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Oviposition and Larval Development of *Neochrysocharis formosa* (Hymenoptera: Eulophidae) inside the Host Larvae, *Liriomyza trifolii*

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Abstract

The biology of the immature stage of *Neochrysocharis formosa*, a larval idiobiont-endoparasitoid of agromyzid leaf miners, was studied to gain a better understanding of *N. formosa* and *in vitro* rearing. Eggs have a smooth surface with a thin, transparent chorion. The length and width are about 190 µm and 60 µm, respectively. Eggs hatched in an average of 32.6 h. Three larval stages are decided by observation of the pre-ecdysis phase. Larvae are hymenopteriform with small mandibles and have 13 body segments. Larval duration at each instar is as follows: 1st instar 35.1 h, 2nd instar 35.7 h and 3rd instar 46.5 h. Mature 3rd-instar larvae escaped from the host and pupated within the mine of the host larvae. Pupal duration was 8 days. The host larvae stopped moving a few minutes after parasitoid oviposition. To examine the oviposition site, longitudinal sections of parasitized host larvae showed all eggs to be within the host's hemocoel. Fewer eggs were oviposited in the anterior part than the middle and posterior part of the host larvae. No encapsulation or melanization were observed around the parasitoid eggs.

Discipline: Plant protection
Additional key words: Agromyzidae, biological control agent, idiobiont-endoparasitoid, leaf miner larvae

Introduction

Neochrysocharis formosa (Hymenoptera: Eulophidae) is distributed in almost all of the countries in the world except for Australasia and South America and the host insects are recorded from more than 100 species in many orders; Coleoptera, Hemiptera, Diptera, Lepidoptera, and Hymenoptera[11]. It is also one of the commonest parasitoids on crops in Japan[1,8,13], and an asexual strain of *N. formosa* was registered as a biological control agent for agromyzid leaf miner pests such as *Chromatomyia horticola* and *Liriomyza trifolii*. The life history and biology of the adult have been extensively studied for pest control[2,12,14], but there are few studies on the immature stage[2,3,9,17]. *Neochrysocharis formosa* is an idiobiont-endoparasitoid, and lays its eggs inside the host, where the larvae develop. This makes the study of the immature stage of this species difficult. Our aim is to obtain a better understanding of the parasitoid and to acquire information that can assist *in vitro* rearing of this species by studying the oviposition site inside the host, *L. trifolii*, and life history of the parasite larvae. *In vitro* rearing of parasitoids is of interest not only to enable detailed studies of parasitoid physiology, but also as a technique for low-cost production[14].

Materials and methods

1. Insects

Liriomyza trifolii (Diptera: Agromyzidae) was provided by Shizuoka Prefectural Experimental Station [the same strain as registered in The NIAS (National Institute of Agrobiological Sciences) Genebank (Tsukuba, Japan)] and reared on kidney bean (*Phaseolus vulgaris*) seedlings[6]. The

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bean plant was grown in a plastic pot (9 cm in diameter and 7.5 cm in height) in a greenhouse. The primary leaves were used for insect rearing 2–3 weeks after germination. The apical bud was cut off, leaving just two primary leaves to grow. Three plants were placed in a sieve-type plastic cage (25 × 31 × 28 cm) and 30 adults of *L. trifolii* were released and allowed to oviposit on the leaves for 24 h in a 16L: 8D photoperiod at 25˚C. The parasitoid *N. formosa* was provided by Miyazaki University (the same strain as registered in The NIAS Genebank). The parasitoids were maintained as follows. Thirty parasitoid adults were put into the plastic cage containing the plant and given access to the three bean plants infested with late second or early third-instar larvae of *L. trifolii*, 5 days after oviposition. Parasitoid adults were fed with a 50% honey-water solution soaked in cotton. The bean plants were changed every 5 days for rearing of successive generations. For experiments, designated numbers of parasitoid adults were put into the cage containing a given number of *L. trifolii* larvae.

2. Development of the parasitoid larvae

Two bean plants whose two primary leaves were infested with 60-70 3rd instar larvae of *L. trifolii* were paced in a plastic cage (25 × 31 × 28 cm) and 30 parasitoids were released in the cage. One hour after the parasitoids were released, each host larvae was taken out from the bean leaves and transferred onto wet cotton in a 35-mm Petri dish. The dishes were then sealed tightly with Parafilm® and incubated under 16L: 8D at 25˚C. Since the host larvae stopped moving a few minutes after parasitism, immobile larvae were dissected in Carlson's solution every hour during the first few hours to observe, under phase-contrast microscopy, the parasitoid egg and host larval physiology. Parasitoid eggs could be found almost in the immobile host larvae. In preliminary experiments, the egg and larval duration of *N. formosa* at each instar were not less than 30 h, respectively. So, starting 30 h after parasitism, 5 host larvae were dissected every hour until 1st instar larvae of the parasitoids were found in all 5 dissected hosts. A further 30 h later, 5 host larvae were dissected every 3 h until all parasitoid larvae were found to have molted to 2nd instar larvae. Another 30 h after larval molting to the 2nd instar, 5 host larvae were dissected every 3 h until all the parasitoid larvae had molted to 3rd instar larvae. Since mature 3rd instar larvae escape from the host larvae to pupate, we observed them until the adults emerged. The pre-ecdysis phase with appearance of a new head capsule and new cuticle beneath the old ones was periodically observed on the parasitoid larvae. We used these developmental characteristics for determination of larval stages. Classification of eggs and larval types was adapted from Clausen[5] (1940) and Hagen[7] (1964).

3. Oviposition site

To examine the oviposition site, the hosts post-parasitism were fixed in Boin's fluid (picric acid: formalin: acetic acid, 15: 5: 1) for 4–6 h. The host larvae were then stained with eosin in 70% ethanol for 20–24 h, dehydrated in 80% to absolute ethanol series, soaked in xylene, and embedded in paraffin. Longitudinal sections of 6 µm were made using a microtome. The sections were deparaffinized, dipped in a series of diluted ethanols from 100% to 70%, transferred to distilled water and stained with hematoxylin. The sections were again dehydrated, covered with Bioleit (Okenshoji Co., Ltd., Tokyo), and observed under an optical microscope. The host larval section was divided into three equal parts and the number of eggs within each part was counted, respectively. Forty-six parasitized larvae were surveyed to study the parasitoid oviposition sites.

Results

Photographs of each immature stage are shown in Fig. 1. Eggs had a smooth surface with a thin, transparent chorion (Fig. 1a). The length and width were about 190 µm and 60 µm, respectively. Duration time of eggs was 32.7 ± 0.4 h (n = 5). Larvae had three instars, and were all hymenopteriform with small mandibles and 13 body segments (Fig. 1b, c, d). Larval duration at each instar was as follows: 1st instar larvae, 35.1 ± 1.3 h (n = 5); 2nd instar larvae, 35.7 ± 1.6 h (n = 5); and 3rd instar larvae, 46.5 ± 0 h (n = 4). Mature 3rd instar larvae escaped from the host and pupated within the mine of the host larvae. Pupal color was dark brown to black. Pupal duration was approximately 8 days. Duration from egg to adult was 14.3 days. The host larvae stopped moving a few minutes after parasitism; however, the midgut remained in motion for 2 h.

A longitudinal section of a parasitized host larva under a light microscope is shown in Fig. 2. All the eggs were found within the host's hemocoel. No encapsulation or melanization were observed around the parasitoid eggs. Fewer eggs were oviposited in the anterior part than the middle and posterior part of the host larvae ($\chi 2 = 6.83$, $P < 0.05$, Table 1).

Discussion

We clearly showed biological information on the immature stage of the idiobiont-endoparasitoid, *N. formosa*, by detailed observation of host larval dissections and embedded sections. Chien and Ku[2] (2001) reported *N. formosa* larvae took 4 larval stages inside the host *L. trifolii* larvae. We decided on 3 larval stages by observation of the pre-ecdysis phase. Duration from egg to adult and larval rearing conditions were almost the same as that of Chien and Ku[2] (2001).

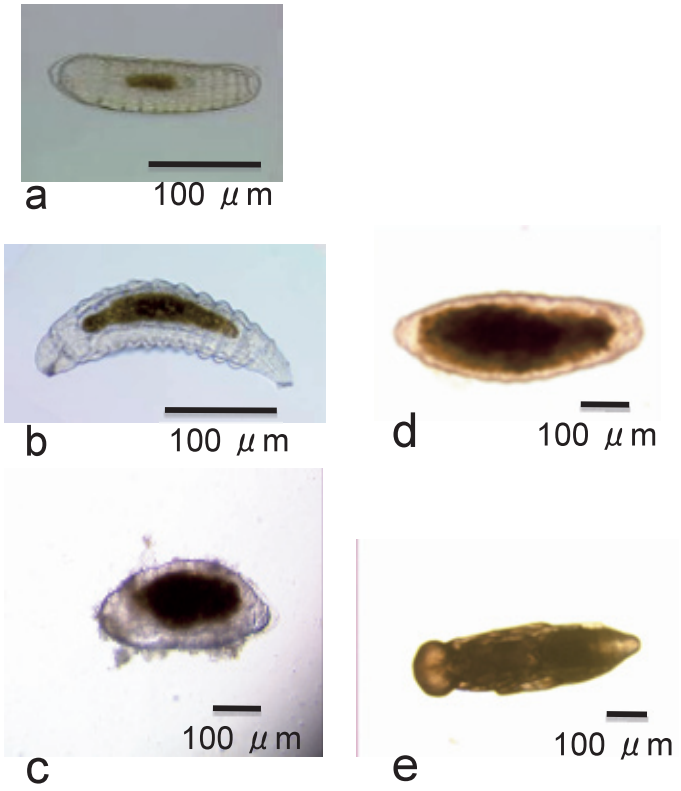

Fig. 1. Immature stage of *N. formosa*
a: egg, b: 1st instar larva, c: 2nd instar larva, d: 3rd instar larva, e: pupa.

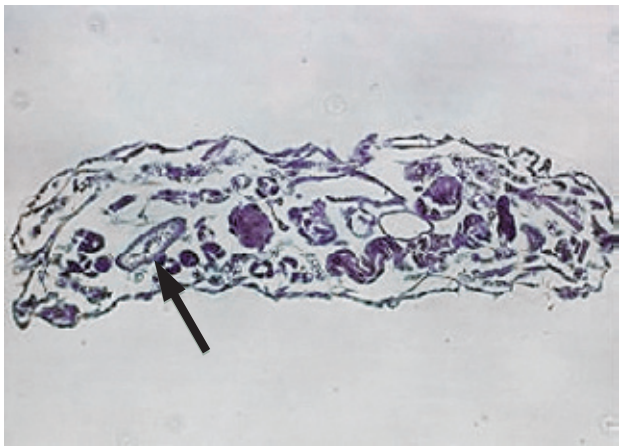

Fig. 2. Longitudinal section of parasitized larva of *L. trifolii*
Arrow represents an egg of *N. formasa.*

Table 1. Distribution of the parasitoid eggs inside the host body

The body parts	anterior	middle	posterior
No. of eggs*	7	19	20
Percentage	15	41	44

*: Significantly different ($\chi 2$ test, $p < 0.05$).

They may have misjudged the larval stage, because they used the head capsule length for judgment. Viggiani[17] (1962) reported *Achrysocharella formosa*, which is now treated as the synonym of *N. formosa*, pupated inside a host pupa, *Phytomyza heringiana* (Diptera: Agromyzidae). Both the observations of Chien and Ku[2] (2001) and this study show parasitized larvae were dead in a few hours, and mature larvae left the host larvae and pupated outside of the host. The species Viggiani[17] (1962) used seems to be a koinobiont parasitoid, which does not kill the host until parasitoid pupation. Biology of the European species needs to be compared with that of the Asian species.

The parasitoid preferred to oviposit eggs in the middle and posterior part of the host larvae rather than in the anterior part. Since the leaf miner larvae retreated when parasitoids tried to attack the head, parasitoid females could thus find it difficult to oviposit in the anterior part of the larva. Some endoparasitoids are known to oviposit in special organs such as the nervous tissue, gut wall or fat body, where the eggs can relatively escape from attack of the host's hemocytes[13]. No oviposition by *N. formosa* was found in such organs. No encapsulation or melanization were observed around the parasitoid eggs. It is supposed that *N. formosa* injects enough venom into the host to inactivate the hemocytes at

oviposition.

The data presented here will give useful information for *in vitro* rearing, as well as the physiological requirements of the parasitoid.

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