Development of a tachinid parasitoid, Gymnosoma rotundatum (Diptera: Tachinidae) on Plautia crossota stali (Heteroptera: Pentatomidae),† and its effects on host reproduction‡

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Abstract
The developmental biology of a tachinid parasitoid (Gymnosoma rotundatum) and the effects of its parasitism on reproduction of the host, the brown-winged green bug (Plautia crossota stali), were studied. The tachinid females laid eggs on the abdominal tergum of the hosts and larvae penetrated into and developed in their body cavities. A single mature larva exited from each of the hosts and pupariated. Even in cases when the fly deposited two eggs onto a host, only one maggot survived to maturity. The hosts died within a day after the maggot emerged. The percentage of larvae emerging from hosts, larval duration, and puparial weight were not affected by the number (one or two) of parasitoid eggs per host. The duration of the larval stage was longer and puparia were bigger in parasitoids that emerged from female hosts than those that emerged from male hosts; G. rotundatum was so variable in body size that larger individuals tended to emerge from larger hosts. Though parasitoids did develop even in unfed hosts, such puparia were much smaller in size than puparia developed in fed hosts. The reproductive abilities of the parasitized bugs gradually reduced as the tachinid maggots matured. In parasitized female bugs, ovaries gradually shrank with a suppression of oviposition. Both the number of laid eggs and the percentage of viable eggs decreased rapidly after the 8th day of parasitization. In parasitized male bugs, the fertility rates decreased rapidly after the 8th day of parasitization.

Key words: Tachinidae; Gymnosoma rotundatum; Pentatomidae; Plautia crossota stali; parasitism

INTRODUCTION

Parasitoids are mainly comprised of hymenopteran and dipteran insects. Some species among them kill or paralyze the hosts at parasitization (idiobionts), and other species develop in the body cavities of live host insects that continue to feed and develop (koinobionts). On the latter hosts, parasitism causes various harmful effects such as the inhibition of development and the atrophy of the reproductive organs (Clausen, 1940).

Tachinid flies are exclusively endoparasitic species that develop in the cavities of live hosts. They have a vast host range that spreads over many taxonomic groups of insects, including the phytophagous species (Belshaw, 1994). Accordingly, tachinids are considered as potentially important biological control agents as are the hymenopteran parasitoids (Greathead, 1986; Grenier, 1988).

A tachinid parasitoid, Gymnosoma rotundatum parasitizes the live adults of the brown-winged green bug, Plautia crossota stali, a serious fruit tree pest in Japan (Yamada and Miyahara, 1979). The impact of the tachinid on P. c. stali populations has been evaluated and the fly has been recognized to be a prominent biological control agent (Yamada and Miyahara, 1979; Oda, 1980). However, there is little known about the basic biology of G. rotundatum and its influence upon its host. Thus, to clarify the traits of the tachinid fly as a natural enemy, the development of G. rotundatum on P. c. stali and the reproductive ability of parasitized hosts were studied.

MATERIALS AND METHODS

Stock culture. The stock culture of G. rotundatum used for the present study originated from

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† The scientific name, Plautia crossota stali is used for this species in the present paper according to its use by Tomokuni et al. (1993), although Plautia stali has been commonly used.
‡ Contribution No. 1288 of the National Institute of Fruit Tree Science.
about 20 adults collected from Ushiku City, Ibaraki Prefecture (36.0°N), in early September 1998. Adult flies were reared in a netted stainless steel cage (30×30×30 cm), and kept at 22.5°C and a photoperiod of 16L–8D. Sugar cubes were given as food, and water-soaked cotton wool was given as the water supply. *P. c. stali* adults that were reared in the laboratory according to the method described by Moriya et al. (1985) were provided as hosts. They were randomized from large culture. Exposure to parasitization was carried out by placing adult bugs to be parasitized into cages which housed male and female flies. Thirty bugs parasitized by *G. rotundatum* were reared in a plastic container (9 cm high×15 cm in diameter). On the top of the container, a hole (6 cm in diameter) covered with nylon gauze served for ventilation. For the hosts, raw peanuts served as food and water-soaked cotton wool served as the water supply. *G. rotundatum* individuals that emerged from the hosts and soon pupariated were kept on layers of moist tissue paper in a plastic cup (4 cm high×5 cm in diameter) until they emerged as adults. This procedure was conducted for maintaining successive generations of *G. rotundatum*. Following experiments were conducted at 22.5°C and a photoperiod of 16L–8D except when otherwise indicated.

**Fecundity and longevity of parasitoid flies.** A male and a female of *G. rotundatum* were paired in a plastic container (9 cm high×15 cm in diameter) on the day of their emergence and were kept at 21–22°C and a photoperiod of 16L–8D. Other rearing conditions were the same as those described in the stock culture. Five stink bug adults were provided as hosts into each container. On the next day, they were removed and five new adult stink bugs were introduced. The number of eggs laid on the bugs was checked daily. This procedure was repeated until the flies died.

**Effects of host sex, host body size, and the number of parasitoid eggs per host on tachinid larval development and puparial weight.** Both sexes of the 10-day-old adult bugs of various body sizes were exposed to parasitization in the cages which housed male and female flies. Of these, the hosts on which one or two parasitoid eggs were deposited were individually weighed by an electric balance and reared in a petri dish (2 cm high×9 cm in diameter). Parasitoid larvae emerging from the hosts were checked daily and puparial weight was measured by an electric balance the day after pupariation. The period from oviposition to larval emergence was noted, and subsequently treated as the larval duration in this study, as the period of the egg stage was difficult to determine without imposing stress on the hosts. On the day of eclosion, adult flies were weighed by an electric balance and their head width was measured under a binocular microscope.

**Effects of hosts’ nutritional condition on tachinid larval development and puparial weight.** Female adult bugs on which one parasitoid egg was deposited on the 14th day of adult stage were divided into three groups; the 1st and the 2nd groups were allowed to feed every day and every five days, respectively, and the 3rd group was kept without food. Water was supplied to all three groups. The parasitized female bugs were paired with mature intact males and reared in petri dishes (2 cm high×9 cm in diameter). Parasitoid emergence from the host and the number of eggs laid by parasitized bugs were checked every day. Puparia were weighed by an electric balance one day after pupariation.

**Effects of parasitization on host fecundity and fertility.** To elucidate the effects of parasitization on host fecundity, female bugs were exposed to parasitization on either the 4th or the 14th day of adult stage. Female bugs are immature on day 4 and mature on day 14 because pre-oviposition period is about 11–12 days (Moriya, 1995). These female bugs on which female flies laid one or two eggs were paired with unparasitized matured males in petri dishes (2 cm high×9 cm in diameter). Other experiments in the present study showed that even if the flies deposited two eggs onto a single host, only one maggot could survive to maturity. Therefore, the female bugs with one or two parasite eggs were pooled in this experiment. The number of eggs laid by the female bugs was examined daily. The percentage of fertilized eggs was also examined because the eye spots of the embryos became visible through the egg shell by the 5th day after oviposition at 22.5°C.

In addition, ovary size and egg size were examined. Pairs of ovaries were extracted by dissection under a binocular microscope and ovaries were individually weighed by an electric balance after having been dried at 80°C for about one day. *P. c. stali*
lays cylindrical eggs as a batch of about 14 eggs; three to five of these eggs were selected randomly from the egg batches laid by parasitized and intact females in order to measure egg diameters under a binocular microscope.

In males, adult bugs were exposed to parasitization on the 11th day of the adult stage when male bugs already mature and often show mating behavior. These males were reared individually in a petri dish. An unparasitized virgin female was introduced into each petri dish for two days, and then was replaced with another virgin female. Removed females were reared individually in petri dishes to examine their fecundity and the percentage of fertilized eggs. This procedure was repeated until the male bug died.

Data analysis. All statistical analyses were performed with Stat View-J version 5.0 (SAS Institute Inc., 1998).

RESULTS

Fecundity and longevity of parasitoid flies

Adults of *G. rotundatum* mated readily in a plastic container and a netted stainless steel cage. Flies were often observed to mate on the day of emergence. Most female flies began to lay eggs on the day after adult emergence. Eggs were laid on the abdominal tergum of the host. Although the longevity of adult parasitoids varied considerably, males tended to survive longer than females; mean (± SE) longevity was 20.1 (± 2.5) days and 15.8 (± 2.0) days in males and females with oviposition, respectively (Fig. 1). Females oviposited almost

![Graph showing egg production and longevity of *G. rotundatum* adults.](image)

Fig. 1. Egg production and longevity of *G. rotundatum* adults. *n* = 17 for each sex.

<table>
<thead>
<tr>
<th>Host sex</th>
<th>No. of hosts parasitized</th>
<th>No. of larvae emerging</th>
<th>Larval duration in days</th>
<th>Fresh weight of puparium in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 egg/host</td>
<td>2 eggs/host</td>
<td>1 egg/host</td>
<td>2 eggs/host</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>50</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>50</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td><em>p</em> (ANOVA)</td>
<td></td>
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</table>

*Mean± SE. Two-way ANOVA with two factors of the host sex and the number of parasitoid eggs was conducted first and showed that only the host sex was responsible for significant differences in larval duration (host sex: *p* < 0.001, the number of parasitoid eggs: *p* = 0.428, the interaction of the two factors: *p* = 0.630) and in fresh weight of puparium (host sex: *p* < 0.001, the number of parasitoid eggs: *p* = 0.305, the interaction of the two factors: *p* = 0.925). Then, one-way ANOVA was performed by regarding the host sex as the main factor, so that significant differences were observed in all columns.*
The total number (± SE) of eggs laid by a single female during their lifetime (14.9 ± 2.0 days) and the total number (± SE) of eggs laid by a single female was 98.1 ± 13.2.

Parasitized hosts died without exception within a day after the maggot emerged. The maggots pupariated within a day after emergence from the hosts. Table 1 shows the effects of host sex and the number of parasitoid eggs per host on larval development and puparial weight of the parasitoid fly. Even if the flies deposited two eggs onto a single host, only one maggot could survive to maturity. Percentage of larvae emerging from hosts was not affected by either the host sex or the number of parasitoid eggs per host. On the other hand, the larval duration and puparial weight of the flies were affected by the host sex but not by the number of parasitoid eggs per host. The larval period of parasitoid tended to be longer in female hosts than in male hosts. Mean puparial weights from female hosts were about 20% larger than those from male hosts.

**Table 1. Effects of host sex and parasitoid sex on developmental traits [mean ± SE (n)] of G. rotundatum**

<table>
<thead>
<tr>
<th>Host sex</th>
<th>Larval duration in days&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>Puparial duration in days&lt;sup&gt;ac&lt;/sup&gt;</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (25)</td>
<td>Female (23)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15.28 ± 0.26 (25)</td>
<td>15.65 ± 0.29 (23)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16.17 ± 0.20 (24)</td>
<td>17.20 ± 0.38 (25)</td>
<td></td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>0.009</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Flies that died in larval or puparial stage were excluded.
<sup>b</sup> Flies that failed to emerge as adults were excluded.
<sup>c</sup> Flies that died within a day after adult emergence were excluded.

**Table 2. Effects of host sex and parasitoid sex on developmental traits [mean ± SE (n)] of G. rotundatum**

<table>
<thead>
<tr>
<th>Host sex</th>
<th>Larval duration in days&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Puparial duration in days&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (24)</td>
<td>Female (25)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12.00 ± 0.13 (25)</td>
<td>12.44 ± 0.14 (23)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12.46 ± 0.12 (24)</td>
<td>12.36 ± 0.10 (25)</td>
<td></td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>0.009</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

<sup>d</sup> Two-way ANOVA (host sex: df = 1, F = 17.792, p < 0.001, parasitoid sex: df = 1, F = 5.930, p = 0.017, the interaction of the two factors: df = 1, F = 1.312, p = 0.255).
<sup>e</sup> Two-way ANOVA (host sex: df = 1, F = 2.483, p = 0.119, parasitoid sex: df = 1, F = 1.910, p = 0.170, the interaction of the two factors: df = 1, F = 4.796, p = 0.031).
<sup>f</sup> Two-way ANOVA (host sex: df = 1, F = 0.001, parasitoid sex: df = 1, F = 0.265, the interaction of the two factors: df = 1, F = 0.271).
<sup>g</sup> Two-way ANOVA (host sex: df = 1, F = 64.473, p < 0.001, parasitoid sex: df = 1, F = 2.095, p = 0.151, the interaction of the two factors: df = 1, F = 5.592, p = 0.020).
<sup>h</sup> One-way ANOVA was performed by regarding the host sex as the main factor, so that the significant differences were observed in the concerned columns.

**Fig. 2.** The relationship between host size and parasitoid size. The fresh weight of the puparium is plotted against the fresh weight of the host. **★★★★:** Significant at 0.1% level.
sitoid sex had meaningful effects on the parasitoid puparial durations. Sex ratios were about 1:1 in parasitic flies that emerged from both sexes of the hosts. *G. rotundatum* was highly variable as regards adult body size: the largest fly was 39% larger in head width than the smallest fly.

The relationship between host size and parasitoid size is shown in Fig. 2. The correlation was significant at 0.1% level in both sexes of host; larger parasitoids tended to emerge from larger hosts.

**Effects of hosts’ nutritional conditions on tachinid larval development and puparial weight**

Percentage of larvae emerging from female bugs and adult emergence, and the means of larval duration were not significantly different among the three nutritional conditions of the hosts (Table 3). On the other hand, mean puparial weight was significantly smaller in the unfed treatment. Most of the parasitized female bugs laid their eggs for about one week, and mating behavior was observed in some parasitized females. Host females that fed freely laid significantly more eggs than those that were restrictively fed and those that were unfed.

**Effects of parasitization on host fecundity and fertility**

Percentages of female bugs that could oviposit were 93% and 100% when the bugs were parasitized on the 4th and the 14th day of the adult stage, respectively. However, ovaries of parasitized bugs gradually shrank after the 6th day of parasitization (Fig. 3), and oviducts coincidentally came to be devoid of eggs. On the other hand, intact females retained mature ovaries for at least two weeks. The loss of functional ovaries was reflected as a suppression of oviposition. Percentage of ovipositing host females decreased rapidly prior to their death, with the result that the mean number of laid eggs decreased (Fig. 4A, B). The percentage of fertilized eggs decreased rapidly (Fig. 4C, D). Clutch size and egg volume decreased (Figs. 5 and 6), and percentage of malformed eggs increased (data not shown). As a result, female bugs parasitized on the 4th and the 14th day of adult stage produced 8.6 and 34.8 viable eggs after parasitization, respectively (Table 4). The number of viable eggs produced by unparasitized females was 170.9 for the lifetime of the insect, and 135.6 after the
Fig. 3. Changes in ovary size of unparasitized and parasitized female bugs. The parasitized female bugs were prepared by exposing female bugs to parasitization on the 14th day of adult stage. Days after parasitization are shown on the right of each figure. Means are indicated by arrows.

Fig. 4. Longevity and fecundity of parasitized female bugs. The parasitized female bugs were prepared by exposing female bugs to parasitization on the 4th day (A and C) or 14th day (B and D) of the adult stage.
In males, the survival rates were high until the 14th day after parasitization, but the number of individuals that were able to fertilize eggs decreased rapidly after the 8th day of parasitization (Fig. 7).

**DISCUSSION**

Tachinid parasitoids often affect the reproductive abilities of pentatomid bugs (Beard, 1940; Clausen, 1940; Shahjahan, 1968; Harris and Todd, 1982). When adult females of *Nezara viridula* were parasitized by *Trichopoda pennipes*, fecundity was not reduced relative to that of unparasitized females. However, the lifetime fecundity of unparasitized females was much higher than that of parasitized females due to the difference in longevity (Shahjahan, 1968; Harris and Todd, 1982). On the other hand, *Nysius ericeae* females parasitized by *Hyalomya aldrichi* can only rarely lay eggs (Clausen, 1940). Thus, the effect of tachinid parasitism upon pentatomid bugs varies from species to species.

Fig. 5. Changes in batch size of unparasitized and parasitized female bugs. The parasitized female bugs were prepared by exposing female bugs to parasitization on the 14th day of the adult stage. Days after parasitization are shown on the left of each figure.

Fig. 6. Changes in egg size of unparasitized and parasitized female bugs. The parasitized female bugs were prepared by exposing female bugs to parasitization on the 18th day of the adult stage. Open and closed circles indicate mean diameters of eggs laid by unparasitized and parasitized female bugs, respectively. Vertical bars indicate standard deviations. The sample size is shown in parentheses. **: Significant difference at 0.1% level between eggs laid by parasitized and intact bugs (ANOVA).
The present study showed that adults of *P. c. stali* parasitized by *G. rotundatum* lost their reproductive abilities prior to death: the number of laid eggs, clutch size, egg size and the percentage of viable eggs began to decrease approximately one week after parasitization (Figs. 4, 5, and 6). It was also clarified by dissection that the ovaries of parasitized females shrank after six days of parasitization (Fig. 3). When mature female bugs were parasitized, they were able to lay egg masses normally several times. On the other hand, when young female bugs were parasitized, they laid considerably fewer eggs (Table 4). These findings suggest that parasitization inhibits egg production by hampering egg development in ovarioles. In contrast, already mature eggs in ovarioles appear to be laid independent of parasitization.

Once the endoparasite gets into the host body, its development must depend on the nutrients in the host. The nutritional condition of *P. c. stali* affected the body size of *G. rotundatum*, though it did not affect the percentage of maggots that matured (Table 3). *G. rotundatum* individuals that emerged from restrictively fed bugs were similar in body size to those that emerged from bugs fed freely, and were significantly larger than those from unfed hosts (Table 3). Thus, when the hosts were fed restrictively, nutrients taken by the host were mostly used for the development of parasitoid larvae, and therefore the egg production of those hosts was reduced.

In contrast to the study of parasitized female bugs, the reproductive ability of parasitized male bugs has been poorly studied. Beard (1940) reported that the testes of *Anasa tristis* males were degenerated by parasitization with *T. pennipes*, but that their reproductive activity was not reduced. However, the reproductive ability of these males was not studied over the duration of a lifetime. The present study demonstrated that parasitized *P. c. stali* males retained their reproductive ability for approximately one week, and this ability was reduced thereafter (Fig. 7). The cause of this reduction is not clear. Testis and mating behavior of parasitized male bugs should be examined in detail.

Thus, the present study showed that parasitization by *G. rotundatum* causes considerable reduction in reproductive ability of *P. c. stali*. These results suggest that this fly potentially has the ability to control the density of *P. c. stali* populations. To quantify the impact of *G. rotundatum* on *P. c. stali* populations, seasonal abundance of this fly and

### Table 4. Effects of parasitization on longevity and fecundity of the host

<table>
<thead>
<tr>
<th>Host age at parasitization</th>
<th>N</th>
<th>Longevity in days</th>
<th>No. of eggs laid</th>
<th>No. of fertilized eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>30</td>
<td>22.63 ± 0.44</td>
<td>14.40 ± 1.39</td>
<td>8.63 ± 1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18.63 ± 0.44)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>35</td>
<td>31.71 ± 0.34</td>
<td>45.26 ± 2.59c</td>
<td>34.80 ± 3.20c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.71 ± 0.36)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>42.45 ± 3.31</td>
<td>212.45 ± 21.73</td>
<td>170.93 ± 18.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(166.48 ± 20.20)c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Mean ± SE.

*b* Longevity after parasitization.

*c* The number of eggs laid after the 14th day of the adult stage.
percentage of parasitism in the field should be estimated.

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