Short Communications

In vitro Rearing of the Egg Parasitoid, Ooencyrtus nezarae Ishii (Hymenoptera: Encyrtidae)\(^1\)

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In vitro culture of many parasitoids has been used to examine physiological requirements for growth and development (House, 1977; Thompson, 1986; Nettles, 1990). Several egg parasitoids have been successfully cultured from the egg to adult on artificial diets in vitro, including Trichogramma spp. (Liu et al., 1979; Strand and Vinson, 1985), Tetrastichus schoenobii (Ding et al., 1980) and Telenomus heliothis (Strand et al., 1988). Ooencyrtus nezarae Ishii is an egg parasitoid of phytophagous bugs which are serious pests of soybean in Japan (Takasu and Hirose, 1985; 1986). As is typical of many encyrtids (Maple, 1947), O. nezarae eggs and larvae are attached to a stalk which protrudes through the host egg chorion (Takasu and Hirose, 1989). Although larval development of O. nezarae in eggs of Ripipterus clavatus Thunberg has been observed by Takasu and Hirose (1989), physiology of the growth and development of this parasitoid is unknown.

In this paper, we describe in vitro development of O. nezarae on tissue culture medium and artificial diets, and discuss factors important for pupation of O. nezarae.

MATERIALS AND METHODS

The parasitoids and hosts were cultured in the same manner as described by Takasu and Hirose (1988). To obtain R. clavatus eggs parasitized by O. nezarae, host eggs were individually exposed to a parasitoid female in a test tube (3 cm dia. x 15 cm) for 3 h. The parasitized eggs were surface-sterilized in 70% ethanol for 2 min and 2% NaOCl for 15 min and then rinsed in sterile distilled water 3 times. Surface-sterilized host eggs were dissected in a sterile Petri dish (9 cm) with forceps, and an insect pin was used to collect the parasitoid eggs. After parasitized eggs were rinsed in sterile physiological saline solution (0.75% NaCl) 3 times, they were transferred to a well of a Cuprasc plate (Nunclon 10 µl) which contained 3 µl of culture medium. O. nezarae usually laid 4–6 eggs in a R. clavatus egg. Eggs obtained from the same parasitized host were reared together in the same well. Several empty wells were filled with sterile distilled water to maintain proper humidity. When parasitoid larvae had grown to the final instar, excess medium was removed with a piece of sterilized filter paper to facilitate pupation of parasitoids. A preliminary test showed that this removal of excess medium allowed O. nezarae to pupate. When O. nezarae developed to the final instar in R. clavatus eggs, the larvae were transferred to wells of a Cuprasc plate containing Mitsuhashi’s MGM-443 tissue culture medium (Mitsuhashi, 1980) and reared for 1 d. Excess medium was then removed. The majority of such larvae developed to adults (Takasu, unpublished). The rearing plates were kept at 25°C under constant darkness except during examination.

In the present study, O. nezarae were cultured in vitro in Grace’s tissue culture medium, Mitsuhashi’s MGM-443, an artificial diet consisting of cow milk (50%) and chicken egg yolk (50%), and an artificial diet with 50% MGM-443 or 50% hemolymph of Bombyx mori (Lepidoptera: Bombycidae). B. mori hemolymph was collected from early final instar larvae. A proleg of larvae was cut and hemolymph collected in a sterile vial. After the sample was kept at 60°C for 10 min, hemolymph was centrifuged for 15 min at 3000 rpm and then the supernatant was frozen. After chicken eggs were surface-sterilized in 70% ethanol for 15 min, the eggs were dissected with forceps in

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sterilized Petri dishes (9 cm). The egg yolk was centrifuged for 15 min at 8000 rpm to obtain the supernatant.

To compare in vitro development with natural development of *O. nezarae*, 3-day-old *R. clavatus* eggs were examined. Forty 3-day-old *R. clavatus* eggs were each exposed to a parasitoid female for 3 h in a test tube (3 cm dia. x 15 cm). Ovipositing behavior was observed and the number of eggs laid into each host was recorded. The parasitized hosts were each kept in a test tube at 25°C. Five parasitized hosts were dissected with insect pins on each of 5 d after oviposition (days 2, 4, 5, 6 and 7). The other parasitized hosts were kept in tubes until parasitoids emerged. The number of adults emerging from the parasitized hosts was recorded. Percent survival of *O. nezarae* from eggs to adults was determined as the number of eggs laid relative to the number of adults that emerged.

To determine whether *O. nezarae* can develop in non-hosts, *O. nezarae* eggs were reared in *B. mori* eggs. Diapausing *B. mori* egg masses consisting of 10–30 eggs were each given to individual *O. nezarae* females in Petri dishes (9 cm); 80 parasitized eggs were obtained in this way. Forty parasitized eggs were dissected to determine the number of eggs in each host. The other parasitized eggs were kept in a test tube at 25°C until the parasitoid adults emerged. Twenty two days after oviposition the parasitized eggs from which no parasitoids emerged were dissected to identify the stage of the dead parasitoids.

RESULTS AND DISCUSSION

*O. nezarae* eggs laid in *R. clavatus* eggs hatched within 2 d after oviposition at 25°C. After consuming all host contents, larvae developed to the final (fifth) instar in 4–5 d. They pupated in 7–8 d and adults emerged in 14–15 d. Percent survival of *O. nezarae* eggs (N=74) to adults was 96.3% (Table 1).

When reared on Grace's tissue culture medium and Mitsuhashi's MGM-443 in vitro, 85% of *O. nezarae* eggs hatched 40–48 h after oviposition (Table 1). The time taken from egg deposition to egg hatching was similar to that in *R. clavatus* eggs (Takasu and Hirose, 1989). This suggest that host hemolymph is not necessary for eggs to hatch. All larvae failed to develop to the final instar on these 2 tissue culture media (Table 1).

Egg parasitoids of Lepidoptera, *Trichogramma* spp., have been reared successfully from eggs to adults on artificial diets containing chicken egg yolk, milk and Lepidoptera hemolymph in vitro (Liu et al., 1979; Xu et al., 1986a; 1986b). In the present study, when *O. nezarae* were reared on an artificial diet containing only milk and chicken egg yolk, 45% of parasitoid eggs developed to final instar larvae (Table 1). Addition of 50% MGM-443 to this medium did not improve development of *O. nezarae*. However, when 50% *B. mori* hemolymph was added to the medium, 70% of *O. nezarae* developed to the final instar. This percentage was significantly (p<0.05) higher than the former (Table 1). When reared on these 3 media in vitro, parasitoid eggs hatched 40–48 h after oviposition and developed to the final instar in 5–6 d. The time taken from the egg stage to the final instar was 1 or 2 d longer than that required when reared in *R. clavatus* eggs. All final instar larvae on these

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of parasitoids</th>
<th>% survival to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First instar</td>
</tr>
<tr>
<td>Grace</td>
<td>25</td>
<td>84.0a</td>
</tr>
<tr>
<td>MGM-443</td>
<td>45</td>
<td>86.7a</td>
</tr>
<tr>
<td>Cow milk + chicken egg yolk (50: 50%)</td>
<td>16</td>
<td>75.0a</td>
</tr>
<tr>
<td>Cow milk + chicken egg yolk + MGM-443 (25: 25: 50%)</td>
<td>18</td>
<td>72.2a</td>
</tr>
<tr>
<td>Cow milk + chicken egg yolk + <em>B. mori</em> hemolymph (25: 25: 50%)</td>
<td>40</td>
<td>85.0a</td>
</tr>
<tr>
<td><em>R. clavatus</em> egg</td>
<td>74</td>
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</tbody>
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The percentage values followed by the different letters in the same column differed significantly (p<0.05) by chi-square test of homogeneity of proportions.
artificial diets died before pupation (Table 1). Other studies of in vitro rearing of egg parasitoids have shown that failure of the parasitoids to pupate is caused by nutritional factors (Xie et al., 1986b; Irie et al., 1987) or non-nutritional factors, e.g., the presence of excess medium (Strand and Vinson, 1985; Strand et al., 1988). Since no nutritional factors in this study appeared to be responsible for the failure of pupation (see MATERIALS AND METHODS), it is likely to have been caused by a general lack of nutrition. In Trichogramma spp., some substances present in Lepidoptera hemolymph are necessary for pupation (Irie et al., 1987). Similarly, pupation of O. nezarae may depend on the presence of some substances in hemolymph of its hosts, Hemiptera eggs (Takasu and Hirose, 1983; 1986). The artificial media used in this study did not contain Hemiptera hemolymph, although some contained B. mori hemolymph.

This idea is supported by the fact that most of O. nezarae laid in B. mori eggs die as third instars. When given masses of B. mori in the laboratory, O. nezarae females laid 2 or 3 eggs (Mean=2.4) into each B. mori egg. Of O. nezarae laid in B. mori eggs, 76% developed to final instar larvae in 4 or 5 d after oviposition. However, most final instars died before pupation. As a result, only 4% of eggs laid in B. mori eggs developed to adults (2♀, 1♂). It appears that B. mori eggs do not contain a sufficient amount of substances necessary for pupation of O. nezarae.

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