Presence of a conspecific increases superparasitism but not infanticide under

self- and conspecific superparasitism in a semisolitary parasitoid,

Echthrodelphax fairchildii (Hymenoptera: Dryinidae)

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Abstract

Female parasitoids are expected to avoid superparasitism (ovipositing in/on parasitized hosts) when unparasitized hosts are available. However, when the supply of unparasitized hosts is restricted, they are expected to self- as well as conspecifically superparasitize. One of the cues of a reduced availability of unparasitized hosts is the presence of a conspecific. Moreover, if the focal species can perform infanticide, after encountering a conspecific female the females are expected to kill eggs existing in/on hosts when superparasitizing, because the eggs are more likely to be laid by others. In this study we investigated whether females of an infanticidal semisolitary parasitoid, *Echthrodelphax fairchildii*, increase their frequencies of superparasitism and infanticide after encountering a conspecific female. *Echthrodelphax fairchildii* females are capable of discriminating between self- and conspecific superparasitism until up to 0.75 h after the first egg was laid (self-superparasitism frequency < conspecific superparasitism frequency). As expected, the female parasitoids were more likely to perform self- and conspecific superparasitism after they had encountered a conspecific. In particular, the self-superparasitism frequency increased highly within a short

period after the first oviposition, so that no difference between the self- and conspecific superparasitism frequencies was found. In contrast, the infanticidal-probing frequency remained extremely low, irrespective of whether or not the female parasitoids had encountered a conspecific. Moreover, when superparasitizing, females usually laid female eggs. Possible causes for the low frequency of infanticidal probing and the female-biased sex ratio are discussed.

INTRODUCTION

Female parasitoids, whether solitary or gregarious, are expected to be more likely to accept a conspecifically parasitized host (i.e. a low-value host) for ovipositing after encountering a conspecific (van Alphen & Visser 1990). This is predicted from many models, most of which are based on an evolutionarily-stable-strategy (ESS) theory (van der Hoeven & Hemerik 1990; Mangel 1992; Visser *et al*. 1992a; Visser 1993; Haccou *et al*. 2003; Hamelin *et al*. 2007). The same prediction is also suggested by a classical diet menu model (Charnov & Orians 1973; Stephens & Krebs 1986), because the presence of a

conspecific suggests that the patch quality, which is determined mainly by the availability of unparasitized hosts, has been reduced or will soon be reduced. However, it should be noted that when a female parasitoid searches in a habitat characterized by multiple patches, the resource value of other patches influences decision-making about the acceptance of superparasitism. A rich habitat contains many patches with abundant unparasitized hosts, and in this situation the parasitoid female should avoid superparasitism and leave the present patch, while the reverse is true in a poor habitat.

This prediction is also applicable to self-superparasitism in solitary and gregarious parasitoids under situations where the total fitness performance of all progenies developing under self-superparasitism is larger than the total fitness performance of a progeny (or progenies) for a single oviposition. Such situations are likely to occur when at least one conspecific is present (Cloutier 1984; van Alphen & Visser 1990; Yamada & Sugaura 2003; Ito & Yamada 2005). In summary, the presence of a conspecific is considered to be likely to induce superparasitism depending on the resource value of the habitat, irrespective of whether it is self or conspecific.

To our best knowledge, four parasitoid species have been investigated

in attempts to determine the effects of the presence of one or multiple

conspecifics on superparasitism: *Leptopilina heterotoma* (Visser *et al*. 1990,

1992b; Visser 1993), *Venturia canescens* (Hughes *et al*. 1994), *Anaphes nitens*

(Santolamazza-Carbone & Cordero Rivera 2003) and *Fopius arisanus* (Wang &

Messing 2008). *Leptopilina heterotoma* and *A. nitens* provide positive evidence

for the induction of superparasitism, but in *V. canescens* the superparasitism

frequency decreases as the number of parasitoids increases, while in *F. arisanus*

the female parasitoid usually avoids conspecific superparasitism irrespective of

whether or not she encounters a conspecific. The reasons for superparasitism

not occurring appear to be the high parasitoid/host ratio in the patch for *V.*

canescens (Hughes *et al*. 1994) and the extremely low survival rate of the

second progeny for *F. arisanus* (Wang & Messing 2003, 2008). Superparasitism

avoidance appears to be adaptive under such situations. Unfortunately, self- and

conspecific superparasitism were not addressed separately or the latter was

only addressed in the above species, with the sole exception of *L. heterotoma*

(Visser *et al*. 1990, 1992b; Visser 1993).

Females of some parasitoid species perform infanticide, including

ovicide and larvicide, under superparasitism (Arakawa 1987; Mayhew 1997;

Yamada & Kitashiro 2002; Goubault *et al*. 2004; Tena *et al*. 2008; Takasuka & Matsumoto 2011). Infanticide increases the fitness performance of the second progeny, because that progeny has a handicap in competing with the first progeny for the host resources under superparasitism (Salt 1961; Visser *et al*. 1992c; Sirot 1996; Field *et al*. 1997; Vinson & Hegazi 1998; Lebreton *et al*. 2009). Gregarious parasitoid females should refrain from performing infanticide under self-superparasitism, provided that the contribution of a large number of emerging adults to fitness gains is greater than the contribution of small emerging adults under superparasitism. Parasitoids that cannot discriminate between self- and conspecifically parasitized hosts are expected to decide to perform infanticide based on a cue/cues for detecting whether the parasitized hosts are parasitized by themselves or conspecifics. The presence of a conspecific is one such cue.

The parasitoid *Echthrodelphax fairchildii* Perkins (Hymenoptera: Dryinidae) is a semisolitary species: two adults can emerge from a single host for first-to-second oviposition intervals of <24 h (Yamada & Ikawa 2003), although the female parasitoid always lays a single egg during the process of catching, ovipositing on, and releasing a host (Yamada & Imai 2000). The female

parasitoid lays an egg between the wing bud and the epimeron of the meso- or metathorax (Yamada & Imai 2000; Yamada & Ikawa 2003). Superparasitism in *E. fairchildii* parasitoids is classified into two types according to whether the first and second ovipositions occur on the same side (S type) or on different sides (D type) of the host: the first progeny is always killed in the former case (Yamada & Ikawa 2005). The female parasitoid often probes the nonoviposition side for infanticide under superparasitism (Yamada & Ikawa 2003); this probing takes <10 s and hence its cost appears to be negligible in terms of time.

We previously (Ito & Yamada 2014) reported that the female parasitoid could discriminate between self- and conspecifically parasitized hosts when the oviposition interval was ≤0.75 h: the superparasitism frequency was lower under self-superparasitism than under conspecific superparasitism. Therefore, the probing frequency under conspecific superparasitism was expected to increase for oviposition intervals of ≤0.75 h, while actually it did not (Ito & Yamada 2014). Moreover, eggs laid under superparasitism were usually female, as were those laid on unparasitized hosts.

The aim of the present study was to determine the frequencies of superparasitism and infanticidal probing and the sex ratio of progenies when the

female parasitoid *E. fairchildii* encounters a self- or conspecifically parasitized host in the presence of a conspecific, and to compare the data obtained in the present study with those obtained in the absence of a conspecific (Ito & Yamada 2014). Then, regarding the frequencies of superparasitism and infanticidal probing, we verified whether the presence of a conspecific induces the above-predicted changes.

MATERIALS AND METHODS

The experimental procedures were the same as those described in Ito and Yamada (2014), except that the parasitoid female was kept with a conspecific before a superparasitism bout. The procedures are therefore described only briefly below.

Insects

Echthrodelphax fairchildii is a synovigenic ectoparasitoid of the following three rice-damaging planthoppers (Homoptera: Delphacidae): *Nilaparvata lugens* (Stål), *Sogatella furcifera* (Horváth) and *Laodelphax striatellus* (Fallén). The female parasitoid often feeds on these hosts. Immature parasitoids are

sedentary at the position where they are laid as eggs. Parasitized hosts continue to feed on host plants, but they do not molt to the next instar.

Echthrodelphax fairchildii and a host species, *L. striatellus*, were collected at two places separated by 10 km during 1992 in Tsu, Mie Prefecture, Japan, and had been reared continuously for 10 years under laboratory conditions: the two parasitoid populations were maintained separately, while the two host populations were reared in mixture. In the experiments for conspecific superparasitism, the use of related individuals was avoided by using a pair of individuals from different populations.

Parasitoid pupae were gathered for experiments from the laboratory populations and kept individually in 10 ml glass vials. After emergence the females were individually reared in 340 ml plastic cages containing a 50% (by weight) honey solution, one fifth-instar host, a combined total of 15 second- and third-instar hosts (usually mainly second-instar hosts), and two male wasps for mating. The amount and instars of supplied hosts were the same as in Ito and Yamada (2014); note that they are described incorrectly in Ito and Yamada (2014). These hosts and the honey solution were renewed every day. The second- and third-instar hosts were provided as food for female parasitoids; this

parasitoid does not oviposit on second-instar hosts in any situation, and sometimes oviposits on third-instar hosts, but only when fourth- or fifth-instar hosts are not available (YY Yamada *et al.*, unpubl. data, 2013). Thus, the females experienced a very low availability of hosts before a superparasitism bout, which would increase the likelihood of superparasitism. Mated females aged 4–15 days were used for superparasitism bouts. The female did not lay two eggs on an unparasitized host during a single oviposition bout or perform ovipositing twice without releasing the host in the presence of a conspecific, which differs from the behaviors exhibited by the females of many parasitoid species (Godfray 1994; Rosenheim & Hongkham 1996). Moreover, almost all first eggs were female irrespective of whether or not a conspecific was present. Observation and rearing were performed in a room at 24–26°C and with a 16 h light : 8 h dark (LD 16:8).

Superparasitism bouts

The following first-to-second oviposition intervals were used in the superparasitism bouts: 0, 0.25, 0.5, 0.75, 1, 1.5 and 2 h. In each first oviposition bout, the female in a rearing cage was moved into a clean 4 ml transparent

plastic vial containing two second-instar hosts for food immediately after the light

was turned on, and kept therein for 1 h before a healthy fifth-instar host was

added. The host was removed immediately after being parasitized, and then

returned after an assigned oviposition interval when self-superparasitism bouts

were performed. An exception was for an interval of 0 h, in which the host was

kept in the vial with the parasitoid instead of being removed. For both self- and

conspecific superparasitism bouts, a single second-instar host was supplied to

the female parasitoid for food during oviposition intervals of 0.5 and 0.75 h, while

two, three and four hosts were supplied during intervals of 1, 1.5 and 2 h,

respectively. This procedure ensured that female parasitoids did not feed on

parasitized hosts.

To expose each female used for superparasitism bouts to a conspecific

(called an opponent hereafter) for 1 h before a superparasitism bout, an

opponent and two second-instar hosts (for food) were put into a 4 ml vial

containing the focal female 1 h before the superparasitism bout. The focal

female, the opponent, or both were marked on the forewing using a blue or red

felt pen (Magic Ink$^®$ No. 500; Teranishi Chemical Industry, Osaka, Japan) so

that they could be discriminated. No difference in behavior between marked and

unmarked females was observed, and the marking also exerted no statistically

detectable effects on the studied parameters. For oviposition intervals of 0.25,

0.5 and 0.75 h, the opponent was picked from the vial at 0.75, 0.5 and 0.25 h,

respectively, after the two females were put together in the vial, and the focal

female was allowed to perform the first oviposition. The opponent was then

returned to the vial containing the focal female immediately after the first

oviposition was completed (it usually took <10 min), and kept therein until a

superparasitism bout occurred.

We observed the ovipositing behavior under fluorescent lighting via a

supersensitive video camera attached to a binocular microscope (40×

magnification), and recorded the oviposition side (right or left) of the host;

however, no preference for the oviposition side was detected, as for cases when

no conspecific is present (Ito & Yamada 2014). Whether the female parasitoid

moved her abdominal tip to the nonoviposition side for infanticidal probing was

also recorded. The sex of eggs laid was identified based on observation of the

movement of the genitalia, particularly sting (Yamada & Imai 2000): observation

was not successful in 0–11.7% of the superparasitism bouts for each oviposition

interval, and the sex was not determined for 0–21.7% of them due to a long

pausing time of the sting (>10 s). The long pausing time suggests that the

female parasitoid had trouble releasing sperm, and consequently often laid male

eggs even though she was considered to have attempted to lay female eggs

(Yamada & Imai 2000). We stopped the observation when the female parasitoid

did not superparasitize a host within 10 min; these cases were categorized as

superparasitism avoidance. When superparasitism avoidance occurred, an

unparasitized fifth-instar host was exposed to the female to examine whether the

avoidance occurred due to the female not being interested in ovipositing. If the

female did not oviposit on this unparasitized host within 10 min, the data were

discarded (such cases were very rare).

Individual females used for conspecific superparasitism bouts were

also used for self-superparasitism bouts, but the same female were not used for

superparasitism bouts in both the presence and absence of a conspecific. We

had planned to allow individual females to perform one self- and one conspecific

superparasitism bout at each interval of 0,0.25, 0.5, 0.75, 1, 1.5 and 2 h (note

that the order of oviposition intervals was selected randomly for each individual),

but this was impossible due to parasitoids dying; about half of the planned bouts

were performed in most cases. In total, between 29 and 44 superparasitism

bouts were performed for each oviposition interval. Because some bouts did not result in ovipositing and the sex of some eggs was not identified, the sample size for each oviposition interval varied from 18 to 37 for the sex ratios, and from 11 to 22 for the probing frequency under D-type superparasitism.

Data analysis

Data obtained in the present experiments were combined with data obtained in experiments conducted in the absence of conspecifics, which have been reported elsewhere (Ito & Yamada 2014), and the combined data were analyzed. Data obtained in the absence of conspecifics were limited to superparasitism bouts performed by parasitoids aged 5–14 days, because superparasitism bouts in the presence of a conspecific were performed by parasitoids aged 5–14 days. These two kinds of experiments were performed concurrently during the same period. The effects of the presence of a conspecific, the type of superparasitism (self or conspecific), and the oviposition interval on superparasitism frequency, infanticidal-probing frequency, and the sex ratio were analyzed. Furthermore, effects of the oviposition sides (D or S type) and the event of infanticidal probing on the sex ratio were analyzed; no effects were detected (as for cases when no

conspecific is present, see Ito & Yamada 2014), so that the analysis results are not presented here. Logistic regression analysis was performed to evaluate the effects of the above factors, using the LogXact® 10 software (Cytel Inc, Cambridge, MA, USA). Significance was tested using exact probability values (Cytel Inc 2012). The logistic regression model included (as a numeric variable) the possible influences of the ages of the ovipositing females. However, the age did not affect the above-mentioned items and so the analysis results for ages of parasitoid females are not presented. In addition to the above-mentioned factors, the influence of individual parasitoids was incorporated in the model as a stratification (random) variable, when possible. All two-way interactions were included in the original model. Nonsignificant interactions were removed one by one to obtain the final model that included only significant interactions; P values for the nonsignificant interactions are reported based on the models that included them.

An analysis of superparasitism frequencies in the absence of a conspecific (Ito & Yamada 2014) revealed a significant two-way interaction between the oviposition interval and the type of superparasitism. Those authors applied separate analyses for oviposition intervals of 0–0.75 and 0.75–2 h in

order to avoid this significant interaction, and such separate analyses were also performed in the present analysis. Moreover, analysis for all oviposition intervals between 0 and 2 h was performed separately for self- and conspecific superparasitism to evaluate more precise effects of the presence of a conspecific and the oviposition interval. An analysis for all oviposition intervals between 0 and 2 h was also performed for self- and conspecific superparasitism in the presence of a conspecific to determine whether the presence of a conspecific eliminated the difference between the two kinds of superparasitism.

RESULTS

For oviposition intervals of 0–0.75 h, the effect of the presence of a conspecific on the superparasitism frequency differed with the type of superparasitism (significant interaction between the type of superparasitism and the presence of a conspecific; Table 1), and hence the analysis was performed separately for self- and conspecific superparasitism bouts. In self-superparasitism bouts the presence of a conspecific increased the superparasitism frequency (Table 1, Fig. 1), and the frequency also increased with the oviposition interval. Meanwhile, in conspecific superparasitism bouts the presence of a conspecific did not affect

the superparasitism frequency (Table 1, Fig. 1), but the frequency increased with the oviposition interval in both the absence and presence of a conspecific, although it should be noted that the superparasitism frequency was similar for oviposition intervals of 0–0.5 h, and increased greatly between oviposition intervals of 0.5 and 0.75 h.

For oviposition intervals of 0.75–2 h, the superparasitism frequency increased in the presence of a conspecific and decreased marginally significantly with the oviposition interval (Table 1), but was independent of the type of superparasitism. The negative relationship between the frequency and oviposition interval can be attributed to the high value for an oviposition interval of 0.75 h; an analysis for 1–2 h revealed that a significant relationship was not present ($P > 0.4$).

Separate analysis for self- and conspecific superparasitism bouts for oviposition intervals of 0–2 h revealed that the presence of a conspecific increased the superparasitism frequency in both self- and conspecific superparasitism bouts. However, note that the significance was marginal in conspecific superparasitism bouts, because the presence of a conspecific caused only a slight increase in the conspecific superparasitism frequency for

oviposition intervals of 0–0.75 h. The superparasitism frequency increased with

the oviposition interval in self-superparasitism bouts, but not in conspecific

superparasitism bouts. However, the oviposition interval had a significant effect

on the conspecific superparasitism frequency when it was defined as a

categorical value (Table 1), with the data suggestive of a small peak at an

oviposition interval of 0.75 h. Such a peak is considered to be also present in

self-superparasitism bouts because the analyses for 0–0.75 h and 0.75–2 h

suggest positive and negative relationships, respectively, between the

superparasitism frequency and oviposition interval, as mentioned above. In the

presence of a conspecific, there was no difference between self- and conspecific

superparasitism frequencies for oviposition intervals of 0–2 h (Table 1).

The probing frequency was extremely low for each oviposition interval

under both self- and conspecific superparasitism irrespective of whether or not a

conspecific was present (Fig. 2). The presence of a conspecific ($P = 0.414$), the

type of superparasitism ($P = 0.147$), and the oviposition interval ($P = 0.688$) did

not significantly affect the probing frequency; no interactions between these

factors are present ($P > 0.2$).

The sex of the progenies was almost always female: the sex ratio for

each oviposition interval under self- and conspecific superparasitism in the presence of a conspecific was at most 5.5% (n = 18), and 0% in 4 of the 14 cases. Consequently, the presence of a conspecific (P = 0.549), the type of superparasitism (P = 0.109), and the oviposition interval (P = 0.314) did not significantly affect the sex ratio of progenies; no interactions between these factors are present (P > 0.2).

DISCUSSION

As predicted, the presence of a conspecific increased both the self- and conspecific superparasitism frequencies in *E. fairchildii* parasitoids, except for conspecific superparasitism at oviposition intervals of ≤0.75 h; as a result, the frequencies in self-superparasitism bouts were as high as those in conspecific superparasitism bouts with the same intervals. This indicates that the female changed her response to parasitized hosts after recognizing the presence of a conspecific, which was considered adaptive, as mentioned in the Introduction. However, as also mentioned in the Introduction, it should be noted that performing superparasitism in the presence of a conspecific is not always adaptive. A low availability of hosts under rearing conditions as well as the

presence of a conspecific was considered to reliably inform the female that she

is in a poor habitat. In addition, *E. fairchildii* females obtain fairly high fitness

gains under both self- and conspecific superparasitism due to two progenies

emerging from one host under superparasitism with a short oviposition interval

(Yamada & Ikawa 2003). These factors appear to have resulted in *E. fairchildii*

females being more likely to remain in the present patch and perform both self-

and conspecific superparasitism.

So far it has been reported that the frequencies of superparasitism

increase in the presence of conspecifics in two parasitoid species, *L. heterotoma*

and *A. nitens* (see the Introduction). However, in *A. nitens* it is unclear whether

the increase is attributable to the increased self-superparasitism frequency or

the increased conspecific superparasitism frequency. Meanwhile, in *L.*

heterotoma increases in both the frequencies of self- and conspecific

superparasitism were found when conspecifics were present (Visser *et al*. 1990,

1992b; Visser 1993); however, those studies did not compare the frequencies of

self- and conspecific superparasitism performed under the same conditions. The

present study is the first to compare the increases in self- and conspecific

superparasitism in the presence of a conspecific: the self-superparasitism

frequency was as high as the conspecific superparasitism frequency in the

presence of a conspecific. However, the frequencies of both self- and

conspecific superparasitism are considered to be influenced by previous

experiences (Visser *et al*. 1990, 1992a,b; Visser 1993), and hence self- and

conspecific superparasitism by *E. fairchildii* are not considered to always exhibit

similar frequencies in the presence of a conspecific.

Interestingly, the presence of a conspecific did not increase the

frequency of conspecific superparasitism with oviposition intervals of ≤0.75 h.

This could have been due to the female laying an egg on a healthy host before a

conspecific superparasitism bout in our experiments. The experience of egg

laying or encountering an unparasitized host increases the probability of

superparasitism avoidance in several parasitoid species (e.g. Henneman *et al*.

1995; Hubbard *et al*. 1999; Babendreier & Hoffmeister 2002; Wang & Messing

2008). The effect of the presence of a conspecific must have been suppressed

for a while after ovipositing on a healthy host; that is, the presence of a

conspecific is ranked lower as a cue for the superparasitism decision than

ovipositing on a healthy host (see Froissart *et al*. 2012).

Moreover, it should be noted that the superparasitism frequency never

reached 100% for any oviposition interval irrespective of whether

superparasitism was self or conspecific, although it was increased by the

presence of a conspecific. This is interesting given that unparasitized hosts are

almost always accepted in *E. fairchildii*. Partial preference has usually been

explained by variations in genotypes, physiological states, and previous

experiences among the individuals studied (Stephens & Krebs 1986). However,

Sirot *et al*. (1997) and Plantegenest *et al*. (2004) proposed the idea that partial

preference is an adaptive strategy, and that an animal makes a stochastic

decision when encountering low- or middle-value prey (hosts). Thus, the partial

preference found in our experiment may be adaptive. In the present

experimental procedure, the female laid an egg on a healthy host before a

superparasitism bout, from which she might have expected to be likely to

encounter healthy hosts soon. It appears to be adaptive for the female to make a

stochastic decision under such conditions to reserve eggs and/or physiological

vigor for the near future (Sirot *et al*. 1997; Plantegenest *et al*. 2004).

  In contrast to the superparasitism frequency, the probing frequency

remained markedly low even in the presence of a conspecific. In particular, for

oviposition intervals of ≤0.75 h, for which self/conspecific discrimination is

possible (Ito & Yamada 2014), the two types of cues of a conspecifically

parasitized host and a conspecific were predicted to induce infanticide, while

actually they did not. We previously suggested three reasons for why the probing

frequency in *E. fairchildii* does not increase under conspecific superparasitism in

the absence of a conspecific (Ito & Yamada 2014): cost of probing, imperfect

self/conspecific discrimination, and different cues for different decisions. The

present experiments suggest no or only a slight contribution of the latter two

mechanisms, because an additional cue, the presence of a conspecific, did not

change the decisions made by the female. An *E. fairchildii* female that is

performing probing must move the host around while holding it away from the

host plant (Yamada & Ikawa 2003), which could be physically demanding work

for the female. However, there remains a slight possibility that encountering

many conspecifics and/or conspecifically parasitized hosts will increase the

probing frequency in *E. fairchildii* (Santolamazza-Carbone & Cordero Rivera

2003; Shuker & West 2004).

  The sex ratio of the progenies was independent of the presence of a

conspecific, the type of superparasitism, and the oviposition interval. Second

eggs laid in the presence of a conspecific were usually female, as were those

laid on healthy hosts in the absence of a conspecific. A possible explanation is described below based on the host-quality model (Charnov 1982) being applicable to sex allocation in *E. fairchildii* parasitoids (YY Yamada & M Masuda, unpubl. data, 2010): females are likely to lay female eggs on fifth-instar hosts but male eggs on third- and fourth-instar hosts. The host-quality model suggests that the parasitoid should respond to relative host values rather than absolute ones; for example, when a female has previously always encountered low-value hosts, she should be more likely to lay female eggs on moderate-value hosts (Charnov *et al.* 1981; Charnov 1982; Ode & Heinz 2002). The females used in the present study were given 15 small (second- and third-instar) hosts and only one large (fifth-instar) hosts before the superparasitism bouts. This resulted in the female parasitoid appearing to regard a parasitized fifth-instar host as having a higher value under the present rearing conditions, and to lay a female egg on it.

In conclusion, the presence of a conspecific increased the frequencies of self- and conspecific superparasitism, except for conspecific superparasitism with short oviposition intervals; in particular, the self-superparasitism frequencies increased dramatically for short oviposition intervals. This suggests that the female parasitoid is able to recognize being in a patch that is or will soon be poor,

and accepts a low-value host instead of leaving the patch. Meanwhile, the presence of a conspecific did not change the infanticidal-probing frequencies or the sex ratio of the progenies under self- and conspecific superparasitism. Possible reasons for the absence of changes are that the infanticidal probing might be substantially costly and a parasitized fifth-instar host might be regarded a high-quality host in the present situations. Moreover, consideration of these results suggests that decision-making by *E. fairchildii* female parasitoids is greatly influenced not only by the presence of a conspecific but also by the rearing conditions. It will be fruitful to investigate the influences of both the previous and current experiences of the female parasitoids on their superparasitism strategy in further experiments.

REFERENCES

van Alphen JJM, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology* **35**, 59–79.

Arakawa R (1987) Attack on the parasitized host by a primary solitary parasitoid, *Encarsia formosa* (Hymenoptera: Aphelinidae): the second female pierces, with her ovipositor, the egg by the first one. *Applied Entomology and Zoology* **22**, 644–645.

Babendreier D, Hoffmeister TS (2002) Superparasitism in the solitary

ectoparasitoid *Aptesis nigrocincta*: the influence of egg load and host

encounter rate. *Entomologia Experimentalis et Applicata* **105**, 63–69.

Charnov EL (1982) *The Theory of Sex Allocation*. Princeton University Press,

Princeton, NJ.

Charnov EL, Orians GH (1973) *Optimal Foraging: Some Theoretical*

Explorations. Department of Biology, University of Utah, Salt Lake, UT.

[Cited 17 June 2014.] Available from URL:

http://hdl.handle.net/1928/1649

Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio

evolution in a variable environment. *Nature* **289**, 27–33.

Cloutier C (1984) The effect of host density on egg distribution by the solitary

parasitoid *Aphidius nigripes* (Hymenoptera: Aphelinidae). *Canadian*

Entomologist **116**, 805–811.

Cytel Inc (2012) *LogXact 10 User Manual*. Cytel Inc, Cambridge, MA.

Field SA, Keller MA, Calbert G (1997) The pay-off from superparasitism in the

egg parasitoid *Trissolcus basalis*, in relation to patch defence. *Ecological*

Entomology **22**, 142–149.

Froissart L, Bernstein C, Humblot L, Amat I, Desouhant E (2012) Facing multiple

information sources while foraging on successive patches: how does a

parasitoid deal with experience? *Animal Behaviour* **83**, 189–199.

Godfray HCJ (1994) *Parasitoids*: *Behavioral and Evolutionary Ecology.*

Princeton University Press, Princeton, NJ.

Goubault M, Fourrier J, Krespi L, Poinsot D, Cortesero AM (2004) Selection

strategies of parasitized hosts in a generalist parasitoid depend on patch

quality but also on host size. *Journal of Insect Behavior* **17**, 99–113.

Haccou P, Glaizot O, Cannings C (2003) Patch leaving strategies and

superparasitism: an asymmetric generalized war of attrition. *Journal of*

Theoretical Biology **225**, 77–89.

Hamelin F, Bernhard P, Wajnberg E (2007) Superparasitism as a different game.

Theoretical Population Biology **72**, 366–378.

Henneman ML, Papaj DR, Figueredova AJ, Vet LEM (1995) Egg-laying

experience and acceptance of parasitized hosts by the parasitoid,

Leptopilina heterotoma (Hymenoptera: Eucoilidae). *Journal of Insect*

Behavior **8**, 331–342.

van der Hoeven N, Hemerik L (1990) Superparasitism as an ESS: to reject or not

to reject, that is the question. *Journal of Theoretical Biology* **146**, 467–

482.

Hubbard SF, Harvey IF, Fletcher JR (1999) Avoidance of superparasitism: a

matter of learning? *Animal Behaviour* **57**, 1193–1197.

Hughes JP, Harvey IF, Hubbard SF (1994) Host-searching behavior of *Venturia*

canescens (Grav.) (Hymenoptera: Ichneumonidae): superparasitism.

Journal of Insect Behavior **7**, 455–464.

Ito E, Yamada YY (2005) Profitable self-superparasitism in an infanticidal

parasitoid when conspecifics are present: self-superparasitism deters

later attackers from probing for infanticide. *Ecological Entomology* **30**,

714–723.

Ito E, Yamada YY (2014) Self-/conspecific discrimination and superparasitism

strategy in the ovicidal parasitoid *Echthrodelphax fairchildii*

(Hymenoptera: Dryinidae). *Insect Science* **21**, 741–749.

Lebreton S, Labarussias M, Chevrier C, Darrouzet E (2009) Discrimination of the

age of conspecific eggs by an ovipositing ectoparasitic wasp.

Entomologia Experimentalis et Applicata **130**, 28–34.

Mangel M (1992) Descriptions of superparasitism by optimal foraging theory,

evolutionarily stable strategies and quantitative genetics. *Evolutionary Ecology* **6**, 152–169.

Mayhew PJ (1997) Fitness consequences of ovicide in a parasitoid wasp. *Entomologia Experimentalis et Applicata* **84**, 115–126.

Ode PJ, Heinz KM (2002) Host-size-dependent sex ratio theory and improving mass-reared parasitoid sex ratios. *Biological Control* **24**, 31–41.

Plantegenest M, Outreman Y, Goubault M, Wajnberg E (2004) Parasitoids flip a coin before deciding to superparasitize. *Journal of Animal Ecology* **73**, 802–806.

Rosenheim JA, Hongkham D (1996) Clutch size in an obligately siblicidal parasitoid wasp. *Animal Behaviour* **51**, 841–852.

Salt G (1961) Competition among insect parasitoids. *Symposia of the Society for Experimental Biology* **15**, 96–119.

Santolamazza-Carbone S, Cordero Rivera A (2003) Egg load and adaptive superparasitism in *Anaphes nitens*, an egg parasitoid of the *Eucalyptus* snout-beetle *Gonipterus scutellatus*. *Entomologia Experimentalis et Applicata* **106**, 127–134.

Shuker DM, West SA (2004) Information constraints and the precision of

adaptation: sex ratio manipulation in wasps. *Proceedings of the National*

Academy of Sciences of the United States of America **101**, 10363–10367.

Sirot E (1996) The pay-off from superparasitism in the solitary parasitoid

Venturia canescens. *Ecological Entomology* **21**, 305–307.

Sirot E, Ploye H, Bernstein C (1997) State dependent superparasitism in a

solitary parasitoid: egg load and survival. *Behavioral Ecology* **8**, 226–232.

Stephens DW, Krebs JR (1986) *Foraging Theory*. Princeton University Press,

Princeton, NJ.

Takasuka K, Matsumoto R (2011) Infanticide by a solitary koinobiont

ichneumonid ectoparasitoid of spiders. *Naturwissenschaften* **98**, 529–

536.

Tena A, Kapranas A, Garcia-Mari F, Luck RF (2008) Host discrimination,

superparasitism and infanticide by gregarious endoparasitoid. *Animal*

Behaviour **76**, 789–799.

Vinson SB, Hegazi EM (1998) A possible mechanism for the physiological

suppression of conspecific eggs and larvae following superparasitism by

solitary endoparasitoids. *Journal of Insect Physiology* **44**, 703–712.

Visser ME (1993) Adaptive self- and conspecific superparasitism in the solitary

parasitoid *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). *Behavioral Ecology* **4**, 22–28.

Visser ME, van Alphen JJM, Nell HW (1990) Adaptive superparasitism and patch time allocation in solitary parasitoids: the influence of the number of parasitoids depleting a patch. *Behaviour* **114**, 21–36.

Visser ME, van Alphen JJM, Hemerik L (1992a) Adaptive superparasitism and patch time allocation in solitary parasitoids: an ESS model. *Journal of Animal Ecology* **61**, 93–101.

Visser ME, van Alphen JJM, Nell HW (1992b) Adaptive superparasitism and patch time allocation in solitary parasitoids: the influence of pre-patch experience. *Behavioral Ecology and Sociobiology* **31**, 163–171.

Visser ME, Luyckx B, Nell HW, Boskamp GJF (1992c) Adaptive superparasitism in solitary parasitoids: marking of parasitized hosts in relation to the pay-off from superparasitism. *Ecological Entomology* **17**, 76–82.

Wang XG, Messing RH (2003) Intra- and interspecific competition by *Fopius arisanus* and *Diachesmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. *Biological Control* **27**, 251–259.

Wang XG, Messing RH (2008) Role of egg-laying experience in avoidance of

superparasitism by fruit fly parasitoid *Fopius arisanus* (Hymenoptera:

Braconidae). *Annals of the Entomological Society of America* **101**, 656–

663.

Yamada YY, Ikawa K (2003) Adaptive significance of facultative infanticide in the

semi-solitary parasitoid *Echthrodelphax fairchildii*. *Ecological Entomology*

28, 613–621.

Yamada YY, Ikawa K (2005) Superparasitism strategy in a semisolitary

parasitoid with imperfect self/non-self recognition, *Echthrodelphax*

fairchildii. *Entomologia Experimentalis et Applicata* **114**, 143–152.

Yamada YY, Imai N (2000) Identification of the sex of eggs and the mating status

of female adults in *Echthrodelphax fairchildii* (Hymenoptera: Dryinidae)

based on oviposition behavior. *Entomological Science* **3**, 579–583.

Yamada YY, Kitashiro S (2002) Infanticide in a dryinid parasitoid,

Haplogonatopus atratus. *Journal of Insect Behavior* **15**, 415–427.

Yamada YY, Sugaura K (2003) Evidence for adaptive self-superparasitism in the

dryinid parasitoid *Haplogonatopus atratus* when conspecifics are present.

Oikos **103**, 175–181.

FIGURE LEGENDS

Figure 1 Frequency of superparasitism in *Echthrodelphax fairchildii* parasitoids

for different first-to-second oviposition intervals in the presence and

absence of a conspecific. Data for the absence of a conspecific are from

Ito and Yamada (2014).

Figure 2 Frequency of infanticidal probing of the nonoviposition side in

Echthrodelphax fairchildii parasitoids for different first-to-second

oviposition intervals in the presence and absence of a conspecific. Data

for the absence of a conspecific are from Ito and Yamada (2014).

Table 1 Statistical results for superparasitism frequency: *P* values for each factor

Factor	Data analyzed						
	Self- and conspecific superparasitism for 0–0.75 h	Self-superparasitism for 0–0.75 h	Conspecific superparasitism for 0–0.75 h	Self- and conspecific superparasitism for 0.75–2 h	Self-superparasitism for 0–2 h	Conspecific superparasitism for 0–2 h [†]	Self- and conspecific superparasitism for 0–2 h, two females
Oviposition interval (A)–	<0.001	<0.001	0.010	0.045	<0.0001	0.494 (0.011 [‡])	0.001
Alone *vs* two females (B)	<0.001	<0.001	0.452	<0.001	<0.0001	0.048 (0.044 [‡])	–
Self- *vs* conspecific superparasitism (C)	<0.001	–	–	0.311	–	–	0.263
A × B	0.322 [‡]	0.333	0.680	0.856 [‡]	0.076 [‡]	0.311 (0.858 [‡])	–
A × C	0.111 [‡]	–	–	0.736 [‡]	–	–	0.915
B × C	0.012 [‡]	–	–	0.815 [‡]	–	–	–

[†]Values in parentheses indicate *P* values for when factor A was defined as a categorical value. [‡]Monte Carlo method.
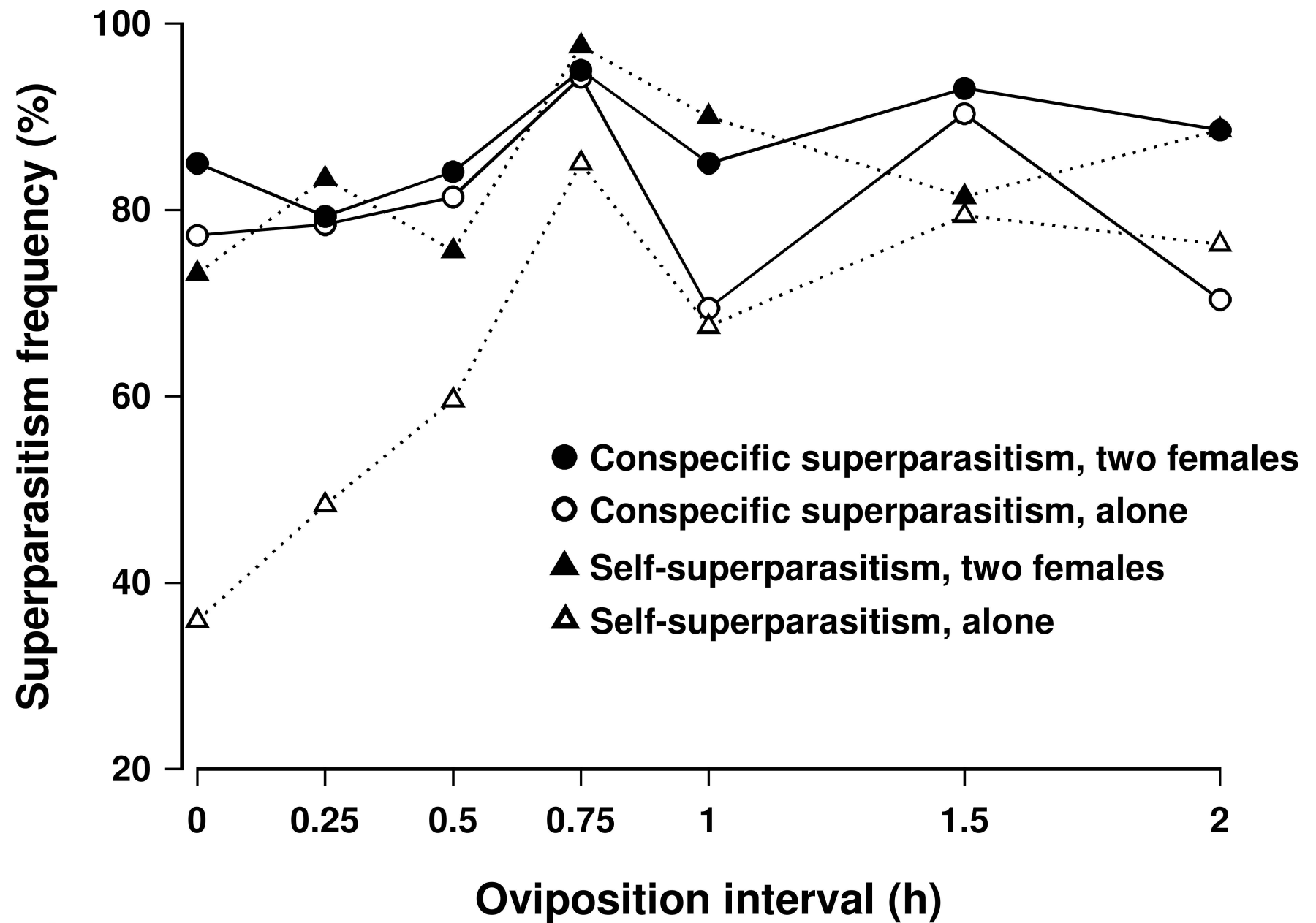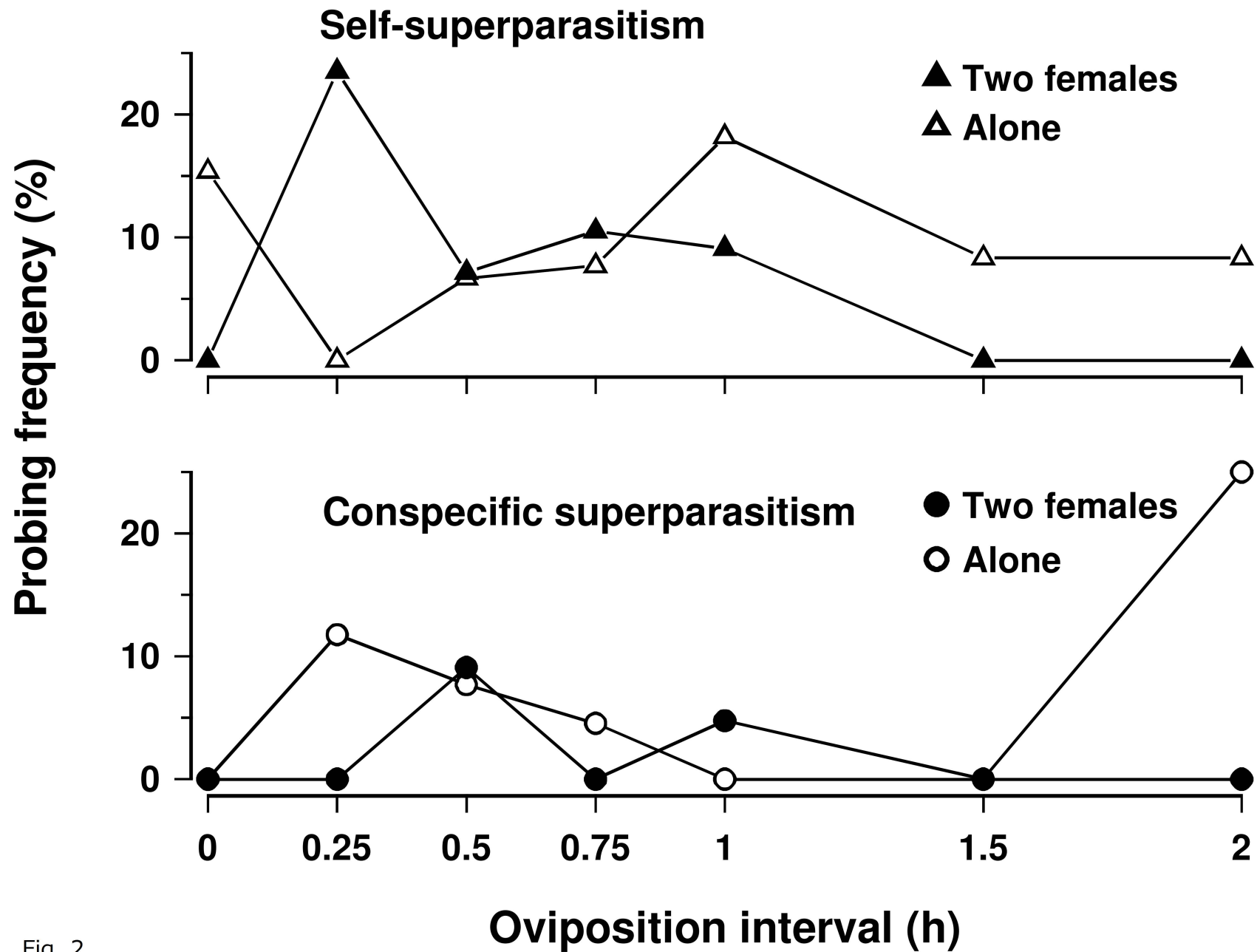
Fig. 1

Fig. 2