

AVOIDANCE OF MULTIPLE OVIPOSITION IN *METEORUS PULCHRICORNIS* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

Meteorus pulchricornis is a solitary endoparasitic koinobiont of free-living lepidopteran larvae. To test the avoidance of multiple oviposition, 20 individuals of 2nd instar larvae of *Spodoptera litura* were put together with a young or old female wasp for 24 hours for oviposition. We examined the distribution pattern of eggs deposited in twenty hosts after 24 hours oviposition in order to test the presence of host discrimination by female wasps to avoid multiple oviposition to the larvae of *S. litura*. The standardized Morisita index of dispersion (I_p) for the number of eggs in a host larva was almost always below zero, and the 95% confidence intervals of the means were significantly lower than zero for both 1–2-day-old ($F = 2.10$, $df = 5$, 14 ; $P = 0.07$) and 10-day-old females ($F = 2.07$; $df = 4$, 9 ; $P = 0.10$), indicating that multiple oviposition tends to be avoided, although it is not completely.

1. Introduction

In solitary endoparasitoids, superparasitism causes the elimination of excessive larvae and wastes offspring, especially in self-superparasitism (Volk and Mackauer, 1990). Superparasitism can be advantageous when eggs from later ovipositions have a chance to outcompete older individuals (van Dijken and Waage, 1987; Bai and Mackauer, 1990; van Alphen and Visser, 1990; van Alphen et al., 1992; Chau and Maeto, 2008), but unparasitized hosts always provide the highest chance of offspring development and are preferred for oviposition (van Alphen and Visser, 1990). Therefore, most solitary parasitoids have abilities for host discrimination to distinguish unparasitized from parasitized hosts, reducing the frequency of wasted eggs, the time spent searching, and the mortality risk of offspring (van Lenteren, 1976, 1981).

To differentiate unparasitized hosts from parasitized ones, insect parasitoids use various

mechanisms, including chemical (Vinson & Guillot, 1972; Hubbard et al., 1987; McBrien & Mackauer, 1991; Ueno & Tanaka, 1996) and physical markers (Takasu and Hirose, 1988). For examples, cornicle secretion of the pea aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae), stimulated oviposition behavior of the endoparasitoid *Aphidius ervi* (Hymenoptera: Braconidae) (Battaglia et al., 1993). A hymenopteran parasitoid *Pteromalus cerealellae* (Pteromalidae), responds to the host-specific chemical cues released from *Callosobruchus maculatus* (Coleoptera: Bruchidae) for host location (Mbata et al., 2004). Also, plants can produce chemicals to mediate the habitat location in parasitic wasps. Indeed, oviposition by *Pieris brassicae* (Lepidoptera: Pieridae) on host plants induces a change in chemical component on the surface of leaves, arresting the egg parasitoid *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) (Fatouros et al., 2004). A study in the tachinid fly *Exorista japonica* has showed that the length, diameter, and velocity

of dummy hosts affect the ovipositional behavior (Yamawaki and Kainoh, 2005). The combination of visual and chemical cues was important in the dipteran parasitoid *Apocephalus paraponerae* (Phoridae), where visual cues help to recognize the body size of ants and species-specific cuticular hydrocarbons for oviposition (Morehead and Feener, 2000). In addition, host discrimination is affected by the time interval between ovipositions (Chow and Mackauer, 1986; Ueno, 1999; Islam and Copland, 2000; Outremen et al., 2001) or by a wasp's previous experience with ovipositions to unparasitized hosts (van Lenteren and Bakker, 1975; van Lenteren, 1981; Klomp et al., 1980; van Alphen and Visser, 1990). Recent studies have indicated that individual-specific chemical marks play an essential role in distinguishing self-parasitized hosts from conspecific parasitized hosts (Hubbard et al., 1987; Volk and Mackauer, 1990; van Dijken et al., 1992; Visser, 1993). Despite that considerable effort has been spent to understand the bionomics and biology of *M. pulchricornis* as a potential biocontrol agent against lepidopteran pests (Askari et al., 1977; Fuester et al., 1993; Nguyen et al., 2005; Liu and Li, 2006; Suzuki and Tanaka, 2006, 2007; Chau and Maeto, 2008; Heping et al., 2008; Shirai and Maeto, in press), the abilities and mechanisms of host discrimination have not been investigated for this species. Therefore, the purpose of this study was to test the ability for self-host discrimination of *M. pulchricornis* from study the distribution pattern of eggs deposited in host larvae.

2. Materials and Methods

2.1. Parasitoid

A uniparental strain of *Meteorus pulchricornis* that originated from Shikoku, Japan, was maintained on *Spodoptera litura* (Noctuidae) in the laboratory at 25°C and a 16L: 8D light regimen. Wasps were fed with cotton saturated in a 20% honey solution. Newly emerged 1–2-day-old and 10-day-old female wasps lacking oviposition experience were used for experiment.

2.2. Host

Spodoptera litura was reared with an artificial diet (Nihon-Nosan-Kogyo, Insecta LFS) at 25°C and a 16L: 8D photoperiod. Second-instar larvae (5–6 days old after hatching) were provided for oviposition in the experiment.

2.3. Experiment: Self-host discrimination in *M. pulchricornis*

In the first trial, 20 unparasitized hosts were introduced with an inexperienced female in a transparent plastic container (BD Biosciences, NH 52; 52 mm in diameter, 10 mm in height) with pieces of artificial diet (Insecta LFS) and left for 24 h at 25°C and 16L:8D light regimen. Then, the female and hosts were separated. All 20 hosts were dissected under a binocular microscope to count the number of eggs in each host. The female was fed with cotton saturated in a 20% honey solution for the next 24 h, after which a subsequent trial commenced with another 20 unparasitized hosts. In total, each 1–2-day-old female was tested during six trials and each 10-day-old female was tested during five trials. Fifteen 1–2-day-old females and ten 10-day-old females were tested

The experiments were carried out at Laboratory of Insect Biodiversity and Ecosystem Science, Kobe, Japan.

2.4. Data analysis

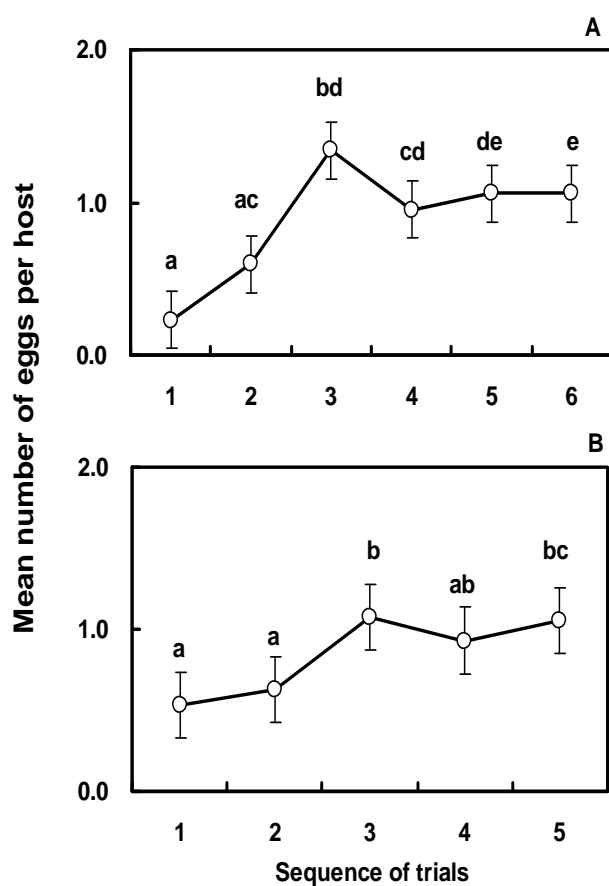
For each trial with every female, the standardized Morisita index of dispersion (I_p) was calculated (Smith-Gill, 1975; Krebs, 1999). I_p ranges from -1.0 to 1.0 ; random patterns give an I_p of zero, positive values result from clumped patterns, and uniform patterns produce negative values. The 95% confidence interval of the random distribution ranges from -0.5 to 0.5 (Smith-Gill, 1975). The mean number of eggs per host and I_p were compared between trials using a one-way ANOVA with a random factor (wasp individuals) followed by the estimation of 95% confidence intervals for the means, and Tukey's honestly significant differences (HSD) test for multiple comparisons.

Software. Statistical analyses were performed with SPSS Ver. 12 (SPSS Inc., Chicago, IL).

3. Results

Superparasitism was observed with at least one host in all cases (140), and up to five eggs were deposited in a single host. The mean number of eggs per host was significantly different between trials for both 1–2-day-old females ($F = 17.92$, $df = 5, 14$; $P < 0.001$) and 10-day-old females ($F = 6.11$; $df = 4, 9$; $P = 0.001$), with the mean number of eggs increasing to approximately 1.0 by the third trial (**Fig. 1**). The standardized Morisita index of dispersion (I_p) was generally negative and

Figure 1. Mean number of eggs per host in sequential trials with 1–2-day-old (A) or 10-day-old (B) females. Error bars indicate 95% confidence intervals. Different letters indicate significantly different means according to a Tukey's honestly significant differences (HSD) test of multiple comparisons ($P < 0.05$).



fell below -0.5 (95% confidence limit of random patterns according to Smith-Gill, 1975) in 25% (21/84; 1–2-day-old females) and 22% (11/50; 10-day-old females) of the cases, indicating a weak but distinct tendency toward a uniform distribution of eggs (**Fig. 2**). Besides, **Fig. 3** shows three examples of egg distributions that differ from a random pattern (A) to a uniform pattern (C) when the mean number of eggs per host is 1.0 for 1–2-day-old females. The index of dispersion (I_p) was not significantly different between trials, both for 1–2-day-old females ($F = 2.10$, $df = 5, 14$; $P = 0.07$) and 10-day-old females ($F = 2.07$; $df = 4, 9$; $P = 0.10$), with the 95% confidence limits of the means always falling below zero (**Fig. 4**).

Figure 2. Relationship between the standardized Morisita index of dispersion (I_p) and the mean number of eggs per host in 1–2-day-old (A) and 10-day-old (B) females. I_p values between -0.5 and 0.5 fall within the 95% confidence limit of a random distribution pattern (Smith-Gill, 1975).

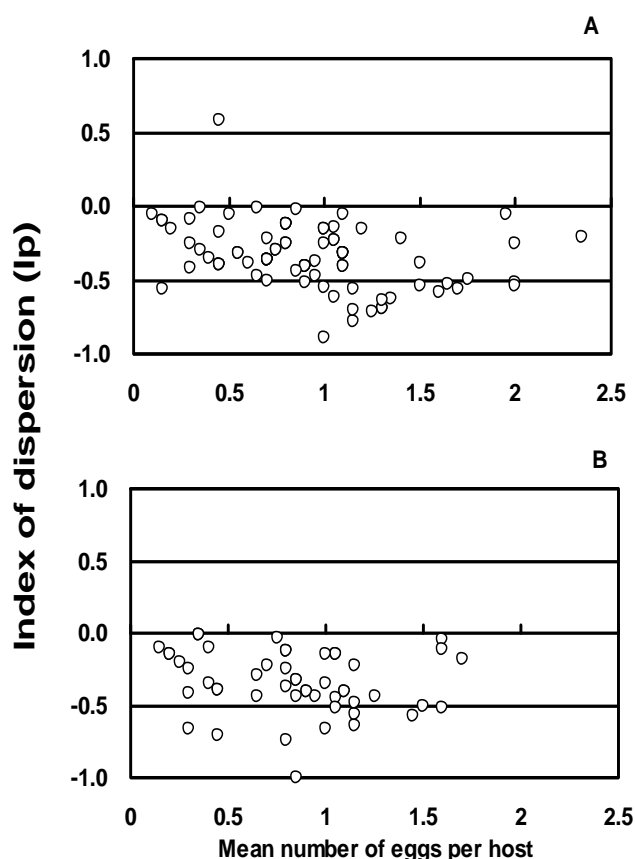


Figure 3. Three examples of egg distributions from a random pattern (A) to a uniform pattern (C) when the mean number of eggs per host is 1.0 for 1–2-day-old females.

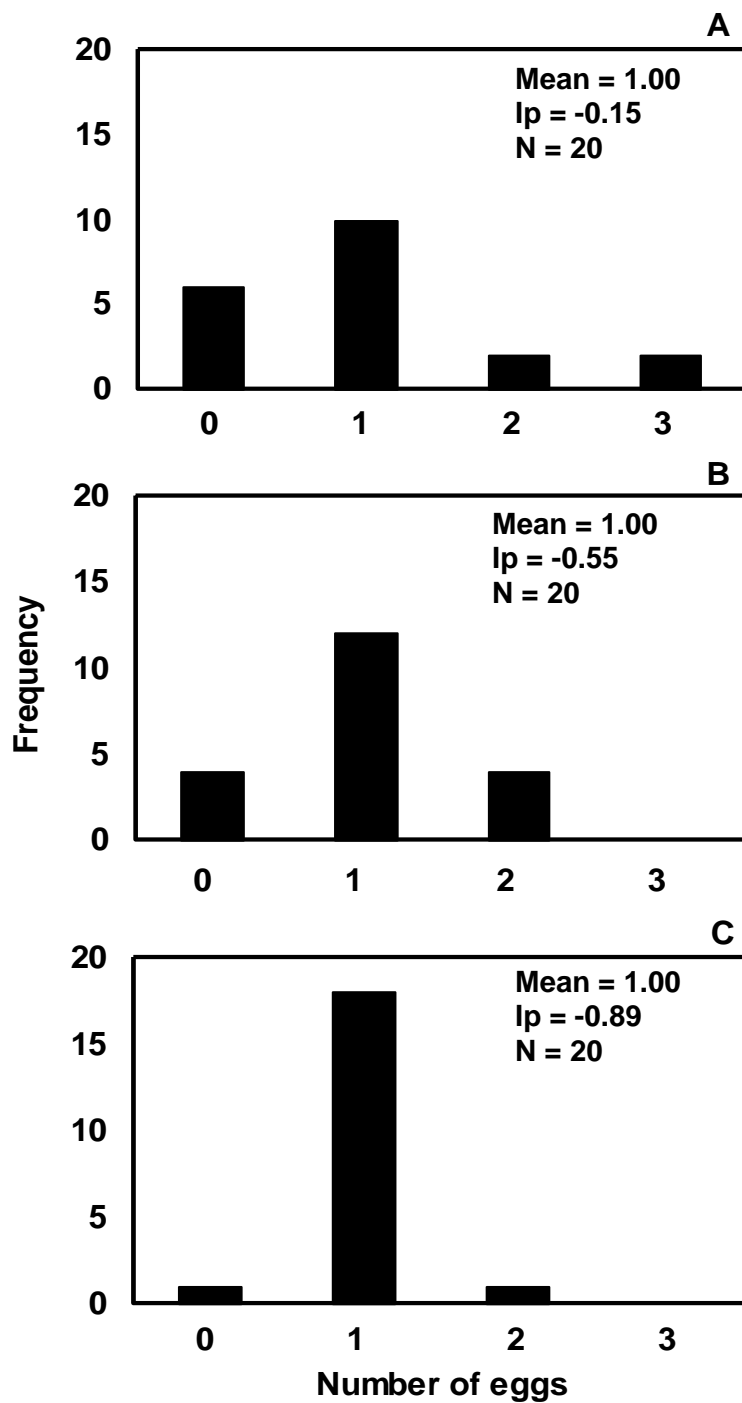
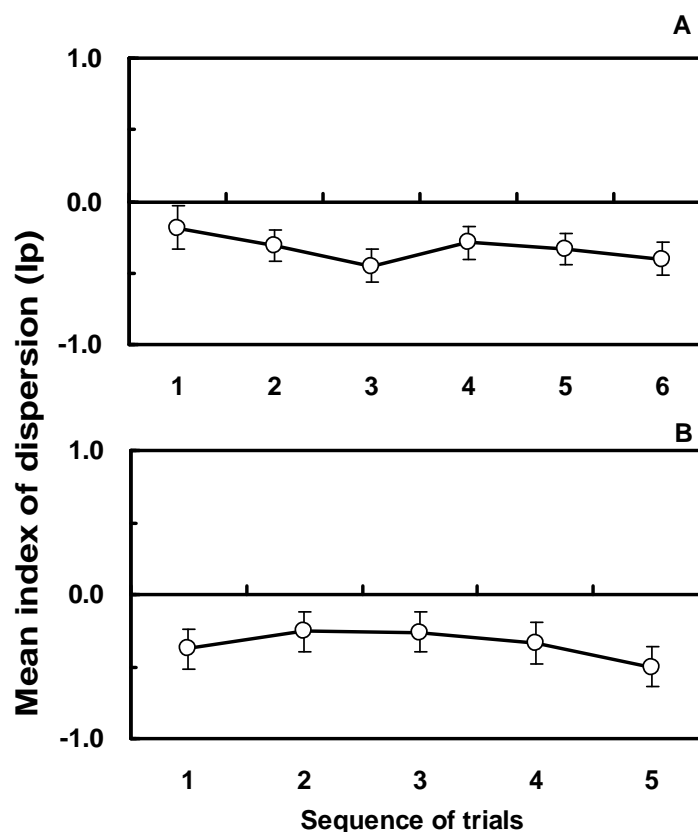


Figure 4. Mean of the standardized Morisita index of dispersion (I_p) in sequential trials with 1–2-day-old (A) or 10-day-old (B) females. Error bars indicate 95% confidence intervals.



4. Discussion

As is expected for solitary parasitoids, multiple oviposition can be avoided, although not completely in *M. pulchricornis* because the egg distribution tended to be uniform in the experiment.

Many studies of host discrimination have shown that inexperienced females often cannot distinguish between parasitized and unparasitized hosts (van Lenteren, 1981; Potting et al., 1997; Santolamazza-Carbone et al., 2004). Other factors such as host density and egg load also affect the intensity of host discrimination (Miura et al., 1994; Outreman et al., 2001; Mohammad et al., 2005), and superparasitism can be adaptive when unparasitized hosts are comparatively rare (Bakker et al., 1985; Visser et al., 1992; Hubbard et al., 1999).

Results show that the index of dispersion (I_p) for egg distribution did not change in

sequent trials for either young or old females, ruling out the possibility that aging and oviposition experience play an essential role in host discrimination for this species, although an exceptionally highly clumped egg distribution was caused by an inexperienced female (this occurred during the first trial of a 1–2-day-old female). Also, relative host density would not have an obvious effect on host discrimination because the distribution pattern of eggs did not change as the number of deposited eggs increased (i.e., the decline of relative host density to eggs).

We found that both naive and experienced female *M. pulchricornis* have the ability of self-host discrimination, but superparasitism was observed even when the number of eggs deposited was small. This may occur because the mechanism for host discrimination does not persist for long periods in this species.

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