Reproductive Modes and Daily Fecundity of Aenasius bambawalei (Hymenoptera: Encyrtidae), a Parasitoid of Phenacoccus solenopsis (Hemiptera: Pseudococcidae)

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Reproductive modes and daily fecundity of *Aenasius bambawalei* (Hymenoptera: Encyrtidae), a parasitoid of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

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Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae), a solitary parasitoid of the invasive mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), was first described and named by Hayat in India (Hayat 2009). Several investigations had shown that this parasitoid has a high parasitism rates on *P. solenopsis* in India (Sharma 2007; Tanwar et al. 2008; Mohindru et al. 2009). In India, various studies focused on observing the parasitization efficiency of *A. bambawalei* under the laboratory conditions, and natural parasitism of *P. solenopsis* by the parasitoid in the field (Kumar et al. 2009; Jhala et al. 2009; Prasad et al. 2011; Sankar et al. 2011). Subsequently *A. bambawalei* was also discovered in Pakistan (Ashfaq et al. 2010; Bodlah et al. 2010) and China (Chen et al. 2010). Previous laboratory experiments have demonstrated that *A. bambawalei* prefers to parasitize the *P. solenopsis* 3rd instars, and the 3rd instars are also best fit for the parasitoid's development, progeny fitness and favorable sex ratio (Fand et al. 2011; He et al. 2012).

The most typical reproductive mode of parasitoids is haplodiploidy, in which unfertilized eggs develop into males and fertilized eggs into females. However, another reproductive mode in some parasitoid species is thelytoky in which unfertilized eggs can produce female offspring (e.g., Wenseleers & Billen 2000; Giorgini et al. 2010; Rabeling & Kronauer 2013). In our experiment, reproductive modes and daily fecundity of *A. bambawalei* were observed, the results provide useful information for understanding the reproductive behavior and for utilizing the parasitoid.

Phenacoccus solenopsis Tinsley were collected from *Hibiscus rosa-sinensis* L. (Malvales: Malvaceae) plants on the campus of South China Agricultural University (SCAU), Guangzhou, Guangdong Province, China. We fed these specimens on 10 cm-tall seedlings of potato (*Solanum tuberosum* L. (Solanales: Solanaceae) planted in 7.5 cm diam plastic pots. Subsequently, the 1st instars were placed on leaves of the potted *H. rosa-sinensis* plants and raised for several generations. *Aenasius bambawalei* Hayat wasps were also collected from the campus of SCAU. Thus, parasitized mealybug nymphs were collected from *H. rosa-sinensis* plants, and then taken to the laboratory, where they were cultured. Parasitoids that emerged from mummified mealybugs were identified and raised for several generations. Third instar mealybug nymphs and newly emerged adults were used for experiments. The mealybug and parasitoid populations were reared in the laboratory at 27 ± 1 °C and 60 – 70% RH. A 10% solution of honey mixed with purified water was supplied for parasitoid adults.

Each mated *A. bambawalei* female was transferred to a group of 30 third instar mealybugs on fresh 10 cm-tall potted *S. tuberosum* seedlings in a clean transparent cylinder (7.5 × 11 cm) with a 10% honey solution for 24 h. The experiment was conducted at 27 ± 1 °C, 70 ± 5% RH and 12:12 h L:D. After 24 h, the parasitoids were removed while the exposed host nymphs remained on the *S. tuberosum* seedlings. The exposed nymphs were checked daily until adult parasitoids emerged from the mummified mealybugs. Number and sex ratio of the parasitoids were recorded, and hind-tibia lengths of males and females were measured for delimiting body sizes. The experiment was replicated 20 times. A parallel experiment was also conducted with unmated parasitoid females.

Each mated female was then provided with 40 host nymphs on fresh potted seedlings of *S. tuberosum* in a cylinder as described above. These circular containers with host nymphs feeding on fresh potted *S. tuberosum* seedlings were held at 27 ± 1 °C, 70 ± 5% RH and 12:12 h L:D. Each day the number and the survival of the offspring were recorded when parasitoid adults emerged from mummified mealybugs. Females were fed daily with 10% fresh honey solution during the experiment.

Data were checked for normality and homoscedasticity before comparison analysis, analyzed by one-way ANOVA and multiple comparisons of means were conducted by the least significant difference (LSD) test (SAS Institute 2004).

We found that mated parasitoid females could oviposit fertilized eggs within 24 h. Their offspring included males and females, and the female fraction of the progeny adults was 0.9. Unmated parasitoid females could oviposit unfertilized eggs within 24 h, but all their offspring was males. The mean generation (*T*) times of female and male progeny of mated parasitoid females were 14.4 days and 13.4 days, respectively. In contrast the mean generation (*T*) time of male progeny of unmated parasitoid females was 16.4 days, which was significantly longer than the mean generation time of male progeny of mated parasitoid females ($F_{1,38}$ = 207.05, $P < 0.0001$) (Table 1). In addition, the parasitism rate (Table 1; $F_{1,38}$ = 25.30, $P < 0.0001$) and the hind-tibia lengths of male progeny (Table 1; $F_{1,38}$ = 41.15, $P < 0.0001$) of the mated females were both significantly higher than the corresponding values pertaining to unmated females. This implies that copulation can be beneficial for the fitness of the parasitoid females. In many parasitoid species the daily production of progeny (fecundity) fluctuates and shows multiple peaks during female lifespans, and females of these parasitoid species can repeatedly mate (e.g., Muegge & Lambdin 1989; Baezalarios et al. 2002; Karamaouna & Copland 2009; Zipporah et al. 2013). We found that *A. bambawalei* adult females could survive 77 days. The oviposition peak occurred on the 2nd day, and then daily fecundity decreased sharply

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Table 1. Developmental parameters of *Aenasius bambawalei* progeny that were the result of sexual (haplodiploidy) vs asexual (arrhenotoky) reproduction.

Reproductive mode	Percent Parasitism (%)	Generation period of female (d)	Generation period of male (d)	Female ratio	Hind-tibia length (10^{-2}mm)	
					Male	Female
Gamogenesis	41.0 ± 0.3 a	14.4 ± 0.1	13.4 ± 1.0 a	0.9 ± 0.7	41.0 ± 0.4 a	53.3 ± 0.8
Parthenogenesis	29.5 ± 0.7 b	—	16.4 ± 0.8 b	0	36.2 ± 0.2 b	—

Means (± SE) within the same column followed by the different letters are statistically different at $P < 0.05$ level according to ANOVA and LSD test.

when females had mated only once (Fig. 1). The female ovipositional period was 21 days. This implies that *A. bambawalei* females may allocate more energy resources to survival than to reproduction during the mid and late ovipositional periods if they mated once. Previous studies showed that increasing mating frequency increases female fitness parameters (Arnqvist & Nilsson 2000; Fox & Rauter 2003; Avila et al. 2011) such as longevity and fecundity (Savalli & Fox 1999). Would the daily production of progeny by *A. bambawalei* females display multiple peaks if mate multiple times? Future work is needed to answer this question. So far, regrettably, there is no evidence that *A. bambawalei* females will mate more than once.

Summary

Reproductive modes and daily fecundity of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), a parasitoid of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), were elucidated in this experiment. *Aenasius bambawale* reproduced mainly by gamogenesis and occasionally by arrhenotokous parthenogenesis. *Aenasius bambawalei* females allocated far more energy sources to their own survival than to reproduction during the mid and late portions of the ovipositional period. Therefore, newly emerged adult parasitoids should be chosen for mass rearing and for use in the biological control of *P. solenopsis*.

Key Words: parastoid; mealybug; gamogenesis; arrhenotoky

Sumario

El modo de reproducción y la fecundidad diaria de *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), un parasitoide de *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), fueron aclarados en este experimento. *Aenasius bambawale* se reproduce principalmente por gamogenesis y de vez en cuando por arrenotoquia, un tipo de partenogénesis. Las hembras de *Aenasius bambawalei* asignan muchas más fuentes de energía para su propia sobrevivencia que a la reproducción durante la parte mediana y final del período de oviposición. Por lo tanto, parasitoides adultos recién emergidos deben ser elegidos para la cría en masa y para su uso en el control biológico de *P. solenopsis*.

Palabras Clave: parasitoide; cochinilla; gamogenesis; arrenotoquia

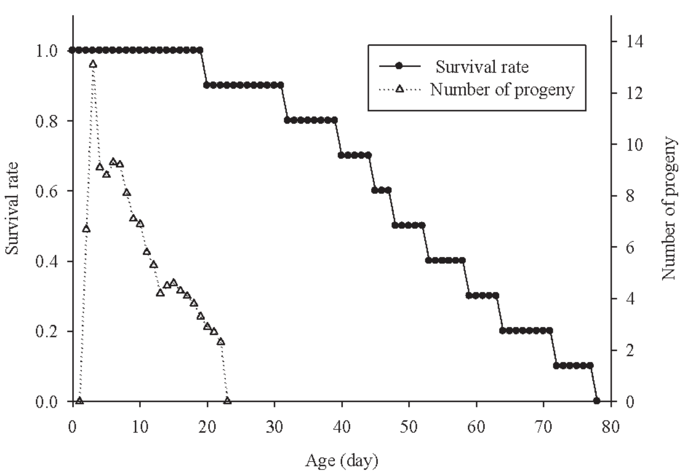

Fig. 1. Daily survival rate and daily fecundity of *Aenasius bambawalei* females.

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