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Development and Host-age Preference of *Gryon philippinense* (Hymenoptera: Scelionidae), a Solitary Egg Parasitoid of the Winter Cherry Bug *Acanthocoris sordidus* (Hemiptera: Coreidae)

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Abstract: The scelionid parasitoid *Gryon philippinense* (Ashmead) is a solitary egg parasitoid of the winter cherry bug, *Acanthocoris sordidus* Thunberg. The thermal effect on the development of *G. philippinense* and *A. sordidus* eggs and the host-age preference of *G. philippinense* were investigated. *G. philippinense* and *A. sordidus* eggs were reared under conditions of 15.0, 20.0, 22.5, 25.0, 27.5 and 30.0°C with LD 16:8. The development of *G. philippinense* and *A. sordidus* eggs was inhibited at 15.0 and 17.5°C, respectively. As the temperature increased from 20.0°C, the emergence rate of *G. philippinense* increased. The developmental period of *G. philippinense* and of *A. sordidus* eggs decreased with the increase of temperature. Both the lower threshold temperature and the effective cumulative temperature of *G. philippinense* were higher than those of *A. sordidus* eggs. Suitable rearing temperature for *G. philippinense* is supposed to be between 22.5 and 30.0°C. When 0-14-day-old host eggs were supplied to *G. philippinense*, hosts 0-2-day-old were all accepted. The emergence rate was higher in younger host eggs than in older ones. The developmental period of *G. philippinense* varied among host eggs of different ages. Host-age had no obvious effect on the body size of the *G. philippinense* male. In the *G. philippinense* female, the body size decreased with increasing host-age. The younger host eggs were supposed to be suitable for mass rearing of *G. philippinense*.

Key words: *Gryon philippinense*, *Acanthocoris sordidus*, lower threshold temperature, effective cumulative temperature, host-age preference

INTRODUCTION

The winter cherry bug *Acanthocoris sordidus* Thunberg is one of the pests of Solanaceae plants like peppers and eggplants[1]. *A. sordidus* is a minor pest but can pose a threat because the use of chemical pesticides is restricted in greenhouses where pollinators and natural enemies are used. In Kochi Prefecture, Japan, the overwintered *A. sordidus* adults sometimes invade such greenhouses from fields in early summer and damage peppers and eggplants (Dasilao, unpublished). Hence, an effective method of biological control to suppress *A. sordidus* is desirable.

Mineo[2] found that a scelionid egg parasitoid, *Gryon philippinense* (Ashmead), emerged from the egg of *A. sordidus*. We found that *G. philippinense* might have the capacity to control *A. sordidus* in Kochi Prefecture[3]. To utilize *G. philippinense* in biological control programs, it is first necessary to investigate its biological characteristics. Reproductive capacity and host handling behavior of *G. philippinense* have already been reported[3]. The present study was carried out to investigate the development of *A. sordidus* egg and *G. philippinense* at different temperatures and the host-age preference of *G. philippinense*. Based on the results, the importance of *G. philippinense* as a biological control agent was discussed.

MATERIALS AND METHODS

Host and parasitoid: The rearing methods for hosts and parasitoids in the laboratory were described in Dasilao and Arakawa[3].

Development of host and parasitoid at different temperatures: To investigate the developmental periods of *A. sordidus* eggs, egg masses (less than 1-day-old) consisting of 15 to 20 eggs were put in individual petri dishes (9.0 cm diameter and 1.5 cm height) with moistened cotton. These egg masses were incubated at 15.0, 17.5,

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20.0, 22.5, 25.0, 27.5 and 30.0°C, respectively, with LD 16: 8. The number of hatching nymphs was recorded daily. The eggs that remained intact were dissected to determine their contents. The total hatching rate at each temperature condition was calculated by dividing the total number of hatched nymphs by the total number of eggs in all egg masses.

To investigate the developmental periods of *G. philippinense*, mated females (1-day-old and no previous experience of oviposition) were put in individual petri dishes with a host egg mass (less than 1-day-old) consisting of 15 to 20 eggs. These dishes were kept in the incubator at 25.0±1°C with LD 16:8. The supplied egg masses were withdrawn after 24 h, put into another petri dish with a piece of moistened cotton and incubated at 15.0, 17.5, 20.0, 22.5, 25.0, 27.5 and 30.0°C, respectively, with LD 16: 8. The emergence of adults of *G. philippinense* was monitored daily. The sex of the progeny was also recorded. It is easy to distinguish between male and female of *G. philippinense* from the shape of the antenna[3]. The host eggs that remained intact were dissected to determine the contents. When a dead adult of *G. philippinense* was found in the host egg, it was included in the count as a parasitized host. The total emergence rate of *G. philippinense* at each temperature condition was calculated by dividing the total number of emerged *G. philippinense* by the total number of parasitized eggs in all egg masses.

These experiments for host and parasitoid were replicated 10 times at each temperature condition. To determine the relationship between the temperature and the duration of development of *A. sordidus* eggs and *G. philippinense*, the formula;

$$k = D\,(t - T_0)$$

was used. In the equation, D is the duration of development (days), t is the temperature (°C), T_0 is the lower threshold temperature and k is the effective cumulative temperature.

Host-age preference of *G. philippinense*: To investigate the host-age preference of *G. philippinense*, mated females (1-day-old and no previous experience of oviposition) were put in individual petri dishes with a host egg mass of 0, 1, 2, 4, 6, 8, 10, 12 and 14-day-old, respectively, at 25.0±1°C with LD 16:8. There were variations in the number of eggs (15-25) in the host egg masses and the host egg density in this experiment was not adjusted. To determine the acceptance of host eggs, the ovipositional behavior of *G. philippinense* was closely monitored through video for 24 h. When the parasitoid showed marking behavior on the host egg at the end of a series of ovipositional behaviors[3], the host was considered to have been accepted and oviposition was completed. Sometimes *G. philippinense* showed marking behavior on the host egg after repeatedly drumming or stinging. In this case, the host egg was also regarded as having been accepted. In the case of rejection, *G. philippinense* usually showed only the drumming behavior and soon left the host. Sometimes *G. philippinense* showed stinging behavior on the host egg and soon left without marking. The fate of the host stung by *G. philippinense* was also observed. The supplied egg masses were withdrawn after 24 h and put into another petri dish with a piece of moistened cotton and incubated at 25.0±1°C with LD 16:8. This experiment was replicated 10 times using different females of *G. philippinense* in each host-age category. The parasitoid emergence or host nymph hatching was observed daily. The emergence rate of *G. philippinense* progeny was computed by dividing the number of adults emerged by the total number of accepted host eggs in each host-age category. The stinging rate was computed by dividing the number of stung eggs by the total number of host eggs in each host-age category.

The forewing length (mm) and head width (mm) of all newly emerged males and females of *G. philippinense* from host eggs of different ages were measured under a microscope with a micrometer.

Statistical analysis: T-test was used for comparing the developmental periods of the males and females of *G. philippinense* at different temperature conditions. Bonferroni Multiple Comparison Test was conducted for comparing the effect of host-age in the developmental periods of *G. philippinense*[4]. Proportions were arcsine-transformed prior to analysis. Tukey-type Multiple Comparison Test was conducted for comparing the host acceptance and emergence rates at different temperatures and on host eggs of different ages[5].

RESULTS

Effect of different temperatures on the development of host and parasitoid: Table 1 shows that no nymph of *A. sordidus* hatched at 15.0 or 17.5°C. The dissection of no-hatched eggs also revealed that no development of nymph occurred at 15.0 or 17.5°C. The hatching rate was significantly higher at 25.0 to 30.0°C than that at 20.0 and 22.5°C (Tukey-type multiple comparison test, $p<0.05$). The developmental period of *A. sordidus* eggs decreased with increasing temperature from 20.0 to 30.0°C.

Table 1: Hatching rate (%) and Mean±SE of developmental period of *Acanthocoris sordidus* eggs at different temperatures with LD 16:8

Temperature (°C)	No. eggs	Hatching rate (%)	Developmental period (days)
15.0	184	0	-
17.5	168	0	-
20.0	207	37.2a	27.5±0.6
22.5	169	91.7b	19.0±0.2
25.0	156	98.7c	15.6±0.4
27.5	164	98.8c	11.9±0.5
30.0	212	99.1c	11.4±0.5

Percentages followed by the same letter within a column were not significantly different by Tukey-type Multiple Comparison Test (p>0.05).

Table 2: Emergence rate (%) and Mean±SE of developmental period of males and females of *Gryon philippinense* emerged from *Acanthocoris sordidus* eggs at different temperatures with LD 16:8

			Male		Female	
Temperature (°C)	No. eggs parasitized	Emergence rate (%)	n	Developmental period (days)	n	Developmental period (days)
15.0	165	0	-	-	-	-
17.5	144	0	-	-	-	-
20.0	140	50.0a	14	48.9±1.1a	56	49.6±1.8b
22.5	186	91.9b	21	25.4±4.2a	150	25.4±5.1a
25.0	163	98.8c	18	18.1±0.1a	143	18.4±0.1b
27.5	233	97.9c	25	16.2±0.3a	203	16.3±0.1a
30.0	244	98.8c	29	14.3±1.7a	212	14.7±1.5b

Percentages followed by the same letter within a column were not significantly different by Tukey-type Multiple Comparison Test (p>0.05)
Means followed by the same letter within a row were not significantly different by T-test (p>0.05)

Table 3: Lower-threshold temperature T_0 and effective cumulative temperature *k* of eggs of *Acanthocoris sordidus* from oviposition to hatching and of males and females of *Gryon philippinense* from oviposition to adult emergence

Species	T_0 (°C)	k (degree-days)
A. sordidus eggs	12.3	194.2
G. philippinense males	13.7	230.8
females	13.6	236.8

Table 4: Final ovipositional behavior pattern of *Gryon philippinense* in host eggs of different ages

		Final ovipositional behavior pattern	
Host-age (days)	No. host eggs	Stinging (%)	Marking (%)
0	212	0	100a
1	163	0	100a
2	246	0	100a
4	248	0	96.0b
6	174	0	69.5c
8	173	1.1a	24.9d
10	130	14.6b	13.1d
12	192	2.6a	41.7d
14	171	14.0b	4.7e

Percentages followed by the same letter within a column were not significantly different by Tukey-type Multiple Comparison Test (p>0.05)

No males or females of *G. philippinense* emerged at 15.0°C (Table 2). The dissection of these host eggs revealed that no development of *G. philippinense* occurred. At 17.5°C, almost no host eggs revealed development of *G. philippinense* and a total of 21 adults of *G. philippinense* were dead in the host; only their heads were shown at the opening of the chorion. As the temperature increased from 20.0°C, the emergence rate increased. The emergence rate was highest at 25.0 and 30.0°C (98.8%) but did not significantly differ from the rate at 27.5°C (Table 2). The developmental period of males and females of *G. philippinense* decreased with increasing temperature. The male progenies of *G. philippinense* emerged earlier than the female progenies at 20.0, 25.0 and 30.0°C (T-test, p<0.05).

It was shown (Table 3) that the lower threshold temperature and the effective cumulative temperature of *G. philippinense* were higher than those of *A. sordidus* eggs, respectively.

Host-age preference of *G. philippinense*: Table 4 shows that hosts 0-2-day-old were all accepted by *G. philippinense*. In this case, all females of *G. philippinense* immediately inserted the ovipositor after drumming. The acceptance rate in 4-14-day-old host eggs ranged from 4.7 to 96.0%. In this case, females of *G. philippinense* often exhibited repeated leaving, drumming, and probing. The rate of being only stung by *G. philippinense* ranged from 1.1 to 14.0% in 8-14-day-old host eggs. No nymph of *A. sordidus* was hatched from a stung host egg.

In 0-2-day-old host eggs, the emergence rates were significantly higher than those of the other aged host eggs (Table 5). The failed emergence rate of *G. philippinense* ranged from 1.2 to 37.5% and was especially high in 14-day-old host eggs.

The males and females emerged from 1-14-day-old host eggs developed significantly earlier than those emerged from 0-day-old host eggs (Table 6). The percentage of female progenies of *G. philippinense* ranged from 82.4 to 91.7% and there was no significant difference in the female sex ratio among host eggs 0-12-day-old. The sex ratio of females emerged from 14-day-old

Table 5: Emergence and failed emergence rates of *Gryon philippinense* from accepted host eggs of different ages

Host-age (days)	No. accepted host eggs	Emergence (%)	Failed emergence (%)
0	212	95.3a	1.4
1	163	98.8a	1.2
2	246	97.2a	1.2
4	238	87.8c	3.4
6	121	91.7b	1.7
8	43	83.7d	0.0
10	17	70.6e	5.9
12	80	92.5b	3.8
14	8	62.5f	37.5

Percentages followed by the same letter within a column were not significantly different by Tukey-type Multiple Comparison Test ($p>0.5$)

Table 6: Mean±SE of developmental period of males and females of *Gryon philippinense* and the percentage of females emerged from *Acanthocoris sordidus* eggs of different ages

	Male		Female		
Host-age (days)	No. individuals	Developmental period (days)	No. individuals	Developmental period (days)	% females
0	24	23.1±1.9a	178	23.5±1.5a	88.1a
1	18	18.1±0.1c	143	18.4±0.1c	88.8a
2	23	18.6±1.9c	216	18.6±1.6c	90.4a
4	23	19.2±3.0c	186	18.8±1.1c	89.0a
6	16	22.3±0.5b	95	22.4±0.5b	85.6a
8	6	19.3±0.3c	30	19.9±0.6c	83.3a
10	1	21.0	11	21.0±0c	91.7a
12	13	20.2±1.6c	61	20.5±1.8c	82.4a
14	2	22. 0±0c	3	22.0±0c	60.0b

Means followed by the same letter within a column were not significantly different by Bonferroni Multiple Comparison Test ($p>0.05$)
Percentages followed by the same letter within a column were not significantly different by Tukey–type Multiple Comparison Test ($p>0.05$)

Table 7: Mean±SE of forewing length and head width of males and females of *Gryon philippinense* emerged from *Acanthocoris sordidus* eggs of different ages

	Male			Female		
Host-age (days)	No. individuals	Forewing length (mm)	Head width (mm)	No. individuals	Forewing length (mm)	Head width (mm)
0	24	1.394±0.003a	0.551±0.001a	178	1.427±0.002a	0.575±0.003a
1	18	1.383±0.007a	0.546±0.002a	143	1.412±0.002b	0.573±0.001b
2	23	1.381±0.004a	0.537±0.001b	216	1.411±0.001b	0.572±0.002b
4	23	1.379±0.005a	0.529±0.003c	186	1.392±0.002d	0.562±0.001c
6	16	1.383±0.004a	0.536±0.004b	95	1.402±0.002c	0.558±0.002c
8	6	1.383±0.006a	0.542±0.004a	30	1.391±0.003d	0.553±0.003c
10	1	1.414	0.550	11	1.365±0.011d	0.545±0.006c
12	13	1.379±0.002a	0.539±0.003a	61	1.379±0.004d	0.553±0.001c
14	2	1.349±0.015a	0.509±0.004d	3	1.254±0.023e	0.550±0c

Means followed by the same letter within a column were not significantly different by Bonferroni Multiple Comparison Test ($p>0.05$)

host eggs was significantly low, but the number of adults emerged was also very low.

In forewing length of the male, there was no significant difference among the host ages (Table 7). Head width of the male varied as the host eggs aged. In females, the forewing length and head width decreased as the host eggs aged and in 0-day-host eggs the forewing length and head width were significantly larger (Table 7).

DISCUSSION

In this study, the calculated lower threshold temperature of *A. sordidus* eggs and *G. philippinense* was below 15.0°C (Table 3). On the other hand, the development of *A. sordidus* eggs and *G. philippinense* was inhibited at 15.0 and 17.5°C (Table 1 and 2). These results suggest that the theoretically calculated value of the lower threshold temperature does not always reflect the biological facts. The emergence rate of *G. philippinense* increased with the increase of temperature from 20.0°C (Table 2), as did that of *A. sordidus* eggs (Table 1).

The developmental period of males and females of *G. philippinense* in *A. sordidus* eggs decreased with the increase of temperature (Table 2). The effective cumulative temperature of *G. philippinense* was higher than that of *A. sordidus* eggs (Table 3), which was revealed by the developmental period of *G. philippinense* being longer than that of *A. sordidus* eggs at the same rearing temperatures (Table 1 and 2). Romeis *et al.*[6] made a similar observation that the developmental period of the parasitoid *G. clavigrallae* Mineo was longer than that of its *Clavigralla gibbosa* (Westwood) (Hemiptera: Coreidae) host eggs.

At 25.0°C, *A. sordidus* eggs hatched about 15.6 days after oviposition (Table 1). Thus, the investigation of the host-age preference of *G. philippinense* was restricted to host ages between 0 and 14-day-old. A higher acceptance rate of *G. philippinense* was shown in younger hosts (Table 4). This result was consistent with several studies that provided evidence of the rejection of older host eggs by egg parasitoids[7-11]. In the case of rejection, *G. philippinense* often showed repeated stinging behavior to the host. Leibee *et al.*[9], Strand and Vinson[12] explained that the change in shape and hardening of the chorion as eggs aged became less acceptable for oviposition by the parasitoids. *G. philippinense* might have difficulty ovipositing on host eggs with increasing age. Change in the host chorion was not investigated.

The emergence rate of *G. philippinense* was higher in 0-2-day-old hosts than in older hosts (Table 5). The lower emergence rate of *G. philippinense* in 14-day-old hosts suggests that the oviposition of *G. philippinense* in older hosts has a slim chance of resulting in emergence. On the other hand, hosts stung by *G. philippinense* died before hatching. This result was similarly cited in Taylor[8], who reported that older host eggs of *Acanthomia tomentosicollis* Stål (Hemiptera: Coreidae) with developing nymphs were not parasitized by *G. gnidus* Nixon (Hymenoptera: Scelionidae).

The developmental periods of *G. philippinense* did not significantly differ in 1-14-day-old hosts at 25.0°C except in 6-day-old host eggs (Table 6). The reason for the exceptionally long developmental period in 6-day-old host eggs is unknown. This result was in contrast with finding in a study by Hirose *et al.*[13] that the developmental period of *G. obesum* became longer in older host eggs of *Euschistus conspersus* Uhler (Heteroptera: Pentatomidae). The developmental period of *G. philippinense* in 0-day-old eggs was significantly longest (Table 6). This long developmental period was probably attributable to the immature condition of the host egg causing a delay in the development of *G. philippinense*.

Host age had no obvious effect on the body size of male *G. philippinense*. On the other hand, the forewing length and the head width of females were significantly larger in 0-day-old host eggs (Table 7). This result suggests that males of *G. philippinense* may not need the same degree of nutrients for body size as females do. Body size may affect the fecundity, longevity and flight activity of a parasitoid[14-17]. In *G. philippinense*, no investigation of the effect of body size on fecundity and flight capacity has yet been tested.

The mass culture method is necessary in considering the effects of temperature on the development of *G. philippinense* in *A. sordidus* eggs. From these experiments it was supposed that the rearing of *G. philippinense* in 0-2-day-old host eggs at 25.0°C might be the optimum condition when there was a sufficient supply of host materials to maintain the stock culture. On the other hand, when newly emerged parasitoids are urgently needed, rearing at 27.5 to 30.0°C might be more useful. For successful biological control of *A. sordidus*, knowledge of other biological characteristics such as the fecundity and longevity of *G. philippinense* from host eggs of different ages and the development of host-storage methods at low temperature condition are necessary.

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