Effects of host age and size on clutch size and sex ratio of *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), a larval-pupal parasitoid of *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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**Abstract**

We investigated the effects of host age and size on clutch size and sex ratio of *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae). The number of eggs laid (primary clutch size) tended to increase with host age and was significantly larger for the late fourth-stadium larvae than for the second- and third-stadium larvae. The number of wasps that emerged from a host (secondary clutch size) tended to increase with host age and was significantly larger for the late fourth-stadium hosts than for the second-stadium hosts. In the late fourth-stadium hosts, secondary clutch size tended to increase significantly with host size. There were no significant differences in sex ratios among host ages or sizes. Where fewer than 10 wasps emerged from a host, most clutches produced only 1 male; larger clusters tended to produce 2 or more males. This implies that *O. sokolowskii* may deposit male eggs in the early eggs of a clutch and place the second male eggs in the tenth or later eggs of a clutch. By this mechanism, *O. sokolowskii* is thought to produce an invariable sex ratio regardless of clutch size.

**Key words:** *Oomyzus (Tetranychus) sokolowskii*, gregarious parasitoid, koinobiont, clutch size, sex ratio

**INTRODUCTION**

Host quality significantly influences the offspring fitness of parasitoid wasps (Charnov and Skinner, 1984; King, 1987; Godfray, 1994). One of the most common qualities studied is host size. It has been frequently found that host size influences the clutch size of parasitoids. Salt (1961) listed 10 studies of different parasitoid species that had shown that female wasps laid larger clutches on bigger hosts, and Godfray (1994) mentioned that a similar list now would probably contain over a hundred species. Host size also influences the parasitoid sex ratio, correlating negatively with the proportion of male progeny (Charnov et al., 1981; King, 1987, 1988, 1993; Heinz and Parrella, 1990; Ueno and Tanaka, 1997; Ueno, 1998). Host size usually increases with host age. However, host size and age may need to be considered independently because of possible physiological differences among host ages. For example, *Trichogramma minutum* deposited more eggs in younger hosts than in older ones of the same size, because old eggs provide fewer resources for the parasitoid larvae than young eggs (Marston and Ertle, 1969). Clarifying the effects of host age and size on parasitoid reproductive characters will not only allow a better understanding of the ecological strategies of parasitoids, but it will also be useful for developing augmenting parasitoids as biological control agents.

*Oomyzus (=*Tetranychus*) sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) is a gregarious larval-pupal endoparasitoid of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). This koinobiont parasitoid is distributed worldwide and is the only chalcidoid to have shown potential for the biological control of *P. xylostella* (Fitton and Walker, 1992). Since this parasitoid seems to be well adapted to high temperature conditions, it has been introduced into tropical and subtropical areas to control *P. xylostella* (Talekar and Hu, 1996). Noda et al. (1996) also reported that parasitism by *O. sokolowskii* in northeastern Japan was highest from August through September (40–80%). In a previous paper (Nakamura and Noda, 2001), we demonstrated *O. sokolowskii*...
could parasitize second- to late fourth-stadium host larvae. This implies the host size has a large variation. In this paper, we describe the results of a laboratory study carried out to investigate whether host age and size affect the clutch size and offspring’s sex ratio of *O. sokolowskii* and discuss the suitability of different host age and size for parasitization.

**MATERIALS AND METHODS**

*Insect resources and general procedures.* The *O. sokolowskii* culture used in this study was established from parasitized host pupae collected in a cabbage field in Morioka, northern Japan, in 1997. The *P. xylostella* culture used for rearing the parasitoid originated from insects collected in Tsu, Mie, in 1983. Host larvae were reared on radish sprouts in a small plastic cup (9 cm diameter and 5 cm depth) at 15°C, 16 h light:8 h dark (LD 16 : 8), until parasitization. Parasitoids were reared by presenting third- and fourth-stadium larvae to a female in a glass tube (2 cm diameter and 10 cm long). Parasitized host larvae were reared at 25°C. Newly emerged *O. sokolowskii* females were kept in glass tubes and fed honey at 15°C, LD 16 : 8 with males to ensure insemination. Only 3- to 7-day-old females with no previous oviposition experience were used for the experiments.

Parasitization experiments were performed in polystyrene tubes (1.5 cm diameter and 6.5 cm long) with radish sprouts at 25°C, LD 16 : 8. One *P. xylostella* larva was presented to one female parasitoid and the oviposition behavior was observed. Immediately after the host was parasitized, the wasp was removed and the parasitized host was reared in a tube with radish sprouts at 25°C, LD 16 : 8. Pupated hosts were singly transferred to 1.5-ml polypropylene microtubes and the emergence of both parasitoids and hosts was checked daily.

**Effect of host age on clutch size and sex ratio of the parasitoid.** Second-, third-, early fourth- (within 24 h after molting), and late fourth-stadium (=prepupa whose cocoon web was removed) *P. xylostella* larvae were each parasitized by a single female parasitoid. Twenty parasitized larvae of each age were dissected after the completion of parasitization to count the actual number of eggs laid per host (=primary clutch size). More than 43 hosts of each age were reared individually in the polystyrene tubes with radish sprouts at 25°C, LD 16 : 8 to count the number of parasitoid progeny that emerged per host (=secondary clutch size) and to calculate their sex ratios.

**Effect of host age on clutch size and sex ratio of the parasitoid.** Late fourth-stadium host larvae (=prepupa whose cocoon web was removed) of different sizes were each parasitized by a single female parasitoid. Only late fourth-stadium larvae were used for this experiment to avoid the influence of any differences in physiological condition of the different host ages. Parasitized host larvae were reared individually in polystyrene tubes at 25°C, LD 16 : 8 to count the number of parasitoid progeny that emerged (=secondary clutch size). After the parasitoids emerged, the body length of the host pupae was measured under a binocular microscope. Because the hosts used in this experiment were prepupae that were no longer feeding, we consider that their size represented the body size when they were parasitized.

**RESULTS**

**Effect of host age on clutch size and sex ratio of the parasitoid**

Primary clutch size tended to increase with host age except in third-stadium larvae and was significantly larger for the late fourth-stadium larvae than for the second- and third-stadium larvae (Table 1). The number of wasps that emerged from a host (=secondary clutch size) also tended to increase with host age, and was significantly larger for the late fourth-stadium hosts than for the second-stadium hosts. There were no significant differences in sex ratio among host sizes.

**Effect of host size on clutch size and sex ratio of the parasitoid**

The number of progeny that emerged per host (=secondary clutch size) increased significantly with host size (regression analysis; \( F=4.421, p=0.0396; \) Fig. 1a). The primary clutch sizes were not measured. There were no significant differences in sex ratio among host sizes (regression analysis; \( F=0.225, p=0.6372; \) Fig. 1b). The smallest clutch size was 3 and the largest was 20. Figure 2 shows the number of males that emerged from a clutch in relation to the secondary clutch size. When the clutch size was less than 10, most clutches produced only 1 male. When the clutch
size was 10 or more, clutches often produced 2 or more males.

**DISCUSSION**

The primary clutch sizes tended to increase with host age (Table 1). Such a positive correlation between host age and parasitoid clutch size has been reported for many parasitoid wasps (Salt, 1941; Klomp and Teerink, 1962). This suggests that a parasitoid can recognize the host size and adjust the clutch size accordingly. In our previous paper (Nakamura and Noda, 2001), we demonstrated that the duration of ovipositor insertion and oviposition of *O. sokolowskii* was significantly longer on fourth-stadium hosts than on second- and third-stadium hosts. We also demonstrated that the time taken for drumming by antennae was slightly longer on fourth-stadium hosts than on other stadia, although not significant. These behavioral differences may indicate a difference in chemical and/or visual conditions between small and large hosts and explain how the parasitoid recognizes host size. The secondary clutch sizes were significantly smaller than the primary ones in all host stadia except for the third-stadium (*t*-test, *p* < 0.05), probably because of larval death. However, the secondary clutch sizes in the fourth-stadium hosts were also larger than those in the other stadia, although not significant. These behavioral differences may indicate a difference in chemical and/or visual conditions between small and large hosts and explain how the parasitoid recognizes host size. The secondary clutch sizes were significantly smaller than the primary ones in all host stadia except for the third-stadium (*t*-test, *p* < 0.05), probably because of larval death. However, the secondary clutch sizes in the fourth-stadium hosts were also larger than those in the other stadia.

Table 1. Effect of host age on primary clutch size, secondary clutch size, and offspring sex ratio

<table>
<thead>
<tr>
<th>Host age (stadium)</th>
<th>Primary clutch size&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. wasps emerged per host&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sex ratio (% males)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>11.4±0.8 a (20)</td>
<td>9.2±0.3 a (63)</td>
<td>10.7</td>
</tr>
<tr>
<td>3rd</td>
<td>10.2±1.5 a (20)</td>
<td>10.1±0.4 ab (60)</td>
<td>12.4</td>
</tr>
<tr>
<td>Early 4th</td>
<td>12.2±0.3 ab (20)</td>
<td>9.6±0.5 ab (43)</td>
<td>13.2</td>
</tr>
<tr>
<td>Late 4th</td>
<td>16.6±1.6 b (20)</td>
<td>11.1±0.6 b (47)</td>
<td>11.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean±SE. Means followed by the same letter do not differ significantly at *p* = 0.05 by Tukey-Kramer’s test. Numbers of hosts used are shown in parentheses.

<sup>b</sup> No significant differences were found in the sex ratio at *p* = 0.05 by R×C tests of independence using chi-square test.

Wang et al. (1999) reported that the number of *O. sokolowskii* progeny per host pupa did not differ significantly among host ages. They put 5 parasitoids and 24 hosts together in a container for 24 h. Such a condition can cause several females to oviposit into the same host and consequently equalize the number of progeny per host. In our study, we presented a single host to a single parasitoid under direct observation. This difference in methods may explain the difference in results.

Host age and host quality may be influ-
enced by the internal physiological condition of the host, such as hormonal balance. Host size and age are mutually confounding factors when larval hosts are used. Therefore, we used late fourth-stadium (=prepupa) hosts of different sizes to test the effects of host size on parasitoid reproduction. Consequently, the secondary clutch size tended to increase significantly with host size (Fig. 1a) as well as with host age. Host size has been frequently found to correlate negatively with the proportion of male progeny (Charnov et al., 1981; King, 1987, 1988, 1993; Heinz and Parrella, 1990; Ueno and Tanaka, 1997; Ueno, 1998). However, we found no significant differences in sex ratio among host sizes. This result suggests that the female wasps can place more male eggs in larger hosts in response to the host size and adjust the offspring sex ratio. In fact, the relationship between the secondary clutch size and the number of males per clutch showed that the female produced only 1 male when the clutch size was 9 or fewer and 2 or more when the clutch size was 10 or more (Fig. 2). This implies that O. sokolowskii may be able to optimize the sex ratio, as some trichogrammatids and scelionids do (Waage, 1982; Waage and Ng, 1982; Noda and Hirose, 1989). Such a sex-ratio control has been found for many parasitoid species in the LMC type situation (Godfray, 1994) where a few males can inseminate many of their sisters. Because O. sokolowskii shows habitually inbreeding or sibmating, it is considered that the sex ratio of O. sokolowskii can be influenced by host size under LMC situations.

In our previous paper (Nakamura and Noda, 2001), we found that fourth-stadium larvae were more suitable hosts for O. sokolowskii than second-stadium larvae, because they produced higher successful parasitism and lower parasite mortality. Our latest results—that clutch size increased with host size—support this conclusion.

REFERENCES


