

Reproductive attributes and functional response of *Anastatus japonicus* on eggs of *Antheraea pernyi*, a factitious host

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Abstract

BACKGROUND: *Anastatus japonicus* Ashmead (Hymenoptera: Eupelmidae) is a solitary egg endoparasitoid that has been studied for inundative biological control of *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in China. In this study, we assessed the reproductive attributes and functional response of *Anastatus japonicus* on a factitious host, *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera: Anthelidae) at 25 ± 1 °C, $70 \pm 5\%$ relative humidity and 16 h:8 h light/dark photoperiod.

RESULTS: The mean lifetime fecundity of *Anastatus japonicus* females was 404.3 progeny produced over an average oviposition period of 42.3 days. The sex ratio of adult progeny was slightly male biased (51.2%), whereas more female progeny were produced before day 20 of a female's life. Single 1-day-old mated *Anastatus japonicus* females exhibited a type II functional response to increasing host densities (1–50 eggs), with an inverse host density-dependent pattern of percent parasitism. The upper limit to the daily attack rate was estimated as 7.6 *Antheraea pernyi* eggs. Furthermore, mutual interference among *Anastatus japonicus* females occurred when increasing densities of parasitoids (1, 2, 4, 8, 16) were exposed to 30 host eggs.

CONCLUSION: Laboratory functional response result revealed that individual *Anastatus japonicus* might be unable to respond effectively to increasing host density in the field, which could be compensated by releasing larger numbers of wasps. Strong mutual interference among foraging *Anastatus japonicus* females should be considered in any future inundative biological control programs for the sustainable management of *Halyomorpha halys* or other host insect pests.

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Keywords: inundative biological control; egg parasitoid; mass rearing; fecundity; sex ratio

1 INTRODUCTION

Biological control with parasitoids, particularly hymenopteran species, is used for sustainable management of agricultural insect pests worldwide.^{1,2} Understanding the reproductive attributes that make parasitoids effective pest control agents, as well as the underlying insect host–parasitoid interactions and the parasitoid's behavioral ecology, is crucial for the successful use of parasitoids in biological pest control programs.^{3,4} The reproductive success of an individual parasitoid depends on a combination of host and parasitoid traits, which in principle can be integrated into a host–parasitoid model to describe the temporal and spatial dynamics of host–parasitoid interactions.⁵ Such host–parasitoid models are driven by the type of functional response, which is the number of hosts attacked or prey consumed in relation to host/prey density over a given time interval.⁶ Functional responses are categorized based on the shape of the response curve as type I (a linear increase), type II (a monotonic decelerating increase), type III (a sigmoidal increase), or type IV (dome shaped).^{5,7–10} A functional response model helps to evaluate two vital parameters affecting host–parasitoid population dynamics, namely handling time and attack rate. Most parasitoids exhibit

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type II functional responses, whereas only a few parasitoids show type III, and type I and IV functional responses are rare.⁵

The functional response of a parasitoid is primarily used to describe the effect of host density on parasitism. However, parasitoids seldom forage in nature as single individuals, and thus the functional response should also consider the interaction of conspecific parasitoids competing for host resources.¹⁰ Parasitoids searching together in a limited space for hosts can adversely affect each other's search efficiency due to behavioral interference and pseudo-interference.^{10,11} To describe the mutual interference between searching parasitoids, Hassell and Varley proposed an empirical model,¹² based on which a few further models were later developed.^{3,11,13,14} Although an argument remains over which of the functional responses is better for modeling purposes,¹⁵ it is equally important to study the functional response of parasitoids to both host and conspecific parasitoid density for a thorough understanding of host–parasitoid interactions to allow for the exploration of the biological control potential of specific insect parasitoids.^{10,16}

Anastatus Motschulsky, 1859 (Hymenoptera: Eupelmidae) is one of the most cosmopolitan genera of Eupelmidae, with about 135 described species, most of which are primary endoparasitoids of the eggs of a wide range of insect, including Blattaria, Hemiptera, Lepidoptera, Mantoidea, Neuroptera, Orthoptera, and Phasmida.¹⁷ Several species of *Anastatus* have been considered or used for biological control of hemipteran pests around the world. In southern China, *Anastatus japonicus* Ashmead has been augmentatively released from egg cards to control the litchi stink bug *Tessaratoma papillosa* Drury (Hemiptera: Pentatomidae) with satisfactory control effects since late 1960s¹⁸; although recent taxonomic research has revealed that this *Anastatus* species was misidentified, and actually is *Anastatus fulloi* Sheng and Wang.¹⁹ In northern China, *Anastatus* sp. was mass released against *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in peach orchards and forests with parasitism rates between 48.7 and 77.2%.²⁰ In Australia, *Anastatus* sp. has been mass reared and released against fruitspotting bugs, *Amblypelta nitida* Stål and *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in macadamia orchards.²¹ In Europe, *Anastatus bifasciatus* (Geoffroy) is the most common native parasitoid of the invasive brown marmorated stink bug *Halyomorpha halys* and has been investigated for potential augmentative biological control against this pest.^{22–25} However, the impact of *Anastatus bifasciatus* on *Halyomorpha halys* eggs in field conditions was deemed low,^{23,24} and natural parasitism rates ranged from 3.6% to 76.1% across the surveyed regions in northern Italy and Switzerland.²⁵ Furthermore, field releases of *Anastatus bifasciatus* in apple and pear orchards in Switzerland and Italy, respectively, did not achieve promising results, causing only 2–16% parasitism of sentinel *Halyomorpha halys* eggs.²⁶

Anastatus japonicus is widely distributed throughout the entire Palaearctic region, attacking over 15 host insects in the Hemiptera and Lepidoptera.¹⁹ The parasitoid, misidentified previously as *Anastatus bifasciatus*, is also present in Canada and the United States (Nearctic region). Its presence in North America is believed to have resulted from introductions associated with biological control of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebididae).^{19,27} In northern China, *Anastatus japonicus* is one of the predominant egg parasitoids of *Halyomorpha halys*, ranked second after *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae).^{19,28} As *Anastatus* species can be mass produced on the factitious host *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera: Anthelidae) at low cost,^{18,29} *Anastatus japonicus* has been selected as a potential candidate for inundative biological control of *Halyomorpha halys* in China.³⁰

In the present study, we assessed the reproductive attributes of *Anastatus japonicus* reared on *Antheraea pernyi*, particularly adult longevity and both age specific and lifetime fecundity. We also evaluated the age-specific functional response of individual *Anastatus japonicus* females to varying densities of *Antheraea pernyi*, as well as their response to conspecific females (i.e. mutual interference) in terms of progeny production and progeny sex ratio. The purpose of this study is to provide baseline information required for mass rearing of *Anastatus japonicus* at high efficiency, providing large numbers of wasps for inundative releases against *Halyomorpha halys* or other host insect pests.

2 MATERIALS AND METHODS

2.1 Insect cultures

Anastatus japonicus were originally obtained from parasitized *Halyomorpha halys* eggs collected in a peach orchard in Beijing, China (N 40° 02' 06", E 116° 12' 41"). The parasitoid colony was maintained in transparent acrylic rearing cages (25 cm × 25 cm × 25 cm). Parasitoids were fed honey twice a week and held under laboratory condition of 25 ± 3 °C, 60 ± 5% relative humidity (RH), and 16 h:8 h light/dark photoperiod.³⁰ To maintain the laboratory colony of *Anastatus japonicus*, frozen eggs of *Antheraea pernyi* from the Beneficial Insect Rearing Facility, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences were provided to the parasitoid for continuous rearing.²⁹ Before use in experiments, the *Anastatus japonicus* population had been continuously reared for over ten generations in the laboratory as described earlier. Newly emerged adult parasitoids < 24 h old were collected and used for the experiments. All bioassays were done in an incubator (BluePard Series, Yiheng Technology Company, Shanghai, China) at 25 ± 1 °C, 70 ± 5% RH, and 16 h:8 h light/dark photoperiod. Frozen eggs of *Antheraea pernyi* were stored at –20 °C for less than 6 months before use in the bioassays. Cold storage of *Antheraea pernyi* eggs has been practiced in commercial mass rearing of *Anastatus* to efficiently use host materials and reduce the production costs.¹⁸ Parasitoid specimens were sent to Dr Lingfei Peng (Fujian Agriculture and Forestry University, China) for confirmation of the identification as *Anastatus japonicus*.

2.2 Adult longevity and lifetime fecundity

Newly emerged males and females of *Anastatus japonicus* were paired and maintained for mating (a female wasp can only mate once, Ali MY, unpublished data) in a transparent plastic bottle (8.3 cm diameter, 6.5 cm height) with an open top covered by a nylon gauze (0.125 mm mesh size). A cluster (20 eggs/mass) of *Antheraea pernyi* eggs attached to a 5 cm² piece of paper with clear liquid glue (Pritt Liquid Glue, Henkei Industry and Trade Co., Ltd, Guangdong, China) was introduced into the plastic bottle and exposed to individual 1-day-old mated females of *Anastatus japonicus* for 24 h each day until the adult died. During the experiment, a cotton wick soaked with a 20% honey water solution was provided as a food source for both male and female *Anastatus japonicus*. A total of 21 pairs were tested. After each 24 h exposure interval, the factitious host eggs were replaced, and the exposed eggs were reared in a plastic Petri dish (6 cm diameter, 2 cm depth) for the emergence of adult parasitoids. The developmental time of individual parasitoids from egg to adult was recorded. The numbers and sex of emerged parasitoid progeny were recorded. The age-specific and lifetime fecundity of *Anastatus japonicus* was measured as the average progeny production achieved by individual females at a specific age during the life span. To

determine adult longevity, adult parasitoids were checked daily for dead individuals and removed from the plastic bottles.

2.3 Functional response at constant parasitoid density and varying host densities

To assess the functional response of *Anastatus japonicus* to host density, a randomly chosen host density of between 1 and 50 eggs of *Antheraea pernyi* was exposed to a single 1-day-old mated female *Anastatus japonicus* in a transparent plastic bottle (8.3 cm diameter, 6.5 cm height) as described earlier for 24 h ($n = 65$, total replicates; some of the randomly chosen densities were measured more than once). *Antheraea pernyi* eggs (1–50 eggs/mass) were attached to a piece of paper as described earlier. During the exposure, a cotton wick soaked with 20% honey water solution was provided as food source for the female parasitoids. After 24 h exposure intervals, the factitious host eggs were collected and reared in a plastic Petri dish (6 cm diameter, 2 cm depth) until the emergence of adult parasitoids. The number and sex of emerged parasitoid progeny was recorded for each host density tested. The number of hosts parasitized for each host density was measured as the number of *Antheraea pernyi* eggs with emerged parasitoids.

2.4 Mutual interference at constant host density and varying parasitoid densities

To understand the impact of intraspecific interference among female parasitoids on their progeny production and progeny sex ratio, a constant number of host eggs were exposed to different parasitoid densities. In a transparent plastic bottle (8.3 cm diameter, 6.5 cm height), 30 *Antheraea pernyi* eggs were exposed to one of five densities of 1-day-old mated *Anastatus japonicus* females (1, 2, 4, 8, 16) for 24 h ($n = 11$ for each parasitoid density). A similar protocol as the functional response experiment was used for the preparation of the host eggs (30 eggs/mass) and provisioning with a 20% honey water solution as food. After exposure, the host eggs were removed and reared as described earlier. The numbers and sex of emerged parasitoid progeny were recorded for each parasitoid density (1, 2, 4, 8, and 16 per test bottle). The number of hosts parasitized for each parasitoid density was measured as the number of *Antheraea pernyi* eggs with emerged parasitoids.

2.5 Data analysis

2.5.1 Adult longevity and lifetime fecundity

The adult life span of male and female *Anastatus japonicus* wasps was calculated by days from emergence to death. Kaplan–Meier estimation method was used to obtain survival curves, and significance between male and female survival curves was determined using a log-rank (Mantel–Cox) test. The age-specific fecundity was measured as the number of progeny produced at a specific age, while the lifetime fecundity was measured as the number of progeny produced by mated *Anastatus japonicus* females during their lifespan. The fecundity data and effect of female age on lifetime fecundity were analyzed with generalized linear models (GLMs) using a normal distribution (log link function). The sex ratio (% female) was analyzed with a GLM using a binomial distribution (logit link function). The relationship between lifetime fecundity (dependent variable) and longevity (independent variable) was analyzed with linear regression.

2.5.2 Functional response

Following Juliano,³¹ the shape (type) of functional response was determined from maximum likelihood estimation of the parameters of a cubic logistic regression model of the proportion of

Antheraea pernyi eggs attacked in relation to host egg density. The significance and sign of the coefficient for the linear term of the model were used to determine response type. A non-significant coefficient is indicative of a type I response, and significant negative or positive coefficients are indicative of type II and type III responses, respectively.

Based on this approach, the functional response of *Anastatus japonicus* was identified as type II and the data were fitted to the random parasitoid equation^{32,33}:

$$N_a = N_0 \left[1 - \exp \left\{ - \frac{aTP}{1 + aT_h N_0} \right\} \right] \quad (1)$$

where N_a is the number of *Antheraea pernyi* eggs parasitized during a specific searching time, N_0 is the density of *Antheraea pernyi* eggs, a is the attack rate (the rate at which a parasitoid encounters hosts), P is the parasitoid density, T is the duration of experiment (i.e. 24 h), and T_h is the handling time (in hours) per host. The random parasitoid equation takes into account that parasitoids can re-encounter host eggs that have previously been parasitized during the course of the experiment. Non-linear least squares regression of the number of *Antheraea pernyi* eggs parasitized in relation to the egg density was used to estimate the parameters of this type II functional response model.

The effect of host egg density on the sex ratio (proportion of females) was analyzed with a GLM using a binomial distribution (logit link function).

2.5.3 Mutual interference

To estimate the parameters of the functional response of *Anastatus japonicus* foraging in a group, the following modification of the random parasitoid equation was used to include the potential for interference among foraging parasitoids:

$$a = QP^{-m} \quad (2)$$

where a is the attack rate, P is parasitoid density, Q is the quest constant, and m is the mutual interference constant.¹² Non-linear least squares regression of the number of *Antheraea pernyi* eggs parasitized in relation to parasitoid density was used to estimate the parameters of this modified type II functional response model. This corresponds to method 3 of DeLong and Vasseur³⁴ for estimating mutual interference for a randomly searching parasitoid.

The effect of parasitoid density on sex ratio (proportion of females) was analyzed with a GLM using a binomial distribution (logit link function).

Statistical analyses were conducted using R (version 4.1.0) for the functional response and mutual interference (functions `glm` and `nls`), and using SPSS (version 22.0) for adult longevity and lifetime fecundity (GLM procedure).

3 RESULTS

3.1 Adult longevity and lifetime fecundity

Mean adult longevity of *Anastatus japonicus* males was 5.4 ± 0.2 standard error (SE) days (minimum = 3, maximum = 9, $n = 21$), and females 44.5 ± 3.0 SE days (minimum = 20, maximum = 67, $n = 21$). Significant differences were found between the adult longevity of males and females ($\chi^2 = 50.70$, $df = 1$, $P < 0.0001$). In general, about 50% of the males died after 4 days and 50% of the females died after 45 days.

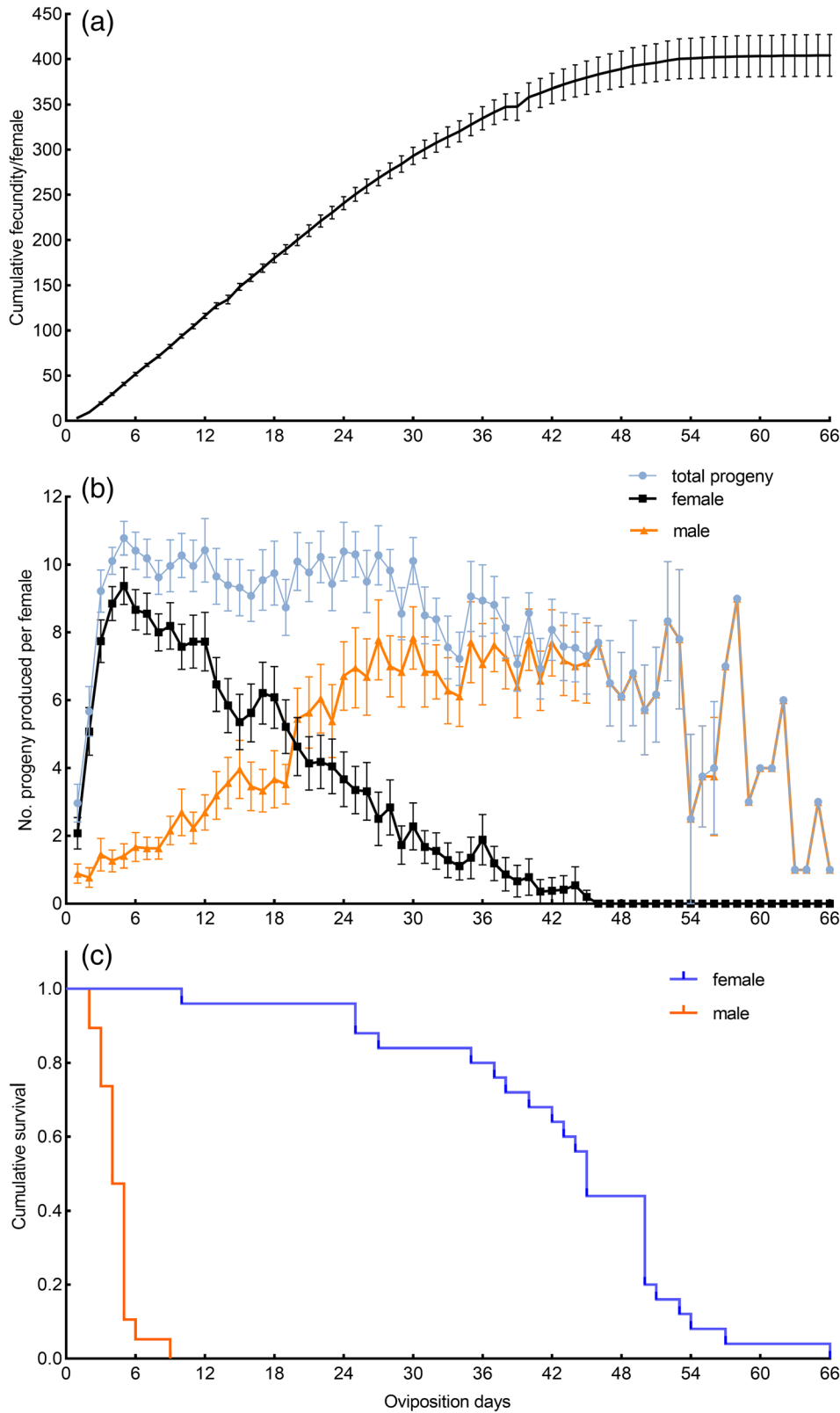


Figure 1. Age-specific reproductive attributes of *Anastatus japonicus*: (a) mean (\pm SE) cumulative fecundity per female, (b) mean (\pm SE) number of female, male, and total progeny produced per female, and (c) cumulative survival of females and males.

The mean number of progeny produced by mated *Anastatus japonicus* females during their lifespan was 404.3 ± 23.2 SE (minimum = 256, maximum = 601, $n = 21$). On average, the total

lifetime fecundity per female plateaued on day 47 at 386.3 ± 19.5 SE progeny, and thereafter there was no significant increase in cumulative fecundity after day 47 ($\chi^2 = 5828.692$,

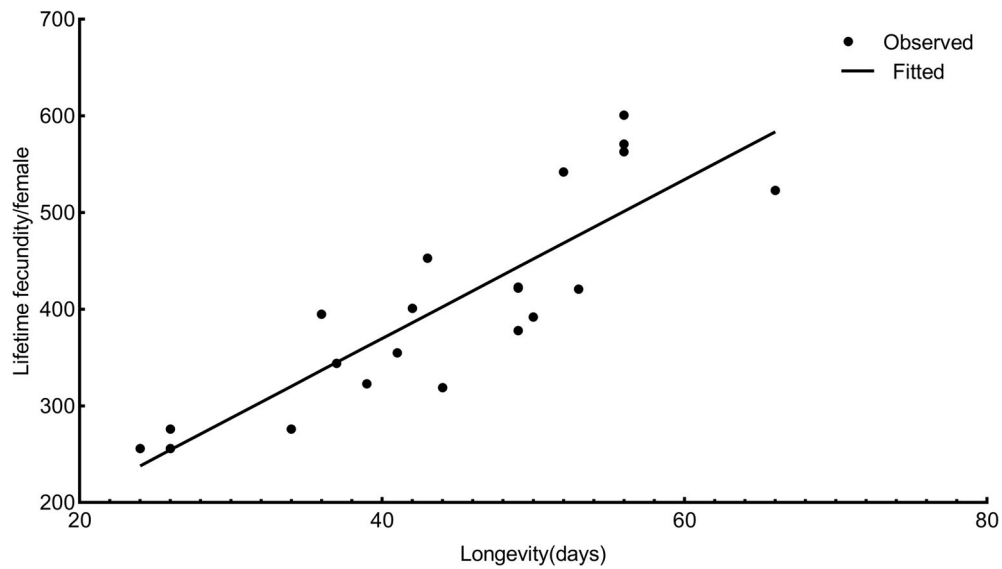


Figure 2. Relationship between the lifetime fecundity per female *Anastatus japonicus* and adult longevity ($y = 8.231x + 40.534$; $R^2 = 0.741$; $F = 54.488$, $df = 1, 19$, $P < 0.001$).

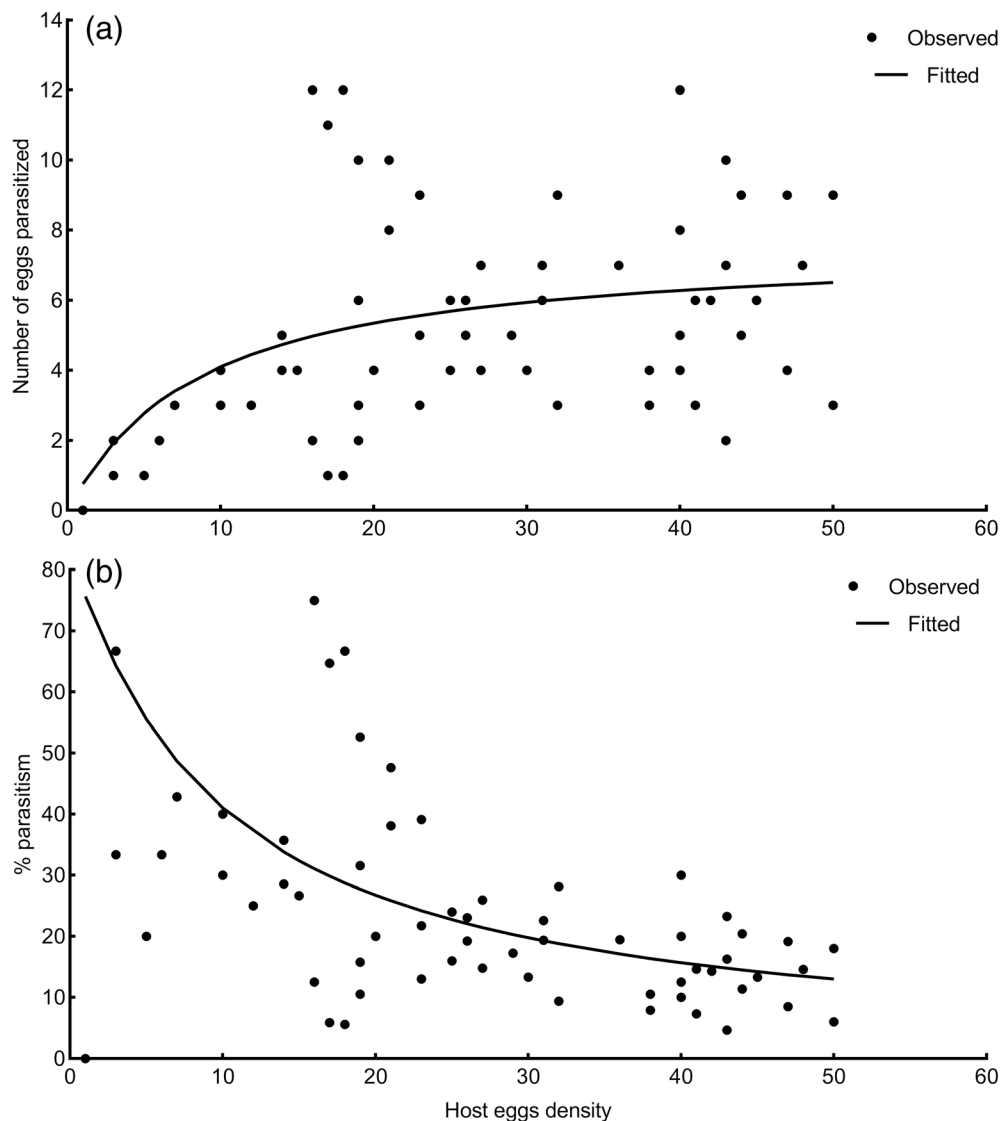


Figure 3. Functional response (a) and parasitism rate (b) of single *Anastatus japonicus* females to varying host densities (between 1 and 50 *Antheraea pernyi* eggs) fitted to the random parasitoid equation with $a = 0.072$ eggs per hour and $T_h = 3.166$ h per host.

df = 65, $P < 0.001$; Fig. 1(a)). The age-specific fecundity per female was significantly affected by the age of the female (GLM, $\chi^2 = 229.9$, df = 65, $P < 0.001$). This parameter first increased steadily with female age before day 6, plateaued until day 30, and then decreased gradually as the age of the female further increased (Fig. 1(b)). The sex ratio of adult progeny was male biased (GLM, $\chi^2 = 50.586$, df = 1, $P < 0.001$); however, the percentage of female progeny still reached $48.8\% \pm 5.0$ SE. More female progeny was produced before day 20, and thereafter more male progeny was produced (Fig. 1(b)).

The oviposition period of *Anastatus japonicus* had a maximum of 66 days and the mean oviposition period per female was 42.3 ± 2.3 SE days ($n = 21$). Over 90% of females were still alive after 27 days when the mean lifetime fecundity was achieved, and 50% of the females were still alive after 44 days during the oviposition period when 93.9% of the lifetime fecundity was achieved (Fig. 1(c)). There was a significant linear relationship between lifetime fecundity and longevity ($F = 54.488$, df = 1, 19, $P < 0.001$) (Fig. 2).

The developmental time of the immature stage from egg to adult of *Anastatus japonicus* was 27.5 ± 0.2 SE days (minimum = 21, maximum = 51, $n = 540$) at 25 °C, 70% RH and 16 h:8 h light/dark photoperiod.

3.2 Functional response at constant parasitoid density and varying host density

A mean number of 5.3 ± 0.4 SE *Antheraea pernyi* eggs were parasitized when *Anastatus japonicus* was exposed to randomly chosen different host densities (between 1 and 50 hosts) with a maximum number of 12 hosts parasitized by a single female. The number of *Antheraea pernyi* eggs parasitized by *Anastatus japonicus* increased with increasing host density (Fig. 3(a)). According to the logistic regression analysis of the proportion of *Antheraea pernyi* eggs parasitized in relation to egg density the estimated coefficient of the linear term was both negative and significantly different from 0 (-3.67 ± 0.76 SE, $n = 65$, $P < 0.001$). This indicated a type II response and that parasitism increased with host density to an upper asymptote representing

the maximum daily *per capita* reproductive capacity of 1-day-old *Anastatus japonicus* females.

The 95% prediction interval of the fitted functional response model was 5.34 parasitized eggs (residual standard error of 2.67, df = 63). Least square estimates for the attack rate (a) and handling time (T_h) of a single *Anastatus japonicus* female were $0.072/h \pm 0.074$ SE and $3.17 \text{ h} \pm 0.55$ SE, respectively. Accordingly, the predicted upper asymptote for the functional response was 7.6 *Antheraea pernyi* eggs parasitized per *Anastatus japonicus* female at higher egg densities (Fig. 3(a)). Consequently, percent parasitism decreased with increasing egg density (Fig. 3(b)).

Furthermore, the progeny sex ratio was independent of egg density (GLM, $\chi^2 = 111.391$, df = 1, $P < 0.001$), with a mean percent female of $87.87\% \pm 2.17$ SE.

3.3 Mutual interference at constant host density and varying parasitoids densities

The average number of *Antheraea pernyi* eggs parasitized (i.e. parasitoid progeny produced) per single *Anastatus japonicus* female decreased from 5.8 ± 0.7 SE to 0.9 ± 0.6 SE when the number of parasitoids per test arena increased from 1 to 16 (GLM, $\chi^2 = 43.739$, df = 4, $P < 0.001$) (Fig. 4). Such reduction in the number of eggs parasitized indicated mutual interference among foraging parasitoids. The 95% prediction interval for the fit of the random parasitoid model with mutual interference was 11.22 parasitized eggs (residual standard error of 5.61, df = 41). The least square estimates of the quest constant (Q), mutual interference constant (m) and handling time (T_h) were 0.21 ± 0.29 SE, 1.66 ± 0.51 SE and $3.09 \text{ h} \pm 0.91$ SE, respectively. The strong mutual interference among 1-day-old *Anastatus japonicus* individuals foraging in groups led to a predicted reduction in the *per capita* number of eggs parasitized from 6.55 for single females to 0.92 for groups of 16 females (Fig. 4). Hence, there were no differences of total progeny production among the parasitoids foraging in groups of 2, 4, 8 and 16, but in all cases the reproductive output was higher than for parasitoids foraging alone (GLM, $\chi^2 = 20.839$, df = 4, $P < 0.001$; Table 1).

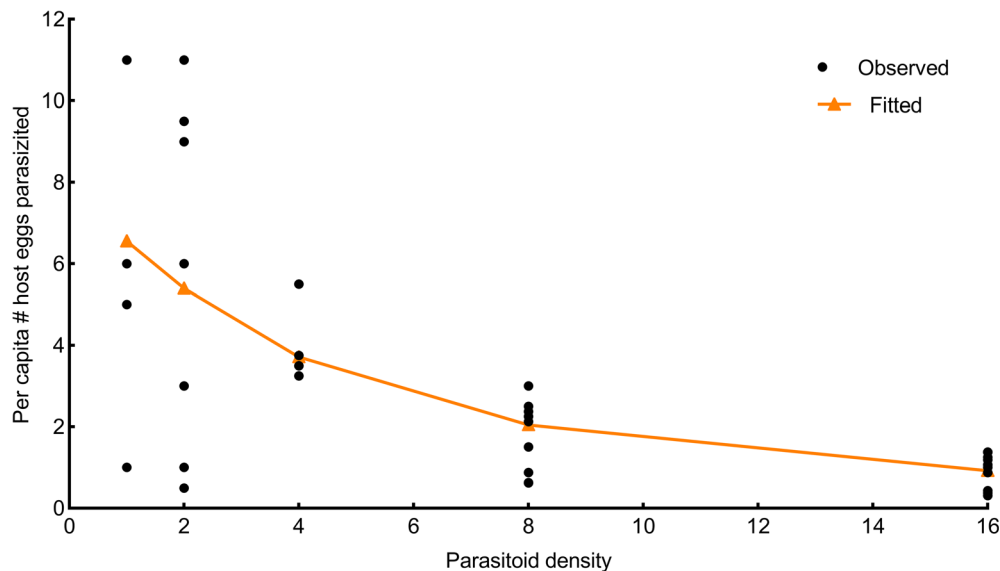


Figure 4. *Per capita* number of *Antheraea pernyi* eggs parasitized by *Anastatus japonicus* females foraging alone or in groups of 2, 4, 8, 16 for 30 host eggs fitted to the random parasitoid equation modified to include mutual interference with $Q = 0.209$, $m = 1.661$ and $T_h = 3.087$ h.

Table 1. Mean number of progeny produced and mean sex ratio of parasitoid progeny produced (\pm standard error) by *Anastatus japonicus* females when foraging in groups of 1, 2, 4, 8 or 16 females

Parasitoid density	Host density	Mean number of progeny	Sex ratio (percent females)
1	30	5.8 \pm 1.9 b	81.3 \pm 6.0 a
2	30	11.5 \pm 1.9 a	82.7 \pm 6.0 a
4	30	14.9 \pm 2.0 a	80.7 \pm 6.4 a
8	30	16.2 \pm 1.6 a	89.2 \pm 5.1 a
16	30	14.8 \pm 1.7 a	93.0 \pm 5.3 a

Means in a column with the same letters are not significantly different (least significant difference, $P > 0.05$).

Furthermore, the progeny sex ratio was not influenced by parasitoid density (GLM, $\chi^2 = 3.729$, $df = 4$, $P = 0.444$), and was female biased (Table 1).

4 DISCUSSION

In the present study, the reproductive attributes of *Anastatus japonicus*, a candidate for inundative biological control of *Halyomorpha halys*, were assessed when reared on *Antheraea pernyi*, a common factitious host used for mass production of *Anastatus* and *Trichogramma* species in China.^{18,35} *Antheraea pernyi* is reared extensively on oak trees in forest regions in north-eastern China for silk production. Its cocoons are harvested in autumn and stored at 1–5 °C for up to 5 months without affecting egg quality.²⁹ Infertile eggs are collected from emerged female moths in the following year and can be stored at –10 °C for up to 6 months for mass rearing of *Anastatus* species.¹⁸ Recent study showed that no significant differences in emergence rate and the proportion of *Anastatus fulloi* females when reared on *Antheraea pernyi* eggs stored at –5 °C and –18 °C for 6 months compared to fresh eggs, whereas the parasitism rate was reduced after 6 months cold storage and still reach over 90% on average.³⁶ Nonetheless, cold storage of *Antheraea pernyi* eggs should not exceed 6 months to have limited negative effects to maintain higher level productivity in mass rearing of *Anastatus* species.^{18,36}

Moreover, manually extracted, infertile and washed *Antheraea pernyi* eggs are most suitable for mass rearing of *Anastatus* species, including *Anastatus japonicus*.³⁷ Our present study showed that *Anastatus japonicus* could successfully develop on *Antheraea pernyi*. The developmental time of *Anastatus japonicus* from egg to adult was 27.5 days on average at 25 °C, which was about 5 days longer than for either *Anastatus japonicus* and *Anastatus bifasciatus* when reared on *Halyomorpha halys* eggs at the same temperature and over 6 days longer than *Anastatus japonicus* in *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae).^{26,30,38} This increase in developmental time might be due to the larger size of *Antheraea pernyi* eggs, which allows the parasitoid larva to feed longer than that in smaller eggs, such as those of *Halyomorpha halys* or *R. pedestris*.

Previously, Lu *et al.*³⁹ showed that *Anastatus* sp. fecundity was positively related to female body size, while the later depended largely on the size of the host in which the female developed. Using the larger eggs presented by *Antheraea pernyi* (110 eggs/g)¹⁸ as the factitious host resulted in mean lifetime fecundity of 404.3 progeny per mated *Anastatus japonicus* female during an average oviposition period of 42.3 days. This level was 26 times

higher than the lifetime fecundity of 15.6 eggs per *Anastatus japonicus* female when reared on eggs of *R. pedestris*,³⁸ and nearly double the lifetime fecundity of 228.2 eggs when reared on the smaller eggs of *Samia cynthia ricini* Boisduval (478 eggs/g).^{18,40} The average lifetime progeny production of *Anastatus japonicus* was also much greater than that of the related species *Anastatus bifasciatus* on eggs of *Halyomorpha halys*, which was only 41.5 progeny per female over an average oviposition period of 46 days at a light/temperature cycle of light 16 h/20 °C and dark 8 h/15 °C.²² Much lower lifetime fecundities have also been observed in other *Anastatus* species, such as *Anastatus ramakrishnae* Mani (36.6) on *Halys dentatus* Fabricius,⁴¹ *Anastatus biproruli* Girault (54.6) on *Biprorulus bibax* Breddin,⁴² and *Anastatus semiflavus* Gahan on *Hemileuca oliviae* Cockerell.⁴³ The higher lifetime fecundity of *Anastatus japonicus* could make its mass rearing using *Antheraea pernyi*-based methods more productive, reducing the production costs for inundative biological control. Moreover, the higher survival rate and long oviposition period of *Anastatus japonicus* could possibly extend its control effects after inundative release, which needs to be investigated in *Halyomorpha halys* field populations.

Functional response type is a basic element to understanding the relationship between host density and the number of hosts attacked.^{6,44} Although laboratory studies of functional responses bear little resemblance to field encounters with hosts,⁴⁵ they provide useful information about the potential efficacy of a biological control agent.^{5,46,47} Li *et al.*³⁸ found that *Anastatus japonicus* displayed a Holling type III response when attacking constant host densities of 1, 5, 10, 15 and 20 *R. pedestris* eggs in a Petri dish, and the maximum number of hosts parasitized was 11.3 eggs per 24 h. In the present study, the number of *Antheraea pernyi* eggs parasitized by a single 1-day-old mated female *Anastatus japonicus* increased with an increasing host density and reached an upper limit when the parasitoid reached its maximum reproductive capacity under a 24 h fixed time period. This result showed an inverse host density-dependent pattern of percent parasitism, reflecting a Holling type II response for *Anastatus japonicus*. According to the response model used in this study, the searching efficiency of *Anastatus japonicus* was rather lower compared to other egg parasitoids such as *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae).¹⁰ Handling time (T_h) was 3.17 h per host, accounting for a maximum parasitism rate of 7.6 hosts per 24 h. This result is comparable with the life-time fecundity bioassay that found there were 6.1 progeny produced on average per day. Considering the higher life-time fecundity discussed earlier, the lower daily parasitism rate of *Anastatus japonicus* on *Antheraea pernyi* eggs appears to have been compensated by a longer oviposition period (42.3 days in average). Being synovigenic, 1-day-old mated female *Anastatus japonicus* with less egg load used in this study would result in the lower parasitism rate in the case of the low host densities. Furthermore, the upper limit to the number of hosts parasitized per female per day suggests that while individual *Anastatus japonicus* might be unable to respond effectively to increasing host density in the field, this could be compensated for by releasing larger numbers of wasps. Although *Anastatus* sp. is usually mass released with egg cards,¹⁸ releases of 6-day-old *Anastatus japonicus* with higher egg loads as indicated in the age-specific fecundity result (Fig. 1 (b)) would be strongly recommended to possibly increase parasitism levels under field conditions.

When releasing large numbers of individual parasitoids for inundative biological control, there are high chances that parasitoids

will interfere with each other while exploiting a host patch in the field, which might affect success of releases.⁴ In our study, mutual interference among foraging *Anastatus japonicus* was measured as the reduction in the number of progeny produced per parasitoid with increasing parasitoid density at constant host density (30 *Antheraea pernyi* eggs). As the density of conspecific females increases, each female parasitoid would spend less time searching for hosts and more time interacting with the conspecifics it encounters.³ Interference among foraging female *Anastatus japonicus* reduced parasitism efficiency and the attack rate with increased numbers of conspecifics. The interference constant (m) was estimated as 1.66 for *Anastatus japonicus* in the current experimental setup, which represents strong interference among conspecifics. This value is greater than the more commonly observed intermediate values ($0 < m < 1$)³⁴ that could be considered a stabilizing factor in a host/prey–parasitoid/predator system,^{3,48} and observed for the *Trissolcus basalidis* (Wollaston)–*Nezara viridula* (L.) system.¹⁶ This might be attributed to the fact that female *Anastatus japonicus* had a much longer handling time (over 10 min per egg) for oviposition,³⁰ which would provide an extended window of opportunity for conspecific foraging females to interfere in the success of an oviposition and thus a reduction in the *per capita* number of eggs parasitized. Therefore, strong mutual interference among foraging *Anastatus japonicus* females should be considered in the context of field releases where it could act as a destabilizing factor,⁴⁹ and higher parasitoid densities should be avoided when mass rearing *Anastatus japonicus* on eggs of *Antheraea pernyi*.

Progeny sex ratio is also important in mass production of natural enemies.⁵⁰ Mating status of the parasitoid, physical conditions during oviposition, size of the factitious host's eggs, host nutritional quality, or host embryo developmental stage may all affect sex allocation of female *Anastatus* sp. during oviposition and thus modulate progeny production.¹⁸ *Anastatus* sp. is parthenogenesis arrhenotokous, producing male progeny by unmated adult females.⁴⁰ The sex ratio of *Anastatus japonicus* progeny was strongly male biased (94.4%) when reared on *Halyomorpha halys*.³⁰ In the present study, even though all the tested *Anastatus japonicus* females were mated, the sex ratio of progeny was only slightly male biased (51.2%) during the life span of *Anastatus japonicus* reared on *Antheraea pernyi*. However, more female progeny were produced before day 20. This result has an important implication that we could shorten the oviposition period to obtain larger numbers of female parasitoids during the mass rearing of *Anastatus japonicus* with *Antheraea pernyi*. Li et al.¹⁸ also suggested a mass-rearing protocol that allowed female *Anastatus* sp. to parasitize host eggs for only 20 days, and a supply of fresh honey solution. Moreover, significantly more female progeny were produced as the density of foraging parasitoids of *Anastatus japonicus* increased, and the sex ratios at different parasitoid densities did not differ significantly (Table 1). This result differs with previous studies that found that female parasitoids produced proportionately more male progeny after encountering conspecific females, which would thus increase the mother's fitness through local mate competition.^{46,51} This difference might be attributed to the larger size of *Antheraea pernyi* eggs, given that female *Anastatus japonicus* can detect the size and quality of the host egg through receptors located on the ovipositor, affecting whether to lay fertilized or unfertilized eggs.¹⁸

Currently, farmer use of biological control is limited in comparison with chemical control, and this disadvantage is often attributed to a wide range of problems or barriers, including lack of

knowledge, questionable effectiveness, high cost, compatibility of the technology with other control practices, and the lack of technical support for the end users.⁵² Among these challenges, production efficiency of biological control agents has a direct impact on the costs of the biological control product. In China, using *Antheraea pernyi* as a factitious host lowered the production costs and enabled the development of mass rearing techniques for *Anastatus* sp. and *Trichogramma* species for large-scale application to control *Tessaratoma papillosa* in litchi orchards and economically important lepidopterous pests on agricultural crops, respectively.^{18,35} However, there are concerns that the ability of *Anastatus* sp. locating and parasitizing host eggs in the field would deteriorate after generations of mass rearing on the factitious host.¹⁸ It is feasible to restore the wasps' ability by learning exercises during mass rearing or prior to field release, e.g. exposing *Anastatus* sp. to natural host or infochemical stimuli released from natural host.^{18,53} Moreover, extensive use of *Antheraea pernyi* would also have a significant environmental impact on oak tree forests in north-eastern China, resulting in possible limitations in mass production.³⁵ Therefore, large-scale and cost-effective *Antheraea pernyi*-based mass rearing and field release testing are needed to evaluate field efficacy and non-target effects before recommending the use of mass releases of *Anastatus japonicus* for control of *Halyomorpha halys* to growers.

5 CONCLUSION

The present study clarified some important aspects of the reproduction biology of *Anastatus japonicus* when reared on *Antheraea pernyi*. Higher lifetime fecundity, longer oviposition period and female-biased progeny production in the beginning of production not only make the rearing system of *Antheraea pernyi*–*Anastatus japonicus* more cost effective but also make it feasible to use *Anastatus japonicus* for inundative biological control against *Halyomorpha halys* or *R. pedestris*.^{30,38} Single 1-day-old mated *Anastatus japonicus* performed a type II functional response to increasing host densities in the laboratory bioassay, indicating individual parasitoid might be unable to respond effectively to increasing host density in the field, which could be compensated by releasing larger numbers of wasps. Strong mutual interference among foraging *Anastatus japonicus* females should be considered in the context of inundative releases against *Halyomorpha halys* or other host insect pests.

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AUTHOR CONTRIBUTIONS

FZ, J-PZ, D-SL, and Q-QM conceived and designed the experiments. Q-QM, J-PZ, MYA, and Y-ZZ conducted experiments. Q-QM, NJM, and Y-ML analyzed data. Q-QM, MYA, and FZ wrote the original draft. FZ and NJM reviewed and edited the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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