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Doctoral Thesis

Fitness performance of immatures under superparasitism and superparasitism strategy in an infanticidal semi-solitary parasitoid (Hymenoptera: Dryinidae): Effects of size of ovipositing females

子殺しを行う準単寄生蜂セグロカマバチにおける過寄生から得る利益と過寄生戦略：産卵雌の大きさの影響

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1. General introduction

Parasitoids, insects whose larvae feed and develop on or in insect hosts, often substantially influence the host population dynamics (Beddington et al. 1978). Therefore, many parasitoids have been used for biological control of pests (DeBach and Rosen 1991; Hajek 2004). In addition, parasitoids have been used to test various hypotheses proposed to explain foraging and sex allocation strategies in behavioral ecology (Godfray 1994; Wajnberg et al. 2008) because they are easy to rear and handle in the laboratory (see for 3.1. in chapter 3 for more details).

Superparasitism–ovipositing in or on hosts that are parasitized by the same species (van Dijken and Waage 1987)–is common in parasitoids (Salt 1961; van Alphen and Visser 1990; Godfray 1994). It had been thought for a long time that superparasitism is a mistake of the ovipositing parasitoid–it occurs due to inability of the ovipositing female to discriminate parasitized hosts from unparasitized hosts–and that it is maladaptive behavior. However, superparasitism has been considered to be profitable, and adaptive under some situations; i.e., the parasitoid can get fitness gains through performing superparasitism, and should perform superparasitism under some situations (see for review van Alphen and Visser 1990; Speirs et al. 1991; Godfray 1994).

Superparasitism is divided into self- and conspecific superparasitism: the former refers to when the first and second offspring (originating from the first and second ovipositions, respectively) come from the same mother, and the latter is when the two offsprings come from different mothers. When the survival rate of the second offspring is higher than zero, conspecific superparasitism rewards the mother with a fitness gain (van Alphen and Visser 1990). Meanwhile, self-superparasitism is generally less profitable than conspecific superparasitism due to the siblings facing competition for limited resources (e.g., Yamada and Miyamoto 1998; Yamada and Watanabe 2002; Yamada and Ikawa 2005; Zhang *et al*. 2014); in particular, it is usually non- or negatively profitable for solitary parasitoids except in the cases where multiple parasitoid immatures guarantee a higher emergence probability of one adult, which are probably created by the greater suppression of the immune systems of the host when multiple individuals are present (Puttler and van den Bosch 1959; Luna et al. 2016; Rasekh et al. 2018) or by the presence of conspecifics (van Alphen and Visser 1990; Yamada and Sugaura 2003; Ito and Yamada 2005, 2016).

Body size has great influences on many physiological and ecological characteristics related to foraging, e.g., metabolic rate (Brown et al. 2004), food returned per foraging trip (Kerr et al. 2019), foraging range (Greenleaf et al. 2007; Weise et al. 2010; Orben et al. 2015), available foraging period (Streinzer et al. 2016), and searching efficiency in a patch (Visser 1994), and consequently prey (food)-foraging strategies are expected to change depending on the body size (Cozzoli et al. 2018; Josens et al. 2018). Prey foraging strategy usually aim to maximum energies obtained per time (Stephens and Krebs 1986), while oviposition strategy for parasitoids aims to maximum life-time fitness performance (Wajnberg et al. 2008). Thus, longevity and fecundity have also great influence on the oviposition strategy, and they are also determined primarily by body size (e.g., Heinz 1991; Visser 1994; West et al. 1996). Moreover, the mother size may have effects of the fitness performance of their offspring (mainly determined by the survival rate). Therefore, it must also influence decision-making related to oviposition. Unfortunately, the effects of the body size on the oviposition strategy and the fitness performance of the offspring have never been investigated so far in parasitoids, to my best knowledge.

Whether superparasitism is adaptive is dependent on the fitness performance of offspring, physical conditions of the parasitoids, such as egg load and expected longevity, and the environmental situations in which the parasitoid is placed, such as host availability (van Alphen and Visser 1990; Speirs et al. 1991; Godfray 1994). Determination of the fitness performance of the first and second offspring under superparasitism is a basic step for understanding superparasitism strategy, including decision-making in superparasitism acceptance, sex allocation, selection of the oviposition place, and infanticide (ovicide and larvicide) for infanticidal parasitoids. However, few studies have revealed the fitness performance of the first and second offspring each under superparasitism (see for details 4.1. Introduction); in particular, the effects of the mother size of it have never been revealed.

Here, I determined superparasitism strategy of the infanticidal parasitoid *Echthrodelphax fairchildii* Perkin (Hymenoptera: Dryinidae), using the fourth instar nymph of a host species, *Laodelphax striatellus* (Fallén) (Homoptera: Delphacidae); in particular, I focused the body-size effects on it. First, I determined the body-size effects of the first and second ovipositing females on the fitness performance of the first and second offspring separately under self and conspecific superparasitism with different intervals of the first and second ovipositions (called oviposition intervals hereafter) for the cases of occurrence and non-occurrence of infanticide. I also explored the possibility that the fitness performance of offspring is influenced by the mother size under single parasitism to understand the above body-size effect under superparasitism. Through these studies, I discovered the strong effects of the body size of the ovipositing females under single parasitism and superparasitism, as seen below. Then, I determined the frequencies of superparasitism acceptance and infanticide, selection of the oviposition place, and sex allocation under self and conspecific superparasitism with different oviposition intervals when the female parasitoid was placed in an environment with low availability of suitable hosts. In particular, I focused on the effects of the parasitoid size of the above decision-making.

2. Materials and methods common to all experiments

2.1. Biology of parasitoid

Echthrodelphax fairchildii Perkin (Hymenoptera: Dryinidae) is an infanticidal koinobiont ectoparasitoid of the following three rice-damaging planthoppers (Homoptera: Delphacidae) in Japan: *Nilaparvata lugens* (Stål)*, Sogatella furcifera* (Horváth) *and Laodelphax striatellus* (Fallén). The parasitized hosts continue to feed on the host but do not molt to the next instar, and consequently the host resources available for the immature are determined primarily, to some extent, by the instar of a host when the female parasitoid oviposits on. The female lays an egg under the wing bud of the host, and the immature stays at the oviposition place till leaving the host to spin a cocoon. When superparasitism occurs on fifth-instar hosts, the following is found (Yamada and Ikawa 2003, 2005). When the first and second oviposition sides (left or right) are the same, while holding the host after catching and lifting it, the female always killed the first offspring and the survival rate of the second offspring remains nearly as high as that for single parasitism. Meanwhile, when the oviposition sides are different, the female often move the abdominal tip to the non-oviposition (i.e., first-parasitized) side to sting the first offspring; this act is called non-oviposition side probing or probing simply hereafter. The probing frequency is low for short oviposition intervals (<1 day) and high for long oviposition intervals (≥1 day). When the probing is performed, the first offspring is mostly (not always) killed, the second offspring attains a survival rate similar to that for single parasitism. Two-adult emerge often occurs, when the first and second females select different sides of the host for oviposition under non-probing superparasitism with short (<24 h) oviposition intervals and the second ovipositing female fails in killing the first offspring under probing superparasitism with short (<24 h) oviposition intervals.

Echthrodelphax fairchildii females are synovigenic, and host feeding is necessary for egg maturation. Female parasitoids use second-instar planthoppers only for feeding, third-instar planthoppers for feeding and sometimes for parasitizing probably because of host unsuitability, and fourth- and fifth-instar planthoppers for both feeding and parasitizing (Y.Y. Yamada and S. Noda, unpubl data 2014).

2.2. Collection and rearing

Echthrodelphax fairchildii and *L. striatellus* were both collected from two locations, 10 km away from each other in Tsu, Mie, Japan in 1992, and reared under laboratory conditions. The two parasitoid populations from the different original collection sites were reared separately, while the two host populations were reared together. Field-collected parasitoids and hosts were added to the laboratory populations every few years after 2005 to maintain the genetic variability.

To obtain mother parasitoids for use in experiments, parasitoid pupae were gathered from the laboratory populations and kept individually in 5-mL plastic vials. After emergence, females were individually reared in 340-mL plastic cages containing a 50% (by weight) honey solution, water, 20 second-instar hosts, 20 third-instar hosts, 1 fifth-instar host, 2 male wasps for mating (the males and female came from populations collected at different sites) and about 20 rice seedlings. Honey solution was food for males and females. The honey solution, water, hosts and rice seedlings were renewed every day. A smaller number of hosts (10 second-instar, 10 third-instar, and one fifth- instar hosts) were supplied for a day before a superparasitism bout in order to enable the female to be likely to perform superparasitism. The insects, including parasitized hosts, were reared, and parasitism and superparasitism bouts were performed in a room at 24–26°C, 40–50% relative humidity, and LD 16:8 photoperiod.

3. Fitness performance under single parasitism

3.1. Introduction

The body size of animals is closely related to their fitness performance (Peters 1983; Shingleton 2011), with large individuals usually—but not always—exhibiting relatively high fitness within a species (Kingsolver and Pfennig 2004). This is also applicable to parasitoid wasps. Larger female parasitoids have a higher lifetime fecundity and/or are better at foraging and ovipositing than smaller female wasps (e.g., Heinz 1991; Visser 1994; West et al. 1996), and consequently, they are likely to find more high-quality hosts over their lifetime. However, there have been no reports of large female parasitoids producing large adult offspring or ensuring high immature survival rates for their offspring. A positive relationship between maternal body size and egg size is often found in insects (Fox and Cresak 2000; Fischer et al. 2002). However, the relationship between maternal body size and the fitness performance of offspring during the immature stage is marginal or unclear in insects (Torres-Vila and Rodríguez-Molina 2002; Kojima 2015); the effects of egg size on the fitness performance during part of the immature stage have been often reported (Fox and Cresak 2000), but the reporting of an egg-size effect on the fitness performance during the whole immature stage is quite rare (Fox and Cresak 2000). Mother size affects offspring adult size in some insects (Kojima 2015; Fox 1994a; Steiger 2013), and the effects are caused by genetic factors (Fox 1994b) or differences in the ability of the mother to care for her offspring (Kojima 2015; Steiger 2013). To the best of my knowledge, the effects of maternal body size on the survival rate to adult emergence have not been reported in insects.

Parasitoids have frequently been used to verify predictions of theoretical models of foraging and ovipositing, including host preference, patch use, and sex allocation (Godfray 1994; Wajnberg et al. 2008). This is because they are easy to rear in the laboratory and their decision-making about ovipositing is directly related to their fitness since the offspring must live on/in the host selected by the mother. The fitness performance of female parasitoid adults is determined mainly by lifetime fecundity and characteristics related to foraging and oviposition strategies, as mentioned above. If large female adults produce large adult offspring and/or ensure high survival rates during the immature stage for their offspring, the size of female parasitoids has a greater influence on their fitness than researchers previously thought, and this would greatly impact our understanding of the foraging and ovipositing strategies of parasitoids. A typical example is found in the host quality model (Charnov et al. 1981; Charnov 1982) for sex allocation, which has been applied to sex allocation in many parasitoids (Godfray 1994); the model predicts that the female should lay female and male eggs on high- and low-quality hosts, respectively. The precondition for the model is that the increase in adult size differentially affects the fitness of the female and male adults; female adults achieve more fitness gains than male adults as the adult size increases. Many researchers have tried to verify the precondition (e.g., van den Assem et al. 1989; Heinz 1991; Kazmer and Luck 1995; Ueno 1998, 1999), but no researchers have addressed the effects of maternal body size on the survival of immatures and the size of emerging adults. If such effects are present, sex differences in the size-effect of fitness are greater than researchers previously thought, and the precondition appears to be satisfied easily.

Here, I investigated the effects of maternal body size on offspring size, survival rate, and developmental period using the parasitoid *E. fairchildii.*

3.2 . Methods

3.2.1. Parasitism and rearing of parasitized hosts

Mated females aged 4–5 days were allowed to lay an egg on a fourth-instar host that was within 24 h of molting. Each female parasitoid was used only for one superparasitism event. In an oviposition event, a female in a rearing cage was moved to a clean 4-mL transparent plastic vial containing four second-instar hosts for food immediately after the light was turned on; she was kept there in for 4 h before a healthy fourth-instar host was added. I observed ovipositing behavior under fluorescent lighting using a supersensitive video camera (WAT-902H, Watec, Yamagata, Japan) attached to a binocular microscope. I did not use a ring lamp attached to the binocular microscope to eliminate the potential effects of exceedingly bright light on parasitoid behavior. The sex of the egg was identified based on observations of the movement of the genitalia (Yamada and Imai 2000). The parasitized host was removed immediately after the end of oviposition so that it was not superparasitized.

Parasitized hosts were reared individually in 30-mL glass vials with five or six rice seedlings. The development of the immature parasitoids was observed daily. The sex of each emerged adult was always the same as the sex identified at oviposition. The head widths of the mothers and their offspring were measured a few weeks after their death using an ocular micrometer (96× magnification).

3.2.2. Data analysis

The effects of maternal body size (head width) on the sex ratio and survival rate of the offspring were examined with a logistic regression analysis (LogXact®10, Cytel Software, Cambridge, MA, USA). Significance was tested by calculating the exact probability (Cytel 2012) based on the exact distribution of a focal variable, not by calculating *P*-values with the assumption that the likelihood ratio statistic follows a chi-square distribution with one degree of freedom (Cytel 2012). The effects of maternal body size on the body size (head width) and developmental period of the offspring were analyzed using a mixed linear model. Significance was tested using the likelihood-ratio test implemented with the “anova” function in the “lme4” package of the R program (version 3.4.3) (R Core Team 2016). When examining the survival rate, body size, and developmental period of the offspring, the sex and maternal body size were included in the model as fixed factors, respectively, and the collection site was included as a random factor. In addition, the strength of the relationship between maternal size and offspring size was assessed separately for males and females by calculating the partial R^2 value using a multiple regression model with the collection site included as a fixed categorical value. This analysis was performed using NCSS (version 11, NCSS Statistical Software, Kaysville, UT, USA). The sample sizes for the survival rate, head width and developmental period were 352, 282 and 282 for males and 112, 44 and 44 for females, respectively.

3.3. Results

3.3.1. Effects of mother size on the sex ratio and survival rate of their offspring

Large female wasps were more likely to lay female eggs ($P < 0.001$, Table 1). The interaction between the sex and maternal body size was significant ($P < 0.001$), and the statistical analysis of the survival rate was performed separately for males and females. The survival rates of both male and female offspring increased with increasing maternal body size (in both males and females, $P < 0.001$; Fig. 1): that of the female offspring increased more steeply than that of the male offspring. When the head width of the mother was <0.60 mm, most of the female offspring did not emerge. Death occurred mainly after the appearance of the larval sac, particularly between the appearance of the larval sac and cocoon spinning in females (Table 2).

3.3.2. Effects of mother size on the body size and developmental period of their offspring

The interaction between the sex and maternal body size was significant (X_1^2 = 5.7, *P* = 0.017), and the statistical analysis of offspring body size was performed separately for males and females. Large female wasps produced large male and female offspring (for males, X_1^2 = 8.2, *P* = 0.004; for females, X_1^2 = 12.9, *P* < 0.001; Fig. 2). The body size of the male offspring increased more steeply with increasing maternal body size than that of the female offspring. The strength of the relationship between maternal body size and offspring body size was similar between males and females: with partial R^2 values of 0.202 and 0.254, respectively. Moreover, maternal body size did not affect the developmental period of the offspring in either sex (size, X_1^2 = 2.3, *P* = 0.131; sex, X_1^2 = 34.9, *P* < 0.001; interaction, X_1^2 = 1.1, *P* = 0.293). The developmental period of the female offspring was a little longer than that of the male offspring: 21.78±0.09 days (M±SE) for males and 22.86±0.17 days for females.

3.4. **Discussion**

This is the first study to find that large mother parasitoids produce large offspring and ensure high survival rates of the offspring during the immature stage. A particularly interesting finding was that the effect of maternal body size on the fitness of the offspring was stronger in female offspring than in male offspring. This difference is probably related to sexual dimorphism, but its underlying mechanisms are unknown at present. Small adults did not produce female offspring and refrained from laying female eggs, whereas large female adults were more likely to lay female eggs. This suggests that females changed the sex of their eggs in response to their own body size; female hymenopterans can determine the sex of their eggs by controlling the release of sperm stored in the spermatheca (Godfray 1994; Quicke 1997). Moreover, the female-biased sex ratio for large females is explained well by the host-quality model (Charnov et al. 1981; Charnov 1982). The females used for the experiment encountered many low-quality hosts (third instars) before encountering fourth-instar hosts; consequently, the large parasitoids were likely to lay female eggs on the host. The host-quality model predicts that a female should respond to the relative sizes of the hosts available to her.

Some parasitoids, including bethylids, guard their immature offspring to protect them from attack by competing parasitoids, predators, and other host individuals (Quicke 1997; Jervis 2007; Wang et al. 2014). This kind of guarding may ensure high survival rates and produce large adults. However, *E. fairchildii* females do not guard their offspring. A possible alternative mechanism for the mother-size effects in *E. fairchildii* is that large females are more likely to succeed in regulating the host's physiology than small females. To succeed in parasitism, the parasitoid should force the host to continue feeding on the plant after the parasitism attack and prohibit it from molting. Large females might do so more successfully. The physiology of the host could be manipulated by parasitoid mothers injecting some compounds while ovipositing and/or by parasitoid larvae releasing some compounds from their mouths. Large mothers are known to lay large eggs in some parasitoid species (Klomp and Teerink 1967; Visser 1994). Large eggs reportedly ensure a high survival rate for a period of time after hatching in many arthropods (Fox and Cresak 2000). Therefore, the following scenario is plausible: large mothers lay large eggs, and the larvae hatching from the large eggs release a large amount of host-physiology regulating agents, leading to increased success in parasitism. Unfortunately, no studies have been conducted to explore the mechanisms for such host-physiology manipulation in Dryinidae. Whether genetic factors are involved in the mother-size effects in *E. fairchildii* also remains to be elucidated in future studies.

While ovipositing, braconid and ichneumonid parasitoids inject some compounds, including venom and polydnaviruses, to suppress the host immune defense (Söller and Lanzrein 1996; Burke and Strand 2012; Strand and Burke 2015). These agents are also considered to be involved in controlling the host physiology. Teratocytes also function to control the immune defense system and physiology of the host in some braconids (Pennacchio and Strand 2006; Burke and Strand 2012). However, teratocytes are released in the host body when the parasitoid eggs hatch, and thus, the ectoparasitoid *E. fairchildii* cannot use them. Larvae of *E. fairchildii* insert only their modified mouth into the host body after hatching (Olmi 1984) and are likely to circumvent the host immune defense. Agents produced by *E. fairchildii* are considered to be involved in regulating the physiological development system rather than suppressing the immune defense of the host. Therefore, the mechanisms for regulation of the host's physiology in *E. fairchildii* may be quite different from those that have been explored in braconid and ichneumonid endoparasitoids. Several researchers have recently reported that some parasitoids manipulate the behavior of the hosts to increase fitness gains (Weinersmith 2019). Symbionts (RNA viruses) injected by adults while ovipositing are responsible for the manipulation of host behavior in the parasitoid *Dinocampus coccinellae* (Dheilly et al. 2015). Such symbionts might be found in *E. fairchildii*.

Gao et al. (2016) recently reported no effects of maternal body size on the size of adult offspring, developmental period of immatures, and sex ratio among adult offspring in the gregarious ectoparasitoid *Sclerodermus pupariae* (Hymenoptera: Bethylidae). This species and *E. fairchildii* belong to the superfamily Chrysidoidea, but the former is an idiobiont, while the latter is a koinobiont. The amount of host sources available for the immature parasitoid is fixed in idiobiont parasitoids when the host is parasitized. Thus, effects of maternal body size on the fitness performance of immatures are likely to be found in koinobiont parasitoids. It is interesting to elucidate how common mother-size effects are in koinobiont parasitoids, including dryinids.

4. Fitness performance under superparasitism

4.1. Introduction

Fitness of a female and male offspring in an environment is expressed by the survival rate and developmental time during the immature stage as well as the lifetime fecundity and total number of females fertilized by the male (lifetime mating number) during the adult stage, respectively. The death during the immature stage means zero fitness of the offspring, and the survival rate is considered to contribute most to the fitness. Lifetime fecundity in female parasitoids is usually positively associated with the body size at emergence (e.g., Heinz 1991; Visser 1994; West et al. 1996) and the former can be estimated by the latter. Meanwhile, in parasitoids the effect of male size on the lifetime mating number is considered to be substantially weaker than the effects of female size of the fecundity (e.g., van den Assem et al. 1989; Heinz 1991; Kazmer and Luck 1995; Ueno 1998, 1999). When *E. fairchildii* females lay eggs on unparasitized and parasitized fourth-instar hosts, they lay mostly males (see the previous and next chapters). Thus, the size of emerging adults contributed little to their fitness compared with the survival rate during the immature stage when male eggs are laid on fourth-instar hosts. Moreover, shorter developmental periods may decrease mortality in the field because it shortens time exposed to predation and parasitism. Short developmental periods may increase the number of yearly generations of the parasitoid in temperate regions. However, the differences in the developmental time in *E. fairchildii* males are small: the developmental period ranged from 20 to 25 days for fourth-instar hosts and the percent frequencies of 20 and 25 days are only 3.7 and 1.2% at 25, respectively under superparasitism. Thus, the developmental period is considered to contribute little to fitness difference compared with the survival rate. Therefore, when determining the fitness performance of *E. fairchildii* offspring under superparasitism on fourth-instar hosts, I focused on the survival rate.

Few studies have successfully determined the survival rates of the first and second offspring under superparasitism because it is usually difficult to identify the emerging adults as the first or second offspring. In this context, the survival rates of both emerging offsprings of parasitoids under conspecific superparasitism have been determined for seven hymenopteran species: *Leptopilina heterotoma* (Thomson) (Eucoilidae) (Visser et al. 1992), *Venturia canescens* (Gravenhorst) (Ichneumonidae) (Sirot 1996), *Trissolcus basalis* (Wollaston) (Platygastridae) (Field et al. 1997), *Pachycrepoideus vindemmiae* Rondani (Pteromalidae) (Goubault et al. 2003), *Cotesia vestalis* (Haliday) (Braconidae) (Chen et al. 2020), *Haplogonatopus atratus* Esaki and Hashimoto (Dryinidae) (Yamada and Miyamoto 1998; Yamada and Watanabe 2002), and *Echthrodelphax fairchildii* Perkins (Dryinidae) (Yamada and Ikawa 2003, 2005). For the aforementioned first four species, researchers used two morphologically different strains for the first and second ovipositing females to distinguish between the first and second offspring. For *C. vestalis*, genetically different strains were used, and the distinction was made using microsatellites. In the two dryinid species, the immature parasitoids remain at the location of oviposition until they leave the host to spin cocoons. Thus, the oviposition behavior of the two dryinid species was observed, the oviposition location was recorded, and the first and second offspring were distinguished.

Studies on the aforementioned parasitoid species, except for the two dryinid species, have shown that a long interval between the first and second ovipositions (hereafter called “oviposition interval”) decreases the survival rate of the second offspring (mostly to zero for oviposition intervals of ≤3 days), while increasing that of the first offspring. This is because the first offspring gains an advantage in the competition for host resources owing to earlier access to the host. In addition, when superparasitism occurs, the first offspring often kills the second offspring using the mandibles (Salt 1961; Ueno 1998; Harvey et al. 2013) or the physiological development of the second offspring is suppressed (Vinson and Hegazi 1998). In contrast, in *H. atratus* and *E. fairchildii*, the survival rate of the second offspring remains higher than that of the first offspring, even for long oviposition intervals, as the second ovipositing female kills the first egg or larva using the sting (Yamada and Kitashiro 2002; Yamada and Ikawa 2003, 2005).

Morphologically and genetically different strains cannot be used to distinguish between the first and second offspring under self-superparasitism. From this point of view, the survival rates of the first and second offspring under self-superparasitism have not been reported for any solitary parasitoid species, except for the two aforementioned dryinid species. The studies of the two species revealed that results vary depending on the parasitoid species and the host instars. When *H. atratus* females superparasitize fourth-instar hosts, the survival rates of both the first and second offspring are slightly higher under conspecific superparasitism than under self-superparasitism (Yamada and Miyamoto 1998), whereas there is no difference in their survival rates under self- and conspecific superparasitism when third-instar hosts are parasitized (Yamada and Watanabe 2002). In *E. fairchildii*, self- and conspecific superparasitism generate similar survival rates of offspring emerging from fifth-instar hosts (Yamada and Ikawa 2003, 2005). However, the survival rates of offspring emerging from fourth-instar hosts have not been determined.

Echthrodelphax fairchildii females lay one egg per oviposition under the wing bud of the host [see Yamada and Imai (2000) for oviposition behavior], and the immature larvae remain and develop at the oviposition location until leaving the host to spin cocoons; thus, the distinction between the first and second offspring under superparasitism is readily possible. The parasitized hosts continue to feed on their host plant but do not molt into the next instar, suggesting that the host physiology is regulated by some agents released by the ovipositing female or the immature parasitoid (3.3. Discussion). When the host is in the fifth instar, two adults often emerge under non-probing superparasitism with short (<24 h) oviposition intervals. Before conducting the experiment, I predicted that only one adult would always emerge from superparasitized fourth-instar hosts regardless of non-probing, as a fourth-instar host provides smaller amount of resources for parasitoid development than a fifth-instar host. Moreover, when two eggs are laid on the same side of fifth-instar hosts, the survival rate of the second offspring is nearly as high as that of the offspring of single parasitism (Yamada and Ikawa 2005, see 2.1. Biology of parasitoid). It is interesting to determine whether the same phenomenon occurs for the four-instar host.

Large *E. fairchildii* mothers ensure high survival rates of their offspring under single parasitism (see chapter 3). Thus, the size of the ovipositing females may affect the survival of the first and second offspring under superparasitism; however, there are no empirical studies that have addressed the effects of the sizes of both ovipositing females on the outcome of superparasitism. Furthermore, I hypothesized that some agents, which are involved in ovipositing-female size effect, especially those regulating host physiology, are released by the ovipositing females or their offspring in *E. fairchildii* (see Chapter 3). As no agents are released by the first offspring when it is killed by probing, I can determine whether the ovipositing female or the second offspring releases these agents by comparing superparasitism with and without probing. My hypothesis is that the agents are released by the second offspring; this will be confirmed if the survival rate of the second offspring is influenced by the body size of the first female under non-probing superparasitism, but not under probing superparasitism.

In this study, I determined the survival rates of the first and second offspring under self- and conspecific superparasitism when *E. fairchildii* females superparasitized and laid eggs on fourth-instar hosts; then, I compared the results with those obtained for fifth-instar hosts (Yamada and Ikawa 2003, 2005). The present study aimed at answering the following questions: (1) Do two adults emerge from the host? If so, which factors determine the proportion of two-adult emergence? (2) Do self- and conspecific superparasitism produce offspring of different survival rates? (3) How do the survival rates of the first and second offspring change with increasing oviposition intervals? (4) When the first and second eggs are laid on different sides, how does the probing influence on the survival rates of the first and second offspring change with the oviposition interval? (5) Is self-superparasitism is profitable: i.e., the total survival rate of the first and second offspring under self-superparasitism is larger than the survival of the offspring for single parasitism? (6) When two eggs are laid on the same side, the survival rate of the second offspring is nearly as high as that of the offspring of single parasitism, as seen for the fifth-instar host? (7) How are the survival rates of the first and second offspring associated with the body sizes of the first and second ovipositing females? (8) Who releases the agents for regulating the physiology of the host—the ovipositing females or the offspring? (9) Who ultimately wins the competition between the first and second offspring?

4.2. Methods

4.2.1. Parasitism and superparasitism bouts

The oviposition intervals investigated were 0 h, 1 h, and 24 h, which were defined as the intervals between the starting times of the first parasitism bout (time of placing a healthy host in a vial with a parasitoid) and the superparasitism bout (time of placing a parasitized host in a vial with a parasitoid) (Yamada and Ikawa 2003, 2005; Ito and Yamada 2014, 2016). When the superparasitism bout began <10 min after the beginning of the first bout, the oviposition interval was categorized as a 0-h interval. The three oviposition intervals were chosen to verify self-/conspecific discrimination for oviposition intervals of ≤45 h and two-adult emergence for oviposition interval of <24 h (found when superparasitism occurs in the fifth-instar hosts). A 0-h oviposition interval was chosen because *E. fairchildii* females exhibit the strongest self-/conspecific discrimination for fifth-instar, freshly parasitized hosts. Additionally, because the offspring survival rate under superparasitism of fifth-instar hosts was determined for the 1-h oviposition interval, but not for the 0-h oviposition interval (Yamada and Ikawa 2003, 2005), it would be interesting to determine whether there would be any differences in offspring survival between 0-h and 1-h intervals.

I used five-day-old copulated females for all superparasitism bouts to eliminate the potential effects of the age of the superparasitizing females. Only copulated females were used to determine the sex allocation strategy exhibited by the females. Thus, I used four-day-old females for the first oviposition for the 24-h-interval superparasitism. Each female parasitoid was used only for one superparasitism bout.

To obtain female parasitoids for the experiments, parasitoid pupae were collected from the laboratory populations and singly kept in 5-mL plastic vials. After emergence, the females were individually placed in 340-mL plastic cages containing 50% (w/v) honey solution; water; 20 second-, 20 third-, and 1 fifth-instar planthoppers, as well as about 20 rice seedlings (food for the planthoppers) whose roots were covered in absorbent cotton soaked in Hyponex® (Hyponex Japan Corp., Osaka, Japan) solution; and 2 male parasitoids for mating (the males and females were obtained from different collection-site populations to avoid inbreeding) for 4 days. The honey solution, water, hosts, and rice seedlings were replaced by fresh ones every day until the start of the first parasitism bout for superparasitism. Thus, the females were reared under conditions in which food availability was high but host availability was low. A reduced number of hosts (10 second-, 10 third-, and 1 fifth-instar planthoppers) were supplied a day before the superparasitism bout to induce the females to perform superparasitism; in the case of oviposition intervals of 0 and 1 h, the hosts were supplied a day before the first parasitism bout for superparasitism because the first parasitism and superparasitism bouts occurred on the same day.

In the first parasitism bout, one copulated female from a rearing cage was placed in a clean, transparent plastic vial (4 mL) containing four second-instar hosts as food immediately after lights were switched on in the morning. One fourth-instar host (<24 h after molting) was placed in the vial with the female for the first oviposition. The host was placed in the vial 3 h and 4 h after lights were switched on for 1-h-interval superparasitism bouts and 0-h- and 24-h-interval superparasitism bouts, respectively. The host was removed immediately after being parasitized and reared independently in a 30-mL glass vial containing 5 or 6 rice seedlings whose roots were covered in absorbent cotton soaked in Hyponex® solution.

Superparasitism bouts were allowed to begin 4 h after lights were switched on to eliminate the potential effects of the time span after lights were on. For each 0-h-interval self-superparasitism bout, the parasitized host was maintained in the vial with the parasitoid instead of being removed. For each 1-h-interval self-superparasitism bout, the parasitized host was returned 1 h later into the same plastic vial used for the first oviposition. For each 24-h-interval self-superparasitism bout, the female parasitoid was returned to the 340-mL cage and transferred the next day into a clean 4-mL vial, and the parasitized host was added (following the same procedure as described for the first oviposition).

For conspecific superparasitism bouts, the employment of genetically related ovipositing females was avoided using a pair of females from different collection-site populations. Two hosts, each parasitized by females from one or the other population, were exchanged and supplied to the other female of the pair after an assigned interval. The other procedures were the same as those used for self-superparasitism. This method allowed the second ovipositing female used for conspecific superparasitism to encounter a healthy host at an assigned time before the superparasitism bout, as did the female used for self-superparasitism.

I observed the ovipositing behavior under fluorescent lighting using a supersensitive video camera (WAT-902H, Watec, Yamagata, Japan) attached to a binocular microscope (40 × magnification) to examine four parameters: oviposition, oviposition side, probing (stinging the first offspring present on the non-oviposition side), and sex of the egg. I did not use a ring lamp attached to the binocular microscope to eliminate the potential effects of exceedingly bright light on parasitoid behavior. The sex of each egg was identified based on the observation of the movement of the parasitoid genitalia, particularly the movement of the sting (Yamada and Imai 2000). I distinguished between the first and second offspring by recording the locations of the first and second ovipositions and observing the mature larvae leaving the host and identifying their cocoons.

I recorded cases where the females did not superparasitize the hosts within 10 min of supplying the parasitized host as superparasitism avoidance. When superparasitism avoidance occurred, I provided the female with a healthy fifth-instar host within 1 day of molting, which is considered to be the best suitable host for parasitism, to verify that the avoidance did not occur owing to lack of mature eggs for oviposition. When the female did not parasitize the fifth-instar host, I discarded the sample: such cases are rare (2,1%).

Detailed analyses of the behavior, including superparasitism acceptance, selection of oviposition side, probing, and sex allocation are described in the following chapter.

The superparasitized hosts were examined daily to record eventual death, appearance of the larval sac (visible part of the parasitoid larva, as the egg covered by the wing bud was not visible; usually 3 or 4 days after oviposition), emergence, and sex of the adult parasitoids. The sex of each emerged adult was almost always the same as the sex identified at oviposition: four of the 25 emerged females came from eggs whose sex was identified male at oviposition, while all the 216 emerged males came from eggs whose sex was identified male.

The head widths of the ovipositing females were measured a few weeks after their death using an ocular micrometer (96 × magnification). The analysis of the survival rates was limited to superparasitism in which both the first and second offspring were males; this was done to avoid the effects of sex difference in the survival under single parasitism and competitive strength on the survival under superparasitism (Godfray 1994). As most first and second eggs were identified as males (see chapter 3), the cases in which the first or second offspring were females were not analyzed. In total, 46, 36, and 65 self-superparasitized and 34, 41, and 52 conspecifically superparasitized hosts were analyzed for survival rates under oviposition intervals of 0 h, 1 h, and 24 h, respectively.

4.2.2. Data analysis

I analyzed the effects of the body sizes (head widths) of the first and second ovipositing females, oviposition interval, superparasitism type (self- or conspecific), and probing on the survival rates of the first and second offspring. Analysis was performed separately for the case in which the first and second eggs were laid on different sides of the host (different-sides superparasitism) and the case in which they were laid on the same side (same-side superparasitism). This is because the first offspring were assumed to be stung to death by the second ovipositing female under same-side superparasitism for the fourth-instar host, as assumed for the fifth-instar host (Yamada and Ikawa 2005).

4.2.2.1. Statistical models

Statistical analyses were usually performed using logistic regression models for binary response variables; a few analyses were performed by using other methods, which were described in detail as needed. The statistical significances of the factors and two-way interactions between them were assessed by calculating the exact probability values (Cytel 2012) using LogXact®10 software (Cytel, Cambridge, MA, USA). The head width of the ovipositing females and the oviposition interval were incorporated as numerical variables, while the other factors were incorporated as categorical variables. The collection site of the second ovipositing females was incorporated in the model as a random factor (stratum) when possible, which was often impossible owing to the small sample sizes. Not incorporating the random factor did not appear to cause a serious problem because the effects of the collection site were not detected in the analysis of the survival rates under single parasitism (see chapter 3).

4.2.2.2. Different-side superparasitism

Analysis for survival rates of first and second offspring

I found a significant interaction between probing and oviposition interval under different-side superparasitism. Thus, further analyses were performed as two separate procedures: separate analysis of superparasitism with or without probing, and separate analysis for superparasitism with different oviposition intervals.

Procedure 1: Separate analysis of superparasitism with or without probing. As the first and second ovipositing females are the same under self-superparasitism, causing high multicollinearity, statistical models incorporating the body size of the first female and that of the second female together could not be used. Thus, I first analyzed self- and conspecific superparasitism independently (Procedure 1-1, Table 3) and, then, analyzed the effect of the superparasitism type using models incorporating the superparasitism type, oviposition interval, and body size of the first or second ovipositing female (Procedure 1-2, Table 3).

In Procedure 1-1, for conspecific superparasitism, I used statistical models incorporating the oviposition interval, body sizes of both ovipositing females, and their two-way interactions, while I excluded the body size of the first female for analysis of self-superparasitism to avoid multicollinearity (Table 3). When I analyzed conspecific superparasitism, I removed some data points (4 and 6 points for 0-h and 1-h oviposition intervals, respectively) to eliminate multicollinearity between the body sizes of the first and second females (Zuur et al. 2010), as the removed data points included the cases in which both the first and second females exhibited the largest/near-largest or the smallest/near-smallest body size (Fig. 3). The multicollinearity probably occurred because of the occasional synchronous changes in the densities of the hosts of the same instar between different cages owing to the supply of host plants on the same day; the female planthoppers may have laid eggs on newly supplied plants for one or two days. Moreover, the parasitized fifth-instar hosts produce larger parasitoids than those that parasitized fourth-instar hosts (see chapter 2; Ito and Yamada 2007).

An additional analysis was performed to detect the potential difference between 0-h and 1-h intervals using data from these oviposition intervals, instead of data from all three. This analysis did not detect a difference between 0-h and 1-h oviposition intervals (the results are not presented in this thesis).

Procedure 2: Separate analysis for superparasitism with different oviposition intervals. I analyzed the effects of probing on the survival rates for each oviposition interval under self- and conspecific superparasitism (Table 3). Statistical models for self-superparasitism incorporated probing and size of the single ovipositing female (the first and second ovipositing females are the same), whereas the models for conspecific superparasitism incorporated probing and sizes of both ovipositing females (Procedure 2-1).

Large females were more likely to perform probing than small females (Fig. 13;

see sample sizes in Figs. 5 and 6), which caused multicollinearity between probing and the body size of the second ovipositing female. This means that, if Procedure 2-1 indicated that probing and body size of the second female were significant and nonsignificant, respectively, or vice-versa, it would be possible that the factor estimated to be nonsignificant actually had a significant effect (Zuur et al. 2010). Thus, in such a case, I verified the results of Procedure 2-1 by performing Procedure 2-2.

In Procedure 2-2, I used two statistical models for self-superparasitism, each containing probing or the body size of the second female; and two models for conspecific superparasitism, one incorporating the body sizes of the first and second females and their interaction, and the other incorporating probing, the body size of the first female, and their interaction (i.e., probing and body size of the second female were incorporated in different models) (Table 3). If Procedure 2-2 indicated that probing (estimated to be nonsignificant in Procedure 2-1) was significant, probing might have some effect (Case 2a in Table 4); however, concluding its significance is difficult. Conversely, if Procedure 2-2 indicated that probing was nonsignificant, probing would be nonsignificant (Case 2b in Table 4).

Analysis for competition between the first and second offspring

I analyzed the effects of the sizes of the first and second females and oviposition interval on which offspring won the competition (i.e., emerged). I addressed cases in which either the first or second offspring emerged from the host under non-probing superparasitism. I used a statistical model incorporating the size of the mothers (the first and second females were the same individual), oviposition interval, and their interaction for self-superparasitism, while using another model incorporating the sizes of the first and second females, oviposition interval, and their interactions for conspecific superparasitism.

Analysis for two-adult emergence rate

I analyzed the effects of the body size of the first and second females, superparasitism type (self or conspecific), and oviposition interval on the two-adult emergence rate, i.e., proportion of superparasitized hosts producing two adult parasitoids. Non-probing superparasitism with 0- or 1-h oviposition intervals was addressed because two-adult emergence did not occur in the other cases. First, I used two statistical models incorporating the superparasitism type, oviposition interval, body size of the first or second female, and their two-way interactions (Procedure 3 in Table 5). As a result, a significant interaction between the superparasitism type and oviposition interval was detected (see Table 10). Thus, I performed statistical analysis separately for self- and conspecific superparasitism and separately for the 0-h and 1-h oviposition intervals (Procedure 4 in Table 5).

Analysis for profitable self-super parasitism

I analyzed whether there is a difference in the number of emerging adults per host between non-probing self-superparasitism and single parasitism to determine whether self-superparasitism is profitable: when the number for self-superparasitism is larger than that for single parasitism, self-superparasitism is profitable. When the oviposition interval was 24 h, a logistic model analysis was performed (see 4.2.2.1.): the size of the mother, single parasitism/superparasitism, and their interaction were incorporated into the model. Meanwhile, when the oviposition interval was 0 or 1 h, the number of emerging adults per host was 0, 1, or 2 (i.e., not binomial variable) under self-superparasitism, which means the above-mentioned analysis is not applicable. Two-adult emergence was found only among mothers with head widths of of ≥0.6 mm. Then, comparison was performed separately for small mothers with head width of <0.6 mm and large mothers with head widths of ≥0.6 mm.

The logistic model analysis was performed for the small mothers because two-adult emergence did not occur. As of the large mothers, first, I verified that there was no significant effect of mother size for self-superparasitism with an oviposition interval of 0 or 1-h, using continuation ratio models for polytomous response variables (Cytel 2012); logistic models for binary response variables was used separately for whether the response variables are 0 or >0 and whether the response variables are 2 or <2 (0-h oviposition interval, $P = 0.091$ for response variables of 0 or >0, $P = 0.462$ for response variables of 2 or <2; 1-h oviposition interval, P = 0.142 for response variables of 2 or <2; note that every host produced 1 or 2 parasitoids for 1-h oviposition interval). All hosts singly parasitized by the large females produced parasitoids, and the effect of mother size was considered to be absent although statistical analysis cannot be applied to such a case. Then, a randomization (permutation) test for comparison between single and self-superparasitism using StatXact® 10 software (Cytel, Cambridge, MA, USA). The randomization test does not assume that the response variables follow a specific distribution.

Analysis for direct-success rate of probing

Although most of the probed eggs failed to develop into adults, I frequently observed a larval sac originating from the first egg after probing, which suggests that the ovicidal probing was not successful. Thus, I analyzed the effects of the body sizes of the first and second ovipositing females, oviposition interval, and superparasitism type on the rate of direct success of probing (as indicated by the proportion of the unhatched first offspring). The proportion of the first offspring that did not survive until the appearance of the larval sacs (first instars) was used for this purpose because hatched larvae were inconspicuous under the wing bud for a few days after hatching. To avoid multicollinearity between the body sizes of the first and second females under self-superparasitism, I first used two statistical models incorporating the body size of the first or second female, oviposition interval, superparasitism type, and their interactions. Then I used a statistical model incorporating the body sizes of the first and second females, oviposition interval, and their interactions for conspecific superparasitism to identify a possible interaction between the body sizes of the first and second females.

4.2.2.3. Same-side superparasitism

I assumed that the first offspring was killed by the second female using the sting, as seen for fifth instar (Yamada and Ikawa 2005).

First, I verified the propriety of this assumption by comparing the oviposition-to-larval-sac-appearance period of the second offspring under superparasitism with a 24-h oviposition interval and that of the offspring under single parasitism. No difference between the two values will indicate the propriety of the assumption. Before comparing the two values, using male eggs under single parasitism I determined the effect of the mother size on the period. Because the period was 3 or 4 day, I analyzed the effect using a logistic regression model for binary response variable. Because such an effect was not present ($P = 0.140$), I performed the above comparison by using Fisher's exact test. Comparison was performed between single parasitism and self-superparasitism and between single parasitism and conspecific superparasitism.

Secondly, I determined the effects of the body sizes of the first and second ovipositing females, oviposition interval, superparasitism type (self- or conspecific) of the survival rate of the second offspring following Procedures 1-1 and 1-2 (see 4.2.2.1. and Table 3): note that probing does not occur under same-side superparasitism.

Thirdly, the survival rate of the second offspring for each oviposition interval under conspecific superparasitism was compared with the survival rate of the second offspring for the corresponding interval under different-side conspecific superparasitism with probing or non-probing. This comparison was made to determine which of the two types of superparasitism (different-side and same-side) is more profitable.

4.3. Results

When two eggs were laid on different sides, two-adult emergence occurred under both non-probing self- and conspecific superparasitism with 0-h and 1-h oviposition intervals (Fig. 4). The survival rates of both first and second offspring were generally higher under conspecific superparasitism than under self-superparasitism for all oviposition intervals when probing did not occur. Additionally, probing did not always result in the killing of the first offspring (Fig. 4), particularly under conspecific superparasitism. Although probing ensured higher survival rates for the second offspring compared with those under non-probing superparasitism when the oviposition interval was 24 h, this probing effect was not observed for the oviposition intervals shorter than 24 h. The survival rate of the second offspring under same-side superparasitism was lower than that that under different-side superparasitism, but not when the oviposition interval was 24 h and probing did not occur. These results were statistically checked by considering the size of the ovipositing females, as described below.

4.3.1. Different-side super parasitism

4.3.1.1. Separate analysis of superparasitism with or without probing

Effects of the oviposition interval and the body size of the ovipositing females on the survival of the offspring

When probing did not occur, the survival rate of the second offspring decreased with increasing oviposition intervals under both self- and conspecific superparasitism, whereas that of the first offspring was not associated with the oviposition interval (Table 6, Figs. 5 and 6). Moreover, large females ensured high survival rates of their offspring under both non-probing superparasitism (Table 6, Figs. 5 and 6), except the survival rate of the second offspring under conspecific superparasitism: it was negatively associated with the body size of the first ovipositing female and was approximately similar for each oviposition interval, irrespective of the mother size. The survival rate of the second offspring under self-superparasitism was associated with the mother size (Table 6, Fig. 6); note that the first and second females were the same under self-superparasitism.

When probing occurred, the oviposition interval had no effect on the survival rates of the first and second offspring under both self- and conspecific superparasitism (Table 6, Figs. 5 and 6). The survival rate of the second offspring was positively associated with the mother size under both self- and conspecific superparasitism, whereas that of the first offspring was independent of the mother size under either superparasitism. The survival rate of either offspring was not related to the body size of the non-mothers under conspecific superparasitism.

No interactions were detected between the body sizes of the first and second ovipositing females under either probing or non-probing conspecific superparasitism (Table 6), which suggests that the effect of the size of one ovipositing female was not influenced by that of the other female.

Effects of self-/conspecific difference on the survival of the offspring

In the absence of probing, superparasitism type had a significant effect on the survival rates of the first and second offspring (Table 7), and interactions between the superparasitism type and the body sizes of both the ovipositing females were observed. This result suggests that the survival rates of the first and second offspring were higher under conspecific than under self-superparasitism when the first and/or second ovipositing females were small (Table 7, Figs. 5 and 6).

The effect of the interaction between the superparasitism type and the size of the first ovipositing female on the survival rate of the first offspring was weak (*P* = 0.070, Table 7). Thus, the self-/conspecific difference existed for the survival rate of the first offspring even when the first ovipositing females were fairly large (Fig. 5). The self-/conspecific differences were generated by the extremely low survival rates among the first offspring from small (head widths of <0.6 mm) and middle-sized (head widths of 0.6–0.65 mm) mothers and among the second offspring from small mothers under self-superparasitism. This reduction was conspicuous when the survival rates of offspring under superparasitism and single parasitism were compared based on the mother sizes; the survival rates of offspring from mothers with head widths of 0.56–0.60 mm and ≥0.60 mm were approximately 80% and 100%, respectively, under single parasitism (Fig. 1). In particular, the total survival rate of the first and second offspring from mothers with head widths of <0.60 mm was <10% for each oviposition interval (Figs. 5 and 6).

As for superparasitism with probing, the effect of the superparasitism type was not significant (Table 7). However, the effect was nearly significant for the survival rate of the first offspring (*P* = 0.077 for the model incorporating HWFF and *P* = 0.052 for the model incorporating HWSF) probably because probing often failed to kill the first offspring.

4.3.1.2. Separate analysis for each oviposition interval

Effect of probing on the survival of the first offspring

Probing and the body size of the second ovipositing female had a significant effect on the survival rate of the first offspring under self-superparasitism with the 1-h oviposition interval (Table 8), suggesting that probing significantly decreased the survival rate of the first offspring (Fig. 5). However, the effect of the size of the second female was significant and that of probing was nonsignificant under self-superparasitism with 0-h and 24-h oviposition intervals. Separate analyses (Procedure 2-2) of probing and the body size of the second ovipositing female denied the possibility of the significance of probing (Table 8).

Under conspecific superparasitism with 1-h and 24-h oviposition intervals, probing was nearly significant and significant, respectively, and the body size of the second female was not significant (Table 8), considering that the effect of the focal factor exists even when the *P*-value for the focal factor is ≥0.05, when there is an interaction between the focal factor and another factor (Agresti 1996). This result was verified by separate analysis (Procedure 2-2) of probing and the size of the second female (Table 8), suggesting that probing decreased the survival rate (Fig. 5). No significant effect of probing or the size of the second female was found for the 0-h interval, but this may be a consequence of the small sample sizes for probing superparasitism (Fig. 5)

A significant interaction between probing and the size of the first female existed under conspecific superparasitism with the 24-h oviposition interval (Table 8). This was because the survival rate of the first offspring was positively associated with the body size of the first female under non-probing conspecific superparasitism, but not under its probing counterpart (Table 6, Fig. 5). Thus, the effect of the body size of the first female depended on the occurrence of probing.

Effect of probing on the survival of the second offspring

Probing and the body size of the second ovipositing female significantly increased the survival rate of the second offspring under self-superparasitism with the 24-h oviposition interval (Table 8, Fig. 6), suggesting a positive effect of probing. When the oviposition intervals were 0 h and 1 h under self-superparasitism, the size of the second female was significant, but probing was nonsignificant (Table 8).

Under conspecific superparasitism with the 24-h interval, probing was nonsignificant and the size of the second female was nearly significant ($P = 0.055$) (Table 8, Fig. 6). However, Procedure 2-2 indicated that the two factors were significant (Table 8), suggesting that it is difficult to determine the significance of probing using the present data set (Table 4). No significant effects of probing or the size of the second female were found for the 0-h or 1-h oviposition interval.

4.3.1.3. Competition between the first and second offspring: Who wins?

Self-super parasitism

Only large females produced two-adult emergence (Fig. 7). Neither oviposition interval nor the size of the mothers affected who won (emerged) (Table 9). The second offspring were likely to defeat the first offspring for 0-h and 1-h oviposition intervals (Binomial test, $P = 0.021$ for each oviposition interval).

Conspecific super parasitism

Two-adult emergence was limited to the cases in which the first ovipositing females were large (Fig. 7). The first offspring from small females were likely to lose the competition to the second offspring for all oviposition intervals (Table 9, Fig. 7); the first offspring from small mothers (head width <0.6 mm) almost always lost, whereas the first offspring from large mothers (head width ≥0.6 mm) almost always won or tied (two-adult emergence). However, the body size of the second female did not influence the competition between the first and second offspring (Table 9, Fig. 7).

4.3.1.4. Two-adult emergence rate

Since an interaction between the superparasitism type and oviposition interval was detected (Table 10), I performed analyses separately for self- and conspecific superparasitism and for 0 and 1 h oviposition intervals. Separate analysis for self- and conspecific superparasitism revealed that the two-adult emergence rate was higher for the 1-h oviposition interval than for the 0-h oviposition interval under self-superparasitism (Table 11, Fig. 8), while there was no difference between the two oviposition intervals under conspecific superparasitism. When the oviposition interval was 0 h, the two-adult emergence rate was higher under conspecific than under self-superparasitism (Table 11, Fig. 8). Meanwhile, when the oviposition interval was 1 h, no difference was detected between self- and conspecific superparasitism. The two-adult emergence rate was influenced by the body size of the common mother under self-superparasitism and the body sizes of both the first and second females under conspecific superparasitism (Tables 10 and 11; Fig. 8).

4.3.1.5. Profitability of self-super parasitism

When the head width of ovipositing female was ≥0.6 mm, the number of emerging offsprings per host was larger under self-superparasitism with the 1-h interval than under single parasitism (Table 12), suggesting that self-superparasitism was profitable. Meanwhile, no difference was detected between single parasitism and self-superparasitism with a 0-h interval. When the head width was <0.6 mm, very few adults emerged under self-superparasitism with a 0-h or 1-h interval (Figs. 5 and 6) and consequently the number of emerging offsprings per host was smaller under self-superparasitism than under single parasitism (Table 12), suggesting that self-superparasitism was negative profitable. When the oviposition interval was 24h, self-superparasitism was negatively profitable irrespective of the body size of the ovipositing female.

4.3.1.6. Direct-success rate of probing

The rates of direct success of probing for 0-h, 1-h, and 24-h oviposition intervals were 25.0% (*N* = 12), 0% (*N* = 5), and 17.2% (*N* =2 9) for self-superparasitism and 33.3% (*N* = 3), 8.3% (*N* = 12), and 27.3% (*N* = 22) for conspecific superparasitism, respectively. The size of the first or second ovipositing female, oviposition interval, or superparasitism type had no significant effect on the direct-success rate of probing (Table 13). It is worth mentioning that the size of the second female had a nearly significant positive effect. The interaction between the body sizes of the first and second females under conspecific superparasitism was not significant (*P* = 0.667).

4.3.2. Same-side superparasitism

The frequency of the oviposition-to-larval-sac-appearance period of the second offspring under same-side self- and conspecific superparasitism was statistically the same as that under single parasitism (Table 14), which suggests that the first offspring was killed. The period under superparasitism was 3 or 4 days, as seen under single parasitism (Table 14). No presence of 5 days under superparasitism strongly suggests that the first offspring was killed.

The oviposition interval had no effect on the survival rates of the second offspring under either self- or conspecific superparasitism (Table 15, Fig. 9). The survival rate of the second offspring was positively associated with the mother size under both self- and conspecific superparasitism (nearly significant under self-superparasitism), but it was not related to the body size of the first females (non-mothers) under conspecific superparasitism. No interactions were detected between the body sizes of the first and second ovipositing females under conspecific superparasitism (Table 15). The effect of the superparasitism type was not present (Table 16). These results for same-side superparasitism are the same as those for probing different-side superparasitism (see Tables 6 and 7and Figs. 5 and 6).

The survival rate of the second offspring under same-side self-superparasitism was lower than that that under different-side self-superparasitism superparasitism, whether or not probing occurred (Table 17, Figs 6 and 9)). The oviposition interval had a significant effect on the survival rate of the second offspring when probing did not occur, while not when probing occurred. As of conspecific superparasitism, the effect of the oviposition side was not present when probing occurred, while it depended on the size of the second female when probing did not occur: a significant interaction between OS and HWSF was present (Table 17). When the second female was small (head width of <0.6 mm), the survival rate of the second offspring under same-side conspecific superparasitism was lower than that that under different-side conspecific superparasitism (Table 18, Figs 6 and 9). Meanwhile, when the second female was large (head width of ≥0.6 mm), the effect of the oviposition side was not present. The oviposition interval had no effect on this tendency.

4.4. Discussion

4.4.1. Mother-size effect

The mother-size effect on the survival rate of the offspring was observed under superparasitism, as well as single parasitism, wherein large mothers ensured higher survival rates for their offspring than did small mothers. The mechanisms underlying the mother-size effect can be explained on the basis of who released the agents for the regulation of host physiology: parasitoid offspring (offspring-releasing hypothesis) or mothers (mother-releasing hypothesis) (see 3.4. Discussion). The offspring-releasing hypothesis was supported by the fact that the size effect of the first female disappeared when probing occurred, i.e., when the first offspring were killed or injured. In addition, this hypothesis also explains the difference in the survival rate of the offspring between self- and conspecific superparasitism, which was observed only under different-side superparasitism without probing; however, the mother-releasing hypothesis does not explain this difference. Offspring from large mothers were considered to release more effective agents for regulating the host physiology than those from small mothers.

An exception to the mother-size effect is the case of the mothers of the second offspring under non-probing conspecific superparasitism. The survival rate of the second offspring was not positively associated with the body size of their mothers, or rather negatively associated with the size of the first ovipositing females (unrelated females) (Table 6, Fig. 6). Conversely, the survival rate of the first offspring was positively associated with their mothers' size. The size of the second female had no effect on the survival rate of either the first or second offspring, and the survival rate of the second offspring from small females was almost as high as that of the second offspring from large females. Therefore, the first offspring from small females were highly likely to lose the competition to the second offspring.

In light of the fact that small second females gain as much fitness benefit as large females, small and large females are expected to be as much likely to accept conspecifically parasitized hosts as each other. In fact, however, large females were more likely to perform conspecific superparasitism than small females (see Fig. 10). It is probably owing to the present experimental situation in which the host availability was low. Under such a situation, large females, i.e., females with large egg load, are expected to accept conspecific superparasitism more frequencly than small ones (see chapter 1).

4.4.2. Self/conspecific difference

When probing was absent under different-side superparasitism, the survival rates of the first and second offspring were higher under conspecific than under self-superparasitism. The difference was mainly observed when the first and/or second ovipositing females were small.

This difference was attributed to extremely lower survival rates among the first and second offspring from small mothers under self-superparasitism than among the offspring from small mothers under single parasitism, suggesting that the first and second offspring from small mothers weaken each other's survival. Some researchers (van Alphen and Visser 1990; Luna et al. 2016) have suggested that the possibility that self-superparasitism of solitary parasitoids guarantees a high probability of one adult emerging from the host because multiple immatures more likely regulate the immune systems and physiology of the hosts than a single immature under single parasitism. However, this hypothesis is not applicable to *E. fairchildii* immatures from small mothers, and self-superparasitism resulted in a decreased probability of one adult emerging. The mechanisms underlying this remain to be elucidated.

No differences in the survival rate between self- and conspecific superparasitism were observed for fifth-instar hosts (Yamada and Ikawa 2003, 2005) probably because large females were selected for the experiment through naked-eye observations. Unfortunately, the head widths of the females used for that experiment were not measured.

The self-/conspecific difference in the survival rate of the second offspring from small females predicts that small females are more likely to distinguish between self- and conspecific superparasitism than large females, provided the females have an ability for self-/conspecific discrimination. This prediction was verified in the next chapter: When the oviposition interval was 0, small females discriminated as predicted, whereas large females did not (see Fig. 10).

4.4.3. Mechanisms underlying regulation of host physiology under superparasitism

The fact that the survival rate of the offspring from small mothers with a head width of 0.56–

0.60 mm was approximately 80% under single parasitism (Fig. 1) motivates the following hypothesis for conspecific superparasitism: the host physiology-regulating agents released by the second offspring can regulate the functions of the agents released by the first offspring from small mothers and decrease the survival rate of the first offspring irrespective of the body size of the second females. When the mothers of the first offspring are large, this phenomenon is absent because the first offspring from large females resist the physiological manipulations caused by the agents released by the second offspring.

This hypothesis is based on the assumption that the agents have an ability for self-/conspecific discrimination. A non-genetic factor may be involved in the process of the discrimination because the first and second offspring (brothers) from small mothers under self-superparasitism suppress the survival rates of each other. In addition, when ovipositing females are small under self-superparasitism, the agents released by the second offspring might poorly regulate the functions of the agents released by the first offspring. Similar to small females under self-superparasitism, when ovipositing females are fairly large, the first offspring might poorly resist the physiological manipulations caused by the agents of the second offspring.

Notably, the survival rate of the first offspring of small mothers was substantially lower than that of the second offspring of small mothers under non-probing conspecific superparasitism when the oviposition interval was 0 h; i.e., the first and second eggs were laid at almost the same time (Figs. 5 and 6). The reason is unknown, but it is unlikely that the host physiology-regulating agents released by the offspring are the only players in this role. The ovipositing female, after stinging the ventral side of the host thorax to paralyze the host (Yamada and Imai 2000), stings the oviposition location under the host wing bud just before laying an egg. When performing the above-mentioned two types of stinging on hosts that are already parasitized—not on healthy hosts—the second female may release, in the host body, agents that suppress the development of the first offspring in cooperation with the agents released by the second offspring. The second-female-released agents may be considered effective only when the first female is small because the first offspring from large females exhibited high survival rates (Fig. 5).

To the best of my knowledge, there have been no reports on agents regulating the host immune defenses or physiology in Dryinidae, nor reports on parasitoid larvae releasing such agents. The mechanisms underlying the parasitoid's regulation of the immune defense and physiology of the host have been investigated using braconid and ichneumonid endoparasitoids, as mentioned in 3.4. Discussion. Venom and polydnaviruses, injected into the host body by mother parasitoids, and teratocytes, released in the host body by hatching eggs, are considered to be involved in such mechanisms (Söller and Lanzrein 1996; Pennacchio and Strand 2006; Burke and Strand 2012; Strand and Burke 2015). However, these agents do not appear to be present in *E. fairchildii* because *E. fairchildii* agents appear to be released by the larvae. Agents used by *E. fairchildii* are considered to be involved in regulating the development physiology of the host, rather than suppressing the attack of the host immune system. This is because *E. fairchildii* is ectoparasitic (the immature parasitoid, including the egg, lives on the host, not in it), and thus, likely avoids the immune attack of the host (see 3.4. Discussion). In addition, *E. fairchildii* belongs to Aculeata, whereas braconids and ichneumonids belong to Parasitica. Therefore, the agents for regulation of the host physiology in *E. fairchildii* are considered completely different from those reported in braconid and ichneumonid endoparasitoids.

4.4.4. Profitable self-superparasitism

When the oviposition interval was 1 h, self-superparasitism was profitable for large females with head width of ≥0.6 mm. Meanwhile, when the ovipositing females are small, or the oviposition interval was 0 or 24 h, self-superparasitism generate a quite low level of or no two-adult emergence, and consequently no or negative profitability. The profitability of self-superparasitism is caused primarily by two-adult emergence. The difference in profitability between small and large females predicts that large females more likely accept self-superparasitism than small females for a 1 h oviposition interval. Moreover, the difference in profitability between oviposition intervals of 0 and 1h predicts that large females exhibit a difference in the rate of self-superparasitism acceptance among the two intervals. The first prediction was verified, but not the second prediction, as seen in the next chapter; details are presented in the next chapter.

4.4.5. Probing

Probing frequently failed to directly kill the eggs, which was also observed for fifth-instar hosts, although the failure rate for the fifth-instar hosts was not as high as that for the fourth-instar hosts (Yamada and Ikawa 2003). Most of the eggs that were probed but hatched failed to develop into adults, suggesting that the embryos inside such eggs were injured by probing. I did not verify the positive effect of probing on the survival of the second offspring under conspecific superparasitism because the probing frequency was positively associated with the body size of the ovipositing females; moreover, probing frequently failed to kill the first offspring. Thus, large samples are needed to distinguish between the effects of probing and body size. However, positive effects of probing on the second offspring for long oviposition intervals may exist under conspecific superparasitism. This is because they also exist under self-superparasitism, as well as for fifth-instar hosts (Yamada and Ikawa 2003); moreover, probing decreased the survival rate of the first offspring under conspecific superparasitism with a 24-h oviposition interval, suggesting that the second offspring was likely to avoid competing with the first offspring.

No effects of probing on the survival rate of the second offspring for 0-h and 1-h intervals appeared to cause low probing frequencies under conspecific superparasitism with these intervals (Fig. 13 and see sample sizes in Figs. 4 and 6); details are presented in the next chapter (5.3.4.). Such an association between the survival rate of the second offspring and probing frequency was also observed for the fifth-instar host (Yamada and Ikawa 2003).

4.4.6. Oviposition side

Same-side superparasitism is always accompanied with killing of the first offspring. Regarding self-superparasitism, however, the survival of the second offspring under same-side superparasitism, was lower than that under different-side superparasitism, irrespectively of whether or not probing occurred. It is also true for conspecific superparasitism when same-side superparasitism was compared with different-side superparasitism without probing using small second females. This was contrary to the results found for fifth-instar hosts, in which the survival of the second offspring under same-side superparasitism was fairly high (usually >50%) and as high as or more than that under different-side superparasitism, irrespectively of the superparasitism type (self or conspecific), oviposition interval and occurrence of probing (Yamada and Ikawa 2005). A possible reason for this host-instar difference is that the space behind the wing bud is too narrow for two eggs when the host is in the fourth instar. The second egg appears to be suppressed physically and to be more likely to be damaged.

Contrary to offspring from small females, offspring from large females exhibited similar survival rates between same- and different-side conspecific superparasitism. Reasons for such a difference between small and large females were not unknown at present.

Lower survival of the second offspring and no occurrence of two-adult emergence under same-side self-superparasitism predicts that the females are likely to avoid same-side self-superparasitism. Indeed, the female parasitoids were likely to avoid the same-side self-superparasitism (Fig. 11 and see sample sizes on the bars in Fig. 4); detailed analysis is presented in the next chapter (5.3.2.). Such preference is not seen when the host is in the fifth instar (Yamada and Ikawa 2005). This difference in behavior is considered to reflect the difference in survival of the offspring. Moreover, small females are predicted to be likely to avoid same-side superparasitism and be more likely to avoid probing than large females. These predictions were checked (Figs. 11 and 12); detailed analysis is presented in the next chapter (5.3.2.). Interestingly, large females as well as small females were likely to avoid same-side conspecific superparasitism, which was not explained by the survival rate of the second offspring. Detail discussion about reasons for it is made in the next chapter (5.4.2).

4.4.7. Oviposition interval

When *E. fairchildii* females superparasitized fourth-instar hosts, the survival rate of the second offspring decreased with increasing oviposition intervals under non-probing superparasitism, as seen in non-infanticidal parasitoids. The decreasing survival rate of the second offspring was mainly attributed to the absence of two-adult emergence for the 24-h oviposition interval. The survival rate of the first offspring did not increase with increasing oviposition intervals when probing did not occur, although it is known to increase in non-infanticidal parasitoid species. This is because the survival rate of the first offspring from large females was approximately 100% for 0-h and 1-h oviposition intervals, owing to two-adult emergence, and could not increase with increasing oviposition intervals, while the survival rate of the first offspring from small females was <10% for all three oviposition intervals. However, when long oviposition intervals (48 h and 96 h) are taken into account, the survival rates of both first and second offspring under non-probing superparasitism might decrease with increasing oviposition intervals, and the survival rate of the first offspring might be higher than that of the second offspring for such long intervals, as verified for fifth-instar hosts (Yamada and Ikawa 2003). This pattern is particularly likely to occur when the first and second females are large, as suggested by the present study (Figs. 5 and 6). The next chapter shows how superparasitism acceptance responded to the changes of the survival rate with increasing oviposition intervals.

5. Superparasitism strategy

5.1. Introduction

When a female parasitoid encounters a parasitized host, she must perform decision-making on whether she accepts superparasitism or not. The decision-making is largely influenced by fitness gains obtained from superparasitism, host availability, and parasitoid physiological conditions, as predicted by optimal foraging theory (Stephens and Krebs 1986; van Alphen and Visser 1990; Godfray 1994). Body size is also considered to influence on superparasitism acceptance as mentioned in General Introduction (chapter 1). When the female parasitoid with full egg load encounters a low-quality (parasitized) host, smaller females are more likely to reject such a low-quality host because they have low egg load and should preserve eggs for future encounter of high-quality hosts (Iwasa et al. 1984). However, if females estimate that the availability of hosts continues for a long time, smaller females are more likely to oviposit on low-quality hosts because smaller females live shorter than larger ones (Mangel 1989; Fletcher et al. 1994).

Conspecific superparasitism rewards the female with more fitness gains than self-superparasitism. Thus, an ability to discriminate between self- and conspecifically parasitized hosts is expected to evolve (van Alphen and Visser 1990). Many parasitoids actually have this ability (van Dijken et al. 1992), but it appears to diminish as the oviposition interval increases (Hubbard et al. 1987; Visser 1993; Ueno 1994), with exceptions including *Dinarmus basalis* (Gauthier et al. 1996) and *Nasonia vitripennis* (King 1992). *Echthrodelphax fairchildii* females are likely to avoid self-parasitized fifth-instar parasitized hosts than conspecifically parasitized ones for oviposition intervals of ≤15 min (Ito and Yamada 2014). However, there were still few parasitoid species studied about the effects of the oviposition interval on the discrimination ability.

In *E. fairchildii*, survival rates of the first and second offspring were lower under self-superparasitism than under conspecific superparasitism when the first and/or second females were small females, when the different-side oviposition occurred without probing (see the previous chapter). Meanwhile, self-superparasitism is sometimes profitable for large females, depending on the oviposition interval, whereas not for small females. It predicts that small females are more likely to prefer conspecific superparasitism to self-superparasitism than large females, if they are capable of making self/conspecific discrimination.

The mated female must decide whether she should lay a male or female egg after accepting superparasitism. Small females of *E. fairchildii* are predicted to lay male eggs because female eggs laid by them cannot grow to adults (chapter 3), and actually small females almost always lay male eggs un unparasitized fourth-instar hosts (chapter 3). Several theoretical models may explain sex allocation strategy that should be by large *E. fairchildii* females. If the local mate competition (LMC) model (Hamilton 1967) is applicable to the target parasitoid, the female should be more likely to lay a male egg under conspecific superparasitism than under self-superparasitism, because under conspecific superparasitism she recognizes that local competition for mates will be more likely to occur between her and another female's offspring (King 1992; Darrouzet et al. 2008). Moreover, when the fitness performance or competitive ability of the second offspring during the immature stage under superparasitism differs according to their sex (this is called asymmetric larval competition; van Baaren et al. 1999; Darrouzet et al. 2003; Sykes et al. 2007; Lebreton et al. 2010), the second female may lay an egg of the sex with a stronger competitive ability under conspecific superparasitism. Alternatively, the female may exhibit the sex allocation predicted by the host quality model (female eggs should be laid on higher-quality hosts, Charnov 1982). The host quality model predicts that the female should change the sex of their eggs depending on the quality of hosts she encountered before encountering the target host; that is, females may lay female eggs on parasitized fourth-instar hosts because they encounter many low-quality (third-instar) hosts before the superparasitism bout under the present experimental conditions.

When the female parasitoid is an infanticidal species, she faces another decision-making dilemma besides superparasitism acceptance and sex allocation; she should always perform infanticide against conspecifically parasitized hosts if the cost of infanticide is negligible (Netting and Hunter 2000; Takasuka and Matsumoto 2011) and/or infanticide ensures the higher survival of the second offspring. On the other hand, when the host is self-parasitized the solitary parasitoid should usually avoid superparasitism, and consequently does not need to decide whether she should perform infanticide. The semisolitary parasitoid—in which emergence of two parasitoids is possible under superparasitism even though a single egg is laid in an ovipositing episode—should accept self-parasitized hosts without infanticide instead of avoiding them when the host availability is low, and the two eggs laid will be likely to develop to adulthood. However, the above predictions are based on the assumption that the female parasitoid can perfectly distinguish between self- and conspecifically parasitized hosts. When the self/conspecific discrimination is not perfect or even completely impossible, the female should base her decision on the degree of accuracy of self/conspecific discrimination and the difference between fitness gains obtained by correct and incorrect decisions (Rosenheim and Mangel 1994; Yamada and Ikawa 2005; Yamada and Ito 2014; Segoli et al. 2009). Whet the host is in the fifth instar, *E. fairchildii* females refrain from performing probing for oviposition intervals of <1 day while they often performed probing for oviposition intervals of ≥1 day. It is interesting whether the same trend is seen for fourth-instar hosts. Moreover, if the infanticidal probing incurs some cost, such as shorten longevity, smaller females are more likely to refrain from performing the probing (Iwasa et al. 1984).

As of the oviposition side for superparasitism, in the light of on the difference in fitness gains, *E. fairchildii* females are expected to prefer the different side to the same side under self-superparasitism, when the host is in the fourth instar. Moreover, small females are expected to do so under conspecific superparasitism, too. However, when the host is in the fifth instar, *E. fairchildii* females does not exhibit such preference (Yamada and Ikawa 2005; Ito and Yamada 2014). It is not only because same-side superparasitism provides higher or as high fitness gains than or as different-side superparasitism but also because it seems difficult to change the oviposition side after catching the host. If the difficulty is so large, the females may not prefer the different side for the second oviposition.

Here, I determined superparasitism strategy of *E. fairchildii*, using the fourth-instar host of *L. striatellus*. I elucidated the effects of the superparasitism type, oviposition intervals, and body size of the superparasitizing females on superparasitism-acceptance rate, selection of oviposition side, sex allocation, and probing rate, and I determined the adaptiveness of the decision made by the parasitoids based on the fitness gains shown in the previous chapter. If the decision is not explained by the fitness gains, I discuss possible reasons for it. Moreover, I compared the results for the fourth-instar host with those for the fifth-instar host.

5.2. Methods

5.2.1. Parasitism and superparasitism bouts

I have already described the experimental procedures in the previous chapter (4.2.1.). Sample sizes for superparasitism acceptance, selection of oviposition side, sex allocation, and probing are shown in Table 19.

5.2.2. Data analysis

The effects of the body size (head width) of the female parasitoids, oviposition intervals, and superparasitism type (self or conspecific) on the rates of superparasitism acceptance and probing, sex allocation (proportion of male eggs), and selection of oviposition side (side with or without the first offspring) were examined using logistic regression analysis (LogXact®10, Cytel Software, Cambridge, MA, USA); see 4.2.2.1. in the previous chapter. The effect of the order of the oviposition (first and second) was also examined for the analysis of the sex allocation. The effect of the collection site was included in the model as a random factor (stratum). The significance was tested by calculating the exact probability (Cytel 2012); see 4.2.2.1.

5.3. Results

5.3.1. Superparasitism acceptance

Since an interaction between the oviposition intervals and body size was detected, separate analysis for different oviposition intervals and different ranges of body sizes was performed (Table 20). Large (head widths of ≥0.65 mm) females always performed both self- and conspecific superparasitism at 0 and 1-h oviposition intervals and did it less frequently at a 24-h oviposition interval (Table 21, Fig. 10), while middle-sized (head widths of 0.6-0.65 mm) and small (head widths of 0.55-0.60mm) females more likely performed self and conspecific superparasitism with increasing oviposition intervals. Moreover, separate analyses for different oviposition intervals using small females revealed that they distinguished between self- and conspecifically parasitized hosts) when the oviposition interval was 0 (*P*-values, < 0.001, 0.066, 0.239 for 0-, 1-, 24-h oviposition intervals, respectively).

5.3.2. Oviposition side

The body size of the ovipositing females, oviposition interval or superparasitism type did not affect the selection of oviposition side (Table 22, Fig. 11). The female parasitoids prefered the different side under self and conspecific superparasitism irrespectively of the oviposition interval (Fig. 11): $P < 0.01$ for each of self and conspecific superparasitism with different oviposition intervals (Fisher’s exact test).

5.3.3. Sex allocation

A male egg was usually laid on a host, but larger females more likely refrained from laying male eggs (Table 23, Fig. 12). The superparasitism type or oviposition interval has no effects on the sex allocation.

5.3.4. Infanticidal probing

Since an interaction between the oviposition interval and body size was detected, separate analysis for different oviposition intervals and different ranges of body sizes was performed (Table 24). The separate analysis revealed that larger females were more likely to perform probing for each oviposition interval (Table 25, Fig. 13). In addition, middle-sized (head widths of 0.60-0.65 mm) and large females (head width of ≥0.65 mm) were more likely to perform probing with increasing oviposition intervals, whereas small females (head widths of 0.55-0.60 mm) almost always refrained from probing irrespectively of the oviposition interval.

5.4. Discussion

5.4.1. Superparasitism acceptance

The response of superparasitism-acceptance rate to the oviposition interval differed among females with different body sizes. Large females always or almost always performed superparasitism while middle-sized and small ones more likely did it with increasing oviposition intervals. Moreover, small females distinguished between self- and conspecifically parasitized hosts when the oviposition interval was 0, while middle-sized and large females did not do for any oviposition intervals. This size difference in superparasitism-acceptance rate appeared to be explained by physiological differences between different sized females as well as the difference in fitness performance of immature offspring between different sized females. Larger females have longer expected life spans and larger egg loads, and they are more likely to accept low-quality hosts compared with smaller females when the host availability is low and the parasitoid is young (Iwasa et al. 1984; Mangel 1989; Fletcher et al. 1994; Sirot et al. 1997).

Regarding no self/conspecific discrimination among large and middle-sized females, the following scenario will be plausible. These females actually distinguished between self- and conspecifically parasitized hosts at an oviposition interval of 0 h. However, the distinguishing ability was not perfect, and difference in fitness gains from self- and conspecific superparasitism were slight; in particular, fitness gains from one host is likely to be larger under self-superparasitism than under conspecific superparasitism: note that fitness gains from self-superparasitism are expressed by the total fitness gains from the first and second ovipositions). When host availability is low, fitness gains from one host may contribute greater to the increase in lifetime fitness gains than the number of parasitized hosts. Consequently, large and middle-sized females did not exhibit self/conspecific discrimination for the 0-h oviposition interval. This is contrary to the results for the fifth-instar hosts (Ito and Yamada 2014): the females (probably middle-sized and large females) exhibit self/conspecific discrimination for the 0-h oviposition interval. When the host is in the fifth instar, female eggs are almost always laid (Ito and Yamada 2005, 2007, 2014). Different-side superparasitism without probing is considered to decrease the size of emerging parasitoids (Yamada and Ikawa 2005). The decrease in adult size reduces greatly the fitness of emerged female adults compared with that of emerged male adults (see Chapter 1). This appears to be a plausible reason for the above difference between the host instars.

Large females decreased superparasitism-acceptance rate a little at a 24-h oviposition interval. This is probably because the survival of the second offspring was lower at a 24-h oviposition interval than at the shorter oviposition intervals under different-side conspecific superparasitism without probing, and the females must perform probing (probably costly) to obtain higher survival.

Small females should avoid low-quality hosts compared with large ones, because small females have a limited number of eggs and should increase fitness gains from one oviposition. However, as the low availability of host continues, small females should be more likely to accept low-quality hosts to increase lifetime fitness gains. This explains that the superparasitism-acceptance rate among small and middle-sized females increased with increasing oviposition intervals. Interestingly, this phenomenon occurred although the fitness gain obtained from conspecific superparasitism by small females for an oviposition interval of 24 h was actually lower than that for the shorter oviposition intervals, as shown in chapter 4 (Table 6, Figs. 5 and 6): it should be noticed that former was mainly represented by the survival rate of the offspring under different-side superparasitism without probing because small females hardly performed costly probing (Fig. 13), The self/conspecific discrimination was found only among small females, as predicted from the difference in the fitness gain between self- and conspecific superparasitism. The discrimination was found for the 0-h oviposition interval, but not for the 1-h oviposition interval. This is probably because the females did not distinguish between self- and conspecific superparasitism for oviposition intervals of > 15 min as seen for the fifth-instar hosts (Ito and Yamada 2014). The female parasitoids are considered to have very little chance to encounter self-parasitized hosts in the field except just after the first oviposition owing to planthoppers jumping and dispersing when disturbed by an enemy. Therefore, the females do not need to evolve the self/conspecific discriminating ability.

5.4.2. Oviposition side

Preference in oviposition side was found for the fourth-instar host under both self- and conspecific superparasitism; the different side was preferred. This preference is expected under self-superparasitism in light of the fitness gains obtained (4.4.6 in chapter 4). The fitness gain from self-superparasitism is expressed by the total of fitness performance of the first and second offspring, because both offsprings come from the same female. The first offspring was always killed under same-side parasitism, and consequently the total survival rates of the first and second offspring were larger under different-side superparasitism than under same-side superparasitism. As of conspecific superparasitism, the fitness gains obtained from superparasitism explains the oviposition-side preference for small females. The survival rate of the second offspring under conspecific superparasitism was higher under different-side superparasitism than under same-side superparasitism, when small females refrained from probing (4.3.2. in chapter 4). Small females actually did so (5.3.4.).

Large females were likely to prefer different-side conspecific superparasitism than same-side conspecific superparasitism although they obtained statistically the same fitness gains from same-side and different-side superparasitism. Three possible reasons for it are as follows: 1. Although a little difference in fitness performance is actually present, I did not detect it due to small sample sizes. 2. The females cannot discriminate between self- and conspecifically parasitized hosts: note that the survival rate of the second offspring was always lower under same-side self-superparasitism than under different-side self-superparasitism. 3. The females do not have a perfect ability to recognize their own sizes.

In contrast to the fourth-instar host, preference in oviposition side was not for the fifth-instar host. When the host is in the fifth instar, the fitness gains from superparasitism suggest that the selection of the different side is adaptive under self-superparasitism, while the reverse is true under conspecific superparasitism (Yamada & Ikawa 2005). However, the female did not exhibit preference in oviposition side. The difference in the selection of oviposition side between the fourth and fifth instars appears to be caused by not only difference in the fitness gains but also cost or difficulty of selecting the oviposition side. In the case of the fourth-instar hosts, the female parasitoids were observed turning round the host to change the oviposition side after catching the host, but such action is not observed for the fifth-instar host (Yamada and Ikawa 2005). These observations suggest that it is difficult to select the oviposition side after catching fifth-instar hosts. A fifth-instar host is considered to be too large and heavy for the female to turn it around. When the host was in the fourth instar, small females were expected to refrain from preferring the different side compared with large females due to the cost for turning around the host, but small as well as large females actually preferred the different side. It is probably because the fitness gain under same-side superparasitism was definitely lower than that under different-side superparasitism (4.3.2. in chapter 4).

5.4.3. Sex allocation

Most of the eggs laid under superparasitism were male in the present study, contrary to result for the fifth-instar hosts: most of offspring were female. As predicted in the Introduction of the present chapter (5.1.), small females almost always laid male eggs. Larger females were more likely to increase the proportion of female offspring. This phenomenon consistent with the prediction of the host quality model (Charnov 1982). This will be verified by an experiment in which the females are supplied with fifth instar hosts before the superparasitism bout. If large females adopt the sex allocation strategy predicted by the host quality model, they will lay male eggs under superparasitism.

5.4.4. Infanticidal probing

The probing rate increased with increasing oviposition intervals under both self- and conspecific superparasitism when parasitoids were middle-sized and large. This pattern is consistent with that for the fifth instar host (Yamada & Ikawa 2003, Ito & Yamada 2014), and adaptive because the probing rewarded the females with increased fitness benefits at longer oviposition intervals, as shown in Yamada and Ikawa (2003) and the previous chapter. The self/conspecific difference in the probing rate was detected in Yamada and Ikawa (2003), but not in Ito and Yamada (2014) or the present study. The reason for it is not unknown at present. Meanwhile, small females hardly perform probing irrespective of the oviposition intervals and superparasitism type. This is probably because the probing incurs a cost (Ito & Yamada 2003) and it is relatively larger for small females. In light of the fact that it takes less than 30s to perform one event of probing (Ito 2009), the cost appears to be related to physiology, probably reduction of longevity (Ito and Yamada 2014).

6. General discussion

Fitness performance of *E. fairchildii* immatures under superparasitism differed between the different host instars and the parasitoid females changed their superparasitism strategy accordingly. The body sizes of the first and second ovipositing females had great effects on the fitness performance of the offspring under superparasitism and superparasitism strategy. This has never been reported in other parasitoids. In particular, the present study has reported for the first time in insects that the mother-size effect on survival of the immature offspring. The size of parasitoid adults varies greatly depending on host size and clutch size (Godfray 1994; Quicke 1997), and consequently a wide variation of adult sizes is common. The mother-size effects on fitness performance of the offspring and the size effect of foraging strategy should be explored using many parasitoids in future studies.

The fitness performance of the first and second offspring differed between self- and conspecific superparasitism when the host was in the fourth instar, whereas not when the host was in the fifth instar. The fitness performance of the first and second offspring under both self- and conspecific superparasitism has never been reported so far except for the two dryinid species (Yamada and Miyamoto 1997; Yamada and Watanabe 2002; Yamada and Ikawa 2003, 2004; Ito and Yamada 2003, 2014). One important point to keep in mind is that the difference in the survival of immatures between self- and conspecific superparasitism was detected when the first and/or second females are small (4.3.1.1. in chapter 4). Therefore, such a difference may be detected for the fifth-instar host in *E. fairchildii,* if different-sized females are used and mother-size effects are analyzed. In addition, analysis of mother-size effects is expected to be made for superparasitism of a dryinid parasitoid, *H. atratus*, in which the survival of immatures is higher under conspecific than under self-superparasitism (Yamada and Miyamoto1998) when the host is in the fourth instar.

The cost of ovipositing, turning around the host after catching it and probing appears to be a critical factor for decision making of these kinds of behavior. The cost has been usually expressed in terms of time, eggs or calories (Stephan and Krebs 1986; Godfray 1994). Besides, physiological cost must be important. Hard work has recently been considered to cause delayed maturation, a low fecundity, or short longevity in animals, including wasps and bees (O'Donnell and Jeanne 1992, 1995; Heinsohn and Legge 1999; Finkel and Holbrook 2000; Nilsson 2002; Williams et al. 2008). Ovipositing, turning round the host and probing are all considered to be hard work for *E. fairchildii* females because these kinds of behavior are performed while holding the host with the mandible and chelae after lifting it up. The hard work appears to incur the above physiological costs, and the relative importance of the costs must change depending on the size of the female parasitoid. Such costs have hardly been addressed in parasitoid foraging studies and remain to be elucidated for future studies.

Echthrodelphax fairchildii females changed behavioral decision-making depending on their sizes. A particularly intriguing phenomenon discovered in the present study is that small females discriminated between self- and conspecifically parasitized hosts while larger females did not. This is presumably because although larger females had an ability for self/conspecific discrimination, they adopted strategy different from small females' strategy and accepted self- and conspecific superparasitism at the same frequency (see 5.4.1.). Therefore, when researchers study about some behavior of an animal, they should use samples with different sizes. Otherwise, they may obtain misleading results.

7. Summary

Echthrodelphax fairchildii Perkin (Hymenoptera: Dryinidae) is an infanticidal koinobiont semi-solitary ectoparasitoid of planthoppers in the paddy fields. The female lays an egg under the forewing bud of the host for one parasitism bout, but two adults often emerge under superparasitism with short first-to-second oviposition intervals. The female often moves the abdominal tip to the non-oviposition side to probe for infanticide before laying an egg. Parasitized hosts continue to feed on host plants, but they do not molt. Here, I explored the fitness performance of immature parasitoids under superparasitism and superparasitism strategy using the fourth-instar host, lower in quality than the fifth-instar host. I examined superparasitism with oviposition intervals of 0, 1, and 24h. I also determined the fitness performance of immature parasitoids under single parasitism for comparison.

1) Survival rates and adult body sizes of both the male and female offspring increased with increasing body sizes of their mother under single parasitism, and consequently small females failed in producing female adults.
2) Survival rates of the first and second offspring was revealed under self and conspecific superparasitism. Two-adult emergence occurred at 0- and 1-h oviposition intervals. The first egg was assumed to be killed by the second female under same-side superparasitism (two eggs are laid on the same side) based on the previous study using fifth-instar hosts. This assumption was proved true by comparing the oviposition-to-larval-sac-appearance period of the offspring under same-side superparasitism with an oviposition period of 24 h with that of the offspring under single parasitism. The survival rate of the second offspring under same-side self-superparasitism was lower than under different-side self-superparasitism. It was true for conspecific superparasitism when second females were small, and the probing did not occur. The probing did not ensure

higher survival of the second offspring except for under self-superparasitism with a 24-h oviposition interval. The effect of oviposition intervals was found only under different-side superparasitism without probing: the survival rate of the second offspring decreased with increasing oviposition intervals. Under different-side superparasitism without probing, the survival rates of the first and second offspring were higher under conspecific than self-superparasitism mainly when the first and/or second females were small. Meanwhile, self/conspecific difference was not found under different-side superparasitism with probing or same-side superparasitism

3) The effect of mother body size was found under superparasitism also: larger mothers ensured higher survival of their offspring. The size of ovipositing females did not influence the survival of non-relative offspring. An exception was different-side conspecific superparasitism without probing, under which the first female's size had positive and negative effects on the survival rates of her offspring and the second offspring, respectively, while the second female's size had no effect on the survival of her offspring or the first offspring. When the probing occurred, the negative effect of the first female's size on the survival of the second offspring was not found, and the second female's size had a positive effect on the survival of her offspring. These suggest that the offspring, not the ovipositing females, release host-physiology regulating agents, and that offspring from larger mothers release a larger amount and/or more effective type of such agents than offspring from small mothers.
4) Large females always or almost always performed superparasitism while middle-sized and small females more likely did it with increasing oviposition intervals. Then, small females distinguished between self- and conspecifically parasitized hosts when the oviposition interval was 0, while middle-sized and large females did not. When parasitoids was large and middle-sized, the probing rate increased with increasing

oviposition intervals with no difference between self- and conspecific superparasitism. Meanwhile, small females hardly perform probing irrespectively of the oviposition interval. Most of the eggs laid under superparasitism were male, but as ovipositing females were larger, the proportion of female eggs increased. Ovipositing females prefer different-side superparasitism to same-side superparasitism. These patterns of behavior were considered to be adaptive in light of the fitness gains from superparasitism, self/conspecific distinguishing ability, difference in egg load, longevity, and relative cost for the focal behavior between different-sized females, and the fact that the females were kept with very little chance to encounter high-quality hosts.

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Table 1 Proportion of male eggs laid by females with different head widths

	Head width(mm)				
	<0.56	0.56–0.58	0.58–0.60	0.60–0.62	≥0.62
Males (%)	70.1	80.5	88.2	45.8	11.1
N	47	164	187	48	18

Table 2 Survival rates (%) of immature offspring during different developmental stages

	Developmental stage							
Sex	Oviposition to larval-sac appearance		Larval-sac appearance to host leaving		Host leaving to cocoon spinning		Cocoon spinning to adult emergence	
Male	97.4	*n* = 352	88.9	*n* = 343	92.7	*n* = 305	86.9	*n* = 283
Female	92.8	*n* = 112	63.4	*n* = 104	54.5	*n* = 66	94.4	*n* = 36

Table 3 Factors incorporated into individual statistical models for analysis of the effects of the oviposition interval, probing, superparasitism type, and body sizes of the first and second ovipositing females on the survival rates of the first and second offspring

				Factors incorporated into statistical models					
Procedure	Probing	Oviposition interval (h)	Self- or conspecific	Pr	SC	OI	HWFF	HWSF	Table no. for analysis results
1–1	Y or N	–	Self	–	–	Y	N	Y	6
	Y or N	–	Conspecific	–	–	Y	Y	Y	6
1–2	Y or N	–	–	–	Y	Y	Y	N	7
	Y or N	–	–	–	Y	Y	N	Y	7
2–1	–	0, 1, or 24	Self	Y	–	–	N	Y	8
	–	0, 1, or 24	Conspecific	Y	–	–	Y	Y	8
2–2	–	0, 1, or 24	Self	N	–	–	N	Y	8
	–	0, 1, or 24	Self	Y	–	–	N	N	8
	–	0, 1, or 24	Conspecific	N	–	–	Y	Y	8
	–	0, 1, or 24	Conspecific	Y	–	–	Y	N	8

Pr, occurrence of probing; SC, superparasitism type (self- or conspecific); OI, oviposition interval; HWFF, head width of first ovipositing females; HWSF, head width of second ovipositing females; Y, yes; N, no. Pr and SC were categorical variables, whereas OI, HWFF, and HWSF were numerical variables. Each line indicates factors incorporated in the statistical models for designated cases, e.g., the first line indicates that OI and HWSF were incorporated into a model for self-superparasitism with or without probing.

As a significant interaction existed between Pr and OI, we performed a separate analysis for superparasitism with or without probing (Procedures 1-1, 1-2) and separate analysis for the three oviposition intervals (Procedure 2-1). Strong multicollinearity existed between HWFF and HWSF under self-superparasitism; hence, we avoided incorporating these multicollinearity-causing factors together in the same statistical model (see 4.2.2. Data analysis for more details). Another multicollinearity between HWSF and Pr was present. As a result, when either HWSF or Pr was significant, the factor that was estimated as nonsignificant might actually have been significant (see 4.2.2. Data analysis for more details). Thus, by using two models, in which the probing and the size of the second female were incorporated separately (Procedure 2-2; see Table 4), we verified the significance of the factor that had initially been estimated as nonsignificant.

Table 4 Verification process of the significance of the effects of Pr and HWSF

	Results of Procedure 2-1		Results of Procedure 2-2		Conclusion for significance	
Case	Pr	HWSF	Pr	HWSF	Pr	HWSF
1	S	S	-	-	S	S
2a	NS	S	S	S	PS	S
2b	NS	S	NS	S	NS	S
3a	S	NS	S	NS	S	NS
3b	S	NS	S	S	S	PS

S, significant; NS, not significant; PS, possibly significant—but it was impossible to ascertain the significance with the present data (see below and the footnote of Table 3). Each line indicates the statistical results of Procedures 2-1 and 2-2 and the conclusion for the significance of Pr and HWSF based on these statistical results.

When Procedure 2-1 indicated that Pr and HWSF were significant and not significant, respectively (cases 3a, b), or vice-versa (cases 2a, b), we verified the results of Procedure 2-1 by performing Procedure 2-2 (see Table 3 and main text). When Pr (or HWSF) was not significant in Procedure 2-1 but significant in Procedure 2-2 (cases 2a, 3b), we could not verify its significance by using the present data, although the effect may have had a significant impact.

Table 5 Factors incorporated into individual statistical models for analysis of the effect of the oviposition interval, self/conspecific superparasitism, and the body sizes of the first and second ovipositing females on the two-adult emergence rate

			Factors incorporated into statistical model				
Procedure	Oviposition interval (h)	Self or conspecific	SC	OI	HWFF	HWSF	Table no. for analysis results
3	–	–	Y	Y	Y	N	10
	–	–	Y	Y	N	Y	10
4	–	Self	–	Y	N	Y	11
	–	Conspecific	–	Y	Y	Y	11
	0	–	Y	–	Y	N	11
	0	–	Y	–	N	Y	11
	1	–	Y		Y	N	11
	1	–	Y		N	Y	11

As the first and second ovipositing females are the same under self–superparasitism, we did not incorporate HWFF and HWSF in the same statistical model as shown above.

Table 6 Results (*P*-values) of the analysis of the effects of the oviposition interval and body sizes of the first and second ovipositing females on the survival rates of the first and second offspring

Factor	Without probing		With probing	
	First offspring	Second offspring	First offspring	Second offspring
Self-superparasitism				
OI	0.999	<0.001	1.000	0.154
HWSF (= HWFF)	<0.001	<0.001	0.667	0.013
OI*HWSF	0.235	0.734	1.000	0.237
Conspecific superparasitism[a]				
OI	0.396	0.001	0.462	0.436
HWFF	<0.001	0.045	0.839	0.332
HWSF	0.683	0.110	0.236	0.001
OI*HWFF	0.429	0.294	1.000	1.000
OI*HWSF	0.188	0.948	1.000	1.000
HWFF*HWSF	1.000	0.416	1.000	1.000

See Procedure 1-1 in Table 1 for models used for the analysis.

[a] Multicollinearity-free data, obtained by removing some data, were used (see Fig. 3).

Table 7 Results (*P*-values) of the analysis of the effects of the superparasitism type, oviposition interval, and body sizes of the first and second ovipositing females on the survival rates of the first and second offspring

Factor	Without probing		With probing	
	First offspring	Second offspring	First offspring	Second offspring
HWFF incorporated in the model				
SC	<0.001	<0.001	0.077	0.764
SC*OI	0.382	0.288	1.000	0.504
SC*HWFF	0.070	<0.001	0.475	0.077
HWSF incorporated in the model				
SC	0.026	0.006	0.052	0.564
SC*OI	0.086	0.194	1.000	0.665
SC*HWSF	0.042	0.012	0.344	0.904

See Procedure 1-2 in Table 3 for the models used in the analysis. As this analysis was performed to detect possible differences between self- and conspecific superparasitism, *P*-values are presented only for SC, SC*OI, and SC*HWFF (or HWSF). See Table 6 for the effects of the body size of the ovipositing females and oviposition interval.

Table 8 Results (*P*-values) of the analysis of the effects of the probing and body sizes of the first and second ovipositing females on the survival rates of the first and second offspring for each oviposition interval

Self or conspecific	Factor	Oviposition interval (h)		
		0	1	24
Survival of first offspring				
Self	Pr	1.000 (1.000)	0.023	0.147 (1.000)
	HWSF (= HWFF)	0.018 (0.018)[a]	<0.001	0.009 (0.163)
	Pr*HWSF	1.000	1.000	0.462
Conspecific[b]	Pr	1.000	0.059 (0.058)	1.000 (1.000)
	HWFF	<0.001	<0.001	0.002
	HWSF	1.000	0.729 (0.542)	0.244 (0.020)
	Pr*HWFF	1.000	1.000 (1.000)	0.001 (<0.001)
	Pr*HWSF	1.000	1.000	1.000
	HWFF*HWSF	1.000	1.000 (0.118)	1.000 (0.018)
Survival of second offspring				
Self	Pr	1.000 (0.296)	1.000 (0.069)	<0.001
	HWSF (= HWFF)	<0.001 (<0.001)	<0.001 (<0.001)	0.001
	Pr*HWSF	0.181	1.000	0.824
Conspecific[b]	Pr	0.400	0.131	0.675 (0.023)
	HWFF	0.119	0.184	0.498
	HWSF	0.092	0.198	0.055 (0.001)
	Pr*HWFF	1.000	1.000	0.546 (0.045)
	Pr*HWSF	1.000	0.333	0.678
	HWFF*HWSF	1.000	1.000	0.103 (0.070)

See Procedures 2-1 and 2-2 in Table 3 for the models used in the analysis.

[a] Values in parentheses indicate *P*-values obtained from Procedure 2-2, executed when either Pr or HWSF was significant or nearly significant in Procedure 2-1.

[b] Multicollinearity-free data, obtained by removing some data, were used (see Fig. 3).

Table 9 Results of the analysis of the effects of the oviposition interval and body sizes of the first and second ovipositing females on the outcome of the competition between the first and second offspring

Self or conspecific	Factor	*P*-value
Self	OI	0.143
	HWSF (HWFF)	0.382
	OI*HWSF	1.000
Conspecific[a]	OI	0.375
	HWFF	<0.001
	HWSF	0.230
	OI*HWFF	1.000
	OI*HWSF	0.822
	HWFF*HWSF	1.000

The case of single emergences was addressed. Competition outcome was defined as the emergence of the first or second offspring.

[a] Multicollinearity-free data, obtained by removing some data, were used (see Fig. 3).

Table 10 Effects of the self/conspecific superparasitism, oviposition interval, and body size of the ovipositing females on the two–adult emergence rate

	P-value	
Factor	HWFF incorporated in model	HWSF incorporated in model
SC	<0.001	<0.001
OI	0.027	0.033
HWFL (or HWSF)	<0.001	<0.001
SC*OI	0.014	0.009
SC*HWFF (or HWSF)	0.779	0.807
OI*HWFF (or HWSF)	0.835	0.755

Non-probing superparasitism with 0 h– or 1 h–intervals was addressed because two-adult emergence did not occur in the other cases. See Procedure 3 in Table 5 for models used for the analysis

Table 11 Results (*P* values) of the analysis of the effects of the self/conspecific superparasitism, oviposition interval, and body size of the ovipositing females on the two–adult emergence rate

	Self or conspecific[b]		Oviposition interval[c] (h)	
Factor[a]	Self	Conspecific	0	1
OI (SC)	0.031	0.188	<0.001	0.695
HWFF	<0.001	<0.001	<0.001	<0.001
OI(SC)*HWFF	0.818	1.000	1.000	0.765
OI (SC)	–	–	0.001	1.000
HWSF	–	0.010	<0.001	<0.001
OI(SC)*HWSF	–	1.000	0.856	0.904
HWFL*HWSF	–	1.000	–	–

See Procedure 4 in Table 5 for models used for the analysis

[a] OI for two left columns, SC for two right columns.

[b] Separate analysis for self and conspecific superparasitism.

[c] Separate analysis for superparasitism with the 0 or 1–h oviposition interval.

Table 12 Results (*P* values) of comparison in the number of emerging adults per host between single parasitism[a] and self-superparasitism

Oviposition interval (h)	Head width of ovipositing female	No. of adults emerging from a host (h) under self-superparasitism[a]			*P*-value[b]		
		0	1	2	Kind of parasitism	Head width of ovipositing female	Interaction
0	<0.6 mm	20	1	0	<0.001	<0.001	0.805
	≥0.6 mm	3	9	1	0.254	–	–
1	<0.6 mm	14	1	0	<0.001	<0.001	0.835
	≥0.6 mm	0	9	7	<0.001	–	–
24	All	31	5	0	<0.001	<0.001	0.483

[a] Data (non-emerging, 0, and emerging, 1) for single parasitism comes from Chapter 3: 70, 249 for head widths of <0.6 mm; 0, 33 for head widths of ≥0.6 mm.

[b] Randomization test was performed for females with head width of ≥0.6 mm for intervals of 0 and 1 h. Logistic model analysis was performed for the other cases. The head width of the ovipositing female and the kind of parasitism (single or self-superparasitism) were incorporated into the model. See *Analysis for profitable self-superparasitism* in 4.2.2.2. for details of analysis methods.

Table 13 Results (*P*-values) of the analysis of the effects of the superparasitism type, oviposition interval, and body sizes of the first and second ovipositing females on the direct-success rate of probing (death rate of the first offspring during the period from oviposition until the appearance of larval sac)

Factor	HWSF incorporated	HWFF incorporated
SC	0.377	1.000
OI	0.436	0.670
HWSF (or HWFF)	0.052	0.180
SC*OI	1.000	1.000
SC*HWSF (or HWFF)	0.935	0.291
OI*HWSF (or HWFF)	0.426	0.586

Table 14 Comparison of the oviposition-to-larval-sac-appearance period of the male offspring under single parasitism with that of the second male offspring under same-side superparasitism with a oviposition interval of 24 h

Type of parasitism	Frequency of period (days) between oviposition and larval-sac appearance		Statistical results (P value) for comparison between single parasitism and superparasitism[a]
	3	4	
Single parasitism	58	294	-
Self superparasitism	5	9	0.074
Conspecific superparasitism	2	8	0.674

[a] Fisher's exact test was used.

Table 15 Results (*P*-values) of the analysis of the effects of the oviposition interval and body size of the first and second ovipositing females on the survival rate of the second offspring under same-side superparasitism

Factor	*P*-value
Self-superparasitism	
OI	0.998
HWFF (= HWSF)	0.052
OI*HWFF	0.069
Conspecific superparasitism[a]	
OI	1.000
HWFF	0.313
HWSF	0.000
OI*HWFF	1.000
OI*HWSF	1.000
HWFF*HWSF	1.000

See Procedure 1-1 in Table 3(1) for the models used in the analysis.

[a] Multicollinearity-free data, which were obtained by removing some data, were used (see Fig. 3).

Table 16 Results (*P*-values) of the analysis of the effects of the oviposition interval, superparasitism type (self or conspecific), and body size of the first and second ovipositing females on the survival rate of the second offspring under same-side superparasitism

Factor	*P*-value
HWFF incorporated in the model	
SC	0.089
OI*SC	0.995
SC*HWFF	0.533
HWSF incorporated in the model	
SC	0.220
OI*SC	0.497
SC*HWSF	0.583

See Procedure 1-2 in Table 3(1) for the models used in the analysis. As this analysis was performed to detect possible differences between self- and conspecific superparasitism, *P*-values are presented only for SC, SC*OI, and SC*HWFF (or HWSF). See Table 15(2’) for the effects of the body size of the ovipositing females and oviposition interval.

Table 17 Comparison of the survival rate of the second offspring under same-side superparasitism with that under different-side superparasitism with or without probing

		P-value	
Self or conspecific	Factor	With probing	Without probing
Self	OS[a]	0.001	0.041
	OI	0.360	0.002
	HWSF (=HWFF)	<0.001	<0.001
	OS*OI	1.000	0.120
	OS*HWSF	0.820	0.057
	OI*HWSF	0.077	0.557
Conspecific[b]	OS	0.470	0.019
	OI	0.742	0.003
	HWFF	0.324	0.037
	HWSF	<0.001	0.141
	OS*OI	1.000	0.300
	OS*HWFF	1.000	0.174
	OS*HWSF	0.354	0.026
	OI*HWFF	1.000	0.481
	OI*HWSF	0.730	0.664
	HWFF*HWSF	1.000	0.041

[a] OS, Oviposition side.

[b] Multicollinearity-free data, which were obtained by removing some data, were used for conspecific superparasitism.

Table 18 Results (*P*-values) of the analysis of the effects of the oviposition side, oviposition interval, and body size of the first female on the survival rate of the second offspring when the probing did not occur: the analysis was performed separately for small and large second females

	Head width of second female	
	<0.6 mm	≥0.6 mm
OS	0.001	0.509
OI	0.012	0.032
HWFF	0.304	0.487
OS*OI	1.000	0.136
OS*HWFF	0.095	0.090
OI*HWFF	0.262	0.924

Multicollinearity-free data, which were obtained by removing some data, were used (see Fig. 3).

Table 19 Sample sizes for superparasitism acceptance, selection of oviposition side, sex allocation, and probing

		Oviposition interval (h)		
Kind of behavior	Self/ conspecific	0	1	24
Superprasititsm acceptance	Self	214	129	133
	Conspcific	99	147	114
Selection of oviposition side, sex allocation, or probing	Self	61	55	79
	Conspcific	45	51	63

Table 20 Analysis results for the effects of the superparasitism type, oviposition interval, and head width on the superparasitism-acceptance rate

Factor	*P-value*
SC	0.750
OI	0.029
HWSF	<0.001
SC*OI	0.130
SC*HWSF	0.131
OI*HWSF	0.045

Table 21 Analysis results (*P*-values) for the effects of the oviposition interval, superparasitism type (self and conspecific), and body size of the ovipositing females on the superparasitism-acceptance rate

Factor	Oviposition Interval (h)			Head width of females (mm)		
	0	1	24	0.55-0.6	0.6-0.65	≥0.65
OI	-	-	-	<0.001	<0.001	0.009
SC	0.032	0.007	0.752	0.694	0.989	0.830
HWSF	0.005	<0.001	<0.001	-	-	-
OI * SC	-	-	-	0.054	0.670	1.000
SC * HWSF	0.017	0.008	0.727	-	-	

Table 22 Analysis results for the effects of the oviposition interval, superparasitism type (self or conspecific), and body size of the females on the election of oviposition side

Factor	*P*-value
SC	0.369
OI	0.072
HWSF	0.083
SC*OI	0.485
SC*HWSF	0.314
OI *HWSF	0.127

Table 23 Analysis results for the effects of the oviposition interval, superparasitism type (self or conspecific), and order of oviposition (first or second) body size of the females on the proportion of male eggs

Factor	*P*-value
HWF	< 0.001
FS	0.373
SC	0.168
OI	0.122
HWF*FS	0.273
HWF*SC	0.587
HWF*OI	0.656
FS*SC	0.219
FS*OI	0.496
SC*INTERVAL	0.098

HWF, Head width of ovipositing females.   FS, First or second oviposition.

Table 24 Analysis results for the effects of the superparasitism type, oviposition interval, and head width on the probing rate

Factor	*P*-value
SC	0.567
OI	0.011
HWSF	<0.001
SC*OI	0.363
SC*HWSF	0.784
OI *HWSF	0.005

Table 25 Analysis results (*P*-values) for the effects of the oviposition interval, superparasitism type (self and conspecific), and body size of the ovipositing females on the probing rate

Factor	Oviposition Interval (h)			Head width of females (mm)		
	0	1	24	0.5-0.6	0.6-0.65	≥0.65
OI	-	-	-	0.704	<0.001	0.022
SC	0.074	0.074	0.626	1.000	0.503	1.000
HWSF	0.017	<0.001	<0.001	-	-	-
OI * SC	-	-	-	0.066	0.650	0.052
SC * HWSF	0.224	0.314	0.277	-	-	

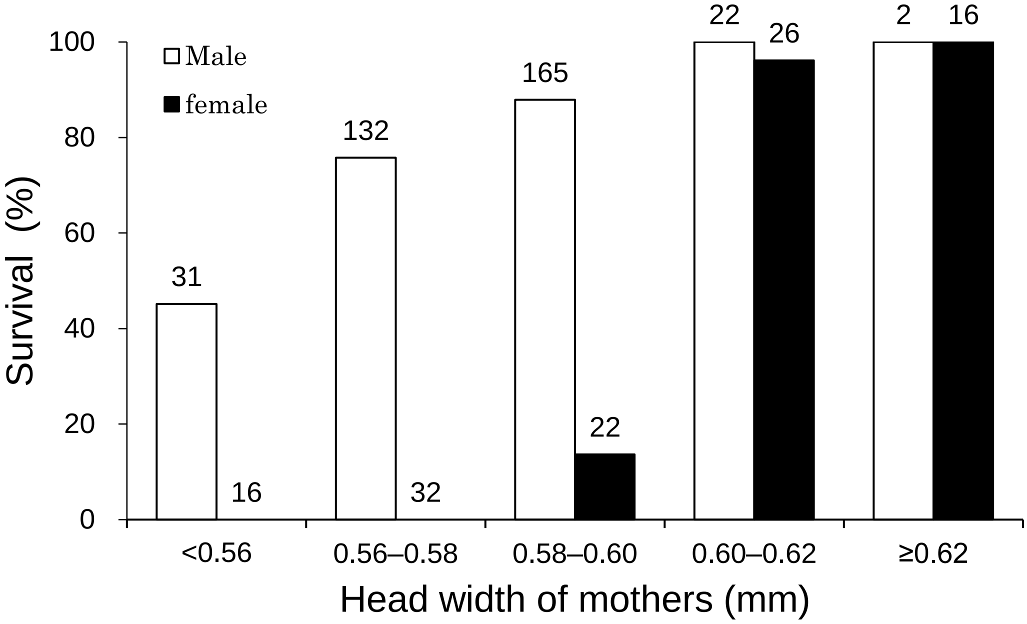

Fig. 1 Effects of the head width of mothers on the survival rates of the male and female offspring. Values on bars indicate sample sizes.

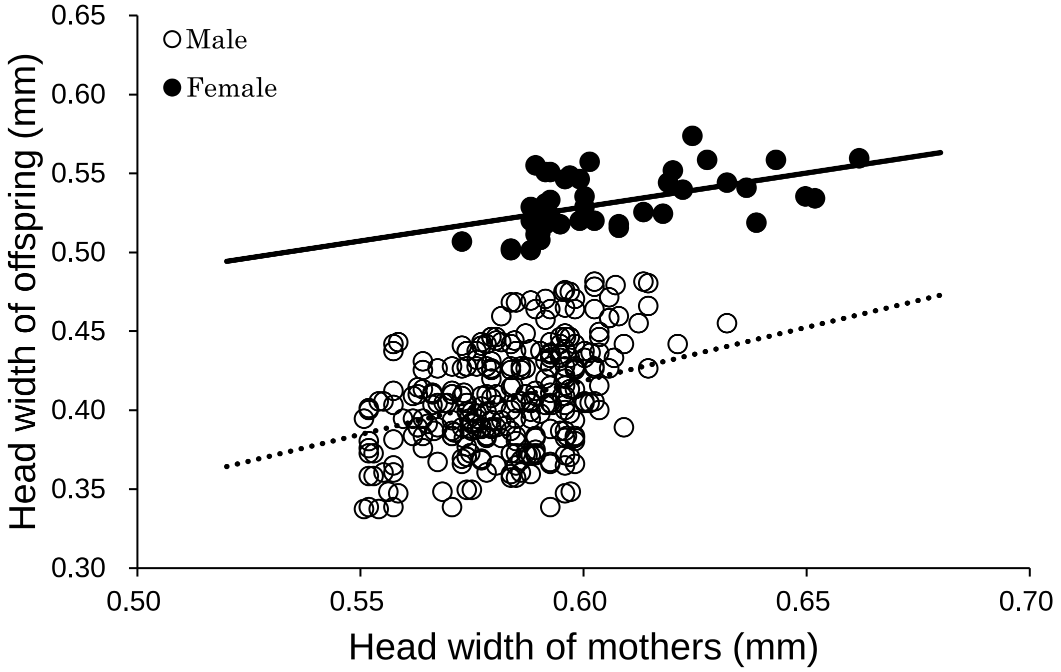

Fig. 2 Effects of the head width of mothers on the head widths of male and female offspring. Lines are from mixed linear model analyses.

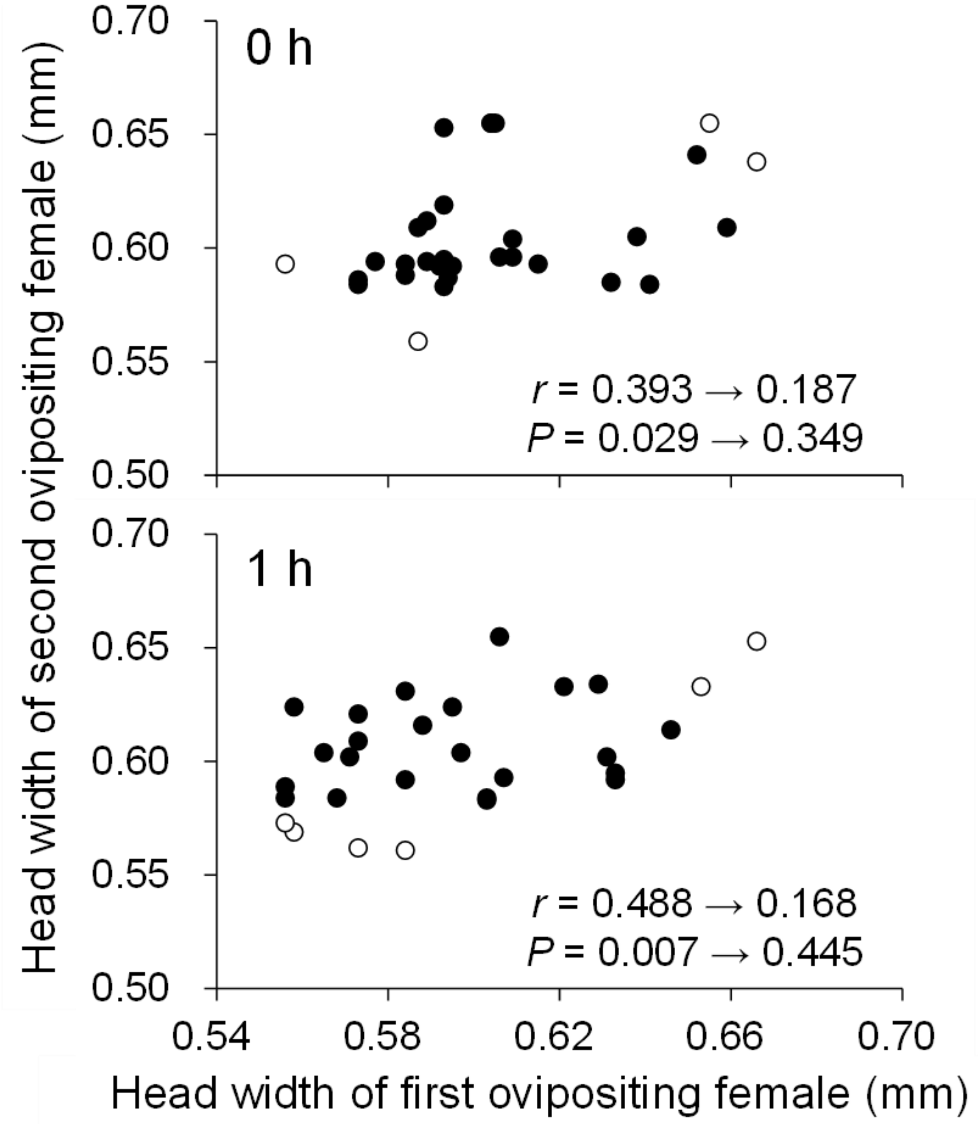

Fig. 3 Relationship between the head widths of the first and second ovipositing females under conspecific superparasitism with oviposition intervals of 0 h and 1 h. To eliminate multicollinearity between the two variables, open circles were not used for many statistical analyses. The *r*- and *P*-values decreased and increased, respectively, by excluding these circles, as shown in the figure.

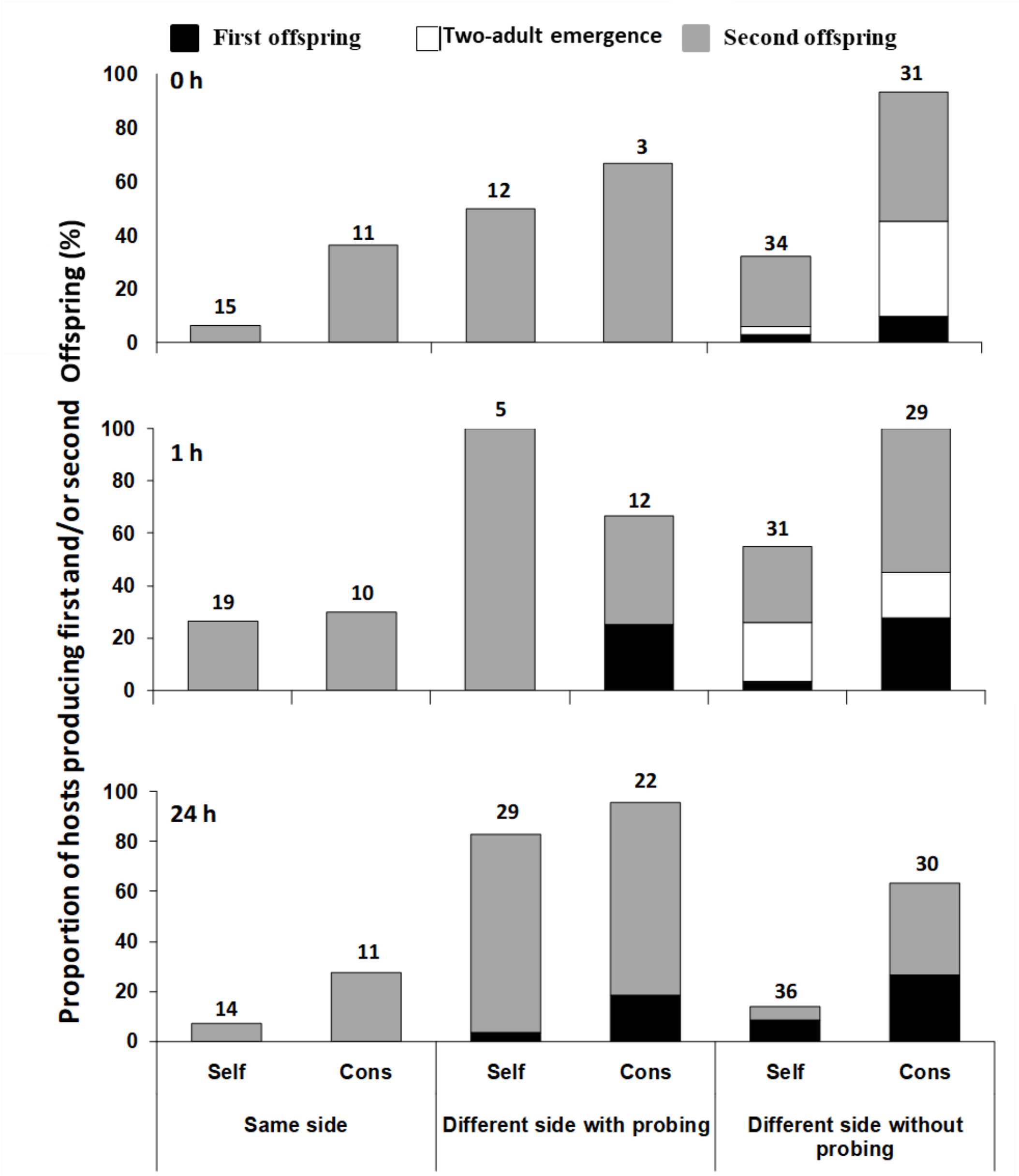

Fig. 4 Proportion of hosts producing first and/or second offspring. Numbers above bars indicate sample sizes.

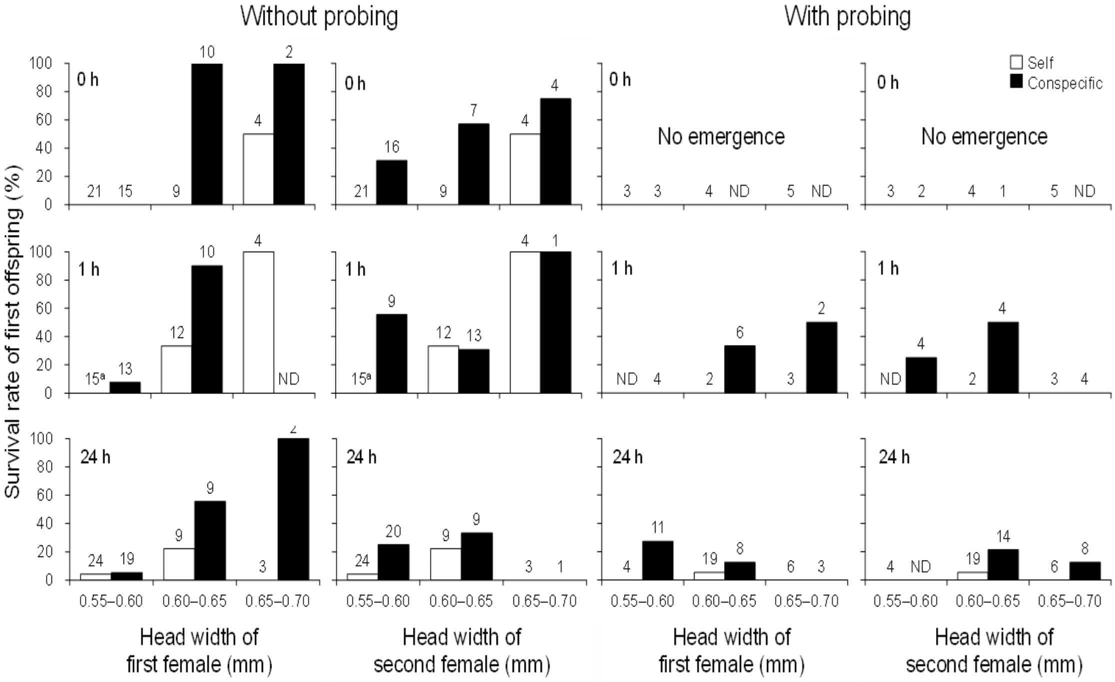

Fig. 5 Effect of body sizes (head widths) of the first and second ovipositing females on the survival rate of the first offspring under superparasitism, with or without probing. Numbers above bars indicate sample sizes. ND, no data; [a], including one female with a head width of 0.533 mm. Samples generating multicollinearity were excluded (Fig. 3). When probing did not occur, the survival rate of the first offspring was positively associated with the mother size and was lower under self-superparasitism than under conspecific superparasitism, mainly when the first and/or second ovipositing females were small. See Tables 6–8 for detailed results of the statistical analysis.

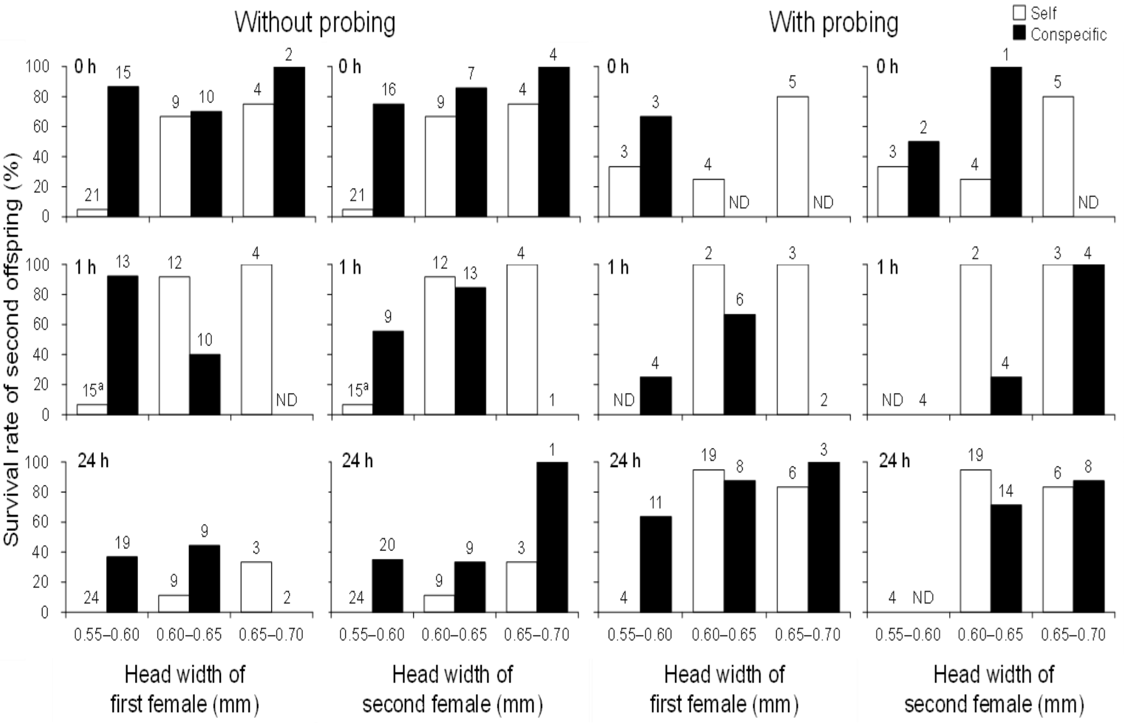

Fig. 6 Effect of body sizes (head widths) of the first and second ovipositing females on the survival rate of the second offspring under superparasitism, with or without probing. Numbers above bars indicate sample sizes. ND, no data; [a], including one female with a head width of 0.533 mm. Samples generating multicollinearity were excluded (Fig. 3). The survival rate of the second offspring was positively associated with the mother size, except for the survival rate of the second offspring under non-probing conspecific superparasitism, which was negatively associated with the first female's size. The survival rate was lower under self-superparasitism than under conspecific superparasitism, when the first and/or ovipositing females were small. See Tables 6–8 for detailed results of the statistical analysis.

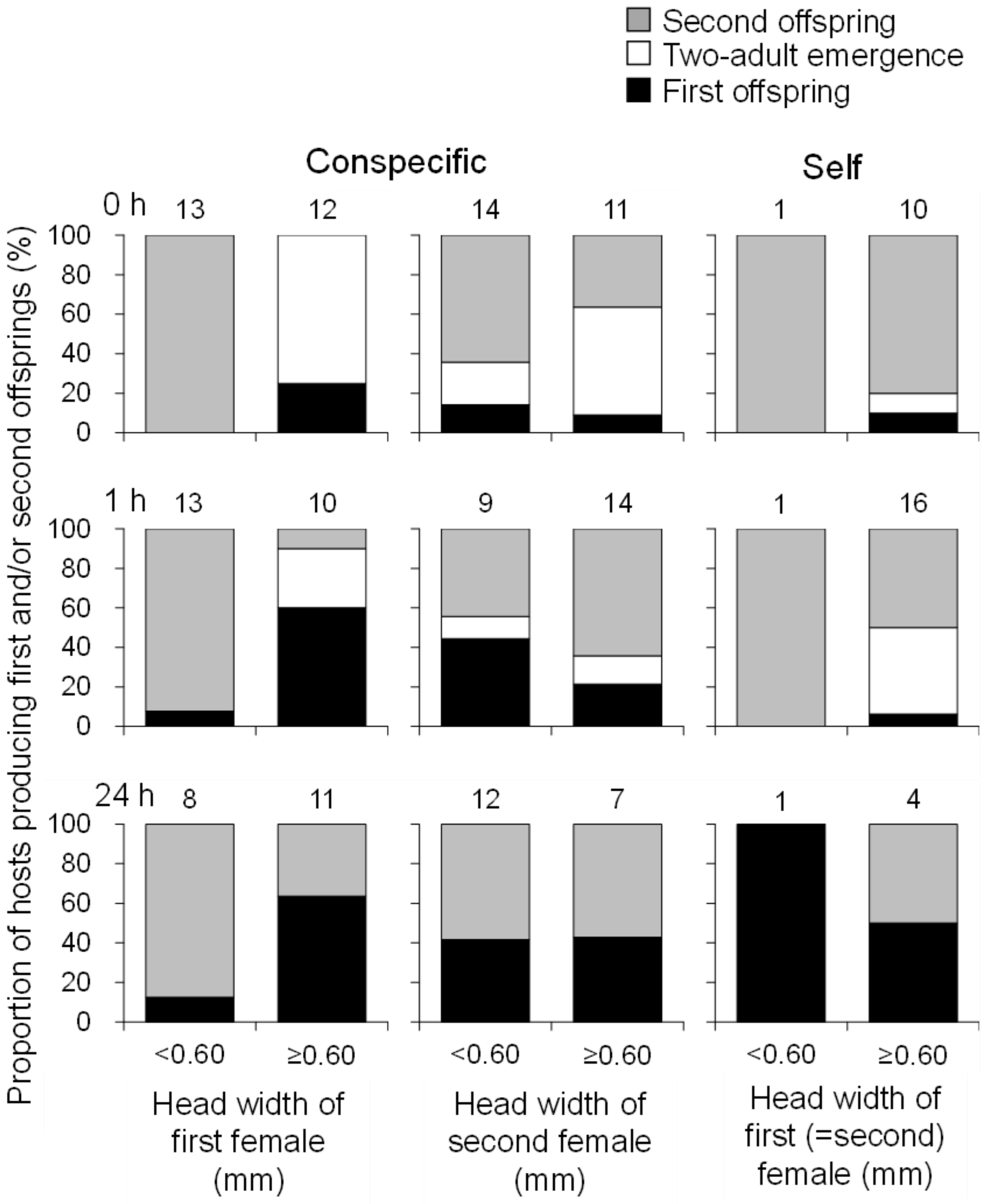

Fig. 7 Effect of body sizes (head widths) of the first and second ovipositing females and oviposition interval on the competition between the first and second offspring (evaluated herein as offspring emergence), under self- and conspecific superparasitism without probing. Numbers above bars indicate sample sizes. Samples generating multicollinearity were excluded (Fig. 3). First offspring from small mothers were highly likely to lose the competition to the second offspring under conspecific superparasitism. See Table 9 for detailed results of the statistical analysis.

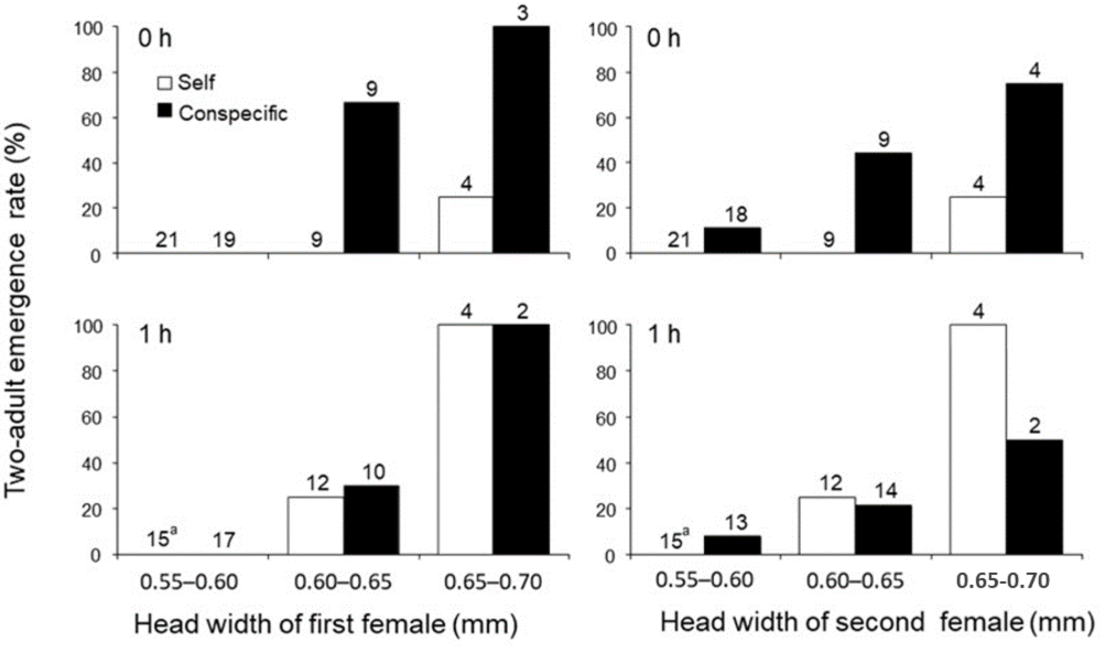

Fig. 8 Effect of body sizes of the ovipositing females on the proportion of two-adult emergence under different side superparasitism without probing. Numbers above bars indicate sample sizes; [a] including one female with a head width of 0.533 mm. When the oviposition interval was 0 h, the two-adult emergence rate was higher under conspecific than under self-superparasitism. See Tables 10 and 11 for detailed results of the statistical analysis.

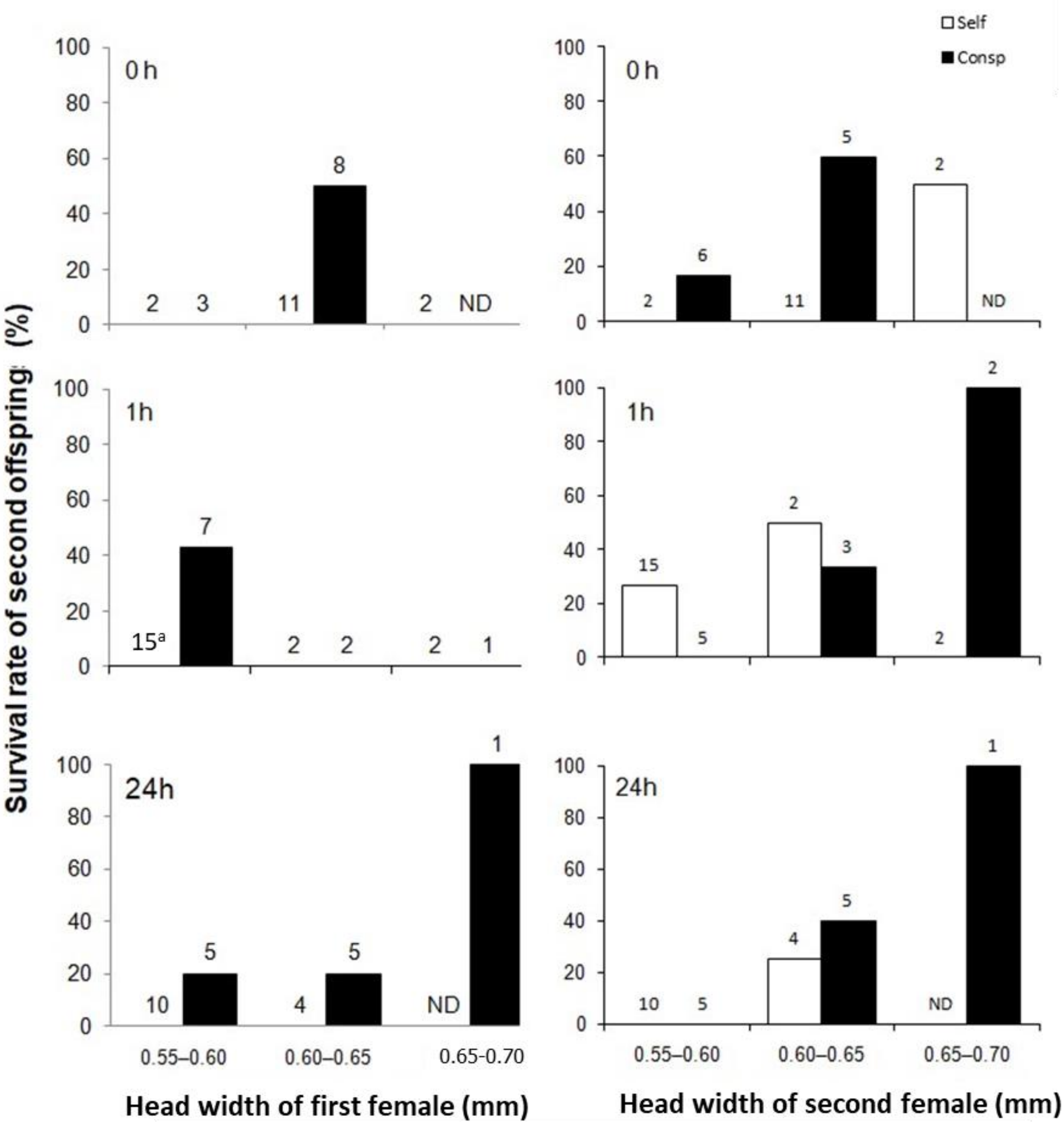

Fig. 9 Effect of body sizes of the first and second ovipositing females on survival rate of second offspring under same-side superparasitism . Numbers above bars indicate sample sizes; ^{a} including one female with a head width of 0.533 mm. See Tables 20 and 21 and the text for detailed results of the statistical analysis. Numbers above bars indicate sample sizes; ND, no data. The effect under same-side superparasitism was similar to it under different-side superparasitism with probing (see Figs. 6 and 7). See Tables 15 and 16 for detailed results of the statistical analysis.

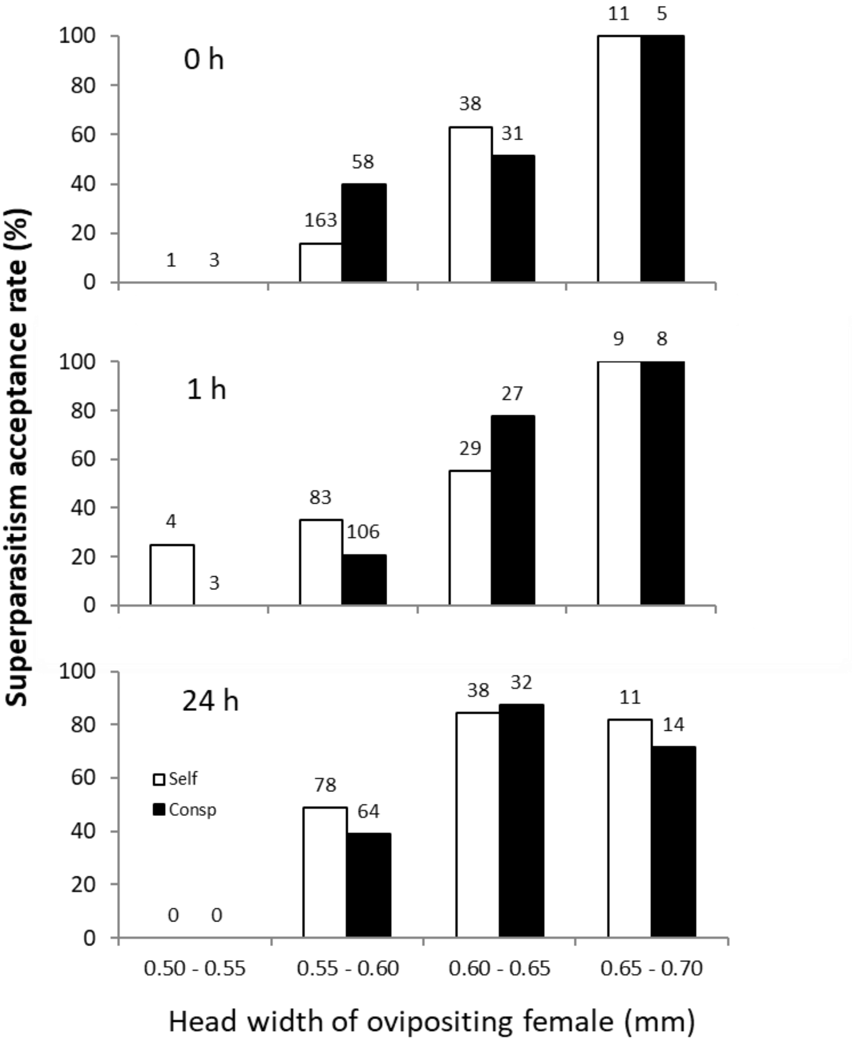

Fig. 10 Effects of the body sizes of the ovipositing females on the superparasitism-acceptance rate for different oviposition intervals. Number above bars indicate sample sizes. Larger females were more likely to accept superparasitism, and females with head widths of 0.55-0.65mm were more likely to accept superparasitism with increasing oviposition intervals. Females with head widths of 0.55-0.60 mm were more likely to accept conspecific superparasitism than self-superparasitism. See Tables 20 and 21 and the text for detailed results of the statistical analysis.

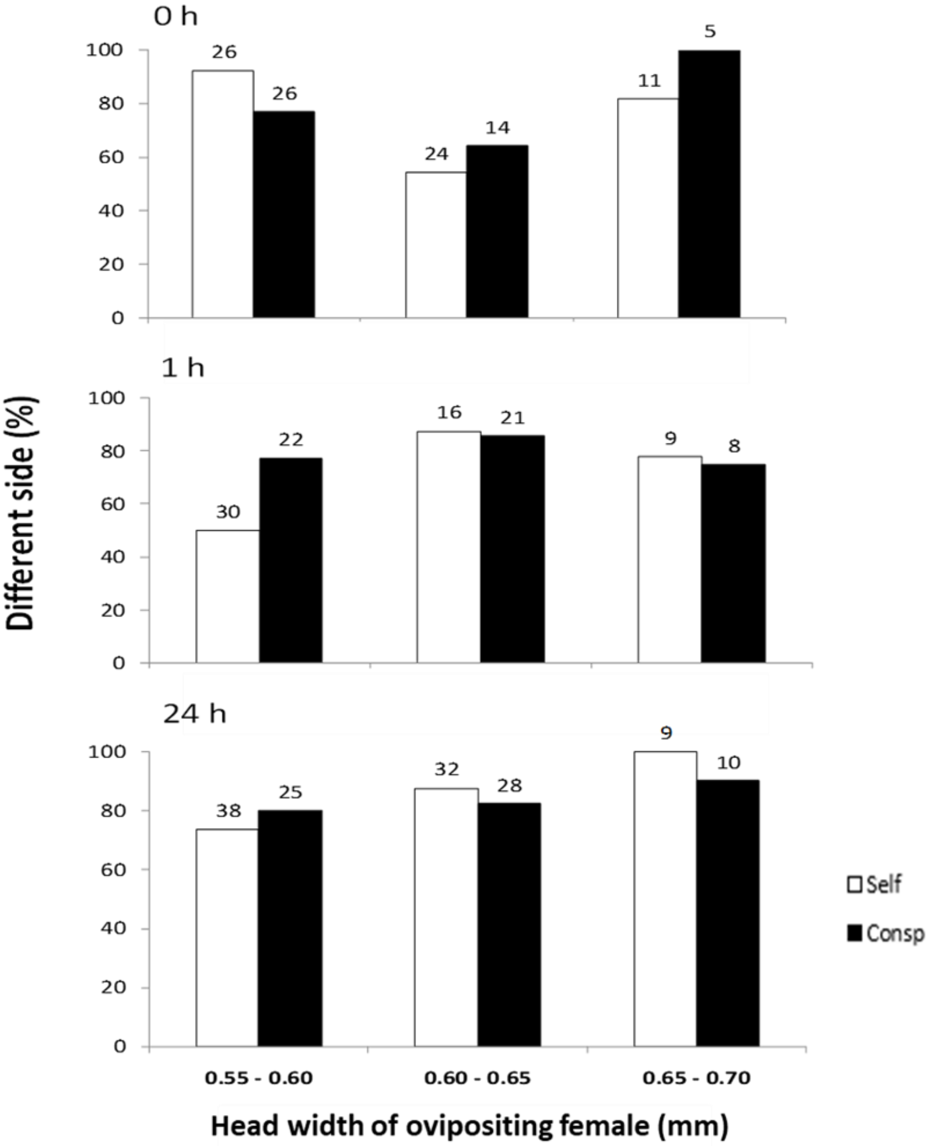

Fig. 11 Effect of the body sizes of the ovipositing females on the selection of oviposition side. Number above bars indicate sample sizes. The different side was preferred. The size of the ovipositing females, oviposition interval, and superparasitism type (self or conspecific) had no effects on the selection of oviposition side. See Table 22 for detailed results of the statistical analysis.

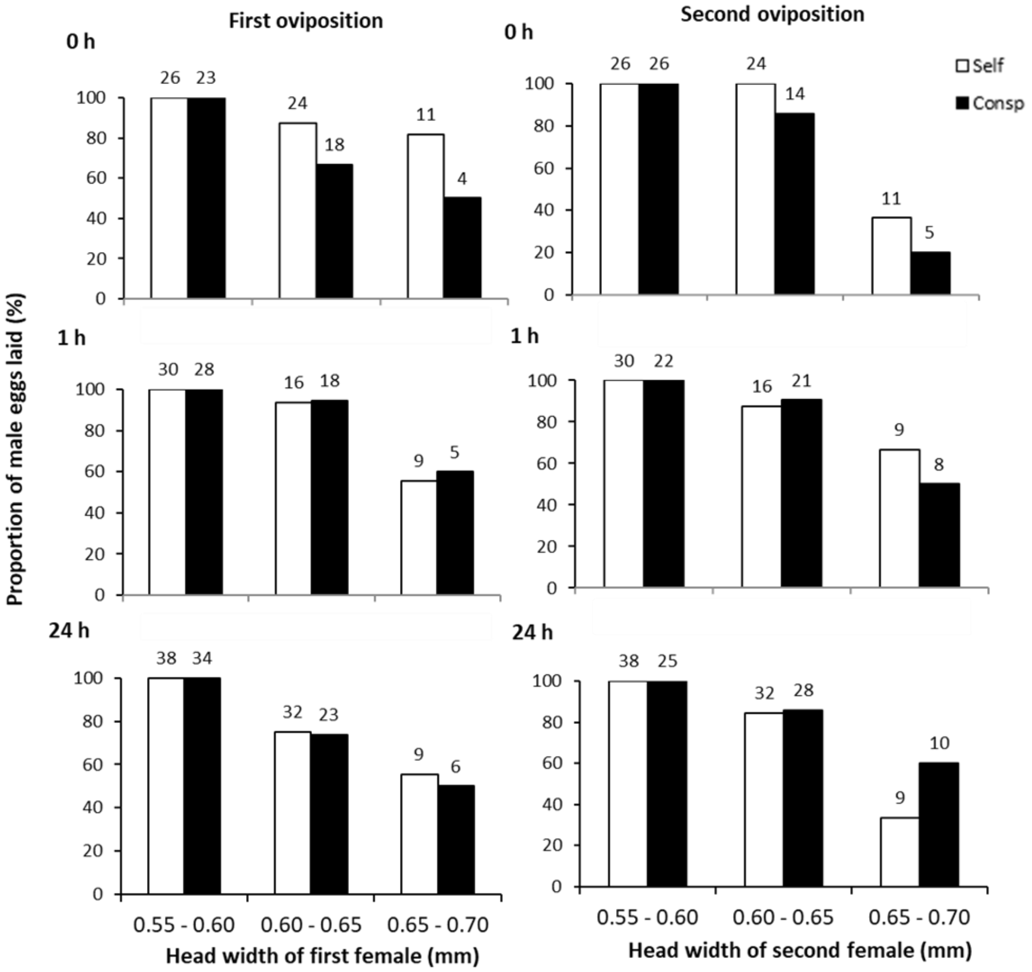

Fig. 12 Effect of body sizes of the first and second ovipositing females on the proportion of male eggs laid. Number above bars indicate sample size. Smaller females were more likely to lay male eggs. See Table 23 for detailed results of the statistical analysis.

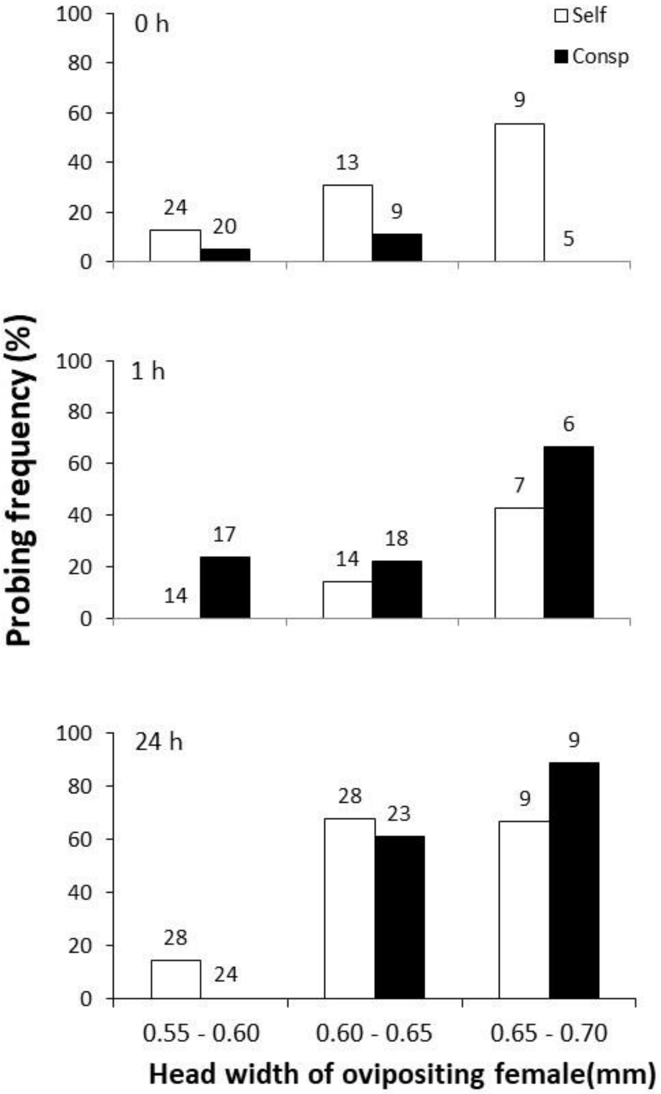

Fig. 13 Effect of the body size of the ovipositing females on the probing frequency for different oviposition intervals under superparasitism. Number above bars indicate sample sizes. Females with head widths of ≥ 0.60 mm were more likely to perform probing with increasing oviposition intervals. See Tables 24 and 25 for detailed results of the statistical analysis.