

Host discrimination modulates brood guarding behaviour and the adaptive superparasitism in the parasitoid wasp *Trissolcus semistriatus* (Hymenoptera: Scelionidae)

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Abstract. Because hosts utilized by parasitoids are vulnerable to further oviposition by conspecifics, host guarding benefits female wasps. The present study aimed to test whether female adults regulate brood guarding behaviour by host discrimination in a solitary parasitoid, *Trissolcus semistriatus* by presenting an intact or parasitized host egg mass to a female adult. Virgin females without oviposition experience had host discrimination ability, which enabled them to adjust the number of eggs laid in the hosts. Mating experience increased superparasitism by female adults, whereas mated females obtained higher discrimination ability by oviposition experience and showed a lower superparasitism rate. As expected, females exhibited brood guard after parasitizing an intact host egg mass, whereas those females visiting a previously parasitized host egg mass, did not do it. Because the survival of eggs in superparasitized hosts is relatively low, regulating brood guarding behaviour by host discrimination is adaptive for female wasps.

Key words. larval competition, oviposition behaviour, Scelionidae, quasi-gregarious parasitoid, sex ratio

Introduction

Hosts utilized by parasitoids are not removed from the habitat, and thus are vulnerable to attack by competitors of the parasitoids or predators of the hosts. Therefore, in some parasitoid species, female wasps stay with their brood after completion of parasitization (Goubault *et al.* 2007; Hu *et al.* 2012), guarding against intruders (Hardy & Blackburn 1991; Wang *et al.* 2014). A host with a parasitoid brood can also be attacked by conspecifics of the parasitoid laying their own brood, i.e. superparasitism, which results in reduced fitness of the primary female (Quicke 1997). Because previously parasitized hosts are also of relatively low value for subsequent parasitoid females, it is advantageous for female wasps to discriminate between parasitized and unparasitized hosts coexisting in the habitat to avoid superparasitism (Bakker *et al.*, 1985). However, adaptive superparasitism occurs when female conspecifics at high population density deplete available hosts and a secondary brood can survive until adult emergence (van Alphen & Visser 1990). The conditions of female wasps influence their decision to superparasitize, in fact, females without oviposition experience have a propensity for superparasitism (van Lenteren & Bakker 1975; Ikawa & Suzuki 1982). In addition, the values of the host and offspring influence the decisions by female wasps to utilize and defend host patches (Goubault *et al.* 2005; Goubault *et al.* 2007).

Many wasps in Scelionidae are solitary endoparasitoids that exploit host egg masses (Austin *et al.* 2005), and are called quasi-gregarious parasitoids (van den Assem *et al.* 1980). An egg mass constitutes a defendable patch of host resources, and female wasps show brood guarding behaviour in this family (Wilson 1961; Waage 1982; Ohno 1999). In solitary parasitoids, offspring of either primary or subsequent females from superparasitized hosts survive larval competition. In addition, female wasps evidently discriminate between parasitized and unparasitized hosts, avoiding superparasitism, although substantial superparasitism occurs in *Trissolcus euschisti* (Ashmead) and *Trissolcus utahensis* (Ashmead) (Okuda & Yeargan 1988; Weber *et al.* 1996). In *T. basalis*, after oviposition a female wasp rubs her ovipositor on the host surface to mark, i.e. performs marking behaviour (Bin *et al.* 1993). In advance of oviposition, a female wasp performs antennal examination of marked hosts before rejecting them (Colazza *et al.* 1996). Conspecific females can also detect an external marking pheromone that enables host discrimination in *T. basalis* (Rosi *et al.*, 2001) and *Telenomus heliothidis* (Strand & Vinson 1983).

Trissolcus semistriatus (Nees) (Hymenoptera: Scelionidae) is a quasi-gregarious parasitoid, which exploits egg masses of *Eurygaster integriceps* Puton in Scutelleridae and

several species in Pentatomidae (Heteroptera) in wheat fields in Turkey (Koçak & Kilinçer, 2003; İslamoğlu, 2011). A female wasp lays 80–100 eggs in her lifetime and survives for 10–20 days at 26°C under laboratory conditions (Kivan & Kilic, 2006; Tarla & Kornoşor, 2009). In Japan, *T. semistriatus* exploits egg masses of the cabbage bug, *Eurydema rugosum* Motshulsky (Pentatomidae) on leaf mustards (K. Matsuo, Y. Todoroki, S. Fujita, J. Endo, H. Numata, T. Mita & Y. Hirose unpublished). Our preliminary observations show that the oviposition behaviour of female adults is similar to that of *T. basalis* described by Field (1988). Female wasps stay on or close to the egg mass that they parasitized, show aggressive behaviour chasing intruders, and occasionally lay eggs in the egg masses of hosts previously parasitized by other females. Because offspring guarding obviously imposes opportunity costs on female wasps, regulation of guarding behaviour benefits them.

The present study aimed to examine the factors that influence whether female adults of *T. semistriatus* parasitize or superparasitize the hosts and guard their offspring. The primary purpose is to test whether female adults regulate brood guarding behaviour by host discrimination. We observed behaviour by a female adult of various types (virgin female, mated female, or mated female with oviposition experience) toward an unparasitized or parasitized host egg mass. By using virgin and mated females, we successfully estimated the primary sex ratios and offspring survivorship. By distinguishing between unparasitized and parasitized hosts, female wasps regulated not only their oviposition behaviour to determine the number of offspring and the proportion of the male offspring, but also guarding behaviour.

Material and methods

Insects

Egg masses of *E. rugosum* were placed artificially in a colony of the leaf mustard, *Brassica juncea*, on the riverside of Takanogawa in Sakyo, Kyoto, Japan (35°02'N, 135°46'E) in May 2013, and collected after 4 days. Adults of *T. semistriatus* emerging from these host eggs and their progeny were reared successively on egg masses of *E. rugosum*. Although occasionally we used eggs of *Plautia stali* Scott (Pentatomidae) as alternative hosts for maintaining the wasps, wasps used for experiments were raised on eggs of *E. rugosum*. All experiments were performed under an LD 16:8 h photoperiod at $25.0 \pm 1.0^\circ\text{C}$ at 95% RH,

unless otherwise stated. Adult wasps were kept individually in cotton-plugged glass tubes (diameter 10 mm, length 75 mm) with honey diluted with water and solidified by agar until used for experiments. Adults of *E. rugosum* were collected from the same leaf mustard colony between March and June in 2014, and reared on dried seeds of rape, *Brassica napus*, at the same temperature and with the same photoperiod to obtain egg masses for parasitization by *T. semistriatus*. Host egg masses consisting of approximately 12 eggs (11.84 ± 0.98 , mean $\pm$ S.D., $n = 50$) were used for experiments 0–2 days after oviposition. The egg number of egg masses did not affect the results significantly (data not shown).

Adult wasps were allowed to mate with their siblings upon emergence from parasitized host egg masses that were kept separately in glass tubes. Then the female wasps were isolated from these glass tubes 1 day or more before they were used for experiments. These females were ensured to be mated because all of them produced female progeny (haplodiploid sex determination). To obtain virgin females, parasitized egg masses were separated into single eggs using forceps and a razor and kept individually in glass tubes until adult emergence. Behaviour of female wasps was examined 1–22 days after adult emergence.

Experimental procedures

A piece of paper (5 mm $\times$ 10 mm) with a host egg mass was cut out from a larger paper that had been placed in a host cage, and was then introduced into a glass tube (diameter 10 mm, length 75 mm) with a female wasp. The behaviour of the wasp was recorded for 3 h using a video camera (HDR-SR12; Sony, Japan) at 25°C. Oviposition behaviour and staying time of female wasps on or close to the egg mass were examined. In *Trissolcus basalis*, marking behaviour of a female wasp after withdrawing her ovipositor indicates that the wasp laid an egg (Wilson, 1961). In *T. semistriatus* also, from host eggs marked by female wasps no host nymph hatched ($N = 84$) in our preliminary observation. Therefore, we observed when and to which host egg the female wasps performed marking behaviour. We counted the replications of marking behaviour in video recordings and regarded them as the number of ovipositions. Parasitism rate was defined as the rate of parasitized host eggs in the host egg mass. In addition, the duration from the last marking behaviour to the time when the females stepped away to a distance of 15 mm from the centre of the egg mass or made a circle along the wall of glass tubes was recorded as the staying time. After 3 h of recording, each egg mass was removed from the glass tube, and immediately introduced into another glass tube with a female wasp.

We examined the behaviour of female wasps in the following five experimental groups (see Fig. 3): First, an unparasitized host egg mass was presented to a virgin female (group A), and then the host egg mass parasitized by the female was subsequently presented to a mated female (group D). Then, the order of virgin and mated females was reversed (groups B and C). Moreover, one unparasitized host egg mass was presented to each of two mated females, and then the host egg masses parasitized by these females were exchanged between the two mated females that have just obtained oviposition experience. Thus, a parasitized egg mass was presented to a mated female adult that had experienced oviposition within 3 h (group E). In other words, unparasitized hosts and virgin females were used in group A, unparasitized hosts and mated females in group B, parasitized hosts and virgin females in group C, parasitized hosts and mated females in group D, and parasitized hosts and mated females that experienced oviposition in group E (each $N = 10$). Therefore, three types of superparasitized hosts were obtained: Hosts successively parasitized by virgin-mated (obtained from groups A and D), mated-virgin (from groups B and C) and mated-mated (from group E) females. The sex ratios of wasps emerging from these three types of host eggs should be different, because virgin females lay only males and mated females lay their progenies with different sex ratios to parasitized and unparasitized hosts. The sex of emerging adults in superparasitized hosts was determined by dissecting the hosts before the wasps emerged from the hosts. Only dissection data from the host eggs verified to have been parasitized twice by two different females were used for analysis.

In all individual eggs in the host egg masses used as parasitized, one (or more) wasp egg had been laid.

Analysis

The data of parasitism rate and the staying time in groups from A to D were analyzed by generalized linear models with binomial distribution and logit link function, and with Gamma distribution and inverse link function, respectively (Agresti 2007). Explanatory variables were wasp condition (mated or virgin) and host condition (parasitized or unparasitized); the age of female wasps was included as a covariate. The significance of the main effects and their interactions was judged by using a likelihood ratio test. Insignificant fixed effects were eliminated backward. Analyses were carried out using R, version 3.0.3 (<http://www.R-project.org>).

Then, following post hoc comparisons were conducted. The parasitism rate and the

staying time of female wasps were compared between pairs of experimental groups using the Wilcoxon rank sum test with Bonferroni correction. Because a single female wasp often laid two or more eggs in the same host egg, i.e. superparasitism, the parasitism rate is not always equal to the number of eggs laid per host egg (total number of ovipositions by a female divided by the number of eggs in the host egg mass). Therefore, the number of eggs laid per host egg was also compared. We conducted a planned comparison of parasitism rate among four pairs of groups (A vs C, B vs D, C vs D, vs D vs E), of staying time between two pairs (A vs B, B vs D), and of the number of eggs laid per host egg between two pairs (A vs C, B vs D) (Zar 2010). Therefore, the Bonferroni-corrected significance levels are 0.0125, 0.025, and 0.025 for parasitism rate, staying time, and the number of eggs laid per host egg, respectively. To examine the effect of host conditions, we compared group A with C and group B with D. In addition, to examine the effect of wasp conditions, we compared group A with B and group C with D. The effect of oviposition experience was examined by comparing group D with E.

The offspring survival rate in superparasitized hosts was estimated based on the method by Ohno (1999) after slight modification. The primary sex ratios when females oviposit to unparasitized or parasitized hosts were also calculated by this method. The sex ratios of wasps emerging in the hosts successively parasitized by mated-virgin (S_a), virgin-mated (S_b), and mated-mated (S_t) female wasps are expressed as:

$$S_a = S_c + V_m(1 - S_c)$$

$$S_b = S_d + (1 - V_f)(1 - S_d)$$

$$S_t = S_c S_d + (1 - V_f)(1 - S_d) S_c + (1 - S_c) S_d V_m$$

Here V_m and V_f are the probabilities that male and female offspring of the subsequent female survive through larval competition in the superparasitized host, respectively. In addition, S_c and S_d are the primary sex ratios when mated females oviposit to unparasitized and parasitized hosts, respectively.

Results

Host discrimination and adaptive superparasitism

To examine whether host conditions, wasp conditions or both affect oviposition behaviour of female wasps, an unparasitized or parasitized host egg mass was presented to a

virgin female, mated female, or mated female with oviposition experience. We examined the proportion of eggs parasitized by a female wasp for each egg mass (parasitism rate). There were significant effects of wasp condition (virgin or mated; $D = 36.59$, $df = 1$, $P < 0.01$) and host condition (unparasitized or parasitized; $D = 183.59$, $df = 1$, $P < 0.01$) on parasitism rate. There was no significant interaction between wasp and host conditions. In addition, there was a significant effect of the age of female wasps on parasitism rates ($D = 4.14$, $df = 1$, $P < 0.05$). As the age of females was higher, the rate of parasitism was lower.

Both virgin and mated females parasitized all eggs in unparasitized host egg masses (Fig. 1A, B). Significantly more eggs received parasitism by virgin females in unparasitized egg masses than in parasitized ones ($W = 100$, $P < 0.001$, Fig. 1A, C). Thus, virgin females discriminated parasitized hosts from unparasitized ones and avoided complete superparasitism. In parasitized egg masses, mated females parasitized more eggs than virgin females, but the difference was marginal ($W = 80$, $P = 0.025$, Fig. 1C, D). Thus, mating experience made females more prone to superparasitism. Mated females with oviposition experience to unparasitized hosts scarcely laid eggs in parasitized egg masses; their parasitism rate was significantly lower than that of mated females without oviposition experience ($W = 100$, $P < 0.001$, Fig. 1D, E).

Apart from the above experimental groups A–E, we presented an additional unparasitized egg mass to a female wasp for 3–5 h immediately after it completed parasitization to an unparasitized egg mass. No host nymph hatched from the egg mass subsequently presented (95 host eggs and 17 female wasps were used). Therefore, female wasps that experienced oviposition maintained motivation and ability for further parasitization, but had reduced propensity for superparasitization.

Comparison of the number of eggs laid by a female wasp per host egg instead of the parasitism rate did not contradict the conclusion that virgin females discriminate parasitized hosts from unparasitized ones. Indeed, virgin females laid significantly more eggs to unparasitized egg masses (mean $\pm$ SEM = 1.03 ± 0.02) than to parasitized ones (mean $\pm$ SEM = 0.50 ± 0.17 , $W = 80$, $P < 0.025$). On the other hand, the number of eggs laid by mated females per host egg to parasitized egg masses (mean $\pm$ SEM = 0.94 ± 0.10) did not differ significantly from that to unparasitized ones (mean $\pm$ SEM = 1.04 ± 0.02 , $W = 63.5$, $P = 0.319$). Nevertheless, the parasitism rate of mated females in parasitized hosts did differ from that in unparasitized hosts ($W = 95$, $P < 0.001$, Fig. 1C, D). These results indicate that mated female wasps lay two or more eggs into a single host egg in a parasitized host egg mass. Therefore, female wasps showed self-superparasitism more often when they utilized

parasitized host egg masses than when they used intact ones. In addition, mated females that had experienced oviposition laid 0.03 ± 0.02 (mean $\pm$ SEM) eggs per host egg (see Fig. 3).

Staying time

To examine under what circumstances female wasps guard their offspring, we examined the staying time of females on or close to the egg masses after they completed parasitization. There were significant effects of host conditions (unparasitized or parasitized, $D = 38.65$, $df = 1$, $P < 0.01$) on the staying time. However, there was no significant effect of wasp conditions (virgin or mated, $D = 2.68$, $df = 1$, $P > 0.05$) and the age of female wasps ($D = 0.44$, $df = 1$, $P > 0.05$) on the staying time.

Mated females stayed significantly longer when they parasitized intact hosts than when they superparasitized ($W = 100$, $P < 0.001$, Fig. 2B, D). We should not attribute this difference to the difference in the total number of eggs laid by female wasps, because the numbers of eggs laid by females per host egg to parasitized and unparasitized hosts did not differ significantly (see above and Fig. 3). In addition, mated females stayed longer than virgin females when they parasitized intact hosts, but the difference was marginal ($W = 79$, $P = 0.029$, Fig. 2A, B).

Survival rate

To examine whether superparasitism results in any reproductive success of the females that subsequently parasitized the hosts, we estimated the offspring survival rate in superparasitized hosts. The sex ratios of wasps emerging from host eggs sequentially parasitized twice by (1) mated and virgin females, (2) virgin and mated females and (3) two mated females were (1) 0.452 ($N = 31$), (2) 0.887 ($N = 71$) and (3) 0.375 ($N = 8$), respectively (see Fig. 3). The primary sex ratios when virgin and mated females laid eggs to unparasitized host eggs were 1.000 (due to haplodiploid sex determination) and 0.119 ($N = 84$), respectively. Substituting these measured values into equations (1), (2), and (3), the survival rates of wasp larvae in superparasitized hosts were calculated. When a male egg and a female egg were laid in a single host egg, therefore, the calculated survival rates of male and female offspring subsequently laid were 0.378 and 0.592, respectively. In addition, the primary sex ratio when mated females laid eggs to parasitized host eggs was estimated to be 0.809, which is fairly high compared to the primary sex ratio from unparasitized hosts (0.119).

Consequently, when parasitization occurred twice within a 3 h interval, the subsequent offspring sometimes won the larval competition in superparasitized hosts. The proportion of superparasitized eggs in which both wasp larvae died was only 2.7 % ($N = 113$).

Discussion

Fig 3 summarizes the experimental procedures and results. The present study demonstrates that the host-discrimination ability of female adults in *T. semistriatus* (Hymenoptera: Scelionidae) plays an important role not only in avoidance of superparasitism and adjustment of the primary sex ratio but also in the regulation of brood guarding behaviour. Female wasps had an ability to discriminate parasitized hosts from unparasitized ones, and laid significantly fewer eggs into the former without experience of mating and oviposition. In *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae), a solitary parasitoid wasp of *Drosophila* larvae, van Lenteren & Bakker (1975) reported that the ability of females for host discrimination needs to be learned through oviposition. However, later studies showed that virgin female wasps without oviposition experience discriminate between parasitized and unparasitized hosts (van Alphen *et al.* 1987; Visser *et al.* 1992). Also in *Trissolcus basalis*, female wasps without oviposition experience can discriminate parasitized hosts from unparasitized ones to avoid superparasitism (Colazza *et al.* 1996).

Mated females of *T. semistriatus* regulate the fertilization of their eggs. Female wasps of many species in Scelionidae adjust the primary sex ratio of their offspring by allocating male eggs in the early part of an oviposition bout (Hardy 1992). This sex allocation sequence inevitably lowers offspring sex ratios as the total number of eggs laid increases. The sex ratio is also affected directly by the density of female wasps per host patch in *Telenomus remus* Nixon (Hymenoptera: Scelionidae) (van Welzen & Waage 1987). In *T. semistriatus*, the progeny emerging in the laboratory show a female-biased sex ratio (Tarla & Kornoşor 2009). In the present study also, emerging adults from once-parasitized host egg masses showed a female-biased sex ratio. Based on the present results, we conclude that female wasps of *T. semistriatus* adjust the primary sex ratio according to host conditions (parasitized or not). Theoretical studies predict that a female wasp would lay more eggs of male offspring to a previously parasitized host than to an unparasitized host (Suzuki & Iwasa 1980). Indeed, mothers of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) produce more sons when they lay eggs to parasitized hosts (Holmes 1972; Werren 1980).

In *T. semistriatus*, female wasps decided to superparasitize based on both internal (mating and oviposition experience) and host (parasitized or unparasitized) conditions (Fig. 3). Although the actual probability that virgin females disperse and encounter parasitized host egg masses in the field remains unclear, laying fewer eggs in parasitized hosts could be adaptive behaviour of virgin females. Virgin females produce only male offspring due to haplodiploid sex determination. In *T. semistriatus*, only one adult wasp emerges from one host egg resulting from larval competition even when parasitization occurs twice. When a virgin female encounters a host egg mass that has already been parasitized by a mated female (initial female), which proportion of host eggs should the virgin female lay eggs in? We calculated the number of females that sons of the virgin female fertilize, under the assumption of the equal survival rate in larval competition and equal mating rate in competition between male adults. If the virgin female parasitize all the host eggs, her male offspring possibly kill the female offspring of the initial female by larval competition, which are potential mates of her sons. The calculation showed that virgin females gain the maximum reproductive success when they parasitize 51.3 % of eggs in a parasitized host egg mass. In general, the optimal primary sex ratio of the subsequent female reaches 1.0, i.e., all offspring are males, when the female lays sufficiently fewer eggs than the initial female (Suzuki & Iwasa 1980). It is, therefore, advantageous for virgin females to adjust the parasitism rate to a previously parasitized host. Virgin females of a scelionid wasp, *Tiphodytes gerriphagus*, also superparasitize, but less often than mated females (Sousa & Spence 2000).

The high rate of superparasitism in mated females without oviposition experience would have adaptive significance, because a substantial proportion of offspring of the subsequent female survived in a superparasitized host in *T. semistriatus*. The experience of oviposition to unparasitized hosts reduced superparasitism in *T. semistriatus*, and this phenomenon was also shown in two scelionid wasps, *Telenomus fariai* Haliday and *Gryon japonicum* (Ashmed) (Bosque & Rabinovich 1979; Noda 1990). The suppression of superparasitism after oviposition experience also has adaptive significance to avoid self-superparasitism (Roitberg & Mangel 1988). It is possible that peripheral influences such as a change in egg load and sensory inputs produced by eggs moving through the ovipositor act as an immediate mechanism of the modulation of superparasitization behaviour through oviposition experience, as Henneman *et al.* (1994) pointed out in *L. heterotoma*. However, such peripheral influences are insufficient to explain the motivational modulation when the effect of oviposition experience to a previously parasitized host differs from that to an

unparasitized host. In two gregarious egg parasitoids, *Trichogramma embryophagum* (Hartig) and *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae), oviposition experience to a previously parasitized host does not suppress superparasitism (Klomp *et al.* 1979; Miura *et al.* 1994), but we did not examine here whether oviposition experience to a previously parasitized host suppresses superparasitism in *T. semistriatus*. However, we discuss the effect of oviposition experience to a previously parasitized host on brood guarding behaviour by *T. semistriatus* females in the following paragraph.

It is worth noting that a parasitized egg mass triggered guarding behaviour in the female that had freshly oviposited in the egg mass (Figs. 2B, 3), but did not trigger guarding behaviour with a long duration in a female that had freshly oviposited in an already parasitized egg mass (Figs. 2D, 3). Because the number of eggs laid was not significantly different between a parasitized and unparasitized egg mass (Fig. 3B, D), we cannot attribute the difference between effects of oviposition experience to parasitized and unparasitized hosts to mere peripheral influences in female wasps. Rather, we attribute this difference to the information that female wasps obtained by antennation to host eggs when they parasitized intact hosts or previously parasitized hosts. In *T. basalis*, female wasps retain the information received during the host examination and make subsequent decisions based on this information, e.g. the contacts with already marked or unmarked hosts (Colazza *et al.* 1996).

Is the flexible brood guarding behaviour of female *T. semistriatus* adaptive? Because in superparasitized hosts the survival of secondary laid eggs is at most 0.5, the reproductive success of offspring in superparasitized hosts is relatively small as compared to that in unparasitized hosts. In a gregarious ectoparasitoid, *Goniozus nephantidis* (Muesebeck) (Hymenoptera: Bethyridae), in which females guard their offspring to prevent killing by adults of other parasitoids, the defensive tendency of female wasps is affected by the offspring value (Goubault *et al.* 2007). In *T. basalis*, female wasps increase their fighting tendency as the females lay more eggs in the host patch (Field & Calbert 1998). However, Wajnberg *et al.* (2004) failed to show an effect of host conditions (parasitized or not) on the tendency of females to leave their parasitized hosts.

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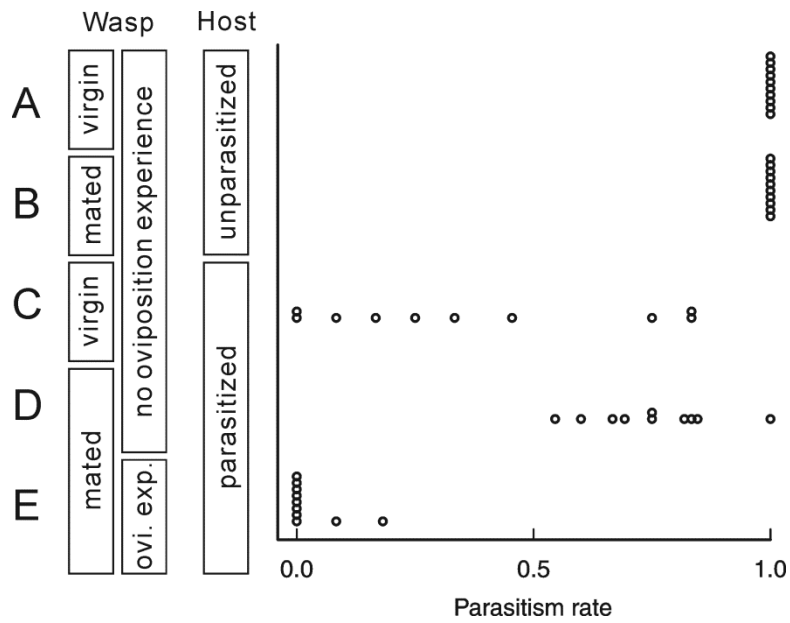


Fig. 1. Proportion of eggs in a host egg mass parasitized by a female adult of *Trissolcus semistriatus*. An unparasitized or a parasitized egg mass of *Eurydema rugosum* was presented to a virgin or mated female adult for 3 h (A–D). A parasitized egg mass was presented to a mated female adult that had experienced oviposition within 3 h also (E). $N = 10$ for each. Female adults were used for experiments 1–22 days after emergence. Each circle indicates a trial.

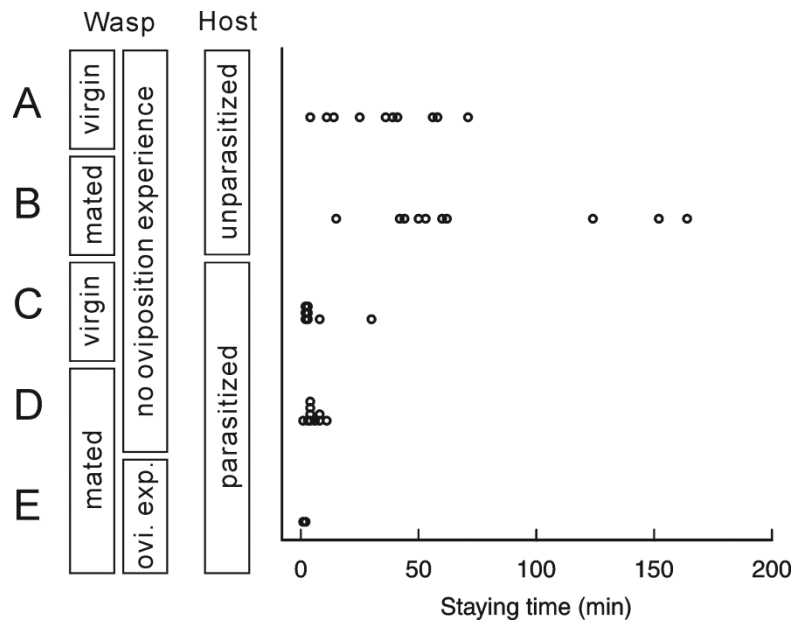


Fig. 2. The staying time of female adults of *Trissolcus semistriatus* within a distance of 15 mm from a host egg mass after completion of parasitization. An unparasitized or a parasitized (P) egg mass of *Eurydema rugosum* was presented to a virgin or mated female adult (A–D). A parasitized egg mass was presented to a mated female that had experienced oviposition within 3 h also (E). $N = 10$ for each. The trials in which female adults did not parasitize host egg masses were not shown. Female adults were used for experiments 1–22 days after their emergence. Each circle indicates a trial.

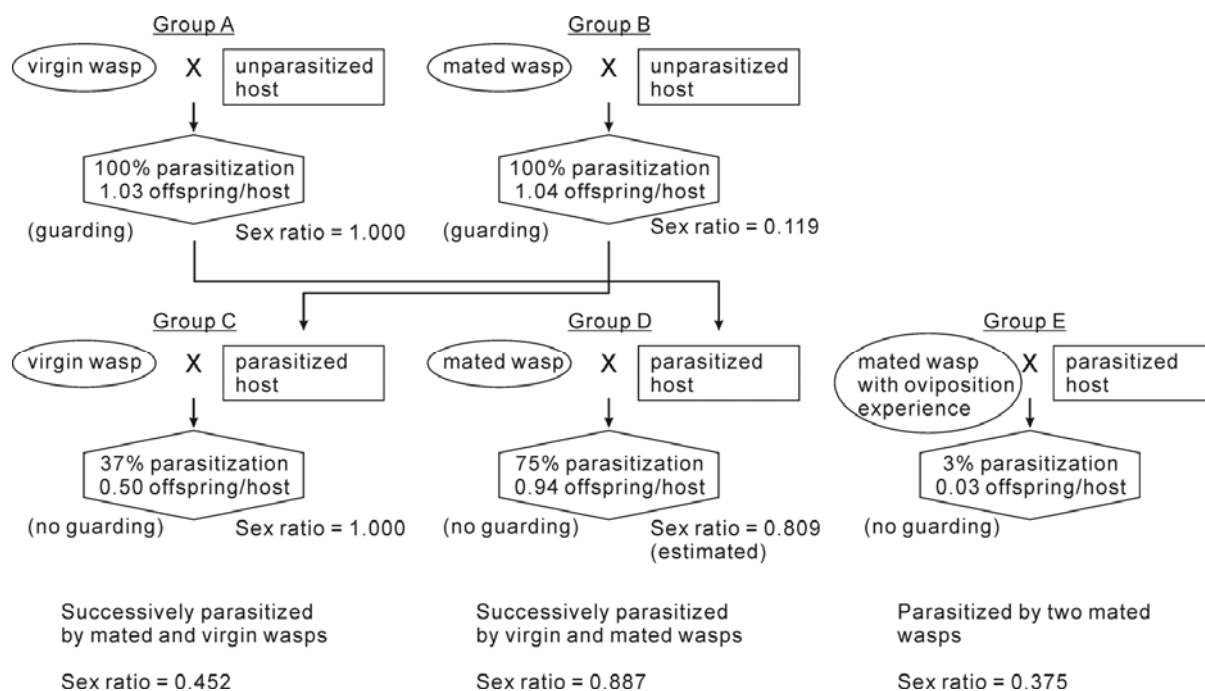


Fig. 3. Experimental procedures and results in parasitism, brood guarding behaviour, and offspring sex ratio in *Trissolcus semistriatus*.