE hosts parasitized by IW females showed that only one egg was laid in a host attacked once, while two eggs in a host attacked twice. Despite of different number of the parasitoid egg laid in a single host egg between hosts attacked once and twice, only one progeny emerged from one host egg...
Table 4. Acceptance of parasitized eggs of *P. xylostella* (PE) by *T. chilonis* females once experienced to oviposit on an unparasitized egg (EWU). The EWU females were divided into three types with different ovipositional intervals

<table>
<thead>
<tr>
<th>Experience condition of females</th>
<th>Interval of oviposition</th>
<th>No. of females tested</th>
<th>% acceptance&lt;br&gt; &lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>EWU-0</td>
<td>&lt;10 min</td>
<td>20</td>
<td>15.0 a</td>
</tr>
<tr>
<td>EWU-1</td>
<td>24 h</td>
<td>12</td>
<td>50.0 b</td>
</tr>
<tr>
<td>EWU-2</td>
<td>48 h</td>
<td>12</td>
<td>58.3 b</td>
</tr>
</tbody>
</table>

<sup>a</sup> Between the time of the first oviposition on UE and the time of the initiation of the test.

<sup>b</sup> Values followed by different letters in the same column differed significantly at 5% level (Fisher's exact probability test).

(Table 3). Thus, superparasitism occurred when a host egg was attacked twice by different females. Female ratio was lower in parasitoids emerging from eggs which were attacked twice by different females than in those from hosts attacked once, but not significantly (Table 3).

Most of the EWU-0 females (17 of 20 females) rejected parasitized host eggs, but half of the EWU-1 and more than half of the EWU-2 females accepted them (Table 4). The percentage acceptance is significantly different between EWU-0 females and EWU-1 or EWU-2 females (p<0.05, Fisher's exact probability test). This indicates that the response of experienced females to parasitized hosts changed after a period of 24 h of no exposure to host eggs.

**DISCUSSION**

Many parasitoids have the ability to discriminate between unparasitized and parasitized hosts and show some degree of restraint in laying eggs in the latter (e.g., Rabb and Bradley, 1970; Van Lenteren, 1976, 1981; Bosque and RabinoVich, 1979; Noda, 1990). Van Lenteren (1976) and Klomp et al. (1980) stated that some parasitoid species lacked the ability to discriminate between parasitized and unparasitized hosts and the discrimination had to be learnt by experience with unparasitized hosts. On the other hand, Van Alphen et al. (1987) and Noda (1990) showed, on the basis of a difference in parasitoid behavior, that inexperienced females of *Trichogramma evanesce*, *Leptopilina heterotoma* and *Gyrion japonicum* have the ability to discriminate hosts, and thought that host discrimination does not need to be learnt in these species.

Suzuki et al. (1984) showed that inexperienced females of *T. chilonis* did not distinguish between parasitized and unparasitized host eggs, *Papilio xuthus*. In our experiment, the difference in the oviposition behavior of inexperienced females with parasitized and unparasitized host eggs was also not obvious.

The percent acceptance of parasitized host eggs, however, varied according to her previous oviposition experience. A female parasitoid which has experienced with an unparasitized host egg is known not to oviposit in a parasitized host egg (e.g., Van Lenteren, 1976; Noda, 1990). In the present study, even when a female had experienced with a parasitized host egg, the percentage acceptance of a parasitized host egg by the female was significantly lower than that of an unparasitized host egg. Moreover, the response of females that had attacked an unparasitized host egg changed
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after a period of 24 h without exposure to host eggs. Therefore the explanation that a female acquires the ability of host discrimination by learning with an unparasitized host egg cannot be given for the fact that the degree of host discrimination of the females varies according to their previous oviposition experience. These results are considered to be the evidence that *T. chilonis* have the ability to discriminate host eggs parasitized by a conspecific female. The mechanism of discrimination is in need of further investigation.

On the other hand, Ikawa and Suzuki (1982) found that the degree of host discrimination varied according to the previous oviposition experiences for *Apanteles glomeratus*, and explained it by the assumption that the parasitoid female throughout her life would behave to maximize her reproductive success, based on the presumed density of the unparasitized hosts. They thought that when available unparasitized hosts were few, parasitoids had better to oviposit both in parasitized and unparasitized hosts in order to maximize the lifetime reproductive success, even though the reproductive success of an egg laid would be smaller in a parasitized host than in an unparasitized one.

The same explanation as Ikawa and Suzuki (1982)'s statement can be given to the present results. In the present study, offspring has a chance of winning the competition for a host because in *T. chilonis* survival rate of progeny laid by a second female in the host egg within 10 min was almost equal as that the first female (Miura, unpublished). *T. chilonis* females emerge with matured ovaries (Hirashima et al., 1990). The female which had never encountered any host eggs after emergence would estimate the density of unparasitized host eggs to be low, so she oviposited in a first-encountered parasitized host egg as readily as she did in an unparasitized host egg. The female which had encountered an unparasitized host egg would estimate the density of unparasitized host egg to be high, so she did not oviposit in a parasitized host egg successively. The female which had encountered parasitized host eggs successively at short intervals would estimate the host density to be high, so she did not more readily oviposit in a parasitized host egg than in an unparasitized host egg. On the other hand, even if a female experienced an unparasitized host egg, she may oviposit in a parasitized host egg after a long-time isolation from hosts. She would estimate the density of host egg to be low. Thus, the oviposition behavior of *T. chilonis* in the present study can be explained by the assumption that the wasp behaves to maximize her own inclusive fitness through her life time.

Superparasitism should affect survival and sex ratio of parasitoids. Iyatomi (1943) reported that biological control program using *Trichogramma japonicum* for a lepidopterous pest had failed by a reason that reproductive rate of *T. japonicum* was decreased with increasing superparasitism. *T. chilonis* would oviposit in a parasitized host after their release under some conditions (e.g., higher parasitoid densities), and the phenomenon should affect the population growth of *T. chilonis*. In the fields, it has to be investigated what extent superparasitism might have an adverse effect on biological control.

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REFERENCES


