Reproductive capacity and host handling behavior of *Gryon philippinense* (Ashmead) (Hymenoptera: Scelionidae), a solitary egg parasitoid of the winter cherry bug, *Acanthocoris sordidus* Thunberg (Hemiptera: Coreidae)

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Abstract

The reproductive capacity and host handling behavior of *Gryon philippinense* (Ashmead), an egg parasitoid of *Acanthocoris sordidus* Thunberg, were investigated under laboratory conditions. The reproductive capacity was studied at 25.0±1°C, LD 16:8. The highest daily average progeny production of the mated *G. philippinense* was recorded on the first and second days after emergence from the host eggs. The fecundity of 1-d and 2-d-old females of *G. philippinense* was 48.5% of the total fecundity. The estimated average survival rate of the immature stage of the female *G. philippinense* was 95.0%. The intrinsic rate of natural increase r_m was 0.174. The females of *G. philippinense* mostly deposited the male egg in the first host found. After every successful oviposition, the females of *G. philippinense* marked the parasitized egg.

Key words: *Gryon philippinense*; *Acanthocoris sordidus*; reproductive capacity; fecundity; sex allocation

INTRODUCTION

The winter cherry bug, *Acanthocoris sordidus* Thunberg, is a pest of eggplants and peppers (Solanaceae) and sweet potatoes (Convolvulaceae) in Japan (Yasunaga et al., 1993). Post-hibernated females and nymphs are gregarious on a host plant (Fujisaki, 1980). They can pose an especially serious threat to vegetable crops in greenhouses where the use of pollinators and natural enemies requires restrictions on the use of chemical pesticides (A. O. Dasilao, unpublished) in Kochi Prefecture, Japan. Mineo (1991) showed that a scelionid egg parasitoid *Gryon philippinense* (Ashmead) emerged from the egg masses of *A. sordidus*. There is no information about the biological characteristics of *G. philippinense* in Kochi Prefecture. We found that *G. philippinense* highly parasitized the egg masses of *A. sordidus* in 2000 and concluded that this species is a major parasitoid of *A. sordidus*. Knowledge of the biological characteristics is of great importance for understanding the effectiveness of the parasitoids as population regulators (Yeargan, 1982). In the present study, we investigated biological characteristics such as the reproductive capacity and host handling behavior of

G. philippinense as a candidate for use as a biological control agent for *A. sordidus* in integrated pest management.

MATERIALS AND METHODS

Rearing of host and parasitoid. Adults and nymphs of *A. sordidus* were reared in an incubator at 25.0±1°C, LD 16:8. About five male and five female adults of *A. sordidus* were reared together in a double layer plastic cage. The first layer (8 cm height and 11 cm width) had a small hole (1 cm diameter) for the soaked sweet potato stems, as their food source, to pass through the second layer plastic cage (4.5 cm height and 9.5 cm width) that contained water. The double layer plastic cage had a ventilation hole (2.5 cm diameter) on the lid and was covered with a fine nylon mesh.

The colonies of *G. philippinense* were established from the egg masses of *A. sordidus* collected from June to August in 2000 and 2001 from the grassland at the Monobe Campus of Kochi University located in the center of Kochi Prefecture, Japan. The adults of *G. philippinense* were reared in a petri dish (9.0 cm diameter and 1.5 cm height) inside an incubator at 25.0±1°C, LD 16:8 and

provided with undiluted honey and moistened cotton. The newly emerged males and females were kept together to ensure that females mated. The females of *G. philippinense* were regularly provided with 1-d-old fresh egg masses of *A. sordidus* collected from the leaves of the sweet potatoes mentioned above.

Reproductive capacity and survival rate. To determine the reproductive capacity and survival rate, 1-d-old mated females of *G. philippinense* ($n=16$) were individually kept in a petri dish inside an incubator at $25.0\pm1°C$, LD 16:8 and provided with eggs ($n=50$) of *A. sordidus* in the first seven days. The number of host eggs provided was reduced to 20 from day 8 to day 14, 10 from day 15 to day 21 and 5 from day 22 and until the females died. The reduction of eggs per egg mass was based on preliminary investigations that *G. philippinense* females laid a higher number of eggs during the earlier days after emergence from the host eggs, and this was followed by a sharp decline. The leaves that contained the egg mass of *A. sordidus* (average clutch size$\pm$SE: 19.2 ± 12.0) were then cut into the desired sizes. To adjust the desired number of eggs in an egg mass, additional eggs of *A. sordidus* were added to the egg masses that contained fewer eggs. The additional eggs were pasted lightly and tightly arranged with the original laid eggs on the leaves. Leaves with a newly formed egg mass were flatly attached with adhesive tape to a semi-circular filter paper (5.5 cm diameter). To reduce the number of eggs in an egg mass, the excess eggs were removed with tweezers. The supplied eggs were withdrawn after 24 h and incubated at $25.0\pm1°C$, LD 16:8. Hatching of the host nymph and emergence of adult *G. philippinense* was observed daily. The host eggs that remained intact after being kept in the incubator for several weeks after emergence of the parasitoid were dissected to determine the condition of the contents. In this experiment, the total numbers of live immatures that emerged and dead immatures of *G. philippinense* inside the eggs of *A. sordidus* were counted to determine the daily oviposition of the female *G. philippinense*. To determine the value of the intrinsic rate of natural increase (r) of females of the *G. philippinense* a calculation using Lotka's (1925) equation

$$\int e^{-rx}l_x m_x dx = 1$$

was carried out. l_x and m_x indicate the specific survival rate of females and the age-specific female fecundity (Birch, 1948). As an approximation it may be written (Birch, 1948):

$$\sum e^{-rx}l_x m_x = 1.$$

The value of r is the innate capacity for increase in numbers (r_m) (Andrewartha and Birch, 1954).

The total number of host eggs supplied to 16 females of *G. philippinense* and the number of emerged *A. sordidus* nymphs were recorded. The survival rate of the immature stage of *G. philippinense* was calculated by dividing the number of emerged *G. philippinense* by the number of parasitized host eggs (the numbers of emerged *A. sordidus* nymphs and dead host eggs were subtracted from the total number of host eggs supplied to 16 females of *G. philippinense*). Parasitized host eggs included the dead adults of *G. philippinense* found in the host that failed to emerge. The contents of *A. sordidus* eggs that could not be identified were regarded as dead eggs.

The sex of the progeny that emerged from the egg masses was determined and recorded. Males and females are distinguishable by the shape of their antennae. Male antennae are black, shaped like a string, and straight from origin to tip, whereas female antennae are black, but have a bulbous tip. Males and females of *G. philippinense* that failed to emerge and were found in the host eggs were included in the count.

Host handling behavior. To investigate host handling behavior, one 1-d-old mated and unoviposited female of *G. philippinense* was kept in a petri dish (9.0 cm diameter and 1.5 cm height) and provided with an *A. sordidus* egg mass. This method was replicated 16 times. The behavior was closely monitored through a binocular microscope and video equipment until all eggs in the egg mass were parasitized. Monitoring of the behavior of female *G. philippinense* when accepting parasitized or unparasitized eggs was conducted at the same time during the investigation of host handling behavior. The duration of inserting the ovipositor, the duration of marking, the duration of drumming and male egg allocation in relationship to the order of

ovipositor insertion by each female of *G. philippinense* were determined with a timer. There were variations in the number of eggs (6–20) in every mass so that the host egg density was not adjusted. Each mated female of *G. philippinense* was then separately provided with different egg masses for oviposition. If the first mated female of *G. philippinense* failed to oviposit within 20 min after the egg mass had been discovered, another mated female was introduced and the first female was taken out. In this study, the number of insertions of the ovipositor by the females of *G. philippinense* in the eggs of *A. sordidus* was investigated. The sequence and time of host handling and host acceptance behavior of each of the females was recorded. Each egg was coded and marked with its respective number in a separate data sheet to identify the order of oviposition. All supplied eggs were withdrawn and kept in a petri dish at 25.0±1°C, LD 16:8. A day before the expected date of emergence of the *G. philippinense*, the supplied eggs were focused on under a dissecting microscope and monitored with a video camera until complete emergence had occurred. The sex of the progeny that emerged from the egg masses in relationship to the order of emergence and ovipositor-insertion was determined and recorded.

RESULTS AND DISCUSSION

Reproductive capacity and survival rate

Figure 1 shows the daily oviposition curve of *G. philippinense* females. The higher daily average progeny production of the mated females of *G. philippinense* was during the first and second days, and was followed by a sharp decline. A similar situation to the scelionid egg parasitoid was reported by James (1988); the bulk of offspring were produced during the first few days of adult life of *Trissolcus biproruli* Girault, a parasitoid of *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae). Hokyo et al. (1966a) also made similar observations in which there was a decrease in the oviposition rate after the second day for both *Telenomus chloropus* Thompson (=*Te. nakagawai* Watanabe) and *Tr. mitsukurii* (Ashmead) (=*Asolcus mitsukurii* Ashmead) in the egg of *Nezara viridula* L. (Hemiptera: Pentatomidae). They observed that *Te. chloropus* and *Tr. mitsukurii* laid small numbers of eggs continually until the sixth and eighth day, respectively,
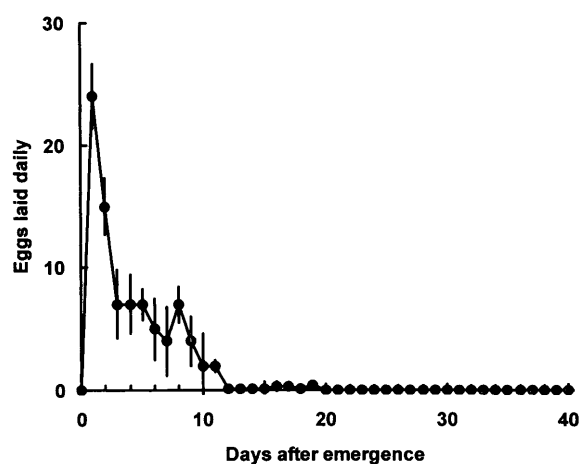

Fig. 1. Daily oviposition of *G. philippinense*.

and lived a short period after the end of oviposition. In this study, 16 *G. philippinense* females lived an average±SE of 21.3±8.0 days when supplied with a streak of honey and moistened cotton. The shortest life span of the females of *G. philippinense* sampled in the study was 11 ($n=1$) days whereas the longest life span was 41 ($n=1$) days, but oviposition stopped on the 20th day. As the ovaries of live and dead females were not dissected after they terminated oviposition in the *A. sordidus* eggs, the reason for the exceptionally long life exhibited by *G. philippinense* females after oviposition in the *A. sordidus* eggs had stopped remains unknown.

One thousand forty eight live and 53 dead immature females resulted from the daily oviposition of the 16 *G. philippinense* females in *A. sordidus* eggs ($n=8,580$). The results show that emergence of *A. sordidus* ($n=7,388$) nymphs from non-parasitized host eggs was 98.8%. The contents of the remaining *A. sordidus* eggs ($n=91$) supplied to 16 *G. philippinense* females could not be identified. The estimated average survival rate of the immature stage of the female *G. philippinense* was 95.0%. The high survival rate may indicate that *G. philippinense* reproduced successfully in host eggs and developed to adult *G. philippinense*.

The total fecundity of the mated females of *G. philippinense* ranged from 65 eggs for an 11-d live female to 122 eggs for a 41-d live female (ave± SE: 82.3±14.7). The fecundity of 1-d (ave±SE: 24.0±5.5) and 2-d-old (ave±SE: 15.9±2.5) females was 48.5% of the total fecundity of *G. philippinense* females. The intrinsic rate of natural

increase r_m was 0.174. In this study, the intrinsic rate of natural increase of *A. sordidus* was not investigated. To verify the r_m values of *G. philippinense* obtained from this study, further studies are necessary to demonstrate whether *G. philippinense* is superior to the host.

Host handling behavior and host discrimination

Two behavioral sequences were observed in females of *G. philippinense* during oviposition: (i) drumming on the eggs with the antennae, insertion of the ovipositor, pulling out the ovipositor and marking the host egg with the ovipositor; and (ii) drumming on the eggs with the antennae, the insertion of the ovipositor, pulling out the ovipositor without marking. In the second case most individuals showed repeated drumming, repeated leaving, and preening and resting near the host eggs.

Figure 2 shows the duration of drumming exhibited by 16 females of *G. philippinense* in egg masses of *A. sordidus*. The duration of drumming increased when almost all eggs (16th–19th) in the egg mass were parasitized. Higuchi and Suzuki (1996) reported a similar situation regarding the increased duration of drumming of *Te. triptus* Nixon in the egg mass of the stinkbug, *Piezodorus hybneri* Gmelin (Hemiptera: Pentatomidae). The 16 females of *G. philippinense* showed an average±SE duration of drumming for 90.1±84.8 s. The continued antennal contact exhibited by 16 females of *G. philippinense* on the eggs of *A. sordidus* during drumming behavior, showed that *G. philippinense* assessed the conditions of the host eggs before ovipositor insertion. In this study, all 16 *G. philippinense* females showed avoidance of ovipositor insertion in already parasitized *A. sordidus* eggs after drumming behavior was exhibited. Strand and Vinson (1983) described the drumming behavior of *Te. heliothidis* Ashmead in the host eggs of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) as an important phase to assess host acceptability. Assessment of host acceptability at this level of foraging is much more time efficient than assessing acceptability after drilling.

Figure 3 shows the duration of ovipositor insertion of 16 females of *G. philippinense* in egg masses of *A. sordidus*. The duration of ovipositor insertion did not change from the 1st to the 15th oviposition bout however it increased in the remaining unparasitized eggs. The reason for the in-
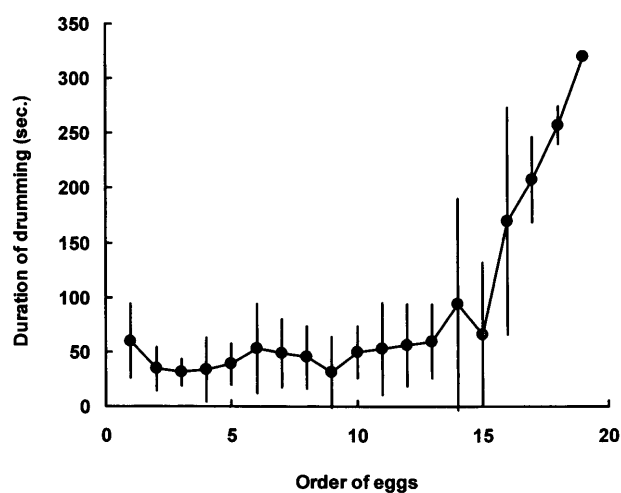

Fig. 2. Average duration of drumming exhibited by females of *G. philippinense* in the egg masses ($n=16$) of *A. sordidus*. Bar indicates SE.
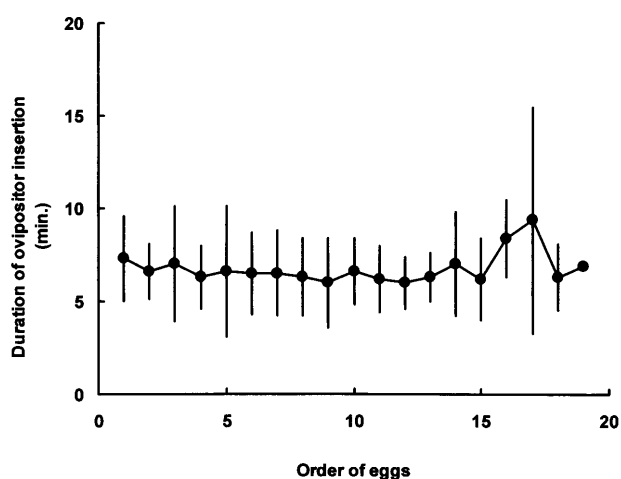

Fig. 3. Duration of ovipositor insertion of females of *G. philippinense* in the different egg masses ($n=16$) of *A. sordidus*. Bar indicates SE.

crease in duration of ovipositor insertion by females of *G. philippinense* in the remaining unparasitized eggs is not known. The duration of oviposition ranged from 4 min to 10 min with an average± SE of 7.0±0.8 min.

Figure 4 shows the duration of marking exhibited by the 16 females of *G. philippinense* in egg masses of *A. sordidus*. The duration (mean±SE) of marking ranged from 8 to 30 s with an average±SE of 17.1±1.6 s. The marking behavior exhibited by the females of *G. philippinense* revealed the scraping on the surface of the parasitized egg with the tip of the ovipositor. The females of *G. philippinense* exhibited an initial backward movement to
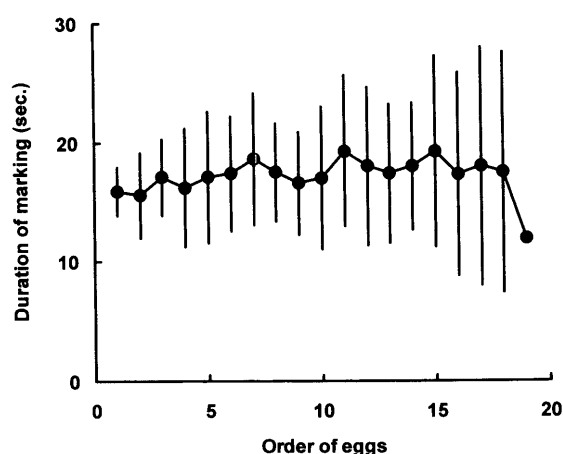
Fig. 4. Duration of marking of females of *G. philippinense* on the egg masses (*n*=16) of *A. sordidus* in relation to order of eggs oviposited. Bar indicates SE.

the opposite side in either a clockwise or in a counter clockwise direction to the top of the eggs of *A. sordidus* for the final marking. Observations from a recorded video showed that 152 eggs of *A. sordidus* were marked by *G. philippinense* and these eggs had successfully developed to adult *G. philippinense*. In three cases by different *G. philippinense* females, the marking behavior was not shown after the ovipositor insertion behavior, however the reason for this behavior was not known. At least these females always returned persistently with an average±SE of 18.3±5.8 s to the same host egg and exhibited ovipositor insertion and marking behavior. The behavior of leaving the host egg without marking suggested an unsuccessful oviposition trial (Higuchi and Suzuki, 1996), as exhibited by *Te. triptus* in the host eggs of *P. hybneri*. The marking behavior was always exhibited by the females of *G. philippinense* after every successful oviposition in the eggs of *A. sordidus*. Egg marking thus serves as a guide for *G. philippinense* females to identify *A. sordidus* eggs that have been self-marked. Higuchi and Suzuki (1996) also reported a similar situation regarding the host marking of *Te. triptus* in the host eggs of *P. hybneri* after every successful attack. Similarly, Rabb and Bradley (1970) described host marking of *Te. sphingis* as a significant marker in which a certain egg has been parasitized. Marking virtually prevents self-superparasitism by the same female in scelionids (Hokyo et al., 1966b; Rabb and Bradley, 1970). Ovipositing females generally mark the host after probing it, leaving a chemical substance on the sur-

face. The female parasitoid marks host eggs following oviposition in order to increase fitness of the female parasitoid (Okuda and Yeargan, 1988). The substance applied to the host egg during marking possesses the characteristics of a pheromone that serves as a chemical messenger (Rabb and Bradley, 1970). Observations from recorded video showed that no *G. philippinense* female showed self-superparasitism. As these females were taken out after all eggs in the egg mass were parasitized, the shortened time exposure of *G. philippinense* females in the egg masses of *A. sordidus* may have caused the avoidance of ovipositor insertion by *G. philippinense* in marked eggs of *A. sordidus*. Although the response was typically avoidance, had we exposed egg masses of *A. sordidus* to a single female of *G. philippinense* for longer periods of time, *G. philippinense* might have oviposited in a parasitized host. This may be adaptive when the likelihood of finding unparasitized hosts is rare (Charnov and Skinner, 1985). Preliminary investigation showed that the presence of more than one female of *G. philippinense* with *A. sordidus* eggs did not elicit any aggressive or territorial behavior. Conspecific superparasitism among solitary parasitoids occurs when different females of the same species each lay one (or more) eggs in the same host (Weber et al., 1996). However, conspecific superparasitism was not investigated in this study. Field studies on ovipositional behavior by *G. philippinense* in the egg mass of *A. sordidus* are also necessary to gain information on the issue of host discrimination by *G. philippinense*.

Figure 5 shows the proportion of male progeny of *G. philippinense* developing in the egg masses of *A. sordidus* in order of the attacked host egg. The male proportion in the first attacked host was 93.7% (*n*=15) whereas in the second host it was 6.3%. The females of *G. philippinense* mostly placed their first male eggs in the first host egg found. Three females deposited the second male egg in the seventh host egg found and one female deposited the second male in the eighth host egg found. Only one female deposited the third male egg in the sixteenth egg found. The 16 egg masses yielded 21 male adults of *G. philippinense* and 18 of them had emerged earlier than the females (*n*=131). One of the 15 male progenies deposited in the first host egg found emerged fourth (three female progenies emerged earlier) from the same egg
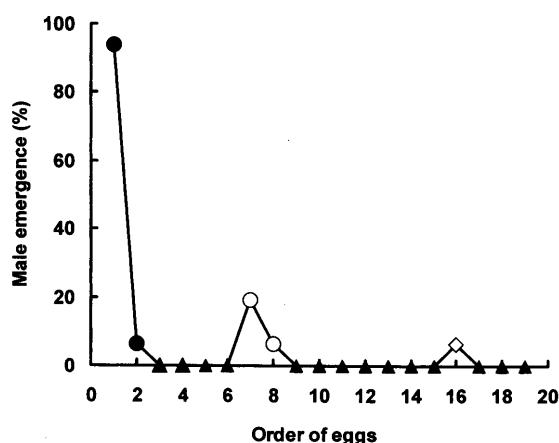
Fig. 5. Proportion of emergence of males of *G. philippinense* in relation to the order of oviposition by female of *G. philippinense* in the egg masses ($n=16$) of *A. sordidus*. Closed circle indicates the first male, open circle indicates the second male and open square indicates third male emergence.

mass. Two other male progenies deposited in the first host egg found failed to emerge. Hokyo and Kiritani (1966) explained the situation in which some solitary scelionid species that attack the egg masses of Heteroptera placed their first male egg in the first host egg found. It was observed that two males of *G. philippinense* emerged as second males in the 7th and 8th egg of a large egg mass of *A. sordidus*. Waage (1982) explained the situation by noting that a second male progeny is sometimes produced towards the end of the oviposition. Suzuki et al. (1984) further explained that if the host is large enough to support the development of more than one offspring, the eggs to be laid may be composed of females and the smallest number of males necessary to inseminate their female siblings. Our study revealed that *G. philippinense* adults emerging from the egg masses were highly female-biased and contained the least number of males to mate with their female siblings. We also observed that males of *G. philippinense* that hatched first always waited on the egg mass of *A. sordidus* for the females of *G. philippinense* to emerge and immediately mated. The earlier emergence of *G. philippinense* males compared to females may be advantageous to ensure that *G. philippinense* females mated in a manner that will maximize production of female progenies, an essential factor for successful parasitism.

There has been no report on the existence of parasitoids of *A. sordidus* other than *G. philippinense*

(Mineo, 1991). A field survey in the central part of Kochi Prefecture showed that *G. philippinense* parasitized *A. sordidus* eggs intensely (A. O. Dasilao, unpublished). We suppose that this species may be a major parasitoid of *A. sordidus*. However, more investigations on the effectiveness of *G. philippinense* in the field must be done. Such studies might provide essential information for the successful control of *A. sordidus* populations. Currently, greenhouse experiments on the dispersal of *G. philippinense* in the *A. sordidus* population are being planned and the results will hopefully provide important information.

ACKNOWLEDGEMENTS

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