Superparasitism strategy in a semisolitary parasitoid with imperfect self/non-self recognition, *Echthrodelphax fairchildii*

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Abstract

To assess the adaptiveness of self- and conspecific superparasitism in the parasitoid *Echthrodelphax fairchildii* Perkins (Hymenoptera: Dryinidae), we measured the rate of superparasitism avoidance and fitness returns from superparasitism for different intervals between the first and second ovipositions. We also tested for any preference in oviposition side and whether any such preference was adaptive. The rate of superparasitism avoidance in both self- and conspecific superparasitism was about 10% for oviposition intervals of 2–8 h, and higher for intervals of 1 and ≥24 h (but did not exceed 35%). When conspecifically superparasitizing (but not self-superparasitizing), females exhibited a slight preference for the side without the first-comer. Under conspecific superparasitism, the survival rate of second comers was independent of the oviposition side and interval, but slightly lower than that of immature parasitoids for single parasitism. The adult size of second-comers on the side harboring the first-comer was larger than that of second-comers on the side without it, when the oviposition interval was <24 or 96 h. These results explained the overall low rate of avoidance of conspecific superparasitism, but not the variation in avoidance rate or the preference for side without the first-comer when conspecifically superparasitizing. Assuming that fitness returns are influenced more by offspring quantity than by their quality, self-superparasitism produced positive fitness returns only when the oviposition interval was <24 h and the side without the first-comer was selected. This suggests that the observed behavior was not adaptive. Possible reasons for the discrepancies between observed and optimal behaviors, including an imperfect ability for self/non-self recognition, are discussed.

Introduction

Superparasitism – ovipositing in or on hosts that are already parasitized by the same species (van Dijken & Waage, 1987) – is common in parasitoids (Salt, 1961; van Alphen & Visser, 1990; Godfray, 1994). Superparasitism is divided into self- and conspecific superparasitism: in the former the first- and second-comers – offspring originating from the first and second ovipositions, respectively – have the same mother, and in the latter the two comers have different mothers. According to optimal foraging theory (Stephens & Krebs, 1986; van Alphen & Visser, 1990; Godfray, 1994), when the second-comer has a non-zero chance of survival, conspecific

superparasitism can be adaptive when unparasitized hosts are scarce.

On the other hand, self-superparasitism for solitary parasitoids usually produces zero or negative fitness returns due to competition between siblings, with some exceptions (Waage, 1986; van Alphen & Visser, 1990; Visser et al., 1990; Rosenheim & Hongkham, 1996; Mackauer & Chau, 2001; Yamada & Sugaura, 2003). However, when the parasitoid is gregarious, self-superparasitism is likely to produce positive fitness returns because the total fitness returns from two ovipositions is likely to be higher than fitness returns from one oviposition on or in an unparasitized host (Vet et al., 1994; Gu et al., 2003).

When superparasitism occurs, the first- and second-comers have to compete for food resources, and the latter are usually at a disadvantage in the competition (Salt,

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Table 1 Decision-making by *Echthrodelphax fairchildii* predicted when encountering a parasitized host

Type of superparasitism	Oviposition interval of <24 h		Oviposition interval of ≥24 h	
	Superparasitism	Oviposition side	Superparasitism	Oviposition side
Self	Accept	Side without the first-comer	Avoid	–
Conspecific	Accept	Side with the first-comer	Accept	Side with the first-comer

1961; Vinson & Hegazi, 1998). Several studies (Strand & Godfray, 1989; Visser et al., 1992; Sirot, 1996; Field et al., 1997; Yamada & Ikawa, 2003) have shown that in the absence of infanticide the survival rate of the second-comers decreases as the interval between the first and second ovipositions increases, often dropping to zero when the oviposition interval is 1–2 days. Therefore, infanticide (including ovicide) is an effective method for overcoming the disadvantage of the second-comer and ensuring their survival (Mayhew, 1997; Yamada & Miyamoto, 1998; Yamada & Kitashiro, 2002; Yamada & Watanabe, 2002; Yamada & Ikawa, 2003), although few species are reported to perform infanticide (Godfray, 1994).

Host and self/non-self discrimination – the ability to distinguish between unparasitized and parasitized hosts, and between self-parasitized and conspecifically parasitized hosts, respectively – are favorable to optimal foraging by parasitoids, but many parasitoids either lack this ability partially or totally (Salt, 1961; van Lenteren, 1981; van Dijken et al., 1992; Godfray, 1994). Parasitoids with imperfect discrimination are expected to adopt different strategies from those adopted by parasitoids with perfect discrimination. Recent studies of behavioral strategies (Dukas, 1998; Flanagan et al., 1998; Shettleworth, 1998; Reece et al., 2004; Shuker & West, 2004; Thiel & Hoffmeister, 2004) have shown that investigating the capability and information constraints provides a better understanding of the evolutionary meaning of the behavioral strategies. However, few studies have addressed foraging strategies for parasitoids with imperfect host discrimination (Yamada, 1988; Rosenheim & Mangel, 1994; Outreman et al., 2001b) or with imperfect self/non-self recognition (Yamada & Ikawa, 2003). Yamada & Ikawa (2003) revealed that the imperfect self/non-self recognition of females of *Echthrodelphax fairchildii* Perkins (Hymenoptera: Dryinidae) explained the seemingly maladaptive frequencies of their infanticidal probing.

Echthrodelphax fairchildii is a semi-solitary ectoparasitoid of the following three rice-damaging planthoppers (Homoptera: Delphacidae) in Japan: *Nilaparvata lugens* (Stål), *Sogatella furcifera* (Horváth), and *Laodelphax striatellus* (Fallén). The female lays an egg in one oviposition, but two adults often emerge when superparasitism occurs (Yamada & Ikawa, 2003). She lays an egg between the forewing bud and the epimeron of the mesothorax (Yamada & Imai,

2000). When she grasps the head of the host with her left pincer and the abdomen with her right pincer, she lays an egg under the right wing bud, and vice versa. Hereafter, the side where the female parasitoid lays an egg is referred to as the oviposition side. Immature parasitoids are sedentary at the place of oviposition. About 1 week after an egg is laid, part of the immature parasitoid – called a larval sac – is visible to the naked eye on the surface of the host.

Yamada & Ikawa (2003) investigated the case in which the first and second eggs are laid on different sides, and found that two adults can emerge from a host for an oviposition interval of <24 h, although these adults are smaller than those for single parasitism. The female parasitoid often probes the non-oviposition side for infanticide, particularly for oviposition intervals of ≥24 h, but the infanticide is not always successful. In contrast, when the first and second ovipositions occur on the same side, the first-comer may be killed and the resulting adult is then expected to be as large as that for single parasitism. Therefore, the optimally parasitizing female is expected to make the decisions listed in Table 1 when encountering a parasitized host in an environment where unparasitized hosts are scarce. This study aimed to test these predictions for fitness returns and decision-making.

First, we describe how often female parasitoids accept parasitized hosts when unparasitized hosts are scarce, and which side they select when laying an egg. Comparing between self- and conspecific superparasitism, we assessed the ability for self/non-self recognition. Second, we reveal fitness returns from self- and conspecific superparasitism on the side harboring the first-comer and those from superparasitism on the side without the first-comer. Third, we reveal which of the first- and second-comers survives when the two are present on the same side. Finally, we discuss the adaptiveness of *E. fairchildii*'s superparasitism and oviposition side selection behavior, and suggest that the absence of self/non-self recognition is responsible for the discrepancy between observed and predicted avoidance rates.

Materials and methods

Insects

Echthrodelphax fairchildii and *L. striatellus* were collected in 1992 from Tsu, Mie, Japan, and reared continuously

in the laboratory. The female parasitoids used were aged 3–20 days. The daily fecundity is 15–25 when female parasitoids are 3–20 days old, and the handling time for oviposition is <1 min even when infanticidal probing occurs (Y.Y. Yamada and E. Ito, unpubl.), and hence the cost for ovipositing appears to be negligibly low.

Superparasitism bouts and estimation of fitness returns from superparasitism

A detailed description of the experimental protocol is available elsewhere (Yamada & Ikawa, 2003), and only an outline is provided here. Experiments were conducted using unmated females, which ensured that all the offspring were male. Unmated females were used because the high degree of sexual dimorphism in this species may influence the competition results. We reared female parasitoids individually in a small cage after their emergence and provided them with first-, second-, and third-instar planthoppers and diluted honey absorbed into cotton wool for food; thus, the females were reared in an environment with a low supply of hosts because they do not usually parasitize these early instar hosts.

The first egg in a superparasitism bout was laid into a fifth-instar larva that was within 24 h of molting. The oviposition interval for superparasitism was assigned to one of seven values: 1, 2, 4, 8, 24, 48, or 96 h. In conspecific superparasitism, female parasitoids were paired and one of each pair was allowed to superparasitize the host that had been parasitized by the other, and the latter was also allowed to superparasitize the host parasitized by the former. For each superparasitism bout, one parasitoid was kept in a clean 10 ml glass vial for 1 h before a parasitized host was added. Moreover, in order to compare fitness returns from superparasitism with those from single parasitism (i.e., ovipositing on an unparasitized host), we let each of the parasitoids parasitize some hosts singly and reared these hosts individually; 302 hosts were treated in this way. We observed the oviposition behavior under a fiber-optic illuminator through a binocular microscope, recording the side on which an egg was laid. We stopped the observation when the female parasitoid did not superparasitize the host within 10 min; these cases are hereafter called superparasitism avoidance. In some bouts, the female parasitoids fed on hosts. These cases are called feeding and are categorized into superparasitism avoidance, because when feeding occurred, superparasitism never took place. In 135 cases of superparasitism avoidance, an unparasitized host was added to ensure that the parasitoids had some mature eggs to lay. In these cases they always laid eggs, and so in the remaining cases of superparasitism avoidance this procedure was skipped. Parasitoids failed to catch the host in two of the superparasitism bouts, and

these data were discarded. Data from 61 superparasitism bouts in which the parasitoids showed no interest in hosts (i.e., did not search for them) were also discarded. We usually allowed each parasitoid to superparasitize once or twice per day and to perform superparasitism bouts a few times for each oviposition interval. Using 103 female parasitoids, we consequently obtained data from 80 to 135 superparasitism bouts for each of the seven oviposition intervals.

We reared parasitized and superparasitized hosts individually in an incubator at 24–26 °C, 40–50% r.h., and L16:D8. The appearance of larval sacs was recorded. After the parasitoids emerged, we measured their head width using an ocular micrometer (under 65× magnification).

Fitness returns from conspecific superparasitism were assessed on the basis of the survival rate to adult emergence, developmental time from ovipositing to adult emergence, and adult body size (head width) for the second-comers. We can estimate fitness returns for self-superparasitism by subtracting the fitness returns for single parasitism from fitness returns for two ovipositions on the same host, which were assessed by the sum of the survival rates, the mean developmental time, and the mean head width for the first- and second-comers. Thus, to compare fitness returns from self-superparasitism between the cases in which the first and second eggs were laid on the same side and the cases in which the two eggs were laid on different sides, we compared fitness returns from two ovipositions on the same host between these two cases. When the first and second eggs were laid on the same side, the second egg was assumed to always win the competition because observation of superparasitizing behavior and the identification of the winner showed this to be the case.

Identification of the winner

Observation of superparasitizing behavior in the above experiment suggested that female parasitoids killed the first-comer in cases where two eggs were laid in the same position. We confirmed this inference on the basis of the egg period of the parasitoid.

We used unmated female parasitoids to eliminate possible sex-related variations in the egg period. We let parasitoids perform self-superparasitism 8 and 24 h after the first oviposition. Experiments were only conducted for self-superparasitism because infanticide was expected to occur more frequently in conspecific superparasitism than in self-superparasitism (Yamada & Ikawa, 2003).

Since we did not determine the egg period due to the difficulty of identifying the egg stage of parasitoids under the wing buds, we observed (through a binocular microscope) the changes in the tip of the parasitoid egg that were visible when the wing bud was lifted with a pin. A change in the

tip from horn shaped to round was found, which suggested the occurrence of egg hatching. Thus, we determined the time span from oviposition to the change in the shape of the egg tip under single parasitism by observing hosts every 30 min at 24–26 °C, 40–50% r. h., and under L16:D8. The time span is hereafter referred to as the apparent egg period.

The apparent egg period was 59.5–65.5 h (see Results); we therefore examined eggs 66 and 74 h after the first oviposition for superparasitism with an 8 h oviposition interval, and 66 and 90 h after the first oviposition for superparasitism with a 24 h oviposition interval. If the shape of the egg tip had changed 66 h after the first oviposition, the first-comer was considered to have survived; if it had not changed, the second-comer was considered to have survived.

Data analysis

The effects of the type of superparasitism (self- or conspecific) and the oviposition interval on the rate of superparasitism avoidance and the preferred oviposition sides were analyzed using a log-linear analysis (see Sokal & Rohlf, 1981). This analysis determined the significance of an interaction based on the significance of the difference between the G-value corresponding to the goodness-of-fit test for the model including the interaction and that for the model excluding the interaction. When oviposition intervals had a significant effect, we tested for homogeneity in order to divide the data into homogeneous sets in which there was no significant difference among samples (Sokal & Rohlf, 1981). Moreover, we examined statistically whether female parasitoids exhibited any preference towards oviposition side by calculating the probability of the occurrence of the observed and lower frequencies whilst assuming that a random choice was made (binomial test); in particular, we examined whether the superparasitizing parasitoids preferred the side with or without the first-comer.

Fitness returns from superparasitism on the side harboring the first-comer were compared with those from superparasitism on the side without it. These two fitness returns were then each compared with fitness returns from single parasitism. When analyzing the survival rate of second-comers under conspecific superparasitism, we used log-linear model analysis for the effect of oviposition side and interval, and Fisher's exact test for comparison with single parasitism. When analyzing head width, developmental time, and the total survival rate of first- and second-comers (i.e., the number of adults emerging per host), we used a Mann–Whitney U-test. Significance was tested using the sequential Bonferroni multiple-comparison procedure (Rice, 1989). ANOVA and t-test were not used due to a lack of normality and/or equal variance.
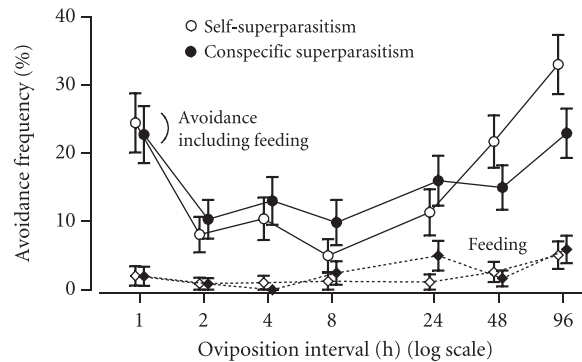

Figure 1 Frequency (mean ± SEM) of avoidance, including feeding, and feeding for different oviposition intervals in *Echthrodelphax fairchildii*. When feeding occurred, superparasitism did not occur.

Results

Superparasitism avoidance

The rate of superparasitism avoidance, including feeding, was about 10% for oviposition intervals of 2–8 h (Figure 1), while it was higher for oviposition intervals of 1 and ≥24 h, in many cases >20% (but it did not exceed 35%). Log-linear model analysis showed that oviposition intervals significantly influenced the avoidance rate, but there was no significant difference between self- and conspecific superparasitism (Table 2). Therefore, the combined values for self- and conspecific superparasitism were used in tests of homogeneity. The avoidance rate for a 96-h oviposition interval was significantly different from that for oviposition intervals of 2–24 h, and the avoidance rate for a 1 h oviposition interval was significantly different from avoidance rates for oviposition intervals of 2 and 8 h. The feeding rate was less than 6%, and was particularly low for intervals of ≤8 h. When feeding occurred, the host died immediately or within a few days. Log-linear model analysis of the feeding rate revealed no difference between self- and conspecific superparasitism, but showed that the oviposition interval was a significant factor (P<0.05; analysis table not presented). The feeding rate for a 96 h oviposition interval was relatively high, but the homogeneity test did not reveal a significant difference between any pairs of samples.

Oviposition side

The proportion of oviposition bouts in which female parasitoids selected the right side for single oviposition was 48.2% (n = 1511), which was not significantly different from 50% (binomial test, P>0.15). Considering that the females did not attempt to change oviposition side when and after catching the host, this indicates that the females selected the oviposition side at random when ovipositing

Table 2 Analysis of superparasitism-avoidance rate in *Echthrodelphax fairchildii* using log-linear models

Model[a]	d.f.	G-value	Goodness-of-fit test (P)	Interaction deleted	d.f.	G-value	Test for effect of deleting the interaction (P)[b]
ABC	0	0.0	1.00	–	–	–	–
AB,BC,AC	6	7.6	0.27	ABC	6	7.6	0.27
AC,AB	12	60.7	<0.001	BC	6	53.1	<0.001
BC,AB	7	8.0	0.33	AC	1	0.4	0.51
BC,AC	12	8.9	0.71	AB	6	1.4	0.97
AC,B	18	61.9	<0.001	BC	6	53.0	<0.001
BC,A	13	9.2	0.76	AC	1	0.3	0.60
A,B,C	19	62.2	<0.001	BC	6	53.0	<0.001

[a]A, Self or conspecific; B, oviposition intervals; C, avoidance. The model [ABC] indicates the inclusion of a three–factor interaction in addition to two-factor interactions. The model [A,B,C] indicates that A, B, and C are independent. The other models in the table indicate the inclusion of 1–3 two-factor interactions.
[b]Significance of an interaction was tested by a difference in G-values for the goodness-of-fit tests between the models with and without the interaction.

on an unparasitized host. Log-linear model analysis showed that the preferred oviposition side did not differ significantly with oviposition interval, but that there was a significant difference between self- and conspecific superparasitism (P<0.05; Figure 2; analysis table not presented). This difference is attributable to female parasitoids preferring to oviposit on sides harboring no first-comer under conspecific superparasitism (binomial test for all intervals combined, P<0.01), while under self-superparasitism they exhibited no preference. However, log-linear model analysis for data excluding those for the 96 h oviposition interval revealed no significant difference between self- and conspecific superparasitism.
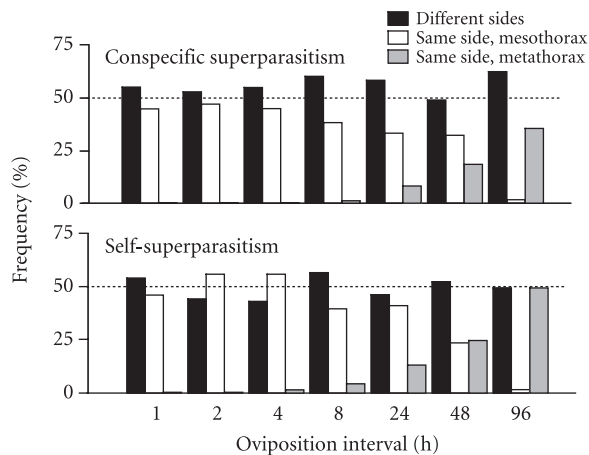

Figure 2 Frequency of cases in which the first and second oviposition sides were different or the same. When *Echthrodelphax fairchildii* oviposition occurred on the same side, the cases were classified by the places on which the second eggs were laid.

Under superparasitism with the first and second oviposition on the same side, the rate at which female parasitoids laid an egg between the hindwing bud and the epimeron of the metathorax increased with oviposition interval. When the interval was 96 h, the parasitoids almost always oviposited under the hindwing buds.

Survival rate

The survival rate of immature parasitoids in singly parasitized hosts was 75.7% (n = 301). When the first and second oviposition sides were the same, no hosts produced two adults, and the sum of survival rates of first- and second-comers under self-superparasitism was similar to the survival rate for single parasitism (Table 3; Figure 3). In contrast, when the first and second oviposition sides differed, two adults often emerged from one host for oviposition intervals of <24 h (Yamada & Ikawa, 2003), and thus the sum of the survival rates of first- and second-comers under self-superparasitism was higher than the survival rate for single parasitism (Table 3; Figure 3). As a consequence, when the oviposition interval was 1 or 4 h, there was a significant difference between the cases in which the first and second oviposition sides were the same and those in which they differed (Table 4).

The survival rate of second-comers under conspecific superparasitism was lower than the survival rate for single parasitism, irrespective of the oviposition side or interval (Table 3; Figure 3). Log-linear model analysis showed that the survival rate of second-comers under conspecific superparasitism did not differ significantly with oviposition side or interval (analysis table not presented).

Adult size

When ovipositions occurred on the same side, the adult size was similar to that for single parasitism, irrespective

Table 3 Statistical comparison between fitness returns from single parasitism and superparasitism in *Echthrodelphax fairchildii*

Fitness component	Self or conspecific[a]	Sides of first and second ovipositions	Oviposition interval (h)[b]						
			1	2	4	8	24	48	96
Survival rate	Self	Same	N	N	N	N	N	N	N
	Self	Different	***	N	***	N	N	N	N
	Conspecific	Same	N	N	M	N	N	M	**
	Conspecific	Different	*	M	M	N	*	N	N
Adult size	Self	Same	N	N	N	N	N	N	N
	Self	Different	***	***	***	***	N	N	N
	Conspecific	Same	N	N	N	N	N	N	N
	Conspecific	Different	***	***	***	*	N	N	**
Developmental time	Self	Same	N	N	N	**	N	*	N
	Self	Different	N	N	N	N	*	N	N
	Conspecific	Same	M	N	N	*	N	N	N
	Conspecific	Different	N	N	*	N	N	N	N

[a]In the case of self-superparasitism the survival rate and means of head widths and developmental times for first- and second-comers combined were investigated, while in the case of conspecific superparasitism the survival rate and means of head widths and developmental times for second-comers were investigated. Mann–Whitney U-test or Fisher's exact test was used for survival; Mann-Whiney U-test was used for head width and developmental time. The significance was determined using the sequential Bonferroni multiple-comparison method (Rice, 1989).
[b] N, $P \geq 0.1$; M, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

of whether the superparasitism was self or conspecific (Table 3; Figure 4). In contrast, when the oviposition sides differed, the emerging adult under superparasitism was significantly smaller than that for single parasitism when the oviposition interval was <24 h. Accordingly, under both self- and conspecific superparasitism, ovipositing on the same side produced significantly larger adults than ovipositing on different sides (Table 4). Moreover,
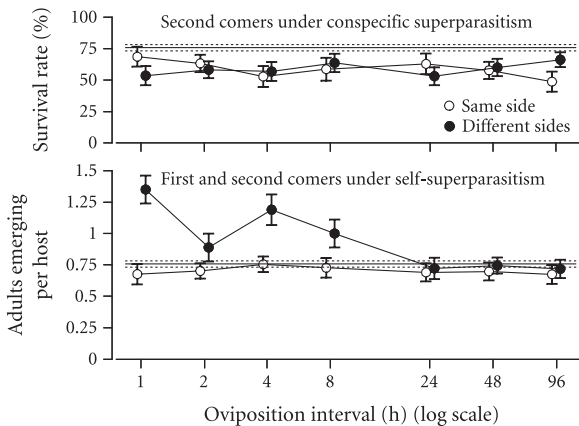

Figure 3 Survival rates (mean ± SEM) of *Echthrodelphax fairchildii* second-comers under conspecific superparasitism and the number (mean ± SEM) of adults emerging per host (circles), i.e., the total survival rate of first- and second-comers, under self-superparasitism. The solid and dotted horizontal lines indicate the survival rate (mean ± SEM) for single parasitism.

under conspecific superparasitism this was also true for an oviposition interval of 96 h.

Developmental time

Overall, the developmental time was a little longer under superparasitism than under single parasitism (Figure 5), but a significant difference was only found for a few cases (Table 3). Furthermore, the difference in developmental time was at most 0.87 days. As a consequence, there was no significant difference in the development time between ovipositing on the same side and ovipositing on different sides, except in one case (Table 4).

Table 4 Statistical comparison in *Echthrodelphax fairchildii* between fitness returns from superparasitism on the side harboring the first-comer and those from superparasitism on the side harboring no first-comer

Fitness component	Self or conspecific[a]	Oviposition interval (h)[b]						
		1	2	4	8	24	48	96
Survival rate	Self	***	N	*	N	N	N	N
	Conspecific	N	N	N	N	N	N	N
Adult size	Self	***	***	***	***	N	N	N
	Conspecific	***	***	*	*	N	N	*
Developmental time	Self	N	N	N	*	N	N	N
	Conspecific	N	N	N	N	N	N	N

[a,b]See the legend of Table 3, except for results for survival rate for conspecific superparasitism, which were obtained using log-linear model analysis.
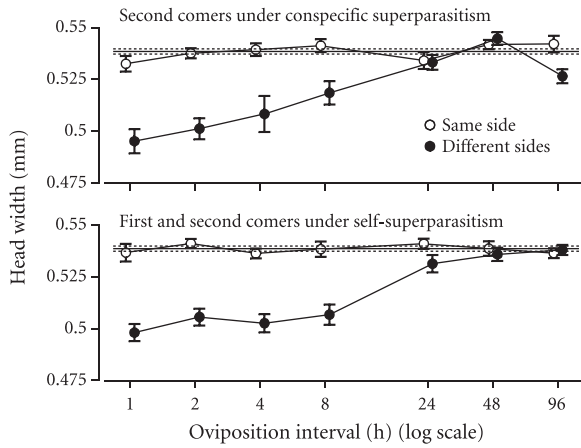
Figure 4 Head width (mean ± SEM) of emerging *Echthrodelphax fairchildii* adults under self- and conspecific superparasitism. The solid and dotted horizontal lines indicate head width (mean ± SEM) for single parasitism.

Superparasitizing behavior when the first and second ovipositions occurred on the same side

After the female parasitoid had inserted the tip of her abdomen between the wing bud and the epimeron of the mesothorax of a host, the movement of her abdominal tip was usually undetectable or unclear. In a few cases, however, it was observed through the translucent wing bud (probably thanks to favorable camera and lighting angles) that the superparasitizing female was repeatedly inserting and withdrawing her stinger. This action suggests that she had stung the first-comer with her stinger, which was
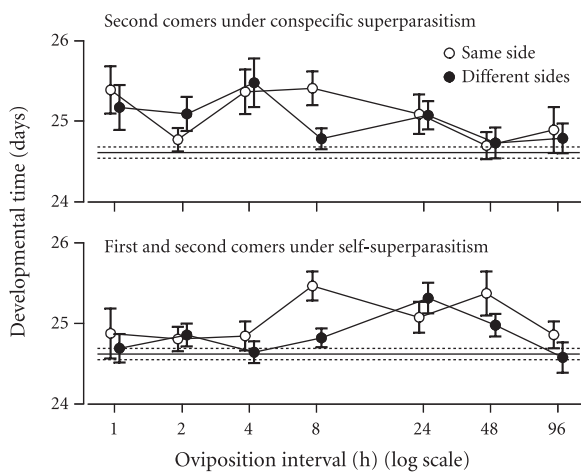

Figure 5 Developmental time (mean ± SEM) from oviposition to adult emergence under self- and conspecific superparasitism in *Echthrodelphax fairchildii*. The solid and dotted horizontal lines indicate developmental time (mean ± SEM) for single parasitism.

Table 5 Percentage of *Echthrodelphax fairchildii* parasitoid eggs with the tip shape changed

Oviposition interval (h)	Hours after first oviposition		
	66	74	90
8	0 (25)[a]	81.8 (11)	–
24	0 (10)	–	100 (5)

[a]Numbers in parentheses indicate sample sizes.

supported by only one of 414 superparasitized hosts producing two larval sacs when the first and second eggs were laid in the same position. Moreover, when a female parasitoid laid a second egg between the hind wing bud and the epimeron of the metathorax, she always moved the tip of the abdomen under the forewing bud for probing before laying the egg. As a result, very few larval sacs (2/139) appeared under the forewing buds. When laying a second egg under the forewing buds, the females never probed under hindwing buds.

Identification of the winner

The apparent egg period was 62.9 ± 1.96 h (mean ± SD) and the range 59.5–65.5 h. The shape change in the parasitoid egg was not detected at 66 h after the first oviposition under superparasitism, but it was detected at 74 and 90 h for superparasitism with an interval of 8 and 24 h, respectively (Table 5). This indicates that first-comers were always killed when the second egg was laid on the side harboring the first-comer.

Discussion

Host discrimination and self/non-self recognition

Echthrodelphax fairchildii females always accept fifth-instar hosts that are within 24 h of molting, as indicated by the fact that female parasitoids that had avoided superparasitism accepted fifth-instar unparasitized hosts. However, *E. fairchildii* females do not always accept fifth-instar hosts that have molted >2 days previously (Y.Y. Yamada, unpubl.). Therefore, host discrimination is thought to occur for oviposition intervals of ≤24 h, but we cannot be certain of this for oviposition intervals of >24 h, based on superparasitism avoidance. However, the following two facts suggest that *E. fairchildii* females perform host discrimination: (1) the frequency of probing of the non-oviposition side was high for these long oviposition intervals (Yamada & Ikawa, 2003), which was not found in ovipositing on unparasitized hosts, and (2) the presence of oviposition under hindwing buds.

The absence of a difference in the rate of superparasitism avoidance between self- and conspecific superparasitism

suggests that *E. fairchildii* females have no ability for self/non-self recognition. However, differences in the preferred oviposition side and in the frequency of probing of the non-oviposition side show that *E. fairchildii* females can recognize their own offspring to some degree (Yamada & Ikawa, 2003). The cause of this discrepancy in self/non-self recognition is unknown at the moment, so more detailed observation of parasitoid behavior is required. However, it should be noted that the preference in the oviposition side was very weak; in particular, for oviposition intervals of ≤48 h there was no preference. This is supported by *E. fairchildii* females never changing the oviposition side when and after catching the host; if they did so, the host might escape.

Adaptiveness of conspecific superparasitism

The overall observed fitness returns from self- and conspecific superparasitism resemble those predicted. In comparison with fitness returns from single parasitism, the survival rate of second-comers under conspecific superparasitism was slightly lower, irrespective of the oviposition side or interval. Furthermore, when an egg was laid on the side without the first-comer for oviposition intervals of <24 h, the emerging adult was smaller than that in single parasitism. The oviposition side did not influence survival rate and developmental time, irrespective of the oviposition interval, while the adults of second-comers on the oviposition side harboring no first-comer were smaller than those on the oviposition side harboring it, when the oviposition interval was <24 or 96 h. This is due to the low frequency of infanticidal probing for oviposition intervals of <24 h, leading to a high frequency of two-adult emergence, and due to the emergence of some small second-comers in the absence of probing for a 96-h interval (Yamada & Ikawa, 2003). Therefore, the optimal decision-making based on fitness returns is as follows. When unparasitized hosts are scarce, female parasitoids should always perform conspecific superparasitism irrespective of the oviposition interval, and for an oviposition interval of <24 or 96 h they should select the side harboring the first-comer. This is almost the same as predicted (Table 1).

The superparasitism-avoidance rate in *E. fairchildii*, particularly the values for long oviposition intervals, is low compared with that for other parasitoids; the avoidance rate increases to more than 60% in *Aphidius rhopalosiphi* and to nearly 100% in *Itoplectis naranyae* and *Pachycrepoideus vindemmiae* as the oviposition interval increases (Ueno, 1994; Outreman et al., 2001a; Goubault et al., 2003). This appears to reflect differences in fitness returns. However, although the avoidance rate was higher for oviposition intervals of 1 and ≥24 h, the fitness returns for these oviposition intervals were not lower than those for the other

intervals. This discrepancy may be mainly attributable to the absence of self/non-self recognition, which is discussed hereafter. Moreover, preferring the side without the first-comer is maladaptive for oviposition intervals of <24 and 96 h.

Adaptiveness of self-superparasitism

When the oviposition sides were the same, superparasitism did not produce positive fitness returns due to infanticide. On the other hand, when the oviposition sides were different, the total survival rate of first- and second-comers for oviposition intervals of <24 h was higher than the survival rate for single parasitism; in particular, the difference was nearly twofold for a 1 h oviposition interval. This is due to a low frequency of infanticidal probing for such short intervals (Yamada & Ikawa, 2003). However, the emerging adult was smaller than that for single parasitism, and hence self-parasitized hosts had a lower value than unparasitized hosts. Here, let us assume that the increase in survival rate more than compensates for the decrease in adult size, which is most likely to be true for male offspring because in parasitoids the effect of size on fitness is generally considered smaller among males than among females (Charnov, 1982; Godfray, 1994). Then, the optimal decision-making based on fitness returns is the same as predicted (Table 1).

The higher superparasitism-avoidance rate observed for oviposition intervals of ≥24 h fits the pattern predicted based on fitness returns, but the avoidance rate was at most 33.1% (i.e., far lower than 100%). The higher avoidance rate observed for a 1-h oviposition interval is unexpected in terms of fitness returns. Moreover, no preference in the oviposition side is maladaptive.

Adaptive superparasitism for parasitoids without self/non-self recognition

Here we consider the optimum superparasitism strategy for *E. fairchildii* females on the assumption that they cannot distinguish between self- and conspecifically parasitized hosts, nor make a free selection of the oviposition side.

The variation in avoidance rate with oviposition interval indicates that when the female parasitoid encounters a parasitized host, she can recognize – to some degree – the time that has elapsed since the first parasitism. By performing self-superparasitism as well as conspecific superparasitism, the female parasitoid will obtain positive fitness returns for short oviposition intervals (of <24 h) if the number of offspring per host contributes more to the fitness returns than does the size of emerging adults. Therefore, she should superparasitize for such short oviposition intervals when unparasitized hosts are scarce. For oviposition intervals of ≥24 h, however, self-superparasitism produces no positive

fitness returns, whereas conspecific superparasitism produces high fitness returns. Hence, in view of the low costs of eggs and oviposition time, female parasitoids in an environment where unparasitized hosts are scarce should superparasitize for oviposition intervals of ≥24 h, but should avoid superparasitizing more frequently than for oviposition intervals of <24 h, other factors being equal. This pattern of superparasitism avoidance is close to the pattern observed in the present study, except for the higher rate of superparasitism for a 1 h oviposition interval. This discrepancy may be attributable to female parasitoids recognizing that hosts parasitized 1 h previously are more likely to be self-parasitized than hosts parasitized ≥2 h previously.

The results of this study indicate that, considering their imperfect recognition abilities, *E. fairchildii* females behave optimally. Many parasitoids are considered to lack self/non-self recognition (van Dijken et al., 1992; Godfray, 1994), and even in those having this capability, the recognition ability may decrease with the time that has elapsed since the first oviposition (Ueno, 1994). Therefore, most parasitoids appear to develop a superparasitism strategy in which imperfect self/non-self recognition is incorporated. Moreover, although parasitoids do not distinguish between self- and conspecifically parasitized hosts, they may indirectly estimate the proportion of self-parasitized hosts among parasitized hosts (see Flanagan et al., 1998; Shuker & West, 2004), e.g., via the time elapsed since the first parasitism and the rate of encountering conspecifics. Such mechanisms also remain to be studied further.

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