1   Self/conspecific discrimination and superparasitism strategy in the ovicidal

2   parasitoid *Echthrodelphax fairchildii* (Hymenoptera: Dryinidae)

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15   **Key words**   infanticide, ovicide, plant hopper, self-recognition, sex ratio

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19   **Abstract**   Superparasitism in solitary parasitoids results in fatal competition

20   between the immature parasitoids, and consequently only one individual can

21   emerge.   In the semisolitary ovicidal parasitoid *Echthrodelphax fairchildii*

22   (Hymenoptera: Dryinidae), two adults can emerge under superparasitism with a

23   short interval (<24 h) between the first and second ovipositions.   We

24   determined the female parasitoid's behavioral responses under self- and

25   conspecific superparasitism bouts with first-to-second oviposition intervals of ≤ 2

26   h.   The self- and conspecific superparasitizing frequencies increased up to an

27   oviposition interval of 0.75 h, with the former remaining lower than the latter,

28   particularly for oviposition intervals of ≤ 0.25 h, suggesting the existence of

29   self/conspecific discrimination.   The superparasitizing frequency plateaued for

30   oviposition intervals of ≥ 0.75 h, with no difference between self- and conspecific

31   superparasitism.   The ovicidal-probing frequency did not differ under self- and

32   conspecific superparasitism, and was usually <20%.   The females exhibited no

33   preference for the oviposition side (i.e., ovipositing on the side with or without the

34   first progeny) and almost always laid female eggs for any oviposition interval

35   under self- and conspecific superparasitism.   The sex ratio was not affected by

36   the type of superparasitism, oviposition sides, or the occurrence of ovicidal

37   probing.   These observed results about the oviposition side, ovicidal probing,

38   and sex ratios differed from the predictions obtained assuming that the females

39   behave optimally.   Possible reasons for the discrepancies are discussed: likely

40   candidates include the high cost of selecting oviposition sides and ovicidal

41   probing, and, for the sex ratio, the low frequency of encountering suitable hosts

42   before superparasitism bouts.

43

44   **Introduction**

45

46   Conspecific superparasitism, which involves ovipositing on or in a conspecifically

47   parasitized host (van Dijken & Waage, 1987), is usually profitable, in that it

48   rewards the parasitoid with a fitness gain, especially when the interval between

49   the first and second ovipositions (i.e., the oviposition interval) is short (e.g.,

50   Visser et al., 1992; Sirot, 1996; Field et al., 1997; Lebreton et al., 2009); however,

51   the gain is less than that from ovipositing on or in a healthy host.   Meanwhile,

52   self-superparasitism is generally considered less profitable than conspecific

53   superparasitism due to the siblings facing competition for limited resources (e.g.,

54   Yamada & Miyamoto, 1998; Yamada & Watanabe, 2002; Yamada & Ikawa,

55   2005); in particular, it is usually non- or negatively profitable for solitary

56   parasitoids except in cases where multiple parasitoid immatures guarantee a

57   higher emergence probability of one adult, which is probably due to greater

58   suppression of the immune systems of the host when multiple individuals are

59   present (Puttler & van den Bosch, 1959).   Thus, an ability to discriminate

60   between self- and conspecifically parasitized hosts is expected to evolve (van

61   Alphen & Visser, 1990).   Many parasitoids have this ability (van Dijken et al.,

62   1992), but it appears to diminish as the oviposition interval increases (Hubbard

63   et al., 1987; Visser, 1993; Ueno, 1994), with the few exceptions including

64   *Dinarmus basalis* (Gauthier et al., 1996) and *Nasonia vitripennis* (King, 1992).

65   Few studies have investigated the effects of the oviposition interval in detail.

66   The mated female must decide whether she should lay a male or female

67   egg after accepting superparasitism.   If the local mate competition (LMC) model

68   (Hamilton, 1967) is applicable to the target parasitoid, the female should be

69   more likely to lay a male egg under conspecific superparasitism than under

70   self-superparasitism, because under the former she recognizes that local

71   competition for mates will be more likely to occur between her progeny and

72   those of another female (King, 1992; Darrouzet et al., 2008).   Moreover, when

73   the fitness performance of the first and second progenies during the immature

74   stage differ according to their sex, the sex of the second progeny may differ

75   between self- and conspecific superparasitism (this is called asymmetric larval

76   competition; van Baaren et al., 1999; Darrouzet et al., 2003; Sykes et al., 2007;

77   Lebreton et al., 2010).

78   When the parasitoid is an ovicidal and/or larvicidal species (both ovicide

79   and larvicide are collectively called infanticide hereafter), it faces another

80   decision-making dilemma besides superparasitism acceptance and sex

81   allocation – the female parasitoid should always perform infanticide against

82   conspecifically parasitized hosts if the cost of infanticide is negligible (Netting &

83   Hunter, 2000; Takasuka & Matsumoto, 2011).   On the other hand, when the

84   host is self-parasitized the solitary parasitoid should usually avoid

85   superparasitism, and consequently does not need to decide whether she should

86   perform infanticide.   The semisolitary parasitoid – in which emergence of two

87   parasitoids is possible under superparasitism even though a single egg is laid in

88   an ovipositing episode – should accept self-parasitized hosts without infanticide

89   instead of avoiding them when the host availability is low and the two eggs laid

90   will be likely to develop to adulthood.   However, the above predictions are

91   based on the assumption that the female parasitoid can perfectly distinguish

92   between self- and conspecifically parasitized hosts.   If the self/conspecific

93   discrimination is not perfect or even completely impossible, the female should

94   base her decision on the degree of accuracy of self/conspecific discrimination

95   and the difference between fitness gains obtained by correct and incorrect

96   decisions (Rosenheim & Mangel, 1994; Yamada & Ikawa, 2005; Segoli et al.,

97   2009).

98   The parasitoid *Echthrodelphax fairchildii* Perkins (Hymenoptera:

99   Dryinidae) is a semisolitary species (Yamada & Imai, 2000; Yamada & Ikawa,

100   2003): two adults can emerge from a single host for oviposition intervals of <24 h.

101   The female parasitoid lays an egg between the wing bud and the epimeron of

102   the thorax (Yamada & Imai, 2000; Yamada & Ikawa, 2003).   Superparasitism in

103   *E. fairchildii* is classified into two types according to whether the first and second

104   ovipositions occur on the same side (S-type) or on different sides (D-type) of the

105   host: the first progeny is always killed by the superparasitizing female in the

106   former case (Yamada & Ikawa, 2005), while two adults may emerge when the

107   oviposition interval is <24 h in the latter case (Yamada & Ikawa, 2003).   The

108   female parasitoid often probes the nonoviposition side for infanticide under

109   D-type superparasitism (Yamada & Ikawa, 2003); this probing takes less than

110   10 s and hence its time cost can probably be ignored.   Our study group has

111   previously (Yamada & Ikawa, 2003, 2005) found that the frequency of

112   superparasitism and the preferred oviposition side among unmated females do

113   not differ between self- and conspecific superparasitism with oviposition intervals

114   of 1–96 h, but that the frequency of probing the nonoviposition side differs

115   slightly but significantly between self- and conspecific superparasitism.   Our

116   group has also determined fitness gains from superparasitism when the first and

117   second eggs are male.   If a larger quantity outweighs the loss of quality (i.e.,

118   small emerging adults), fitness gains are obtained from D-type

119   self-superparasitism with oviposition intervals of <24 h due to the emergence of

120   two adults.   Meanwhile, fitness gains from S-type conspecific superparasitism

121   are similar to those from parasitism on a healthy host because the first progeny

122   is killed, and are higher than those from D-type conspecific superparasitism

123   because probing does not always occur or is successful under the latter

124   (Yamada & Ikawa, 2003).   However, experiments have never been performed

125   for oviposition intervals of <1 h, and it is possible that the female parasitoid is

126   capable of accurate self/conspecific discrimination for such short oviposition

127   intervals, as seen in *Itoplectis naranyae* (Ueno, 1994). Moreover, sex

128   allocation has not previously been compared between self- and conspecific

129   superparasitism in *E. fairchildii*, although our group has suggested that eggs laid

130   under superparasitism are more likely to be female (Ito & Yamada, 2005).

131   Assuming that the effects of superparasitism on survival rates and adult

132   sizes are similar for female and male progenies, we predict that

133   self-superparasitism is less frequent than conspecific superparasitism among

134   parasitoids exhibiting perfect self/conspecific discrimination. Assuming that

135   ovicidal probing of the nonoviposition side incurs no cost, female parasitoids

136   would then be more likely to perform ovicidal probing on the nonoviposition side

137   under conspecific superparasitism than under self-superparasitism. Moreover,

138   females with self/conspecific discrimination may prefer D-type superparasitism

139   under self-superparasitism in order to ensure that two adults emerge, while

140   S-type superparasitism may be preferred under conspecific superparasitism

141   because ovicidal probing does not always succeed (Yamada & Ikawa, 2003).

142   The LMC model (Hamilton, 1967) may be applicable to *E. fairchildii* when

143   considering the sex allocation under superparasitism, since the host population

144   is distributed contagiously (Kuno, 1963). The LMC model was primarily

145 developed for gregarious species, but it can also be applied to solitary

146 parasitoids that are parasitic on hosts distributed in clumps (Godfray, 1994).

147 Then, conspecifically parasitized hosts are cues for the existence of conspecifics

148 and it is expected that the female is more likely to lay male progenies, but not in

149 response to self-parasitized hosts (King, 1992; Darrouzet, 2008). Moreover,

150 asymmetric larval competition may cause the sex ratio to differ between self-

151 and conspecific superparasitism: under self-superparasitism the female may be

152 more likely to lay the sex that maximizes the total fitness performance of both the

153 first and second progenies, while under conspecific superparasitism she may be

154 more likely to lay the sex that maximizes the fitness performance of the second

155 progeny. However, this scenario is effective only when D-type superparasitism

156 occurs and ovicidal probing does not occur; that is, when the first egg is not

157 killed by the superparasitizing female. Therefore, the sex ratio may vary with

158 the type of superparasitism (self- or conspecific superparasitism), oviposition

159 sides, and the occurrence of ovicidal probing. In addition, the female may be

160 likely to lay a male egg under superparasitism following the host-quality model

161 (Charnov, 1982), because the second progeny is likely to emerge as a small

162 wasp due to the small amount of food available (Yamada & Ikawa, 2005). This

163   model is applicable only when the first egg is not killed by the superparasitizing

164   female.   However, it should be noted that the host-quality model does not

165   predict a difference between self- and conspecific superparasitism (King, 1992).

166   The first aim of the present study was to determine the ability for

167   self/conspecific discrimination in the parasitoid *E. fairchildii* by comparing

168   superparasitizing frequencies, probing frequencies, the sex ratio of progenies,

169   and preference for the oviposition side between self- and conspecific

170   superparasitism for oviposition intervals in the range of 0–2 h using mated

171   females.   The second aim was to present possible reasons for any differences

172   between the above-predicted behaviors and those adopted by real female

173   parasitoids.

174

175   **Materials and Methods**

176

177   *Insects*

178

179   *Echthrodelphax fairchildii* is a synovigenic ectoparasitoid of the following three

180   rice-damaging plant hoppers (Homoptera: Delphacidae): *Nilaparvata lugens*

181 (Stål), *Sogatella furcifera* (Horváth), and *Laodelphax striatellus* (Fallén). The

182 female parasitoid often feeds on these hosts. Parasitized hosts continue to

183 feed on host plants, but they do not molt to the next instar. Immature

184 parasitoids are sedentary at the place where ovipositing occurs.

185 *Echthrodelphax fairchildii* female adults usually live for 2–4 weeks. The

186 daily fecundity is 15–25 eggs when the female parasitoid is 3–20 days old

187 (Yamada YY & Yamaguchi S, 1997, unpublished data), and the handling time for

188 laying an egg is usually less than 100 s even when probing for infanticide occurs

189 (Ito, 2009).

190 *Echthrodelphax fairchildii* and *L. striatellus* were collected at two places

191 during 1992 in Tsu, Mie, Japan, and reared separately and continuously under

192 laboratory conditions. Parasitoid pupae were gathered for experiments from

193 the laboratory populations and placed individually in 10-ml glass vials. The use

194 of related individuals for conspecific superparasitism was avoided by ensuring

195 that all pairs comprised two females that originated from different populations

196 that had been reared separately. After emergence, females were individually

197 reared in 340-ml plastic cages containing a 50% (by weight) honey solution, 1

198 fifth-instar host for parasitism or food, 15 first- or second-instar hosts for food,

199   and 2 male wasps for mating.   These hosts and the honey solution were

200   renewed every day.   Since females of *E. fairchildii* seldom parasitize first- and

201   second-instar hosts, this rearing method resulted in a very low availability of

202   hosts, which increased the likelihood of the superparasitizing by parasitoids.

203   Mated females aged 4–20 days (mostly <13 days) were used for

204   superparasitism bouts.   The females were allowed to lay the first egg for

205   superparasitism on fifth-instar nymphs that were within 24 h of molting; single

206   parasitism at this stage maximizes the survival rate of immature parasitoids

207   (Yamada YY & Takayama T, 1996, unpublished data).   Observations and

208   rearing were performed in a room at 24–26°C and with a light:dark photoperiod

209   of 16:8 h.

210

211   *Behavioral options in superparasitism bouts*

212

213   The oviposition interval for superparasitism was defined in the present study as

214   the interval between the time that the first oviposition occurred and the time that

215   a parasitoid and a parasitized host were placed together in a small vial; the

216   following periods were assigned: 0, 0.25, 0.5, 0.75, 1, 1.5, and 2 h.   In each first

217 oviposition bout, the female in a rearing plastic cage was moved into a clean

218 4-ml transparent plastic vial containing two second-instar hosts for food

219 immediately after the light was turned on, and kept therein for 1 h before a

220 healthy fifth-instar host was added.   The host was removed immediately after

221 being parasitized, and then returned after an assigned oviposition interval for

222 self-superparasitism bouts.   The exception was for an interval of 0 h, in which

223 the host was kept in the vial with the parasitoid instead of being removed.   For

224 conspecific superparasitism bouts, two hosts each parasitized by one or the

225 other of a pair were exchanged and given to the partner of the pair after an

226 assigned interval.   An interval of 0 h for conspecific superparasitism was not

227 usually realized because two individuals of a pair rarely oviposited at the same

228 time, and so intervals of <8 min were categorized as 0 h.   Consequently,

229 parasitoids used for conspecific superparasitism bouts were allowed to

230 parasitize an unparasitized host before an assigned interval, as were those used

231 for self-superparasitism bouts, which precluded the possibility that differences in

232 the experience of ovipositing would lead to the behavioral differences between

233 self- and conspecific superparasitism bouts.   For both self- and conspecific

234 superparasitism bouts, a single second-instar host was supplied to the female

235 parasitoid for food during oviposition intervals of 0.5 and 0.75 h, while two, three,

236 and four hosts were supplied during intervals of 1, 1.5, and 2 h, respectively; this

237 experimental procedure ensured that female parasitoids did not feed on the

238 parasitized hosts.   We observed the ovipositing behavior under fluorescent

239 lighting via a supersensitive video camera (WAT-902H, Watec, Yamagata,

240 Japan) attached to a binocular microscope (magnification, 40×), and recorded

241 the oviposition side (right or left) of the host.   Whether the female parasitoid

242 moved her abdominal tip to the nonoviposition side for probing was also

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

244 movement of the genitalia (Yamada & Imai, 2000): these observations were not

245 successful for 0–10.6% of the superparasitism ovipositions for each oviposition

246 interval, and the sex was not determined for 0–11.4% of them due to a long

247 pausing time (>10 s), which suggests that the female parasitoid had trouble

248 releasing sperm, and consequently often laid male eggs even though she was

249 considered to have tried to lay female eggs (Yamada & Imai, 2000).   We

250 stopped the observation when the female parasitoid did not superparasitize a

251 host within 10 min; these cases were regarded as superparasitism avoidance.

252 When superparasitism avoidance occurred, an unparasitized host was exposed

253   to the parasitoid to examine whether the avoidance occurred due to the female's

254   general lack of interest in ovipositing.   If the female did not oviposit on the host

255   within 10 min, the data were discarded (such cases were very rare).

256   Individual females used for conspecific superparasitism were also used

257   for self-superparasitism.   We had planned to allow individual females to perform

258   one self- and one conspecific superparasitism bout at each interval of 0,0.25, 0.5,

259   0.75, 1, 1.5, and 2 h (the order of oviposition intervals was selected randomly for

260   each individual), but this was impossible due to parasitoids dying; about half of

261   the planned bouts were performed in most cases.   In total, between 36 and 64

262   superparasitism bouts were performed for each oviposition interval.   Because

263   some bouts did not result in ovipositing, the sample size for each oviposition

264   interval varied from 23 to 51 for the oviposition sides and sex ratios, and from 9

265   to 26 for the probing frequency under D-type superparasitism.

266

267   *Data analysis*

268

269   The effects of the type of superparasitism and oviposition intervals on

270   superparasitizing frequencies, ovicidal-probing frequencies, preferred

271   oviposition sides, and sex ratios were analyzed.　The cases in which the sex of

272   eggs was identified were used for analyzing sex ratios, and the effects of

273   oviposition sides (same or different) and the occurrence of probing on sex ratios

274   were also analyzed: Cases in which ovipositing occurred on the same side and

275   cases in which it occurred on different sides with no ovicidal probing were first

276   compared, and then the latter were also compared with cases of different

277   oviposition sides in which ovicidal probing occurred.　In the latter comparison,

278   the effect of oviposition intervals was not included in the statistical models due to

279   the frequencies of ovicidal probing being too low.　Logistic regression analysis

280   was performed using the LogXact[®]9 software (Cytel Software, Cambridge, MA,

281   USA).　Significance was tested by determining exact probability values (Cytel

282   Inc., 2010).　The logistic regression model included (as a numeric variable) the

283   possible influences of the age of the ovipositing females.　However, the

284   analysis revealed that the age did not affect the above-mentioned items, and so

285   the analysis results for ages of parasitoid females are not presented.　In

286   addition to the above-mentioned factors, which are all fixed ones, the influence

287   of individual parasitoids was incorporated in the model as a random factor.　The

288   sex ratio was also compared between the first and second eggs under self- and

289   conspecific superparasitism with different oviposition intervals using Fisher's

290   exact test with serial Bonferroni correction (Rice, 1989).   Moreover, whether or

291   not the preference for the oviposition side was random was determined using the

292   binomial test with serial Bonferroni correction.

293   The analysis of superparasitizing frequencies revealed a significant

294   two-way interaction between oviposition intervals and the type of

295   superparasitism ($P = 0.004$).   Therefore, the analysis was performed separately

296   for oviposition intervals of 0–0.75 and 0.75–2 h, for which no two-way interaction

297   was detected.   No significant interactions were detected in the other analyses,

298   including ones for ovicidal-probing frequencies, preferred oviposition sides, and

299   sex ratios, and hence we do not refer to interactions of the factors in the Results.

300   Moreover, a significant difference was detected between self- and conspecific

301   superparasitizing frequencies for oviposition intervals of 0–0.75 h, as described

302   in the Results, and so we evaluated the extent of the difference at individual

303   oviposition intervals by using Fisher's exact test with serial Bonferroni correction.

304

305   **Results**

306

307   The effects of oviposition intervals and the type of superparasitism on

308   superparasitizing frequencies were significant for oviposition intervals of 0–

309   0.75 h (both $P < 0.00001$; Fig. 1).   However, it is should be noted that the

310   conspecific-superparasitizing frequencies at oviposition intervals of 0–0.5 h were

311   similar, and increased abruptly between oviposition intervals of 0.5 and 0.75 h.

312   Comparison between the self- and conspecific superparasitizing frequencies at

313   individual oviposition intervals revealed a significant difference for oviposition

314   intervals of 0 and 0.25 h ($P < 0.001$ and $P < 0.01$, respectively), but not for those

315   of 0.5 and 0.75 h.   Meanwhile, neither the oviposition interval nor the type of

316   superparasitism had significant effects for oviposition intervals of 0.75–2 h ($P =$

317   0.10 and $P = 0.34$, respectively).

318   The probing frequency was usually <20% (Fig. 2), and was independent

319   of the type of superparasitism ($P = 1.00$) and oviposition intervals ($P = 0.65$).

320   There was no preference for the oviposition side for any oviposition interval

321   (Fig. 3; $P > 0.05$, binomial test with serial Bonferroni correction); consequently,

322   the preference for the oviposition side was also independent of the two factors

323   ($P = 0.85$ for oviposition intervals; $P = 0.67$ for the type of superparasitism).

324   Most of the progeny produced were female (Fig. 4).   The sex ratio was

325   independent of oviposition intervals (P = 0.91), the type of superparasitism (P =

326   0.43), and the oviposition sides (P = 0.46).   When ovicidal probing occurred, the

327   sex ratio (i.e., the proportion of males) was 0.0% for both self- and conspecific

328   superparasitism (n = 10 and 5, respectively); this value did not differ from those

329   when ovicidal probing did not occur (0.0% [n = 93] and 5.4% [n = 93] for self- and

330   conspecific superparasitism; P = 1.0 for both).   The sex ratio for ovipositing on

331   healthy hosts was 0.35% (n = 575); this value did not differ from those under

332   self- and conspecific superparasitism for the different oviposition intervals,

333   irrespective of the oviposition sides (P > 0.1, Fisher's exact test with serial

334   Bonferroni correction).

335

336   **Discussion**

337

338   A difference in superparasitizing frequencies for a short oviposition interval

339   indicates that *E. fairchildii* females exhibit self/conspecific discrimination when

340   ovipositing.   The superparasitizing frequency increased with the oviposition

341   interval, irrespective of whether the superparasitism was self- or conspecific

342   superparasitism.   The increase in self-superparasitism is considered to be

343   caused mainly by an impaired ability for self-recognition.   However, it should be

344   noted that the increase in self-superparasitism could have occurred because the

345   parasitoid did not encounter healthy hosts for a while; when healthy hosts are

346   abundant the parasitoid should conserve eggs and time in the hope of

347   encountering more profitable hosts in the near future.   Not encountering hosts

348   for a longer period reportedly increases the probability of a parasitoid

349   superparasitizing (e.g., Hughes et al., 1994; Henneman et al., 1995; Hubbard et

350   al., 1999; Babendreier & Hoffmeister, 2002).   However, it is considered that this

351   factor played only a minor role in our experiments since there was only a very

352   small increase in superparasitizing frequencies for oviposition intervals of

353   ≤ 0.75 h under conspecific superparasitism and no increase in superparasitizing

354   frequencies was found for oviposition intervals of 0.75-2 h under self- and

355   conspecific superparasitism.

356   The conspecific-superparasitizing frequency hardly increased for

357   oviposition intervals within the range of 0−0.5 h, but increased abruptly between

358   oviposition intervals of 0.5 and 0.75 h.   Such a pattern of increase was also

359   found in self- and conspecific superparasitizing frequencies in another

360   experiment where the same procedures were performed except for the use of

361 parasitoids that encountered a conspecific before a superparasitism bout (Ito,

362 2009). We currently cannot explain why such a pattern of increase occurred,

363 but this could be addressed by performing the same procedures using a

364 parasitoid that encounters many unparasitized hosts before a superparasitism

365 bout. These females are less likely to accept superparasitism for shorter

366 oviposition intervals, and hence such an experiment would reveal more clearly

367 how the superparasitizing frequencies change with increasing oviposition

368 intervals.

369 The probing frequencies did not differ between self- and conspecific

370 superparasitism and were very low, even for the oviposition intervals for which

371 the superparasitizing frequency differed, although high probing frequencies had

372 been predicted for the intervals. There are three possible reasons for this,

373 which are not mutually exclusive. The first is that even when probing takes only a

374 short time (<10 s), it may cause unrecoverable fatigue or physiological damage,

375 and consequently the female parasitoid does not obtain net fitness gains by

376 probing conspecifically parasitized hosts. When ovipositing the female

377 parasitoid must hold the host away from the plant on which it is feeding.

378 Moreover, when probing, the female must move the host around while holding it

379   (Yamada & Ikawa, 2003).   This procedure appears to represent hard work for

380   the female, and hard work has been recently considered to cause delayed

381   maturation, a low fecundity, or a short longevity in animals, including wasps and

382   bees (O'Donnell & Jeanne, 1992, 1995; Heinsohn & Legge, 1999; Finkel &

383   Holbrook, 2000; Nilsson, 2002; Williams et al., 2008).   Our group's recent work

384   has revealed that repeated probing on many hosts leads to hesitation in future

385   probing (Yamada YY & Katsuyama H, 2010, unpublished data), suggesting that

386   ovicidal probing incurs a substantial physiological cost.   The second possible

387   reason is that the female parasitoid discriminates the hosts that she has

388   parasitized just a short time previously from conspecifically parasitized hosts, but

389   not from the other self-parasitized hosts.   This means that the parasitized hosts

390   that the female parasitoid does not recognize as self-parasitized include some

391   self-parasitized hosts, unless she can estimate perfectly the amount of time that

392   has passed since the first oviposition.   In addition, not all hosts that have been

393   self-parasitized a short time ago are considered to be recognized as

394   self-parasitized.   The inability to perfectly discriminate self-parasitized hosts

395   from conspecifically parasitized hosts will make her concerned about the risk of

396   accidentally killing her own progeny.   In other words, self-recognition does not

397   lead to conspecific recognition when it is not perfect.   It is considered that the

398   above phenomenon is likely to occur if self-recognition is achieved by using the

399   temporary memory of the smell (chemicals) of self-parasitized hosts (Ueno &

400   Tanaka, 1996) or by comparing her own smell with that left on the host

401   (self-matching; Dawkins, 1982: Wyatt, 2003).   The third possible reason is that

402   cues for discriminating between self- and conspecific superparasitism can differ

403   between when accepting superparasitism and when performing ovicidal probing.

404   King and Skinner (1991) found that the cues used by *N. vitripennis* females for

405   discriminating between unparasitized and parasitized hosts differ between when

406   deciding the clutch size and when performing sex allocation of progenies.

407   The absence of a difference in probing frequency between self- and

408   conspecific superparasitism is inconsistent with our previous findings (Yamada &

409   Ikawa, 2003) of the probing frequency being slightly but significantly higher

410   under conspecific superparasitism than under self-superparasitism for

411   oviposition intervals of 1–96 h.   The reasons for this discrepancy are unclear,

412   but could be associated with differences between the experimental conditions:

413   the previous experiment (1) used unmated females that were reared with first-,

414   second-, and third-instar hosts (third-instar hosts are often used for ovipositing

415 when host availability is low [Yamada YY & Noda S, 2012, unpublished data]),

416 (2) kept the females in a vial without hosts for 1 h before superparasitism bouts,

417 and (3) did not use a constant time between the first oviposition and when the

418 light was turned on.

419   Most of the eggs laid under superparasitism were female in the present

420 study, and the sex ratio was independent of the type of superparasitism,

421 oviposition sides, and the occurrence of ovicidal probing.   Therefore, the LMC

422 model, host-quality model, and asymmetric larval competition do not appear to

423 be applicable to these results.   However, we cannot conclude that these three

424 theories do not apply at all to *E. fairchildii* females.   Encountering a single

425 conspecifically parasitized host is insufficient to induce the female to change the

426 sex ratio, but this ratio may change after encountering many conspecifically

427 parasitized hosts and/or female adults (see Shuker & West, 2004).   Moreover,

428 the host-quality model predicts that the female adult should adjust the sex ratio

429 based on the quality of the target host relative to the hosts she has encountered

430 previously (Charnov, 1982); evidence for this is found in several species,

431 including *Lariophagus distinguendus* (Charnov et al., 1981) and *Diglyphus isaea*

432 (Ode et al., 2002).   All but one of the hosts that the female encountered in a

433   rearing cage before superparasitism bouts in the present study were first- and

434   second-instar hosts that were unsuitable for oviposition (the exception was a

435   fifth-instar host).   The females may regard even parasitized hosts as being of

436   high quality under this rearing condition.   As for asymmetric larval competition,

437   if the female is not only a stronger competitor but also produces the total fitness

438   performance of the first and second progenies, which appears to be rare, the

439   phenomena obtained in the present study can be seen.   Further investigation is

440   required for understanding the sex allocation strategy in *E. fairchildii.*

441   *Echthrodelphax fairchildii* females exhibited no preference for the

442   oviposition side, which is probably due to the difficulties of detecting the side with

443   the first progeny before capturing the host and of changing the side after

444   capturing, as discussed by Yamada and Ikawa (2005).

445   Five species, including *E. fairchildii* (in this study), have been reported to

446   exhibit self/conspecific discrimination for only certain oviposition intervals when

447   facing the dilemma of whether to accept superparasitism.   In four of the five

448   species, self/conspecific discrimination is possible up to a threshold oviposition

449   interval.   This interval is between 30 and 60 min for *I. naranyae* (Ueno, 1994),

450   as in *E. fairchildii*; between 3 and 22 h for *Leptopilina heterotoma* (Visser, 1993)

451   (experiments were performed only for the two oviposition intervals); and

452   between 1 and 2 days for *Venturia canescens* (Hubbard et al., 1987; see also

453   Rogers, 1972; Hubbard et al., 1999). Meanwhile, *D. basalis* females show a

454   strange response: they exhibit self/conspecific discrimination for oviposition

455   intervals of 8, 42, and 72 h but not for oviposition intervals of 0.5, 16, and 24 h.

456   This variation in threshold oviposition intervals appears to reflect variations

457   among the species in the probability of encountering self- and conspecific

458   parasitized hosts in the field and in fitness gains from self- and conspecific

459   superparasitism. This assumption remains to be verified in future studies.

460

461

462   **Disclosure**

463

464   Neither author has any competing financial interests to declare.

465

466   **References**

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614

615 **Figure legends**

616 **Fig. 1** Frequency of superparasitism for different oviposition intervals in

617 *Echthrodelphax fairchildii*.

618 **Fig. 2** Frequency of probing of the nonoviposition side for different oviposition

619 intervals in *Echthrodelphax fairchildii*.

620 **Fig. 3** Proportion of superparasitism events in which the first and second

621 ovipositions were on the same side in *Echthrodelphax fairchildii*.

622 **Fig. 4** Proportion of male second progenies laid under superparasitism in

623 *Echthrodelphax fairchildii*, indicated according to whether the first and

624 second progenies were laid on the same side or different sides. When

625 the ovipositions were on different sides, the cases in which ovicidal
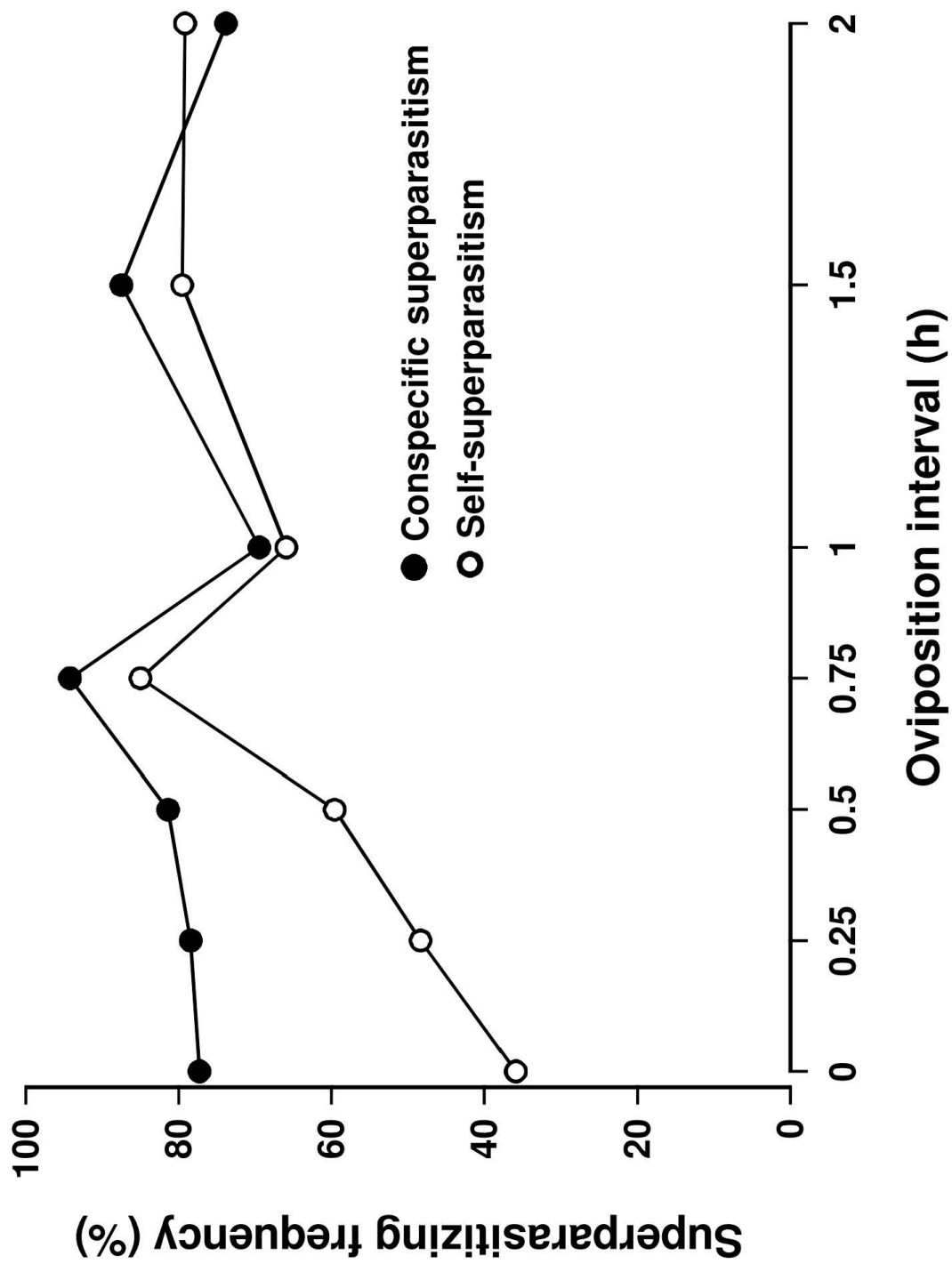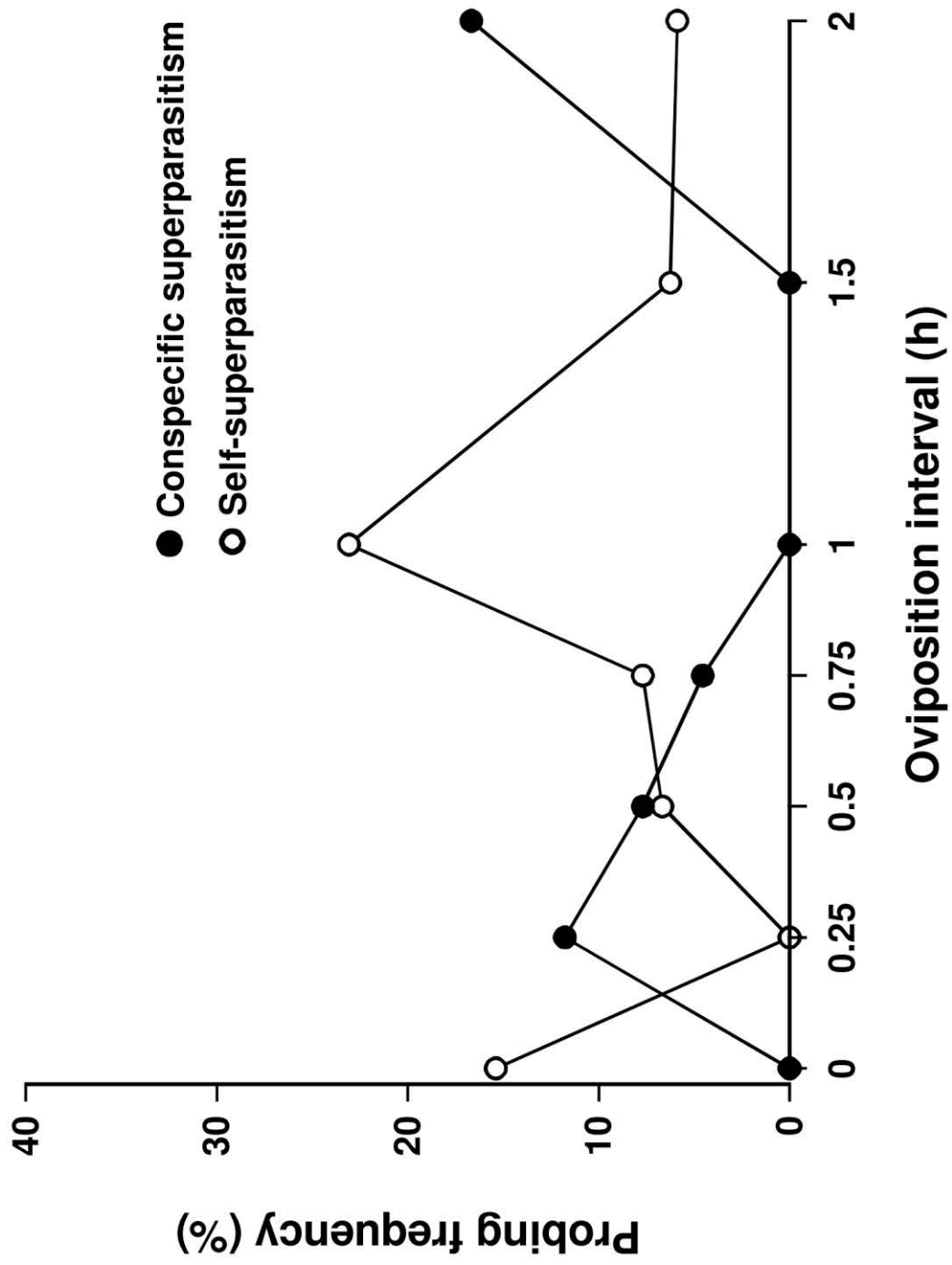
626 probing occurred were excluded.
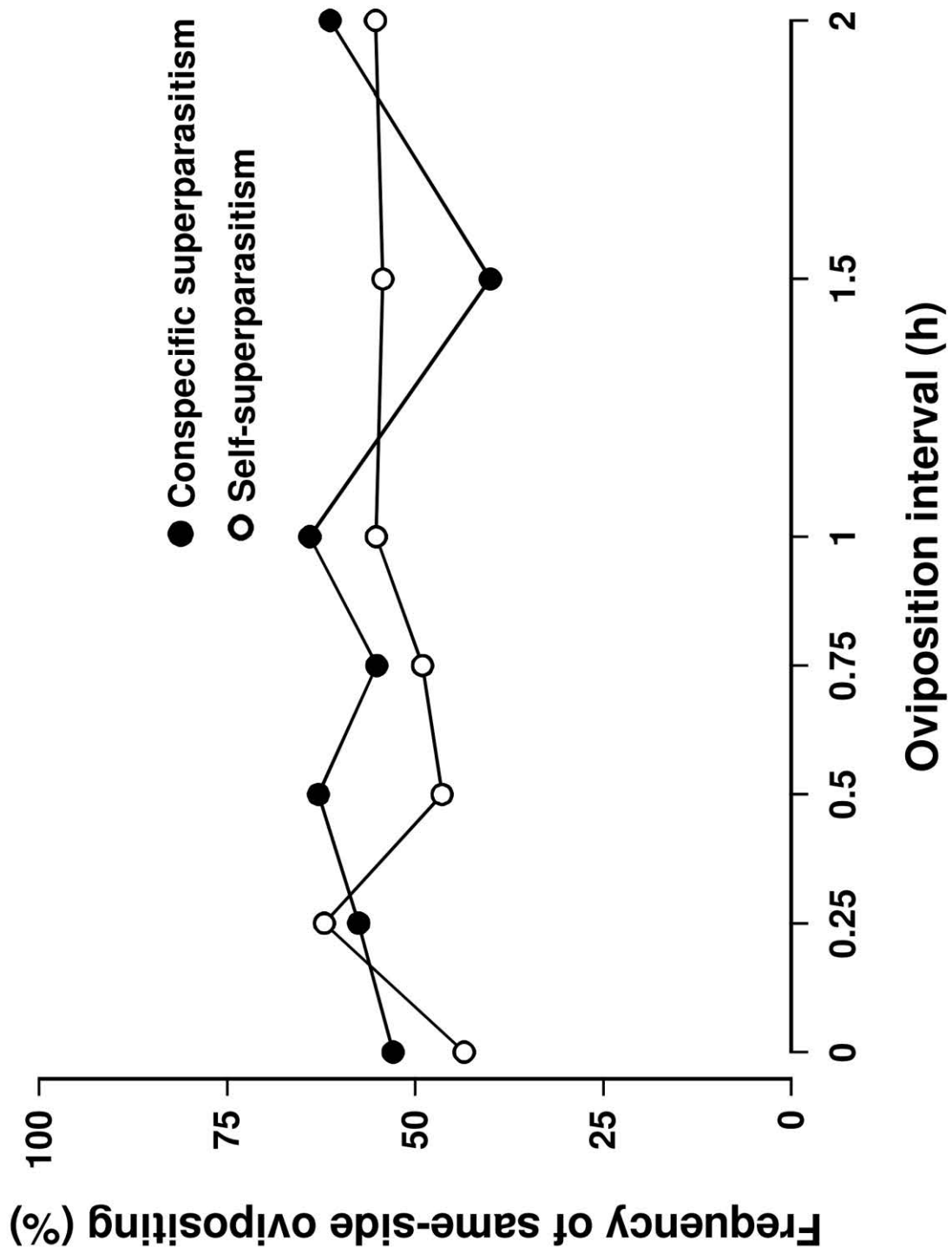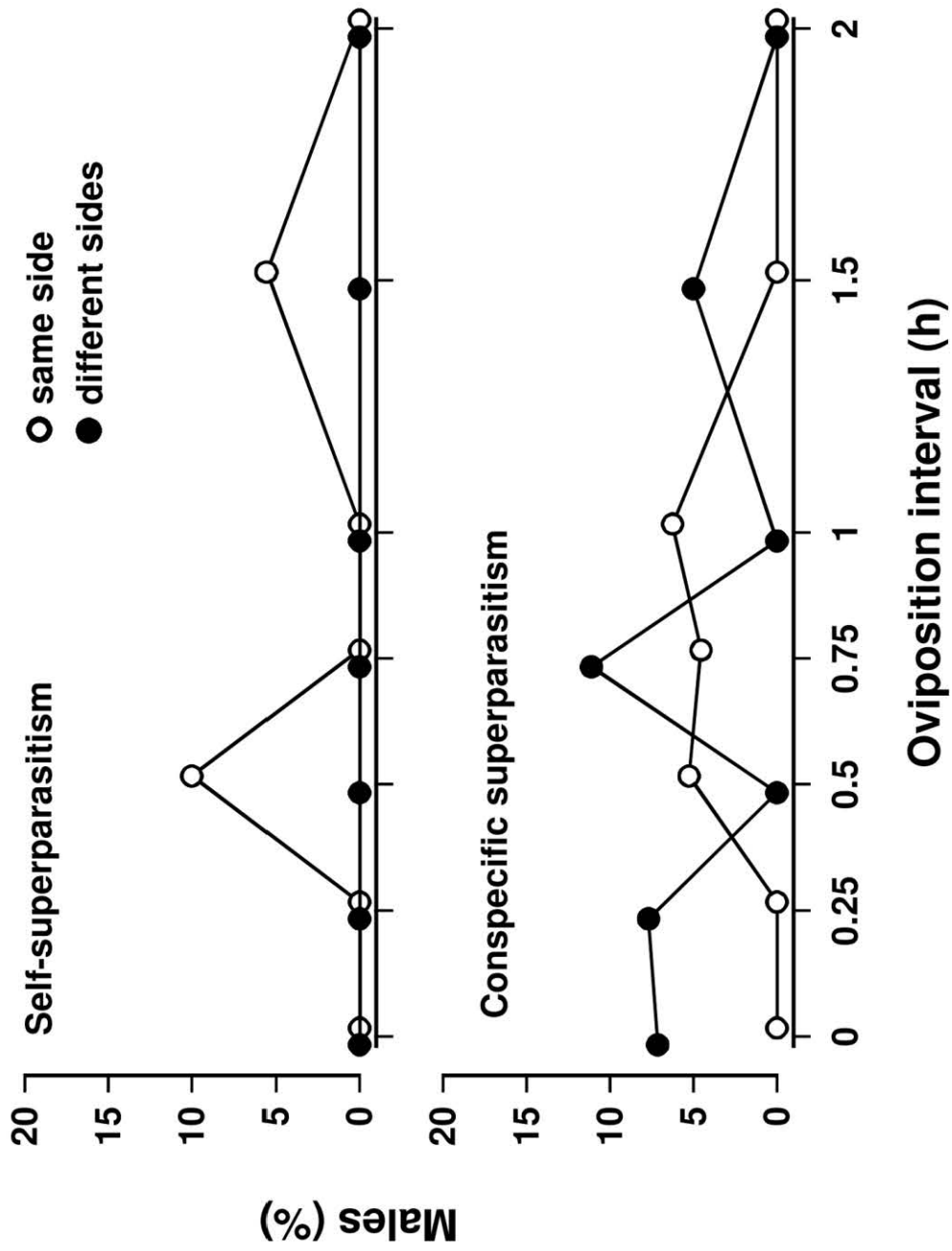
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Figure 1

Figure 2

Figure 3

Figure 4