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The parasitoid *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae) prefers hosts parasitized by conspecifics over unparasitized hosts

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Summary. Two laboratory experiments were conducted to examine the ovipositional preferences of the egg parasitoid *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) for parasitized and unparasitized *Megacopta punctatissimum* Montandon (Hemiptera: Plataspidae). Females that had never oviposited or that had not oviposited for 3 days preferred recently parasitized hosts more than unparasitized hosts. The preference for recently parasitized hosts appeared to be mediated by the punctures in already parasitized hosts made by the ovipositor of the first female. Survival of the parasitoid progeny was lower in recently parasitized hosts than in unparasitized hosts. However, handling time of parasitized hosts was extremely short relative to that of unparasitized hosts, because the superparasitizing female could use the punctures made by the previous females. It is concluded that the females preferred the parasitized hosts over unparasitized hosts because the benefit of saving time and energy for drilling was more than the cost of progeny survival.

Key words: *Ooencyrtus nezarae* – Parasitoid – Optimal foraging – Host discrimination – Handling time

Optimal foraging theory predicts that parasitoid females should exhibit preferences for the more profitable hosts (Charnov and Skinner 1985; Pak 1986). Although the females usually prefer unparasitized hosts to hosts parasitized by conspecifics (van Lenteren 1976, 1981), a preference for parasitized hosts could arise if parasitized hosts were more profitable. However, it has never been shown that parasitoid females have a preference for parasitized hosts over unparasitized hosts, except for aphelinids which hyperparasitize their own or another species (Flanders 1936, 1953).

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Ooencyrtus nezarae is an egg parasitoid of several phytophagous bugs including *Megacopta punctatissimum* in Japan (Takasu and Hirose 1985). Although this parasitoid is gregarious in a large host, such as *Riptortus clavatus* Thunberg, it is usually solitary in eggs of *M. punctatissimum*. In the course of a study on host discrimination by *O. nezarae* attacking *M. punctatissimum* eggs, we observed that parasitoid females that had never oviposited before or had not oviposited for a long time showed a preference for hosts parasitized recently by conspecifics over unparasitized hosts. In this paper, we describe this unusual behavior, and discuss its ecological significance.

Materials and methods

M. punctatissimum adults were collected from several communities of kudzu, *Pueraria lobata* (Willd.) near Fukuoka in April and May 1987. The bugs were kept in cages (22 × 16 × 20 cm) at 25° C and 16 L:8 D, and provided with kudzu vines. Pieces of polyester organdy were introduced into the cages every day, and *M. punctatissimum* laid their egg masses on these ovipositional substrates. *O. nezarae* females were obtained from a laboratory culture maintained at the Institute of Biological Control, Faculty of Agriculture, Kyushu University (Takasu and Hirose 1988).

The following two laboratory experiments were carried out at 25° C, using 3-day-old hosts and 4-day-old, mated parasitoid females. The first experiment was designed to determine whether *O. nezarae* females preferred unparasitized or parasitized eggs in a host egg mass. The interior of parasitized hosts changes after parasitism, and thus it was suspected that the behavior of parasitoid females toward parasitized hosts might also change with increasing host age. Thus, we examined the behavior of females on two types of host egg masses: Type A, an egg mass consisting of unparasitized eggs and eggs that had been parasitized 1 h previously; and Type B, an egg mass consisting of unparasitized eggs and eggs that had been parasitized 24 h previously. To make these two types of host egg masses, an egg mass consisting of 10 unparasitized eggs fastened with a vinyl-acetate glue on a piece of filter paper (3 × 2 cm) was exposed to a female in a test tube (20 × 3 cm). After the female had oviposited in 5 of the 10 eggs, the egg mass was removed and held at 25° C until use in an experiment. Oviposition was verified by the presence of egg stalks protruding from the host chorion (Takasu and Hirose 1988). To examine the behavior of females with different oviposition experience, egg masses of each type were presented to females that had: (1) not oviposited previously (inexperienced females), (2) oviposited 1 h previously into 3–5 unparasitized hosts, or (3) not oviposited for 3 days after oviposition into 3–5 unparasitized hosts. After a female encountered a host egg mass, her behavior toward parasitized and unparasitized eggs was observed until she laid 5 eggs. In the case of inexperienced females, time taken for each behavioral event during oviposition was also measured with a stop watch. For every combination of the three types of females and the two types of egg masses, 20 females were tested.

Because the available data indicated that inexperienced females or females that had not oviposited for 3 days preferred to oviposit in hosts parasitized 1 h earlier, a second experiment was designed to determine if their preference was due to the presence of oviposition punctures. Two unparasitized hosts that had been contiguous to each other were fastened on a piece of filter paper (1 × 1 cm). These two hosts were exposed to a female in a petri dish (6 cm in diameter) under the binocular microscope. *O. nezarae* females usually feed on host fluid exuded from punctures on the host chorion before laying their eggs into the hosts. When the female finished drilling one of the two hosts and began to feed on its fluid, she was removed. This host did not contain any parasitoid egg but had been punctured by the female's ovipositor. The piece of filter paper with punctured host and unparasitized host was exposed to an inexperienced female or a female that had not oviposited for 3 days. Her behavior toward these two types of hosts was observed until she oviposited in one of the two. For each of the two types of females, 15 females were tested.

Results

On encountering host egg masses of type A in the first experiment, inexperienced females and females that had not oviposited for 3 days oviposited significantly more frequently in parasitized hosts than in unparasitized hosts (Table 1). Immediately after encountering host egg masses, the female mounted the egg masses and walked over several eggs while drumming. When a female came into contact with a parasitized host in the course of drumming, she usually stopped and drummed the host surface near the protruding egg stalks for 1–3 sec. She then inserted her ovipositor into the puncture made by the previous female and fed on the fluid of the parasitized host. She then laid an egg in the host through the puncture.

In contrast, when a female that had laid 1 h previously encountered the egg masses of type A, she usually rejected the parasitized hosts, and oviposited significantly more frequently in unparasitized hosts (Table 1). Before rejecting the parasitized hosts, she drummed the stalks or the host surface near the stalks for a few seconds.

On encountering the egg masses of type B, inexperienced females and females that had not oviposited for 3 days oviposited in unparasitized hosts as often as in parasitized hosts. For both types of females, there was no significant difference in the number of parasitized and unparasitized hosts attacked (Table 1). When a female oviposited in the 24-h parasitized host, she did not use the punctures made by previous females. Instead she drilled the hosts in the same manner as unparasitized hosts. When females that had oviposited 1 h previously encountered the egg masses of type B, they rejected 24-h parasitized hosts and oviposited significantly more frequently in unparasitized hosts (Table 1).

To determine whether females changed their ovipositional preference after oviposition on the egg masses, the host preference of females with oviposition experience on type A or B egg masses was examined (Tables 2, 3). These two tables include only data from 10 or more females in each type of oviposition experience, because the data from less than 10 females could not be statistically analyzed. On egg masses of type A, after ovipositing in one parasitized host, inexperienced females often oviposited in parasitized hosts. The frequency of oviposition in parasitized hosts by this type of female differed significantly from the frequency that would have been expected if the wasps had no ovipositional preference for unparasitized hosts or parasitized hosts. However, after ovipositing in two parasitized hosts, this type of female oviposited in parasitized hosts significantly less frequently than would have been expected if they had no preference for any type of host (Table 2). Females that had not oviposited for 3 days at the start of the experiment also sometimes rejected parasitized hosts after ovipositing in one parasitized host. The frequency of oviposition in parasitized hosts by this type of female did not differ significantly from the frequency that would have been expected if they had no preference for any type of host (Table 2).

On the egg masses of type B, inexperienced females often rejected parasitized hosts after ovipositing in one parasitized host. The frequency of oviposition in parasitized hosts by this type of female differed significantly

Table 1. Ovipositional preference for unparasitized and parasitized hosts by parasitoid females in two egg mass types: A, an egg mass consisting of unparasitized eggs and eggs that had been parasitized 1 h previously; and B, an egg mass consisting of unparasitized eggs and eggs that had been parasitized 24 h previously

Type of females	Type of hosts	No. females that first oviposited in unparasitized or parasitized hosts on a host egg mass of	
		Type A	Type B
Inexperienced	Unparasitized	3[a]	8[a]
	Parasitized	17[b]	12[a]
Oviposited 1 h previously	Unparasitized	16[a]	19[a]
	Parasitized	4[b]	1[b]
Not oviposited for 3 days	Unparasitized	4[a]	11[a]
	Parasitized	16[b]	9[a]

[a, b] For each type of female, figures followed by different superscript letters in the same column differed significantly ($P<0.05$); χ^2

Table 2. The effect of oviposition experience of parasitoid females on their host preference on egg masses of type A

Type of females at start of experiment	Oviposition experience during experiment on egg masses[a]	No. of females examined	% females ovipositing in parasitized hosts	
			Observed	Expected[b]
Inexperienced	P	17	76.5*	50.0
	P–P	13	15.4*	50.0
Oviposited 1 h previously	U	16	6.2*	60.0
	U–U	15	0*	70.0
Not oviposited for 3 days	P	10	41.2	50.0

[a] P and U show oviposition in a parasitized and an unparasitized host, respectively. P–P and U–U show successive oviposition in two parasitized and two unparasitized hosts, respectively
[b] Percentages expected if parasitoid females had no ovipositional preference for unparasitized or parasitized hosts
* Significantly ($P<0.05$) different from expected percentages, χ^2

Table 3. The effect of oviposition experience of parasitoid females on their host preference on egg masses of type B

Type of females at start of experiment	Oviposition experience during experiment on egg masses[a]	No. of females examined	% females ovipositing in parasitized hosts	
			Observed	Expected[b]
Inexperienced	P	12	25.0*	50.0
Oviposited 1 h previously	U	19	10.5*	60.0
	U–U	17	0*	70.0
Not oviposited for 3 days	U	14	7.7*	60.0
	U–U	13	0*	70.0

[a, b] see Table 2
* Significantly ($P<0.05$) different from expected percentages, χ^2

Table 4. Mean time taken for each behavioral event of an inexperienced parasitoid female attacking a host

Type of hosts	No. of hosts examined	Mean time (sec) taken for indicated behavioral events*				
		Drumming	Drilling	Host feeding	Ovipositing	Total
Unparasitized	30	51.5[a]	624.6[a]	385.3[a]	94.0[a]	1159.3[a]
Parasitized 1 h previously	18	25.0[b]	42.6[b]	361.2[a]	111.0[a]	539.8[b]
Parasitized 24 h previously	10	43.2[a]	561.3[a]	330.7[a]	98.4[a]	1033.6[a]

* Figures followed by the different superscript letters in the same column differed significantly ($P<0.05$) by Kruskal-Wallis test

from what would have been expected if they had no preference for any type of host (Table 3). Similar findings were obtained from females that had oviposited 1 h previously and females that had not oviposited for 3 days (Table 3).

The mean time for drumming or drilling by inexperienced females was significantly shorter in hosts that were parasitized 1 h previously than in unparasitized hosts or hosts parasitized 24 h previously, because the female inserted her ovipositor through the puncture made by a previous female. As a result, the mean time for oviposition by an inexperienced female on a host parasitized 1 h previously was significantly shorter than that for unparasitized hosts or hosts parasitized 24 h previously (Table 4).

When inexperienced females were provided with a host with a puncture made by a previous female and an unparasitized host in the second experiment, 14 of the 15 females first oviposited in the hosts with the punctures. In the case of females that had not oviposited for 3 days, 13 of the 15 females also oviposited in punctured hosts. For both types of females, there was a significant difference ($P<0.01$, χ^2 test) in the number of ovipositing females between the two types of hosts. On encountering the two types of hosts, both types of females mounted and drummed the hosts before they discovered the punctures. Then, they fed on host fluid exuded from the punctures and thereafter oviposited in the hosts in the same manner as in recently parasitized hosts.

Discussion

In the first experiment, an inexperienced female or a female that had not oviposited for 3 days preferred to oviposit in recently parasitized hosts over unparasitized hosts immediately after encountering an egg mass consisting of both host types. Unmated females of certain aphelinid species oviposit only in hosts parasitized by its own or another species (Flanders 1936, 1953). The unmated females of these species lay only male eggs in the parasitized hosts, because males can develop only as hyperparasitoids of the larvae of their own or another species. Except for such hyperparasitoids, this is the first report of a parasitoid preferring to oviposit in parasitized hosts over unparasitized hosts.

The preference for parasitized hosts by *O. nezarae* females is a result of a laboratory experiment, but it is probably shown in the field. *M. punctatissimum* females usually lay egg masses consisting of 20–50 eggs. In the laboratory, *O. nezarae* females each leave the egg mass consisting of 20 or more eggs before they completely oviposit in all the eggs in it (Takasu and Hirose in prep.). Thus, in the field the females are more likely to encounter host egg masses consisting of unparasitized and parasitized eggs.

In the second experiment, inexperienced females or females that had not oviposited for 3 days preferred hosts with punctures from previously attempted oviposition. This result suggests that the preference for recently parasitized hosts by these two types of females is due to the presence of punctures made by previous females on the parasitized hosts. Such a hypothesis is also supported by the fact that in the first experiment, inexperienced females or females that had not oviposited for 3 days showed no ovipositional preference for hosts 24 h after parasitism. They could not use the punctures in ovipositing on the hosts 24 h after parasitism, probably because the punctures on the parasitized hosts are sealed up by the exudate from the punctures. It was observed that host fluid exudes from the punctures after oviposition.

Inexperienced females at the start of the experiment ceased preferring to oviposit in recently parasitized hosts after ovipositing in two parasitized hosts. Females that had not oviposited for 3 days at the start of the experiment also did so after oviposition in one parasitized host. The loss of the preference for parasitized hosts by these females is because they discriminate between parasitized and unparasitized hosts on the basis of a stalk protruding from host chorion (Takasu and Hirose 1988). Shortly after oviposition in unparasitized hosts, *O. nezarae* females reject parasitized hosts though the inexperienced females readily oviposited in parasitized hosts. In the present study, after inexperienced females or females that had not oviposited for 3 days had oviposited in two or one parasitized hosts, respectively, they also tended to reject parasitized hosts. This suggests that females that have oviposited in parasitized hosts also discriminate between parasitized and unparasitized hosts due to the stalk.

Although solitary parasitoids should avoid ovipositing in parasitized hosts containing their own eggs, they could benefit by ovipositing in parasitized hosts containing other female's eggs under certain conditions (van Alphen and Nell 1982; Bakker et al. 1985). A recently parasitized host which an inexperienced female first encounters clearly contains other females' eggs. Likewise, for females that have not oviposited for a long time, recently parasitized hosts could be hosts containing other females' eggs. Optimal foraging theory predicts that parasitoids should have a preference for the more profitable hosts to maximize the rate of gain of fitness with time (Charnov and Skinner 1985). Since these recently parasitized hosts are more profitable for inexperienced females and females that had not oviposited for 3 days than unparasitized hosts, these two types of females might prefer the recently parasitized hosts. Profitability of a host for parasitoids depends on the number and quality of parasitoid progeny that host sustains and handling time of the parasitoids (Pak 1986). For *O. nezarae* females, profitability of a *M. punctatissimum* egg mainly depends on survival of parasitoid progeny and the handling time associated with oviposition. An adult of *O. nezarae* usually emerges from a host egg even if two or more parasitoid eggs are laid, and the size of the emergent adults do not vary with number of parasitoid eggs laid per host (Takasu and Hirose unpublished). In *M. punctatissimum* eggs, survival of *O. nezarae* progeny is lower in recently parasitized hosts than in unparasitized hosts: 75% and 95% in hosts parasitized 1 h previously and unparasitized hosts respectively (Takasu and Hirose unpublished). However, handling time of *O. nezarae* females was much shorter on recently parasitized hosts than on unparasitized hosts. It took over 10 min for each female to drill the chorion of a *M. punctatissimum* egg (Table 4). This drilling time is much longer than that for other solitary egg parasitoids, such as *Allophanurus indicus* Subba Rao et Chacko (Subba Rao and Chacko 1961), *Telenomus heliothidis* Ashmead (Strand and Vinson 1983), *Trichogramma embryophagum* Htg. (Klomp et al. 1980) and *Trissolcus plautiae* (Watanabe) (Ohno 1987). The use of the punctures by *O. nezarae* females on parasitized hosts would save more time and energy for drilling. So, the benefit from a reduction in handling time could be more than the cost of progeny survival.

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