Biology of Neochrysocharis okazakii (Hymenoptera: Eulophidae), A Parasitoid of The Stone Leak Leafminer Lifiomyza chinensis (Diptera: Agromyzidae)

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Biology of *Neochrysocharis okazakii* (Hymenoptera: Eulophidae), A Parasitoid of The Stone Leak Leafminer *Liriomyza chinensis* (Diptera: Agromyzidae)

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Neochrysocharis okazakii Kamijo (Hymenoptera: Eulophidae) is endoparasitoid capable of developing on several *Liriomyza* leafminer species, and a dominant parasitoid associated with the stone leek leafminer *Liriomyza chinensis* (Kato) (Diptera: Agromyzidae) in Vietnam. Its biology on *L. chinensis* was studied in the laboratory at a constant temperature of 25 °C and a photoperiod of 16L: 8D. Total developmental time from egg to adult emergence was 12.1 and 12.2 days for males and females, respectively. Pupal development lasted slightly shorter than the combined egg and larval stages. The females laid a mean of 60.1 eggs and caused other 36.4 host larvae died during an average lifespan of 20.1 days. Fecundity peaked at age 3 days. The offspring sex ratio was female–biased as 27.8% males. The intrinsic rate of natural increase (r_m) (day^{-1}), net reproduction (Ro), and generation time (T) (day) were 0.219, 17.7 and 40.3, respectively.

INTRODUCTION

Agromyzid leafminers are known to have rich natural enemy communities, particularly insect parasitoids in both their native and invaded ranges (Waterhouse and Norris, 1987; Konishi, 1998; Murphy and LaSalle, 1999, Chen *et al.*, 2003; Tran *et al.*, 2006). Important factors that have encouraged the use of natural enemies for control of the leafminers are their development of resistance against pesticides and the interference of chemical control with biological control with other pests (van der Linden, 2004).

The stone leek leafminer *Liriomyza chinensis* (Kato) has become a serious pest on *Allium* spp. in many countries including China, Japan, Malaysia, Singapore, Thailand (Spencer, 1973, 1990, Chen *et al.*, 2003), Korea (Hwang and Moon, 1995), Vietnam (Andersen *et al.*, 2002, Tran and Takagi, 2005a), and Taiwan (Shiao, 2004). Recently, outbreak of the leafminer has been found in onion crops across Vietnam, and it treated by a wide range of conventional insecticides, which are ineffective (Tran and Takagi, 2005a). It is necessary to consider a biological control program based on the use of parasitoids against this pest.

Neochrysocharis okazakii Kamijo is endoparasitoid capable of developing on several *Liriomyza* leafminer species, including *L. trifolii* (Saito *et al.*, 1996; Arakaki and Kinjo, 1998; Konishi, 2004), *L. sativae* (Konishi, 2004, Tran *et al.*, 2006), and *L. brassicae* (Bjorksten *et al.*, 2005). This wasp species is also predominant among the parasitoids attacking *L. chinen-*

sis in onion, and appears to be a good biological control agent against the leafminer in Vietnam (Tran *et al.*, 2006).

Information on basic biology of a parasitoid species (e.g. developmental time, fecundity, sex ratio) is fundamentally necessary to evaluate its effectiveness as a biological control agent. However, the biology of *N. okazakii* developing on *L. chinensis* has not been studied. The objectives of the present studies are to determine development time for immature developmental stages, and longevity, fecundity, host mortality caused by non–reproductive killing and offspring's sex ratio of female *N. okazakii* reared on *L. chinensis*. The results would contribute to the knowledge of the biology of this parasitoid and to optimize biological control program against *L. chinensis*.

MATERIALS AND METHODS

Insect rearing

Liriomyza chinensis used for the present study originated from a culture reared by the Fukuoka Agricultural Research Center, Fukuoka, Japan. Colonies of the leafminers was reared on Japanese bunching onion, *Allium fistulosum* L. in an environmental chamber at a constant temperature of 25 °C and a photoperiod of 16L: 8D (Tran and Takagi, 2005b).

The wasp parasitoid *N. okazakii* was originated from Hue city, Vietnam. This parasitoid was maintained with the final–instars of *L. chinensis* under the condition of 25 °C, 60–70% humidity and 16L: 8D photoperiod. Each leaf of onion plants (30–40 cm in height with 2–3 leaves) had been infected with 20–40 of second and third instars *L. chinensis*. For parasitization, the 4 host–infested plants and a piece of tissue paper (2 cm × 2 cm) saturated with a honey solution were placed in a plastic cage (45 cm× 30 cm× 32 cm) covered with a

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fine nylon mesh. About 100–200 parasitoids were introduced into the cage. After the exposure for 24 h, these plants were replaced into a plastic container (60 cm× 50 cm× 40 cm) until pupation of the parasitoids (approximately 6 days after parasitism). The onion leaves with parasitoid pupae were removed from the plant stems and placed into a polyethylene terephthalate (PET) bottle (1,5 *l* in volume). Emergence of the parasitoid was checked daily. Females were provided with honey immediately after emergence.

Immature development

Four potted onion plants at 2–3 leave stage, 30–40 cm in height were exposed to 50 mixed sex *L. chinensis* adults (approximately 1:1 sex ratio) in plastic cages (45 cm× 30 cm× 25 cm) covered with a fine nylon mesh for an ovipostition access period of 2–4 h. After oviposition access period, the plants removed and held in environmental chambers at a constant temperature of 25 °C and a photoperiod of 16L: 8D until all leafminer larvae feeding on the plants reach the last instar.

The potted onion plants infested with the last instars of the leafminer were placed in a plastic cage (45 cm× 30 cm× 25 cm) covered with a fine nylon mesh. About 100–200 mixed sex 2 day–old *N. okazakii* adults were introduced into the cage. Female parasitoids were allowed to attack and parasitized leafminer larvae for 6 h. After parasitization access period, the plants were removed and the leaves were then dissected using a microscope to check for paralyzed larvae. The paralyzed larvae were carefully removed and placed into Petri dishes (6 cm in diameter). A piece of cotton saturated with distilled water was laid on each dish, and then a piece of filter paper (5.5 cm in diameter) was placed on the piece of cotton. The paralyzed larvae were placed on the filter paper and then covered with another piece of filter paper. The dishes with paralyzed larvae were maintained in the environmental chamber set at 25 °C and a 16L: 8D photoperiod until pupation of parasitoids. Parasitoid pupae were collected only once per day in the afternoon. The development time of combined egg and larva stages was defined as the time from oviposition until pupa collection. The pupae were individually placed in centrifugal tubes (1.5 *ml* in volume). These tubes were placed at the same experimental conditions. Adult emergence for each pupa and its sex were daily recorded to determine mean development time.

Longevity, fecundity and host mortality due to non–reproductive killing

Parasitoid pupae were randomly removed from the insect rearing cages and placed singly in centrifugal tubes (1.5 *ml* in volume), and maintained in the environmental chamber. The base of the stem of Japanese bunching onion plants infested with approximately 30–40 second and third instar larvae of *L. chinensis* was immersed in water in a 50 ml glass vials. The vials were placed in the PET bottle (1.5 *l* in volume). The bottles had two holes (5 cm× 5 cm) covered with a fine nylon mesh for air circulation. Undiluted honey was streaked

on a piece of Sealon film (Fuji Photo Film Co., Ltd.). The streaked honey film was attached to the top of the bottles, and replaced daily to provide wasps with fresh food. Thereafter, one pair of newly emerged wasps was released in the bottles. These bottles were kept in the environmental chambers. After the exposure for 24 h, the plants were removed and the leaves were then dissected under microscope to check for paralyzed larvae. The paralyzed larvae were maintained in the environmental chamber set at 25 °C and a 16L: 8D photoperiod in the same manner as described above. Plants were exchanged daily until the females died. The number of parasitoid pupae was recorded as fecundity capacity, and longevity of females was determined. Host mortality due to non–reproductive killing (e.g. host feeding, host stinging) was calculated as a difference between number of paralyzed larvae and number of parasitoid pupae. A total of 9 females (0.299± 0.0052 mm in hind tibial length) were used for test.

Sex ratio and body size of offspring

The pupae collected from the second experiment were individually placed in centrifugal tubes (1.5 *ml* in volume) maintained at the same experimental condition until wasp emergence. All offspring wasps were sexed. The sex ratio is expressed as the proportion of males among the offspring (Godfray, 1994). The hind tibial lengths (as indices of body size) of 100 randomly selected wasps of each sex were measured under a binocular microscope.

Rate of population increase

The wasp offspring emerged for each female at a defined age were daily recorded and sexed. The net reproduction rate (Ro), mean generation time (T) and intrinsic rate of natural increase (r_m) were calculated according to the equations given by Birch (1948)

$$Ro = \sum l_x m_x; \quad T = \sum x l_x m_x \Big/ \sum l_x m_x; \quad \sum (\exp(-r_m x) l_x m_x) = 1$$

where, x is female age, l_x is the proportion of females surviving to age, x, m_x is the expected number of daughters produced per female alive at age x

Data analysis

Comparison of developmental time among immature stages (e.g. egg–larva, pupa), and hind tibial length between males and females were analyzed using unpaired *t*–test. A binomial test was conducted to determining whether the sex ratio parasitoid offspring differs from 1:1 ratio. All statistical procedures were carried out using SPSS ver. 12.0 (SPSS Inc., 2003)

RESULTS

Immature development

Developmental time for immature stages of *N. okazakii* is summarized in Table 1. Total developmental time from egg to adult emergence was not significant

Table 1. Developmental time (days) of *N. okazakii* at 25 °C

| Stage	Male		Female	
	Mean± SE	Range	Mean± SE	Range
Egg larva	6.1± 0.07	6–7	6.3± 0.08	6–8
Pupa	5.9± 0.05	5–6	5.9± 0.08	5–7
Total	12.1± 0.05	12–13	12.2± 0.09	12–15
N	19		42	

difference between the sexes (t test, P 0.05), and lasted 12.1 days for males and 12.2 days for females. The duration of combined egg and larva period was 6.1 and 6.3 days for males and females, respectively. The pupal development lasted an average of 5.9 days for both sexes, and was shorter than the combined egg and larva stage for males (t test, t 1.77, n 38, P 0.05) and females (t 3.03, n 84, P 0.05).

Longevity, fecundity and host mortality caused by non–reproductive killing

The females produced a mean of 61.8 offspring pupae and caused other 36.4 host larvae died during an average lifespan of 20.1 days (Table 2). Age–specific survival and fecundity daily distribution was shown in Figure 1. The daily fecundity distribution showed an increase with a peak at day 3 (11.1± 1.74 progeny/female/day), after which a slow decrease followed until females' death (Fig. 1). There was a similar tendency in activities of oviposition and non–reproductive host killing. The peak of host mortality caused by non–reproductive killing activities of adult females was attained at the 4th day from emergence, with 5.3± 1.72 host larvae killed per female (Fig. 2).

Table 2. Longevity (days), fecundity (no. pupae) and host mortality caused by non–reproductive killing (no. dead larvae) of females *N. okazakii* at 25 °C

	Mean± SE	Range
Longevity	20.1± 3.68	5–37
Fecundity	61.8± 5.59	43–95
Host mortality	36.4± 5.59	13–70
N	9	
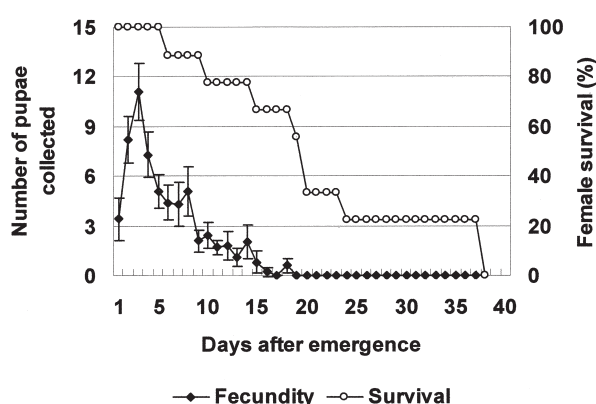
Fig. 1. Daily distribution of fecundity and percentage survival of female *N. okazakii* at 25 °C (mean± SE).
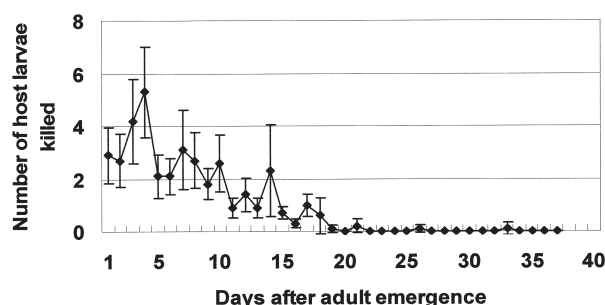
Fig. 2. Daily distribution of host mortality caused by non–reproductive killing of *N. okazakii* at 25 °C (mean± SE).

Table 3. Hind tibial length and sex ratio of offspring *N. okazakii* at 25 °C (Mean± SE)

	Hind tibial length (mm)	Sex ratio (% males)
Male	0.209± 0.0028a	27.8± 2.84*
Female	0.291± 0.0037b	

Mean with the same letter within the same column was not significantly different by t–test, t 17.72, n 200, P 0.00001
* (Binomial test, n 524, P 0.0001)

Hind tibial length and sex ratio

Hind tibial length of females was significantly longer than males (P 0.00001) (Table 3). The offspring sex ratio was female–biased (P 0.0001) (Table 3).

Rate of population increase

The population growth of *N. okazakii* at 25 °C was shown by the intrinsic rate of increase value (r_m) of 0.219 day^{-1}. Mean generation time (T) was 17.7 days. Mean net reproductive rate (Ro) was 40.3.

DISCUSSION

Among the members of *Neochrysocharis*, *N. formosa* has been reared on *L. trifolii* in the laboratory (Maryana, 2000, Tran *et al.*, 2004; Hondo *et al.*, 2006). However, no data on the developmental biology of *N. okazakii* on *Liriomyza* leafminers are available. This is the first report about rearing of *N. okazakii* on *L. chinensis*. The present study indicated that total developmental time from egg to adult emergence of *N. okazakii* was about 12 days. The development is faster than that recorded for *N. formosa* on *L. trifolii* (Maryana, 2000; Hondo *et al.*, 2006).

Male *N. okazakii* developed faster and hence emerged earlier than females did. This phenomenon is called protandry, and protandry is known for *N. formosa* (Maryana, 2000) and other eulophid parasitoids reared on *L. trifolii* (e.g. Minkenberg, 1990, Ho and Ueno, 2002; Bazoocchi *et al.*, 2003; Hondo *et al.*, 2006).

The females produced a mean of 61.8 offspring pupae during an average lifespan of 20.1 days. The fecundity of *N. okazakii* on *L. chinensis* was apparently smaller than other parasitoids of various leafminers

(Minkenberg, 1990; Maryana, 2000), but our results could have been influenced by the experimental manipulation (e.g. dissection, placement), since parasitoid larvae could not pupated due to hatching mortality. Therefore, the realizable fecundity of *N. okazakii* females could be probably higher. The average of net reproduction rate (Ro) suggests that *N. okazakii* population would increase 40.3 times during each generation. The average intrinsic rate of natural increase (r_m) was 0.219 per individual per day at 25 °C. Although such data are necessary to predict the reproductive potential of *N. okazakii* population in greenhouses or open fields, further experiments at different temperature are required, since the net reproductive rates of various parasitoids of leafminers were variable with temperature (Minkenberg, 1990; Hondo *et al.*, 2006).

Non–reproductive killing behavior of various parasitoids of leafminers includes host feeding and host stinging without oviposition (Bernardo *et al.*, 2006). Our results showed that host mortality by adult females without parasitization was a mean of 36.4 larvae, accounting for 37.1% of total host mortality caused by the females. For synovigenic parasitoids, there is a tight link between feeding by adults and subsequent reproduction. Since host blood is superior as a source of proteinaceous material, essential vitamins and salts for egg development, and a valuable source of nutrition for maintaining metabolism in host–feeding species, host–feeding parasitoids generally have reduced life time fecundity and longevity without host–feeding (Lervis and Kidd, 1986; Heimpel and Collier, 1996). In our experiments, only mature host larvae were used both for ovipositing and host–feeding. Since greater consumption of younger instars may be expected simply because they provide less source to host–feeding female parasitoids, the mortality by host–feeding in nature habitats could be probably higher (Bernardo *et al.*, 2006).

Host–stinging behavior has been frequently observed in parasitoids of leafminers (Heinz and Parrella, 1989; Patel and Schuster, 1991; Patel *et al.*, 2003; Bernardo *et al.*, 2006). Proportion of stung host varied depending on the host size distribution (Heinz and Parrella, 1989), host density (Patel *et al.*, 2003) and temperature (Patel and Schuster, 1991). The hosts stung may be a mechanism for limiting the density of leafminer larvae on invididual leaves, thus ensuring the parasitized larvae will not be lost due to the leafmining of survival, non–parasitized larvae on the same leaflet (Patel *et al.*, 2003). Although there is no individual data on host–stinging and host–feeding of *N. okazakii* on *L. chinensis*, total hosts killed by parasitoid feeding and stinging without oviposition should also be considered as a source of mortality due to the parasitoid.

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