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Research

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Unsuccessful Host Stinging by *Aphelinus asychis* (Hymenoptera: Aphelinidae) Impacts Population Parameters of the Pea Aphid (Hemiptera: Aphididae)

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Abstract

The biocontrol values of natural enemies are strongly correlated to their ability to regulate the density of their host/prey. For parasitoids, apart from parasitism and host feeding, unsuccessful host stinging (i.e., stings that were aborted, abandoned, or discontinued without oviposition or host feeding) can also negatively affect their hosts and host populations. Although several studies have reported unsuccessful host stinging and its impacts on hosts, the effects of this type of attack on host life table parameters are still unclear. In the present study, we used the parasitoid *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) and its host *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) to investigate the influence of unsuccessful host stinging on host populations under laboratory conditions at. Biological parameters of *A. pisum* were analyzed using an age stage, two-sex life table. The results of this study showed that unsuccessful host stinging was prevalent under laboratory conditions, and the frequency of this type of attack on third- and fourth-instar hosts was higher than the frequencies of parasitism and host feeding. Unsuccessful host stinging adversely impacted aphid populations, by decreasing aphid survival and reproduction, and impacts were greatest in hosts attacked at the first and fourth instars. These results indicate that unsuccessful host stinging enhances the biological control impact of *A. asychis* attacking *A. pisum*, and its effect on host populations should also be considered when selecting and mass rearing of parasitoids for biological control.

Key words: unsuccessful host stinging, nonreproductive effect, life table, aphid, parasitoid

Parasitoids are invaluable biological control agents, and are used increasingly in most agroecosystems to control pests (Waage and Hassell 1982, Hassell and Waage 1984, Godfray 2016). Parasitoids can kill a significant number of hosts through parasitism and host feeding (Jervis and Kidd 1986, Heimpel and Collier 1996, Zang and Liu 2008, Cheng et al. 2017, Jia and Liu 2018). However, the impacts of parasitoids on host populations also include stings that were aborted, abandoned, or discontinued, hence unsuccessful if they were intended for oviposition or host feeding. This unsuccessful stinging was considered as one means by which parasitoids can reduce host populations (Heinz and Parrella 1989, Bernardo et al. 2006, Cheng et al. 2017, Zhu and Yefremova 2018, Abram et al. 2019). Although such unsuccessful host stinging is common in hymenopteran parasitoids (Jones 1985; Jones et al. 1986; Münster-Swendsen 1994, 2002; Münster-Swendsen and Berryman 2005; Cebolla et al. 2018; De Pedro et al. 2018), its impacts on host populations has been largely neglected in entomological research (Abram et al. 2019).

There is evidence in previous studies that unsuccessful host stinging results in an increase in host mortality, or at least a reduction in fecundity or fertility (Abram et al. 2019). Jones (1985) observed that the cabbage looper *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), when stung but not successfully parasitized by *Chelonus* sp. (Hymenoptera: Braconidae), showed the same symptoms (precocious initiation of metamorphosis) as parasitized hosts. Unsuccessful host stinging can also induce short-term paralysis and changes in aphid behavior (Calvert 1972, Gardner et al. 1984). In addition, host sterilization was evident in *Epinotia tedella* (Clerck) (Lepidoptera: Tortricidae) moths stung by the parasitoid *Apanteles tedellae* Nix. (Hymenoptera: Braconidae) without laying an egg (Münster-Swendsen 1994). Moreover, several host species that were not detectably parasitized suffered from suppressed gonad development as shown by dissection results (Reed-larsen and Brown 1990, Brown and Kainoh 1992, Barratt and Johnstone 2001). Another study was conducted on *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae)

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and *Aphytis chrysomphali* Mercet (Hymenoptera: Aphelinidae), and overstinging was reported to reduce the survival and fecundity of their host *Aonidiella aurantii* (Maskell) (Cebolla et al. 2018). De Pedro et al. (2018) reported that in the parasitoid *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae), unsuccessful host stinging is more frequent male versus female pupae of its host the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), though males emerging from these pupae were smaller, and their mating success was lower. Furthermore, several parasitoids of dipteran leafminers kill their hosts by unsuccessful host stinging, and this behavior contributes to the biological control of leafminers (Bernardo et al. 2006, Kaspi et al. 2011, Liu et al. 2014, Cheng et al. 2017, Ye et al. 2018).

Aphelinus asychis Walker (Hymenoptera: Aphelinidae) is a widespread endoparasitoid of aphids, which has been used in biological control of at least six aphid species (Shirley et al. 2017). For example, *A. asychis* was released in the field for biological control of the Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) in the United States (Brewer et al 2001, Michels and Whitaker-Deerberg 1993). Cate et al. (1973) reported a total of 4,655 aphids were successfully parasitized by 20 females in their entire lifespan, which suggested that *A. asychis* was a valuable biological control agent of the greenbug *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). At the same time, the 20 parasitoid female adults killed a total of 608 greenbugs throughout their lifetime by feeding (Cate et al. 1973). Generally, parasitoid females host feed through punctures made especially for feeding by their ovipositors (Hartley 1922). Aphids intended for host feeding were permanently paralyzed and then died even when feeding did not take place (Cate et al. 1977, Bai and Mackauer 1990b). Additionally, the duration of ovipositor insertions was considerably longer in host-fed aphids than other aphids (Cate et al. 1973).

Unsuccessful host stinging is common in *A. asychis*, and may be due to host rejection or host-defensive behaviors (Gerling et al. 1990, Bai and Mackauer 1991, Liang et al. 2017). Despite the prevalence of unsuccessful host stinging by *A. asychis*, the effects, if any, of this behavior on aphid population dynamics have not been studied. Previously, we found that unsuccessful host stinging was common when *A. asychis* attacked the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). Moreover, *A. asychis* could attack and successfully develop in all instars of *A. pisum* (Gerling et al 1990). In the present study, the effects of unsuccessful host stinging by *A. asychis* on *A. pisum* were assessed under laboratory conditions.

Materials and Methods

Laboratory Culture of Aphids and Parasitoids

Acyrthosiphon pisum were collected from Yuxi, Yunnan, China, and were reared on the broad bean (*Vicia faba* L., cv. 'Jinnong') in a climate chamber (20 ± 1°C, 16: 8 L: D photoperiod) before the start of the experiment. The *Aphelinus asychis* colony, established using pupae and adults obtained from the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in Northwest A&F University (Yangling, Shaanxi, China), had been maintained on *M. persicae* which were bred on cabbage sprouts for more than 4 yr and over 40 generations (25 ± 1°C, 60 ± 5% RH and 16: 8 L: D photoperiod). Females of *A. asychis* were transferred to *A. pisum* for at least five generations prior to the experiments (rearing conditions: 20 ± 1°C, 60 ± 5% RH, L: D 16: 8 photoperiod).

Plexiglas Petri dishes (3.5 cm in diameter and 1 cm in height) filled to about 0.5 cm depth with 1% agar gel and fresh broad bean leaf disks (3 cm in diameter) were used in the experiment and changed daily. Nonattacked adult aphids were individually maintained and monitored in leaf disks to give birth to nymphs. Newborn aphid nymphs were individually transferred to another leaf disk, and the molting of these aphids were monitored daily and a particular instar was used in specific experiment. The mummified aphids collected from broad bean plants were kept separately in PCR tubes (0.2 ml), and were checked regularly to obtain newly emerged parasitoids. Newly emerged parasitoids were caged together in tubes (5 cm in length, 1 cm in diameter) for 2–3 h to allow mating. Food and water were not provided for them. Females were then individually exposed to about 20 mixed-instar aphids (about 5 for each nymphal instar) for 48–60 h in a leaf disk to gain experience before the experiment.

Host Stinging and Life Tables

Mated, experienced females were individually transferred on to a leaf disk containing one aphid of a particular instar. The duration of stinging was observed under a binocular microscope and recorded by a manual stopwatch. Females were then transferred to another leaf disk with an aphid once they finished stinging, and they were allowed to sting successive hosts until they ceased searching for hosts. One hundred and two parasitoid females were used to attack aphid nymphs (31, 29, 18, and 24 for the first, second, third, and fourth instars). The stung aphids were individually reared on a leaf disk until death. The instars of these aphids were recorded daily, and when they reached adulthood their newborn offspring were also counted. Newborn nymphs were removed daily.

All the nonmummified aphids exposed to parasitoids were dissected when they died. Aphids were classified as 'parasitized' if they were mummified or there was an egg or a larva of parasitoid in dissected aphids. Eggs of *A. asychis* are banana-shaped and are large enough to be detected immediately after oviposition (Hartley 1922, Bai and Mackauer 1990b). For this study, the shape of parasitoid eggs or larvae was distinct from the tissues or embryos inside *A. pisum* body (Fig. 1). Host feeding could be distinguished by the states of aphids and the considerably longer ovipositor insertions (Cate et al. 1977, Bai and Mackauer 1990b). When an attacked aphid was not intended for host feeding and there were no eggs or larvae in dissected aphids, it was classified as 'unsuccessfully attacked host' or as a case of 'unsuccessful host stinging'.

The nonattacked aphids, which were not exposed to parasitoids, were reared under the same condition as the stung aphids. Similarly, the development, survivorship, lifespan, and reproduction of these aphids were recorded daily until they died. These nonattacked aphids were used as control. Life table were analyzed to compare the aphids that were stung and those were not attacked.

Life Table Analysis

The raw data for individual *A. pisum*, including survival rate, lifespan, and female daily fecundity, were analyzed by applying the age-stage, two-sex life table theory (Chi and Liu 1985, Chi 1988) using the TWOSEX-MSChart software (Chi 2015). The following data were calculated: the age-stage-specific survival rate (s_{xj}, whereby × is age and j is the stage), the age-specific survival rate (l_x), and the age-specific fecundity (m_x). The net reproductive rate (R_0) was calculated using the following equation:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The intrinsic rate of increase (r) was estimated using the iterative bisection method and the Euler-Lotka equation with the age indexed from 0 (Goodman 1982):

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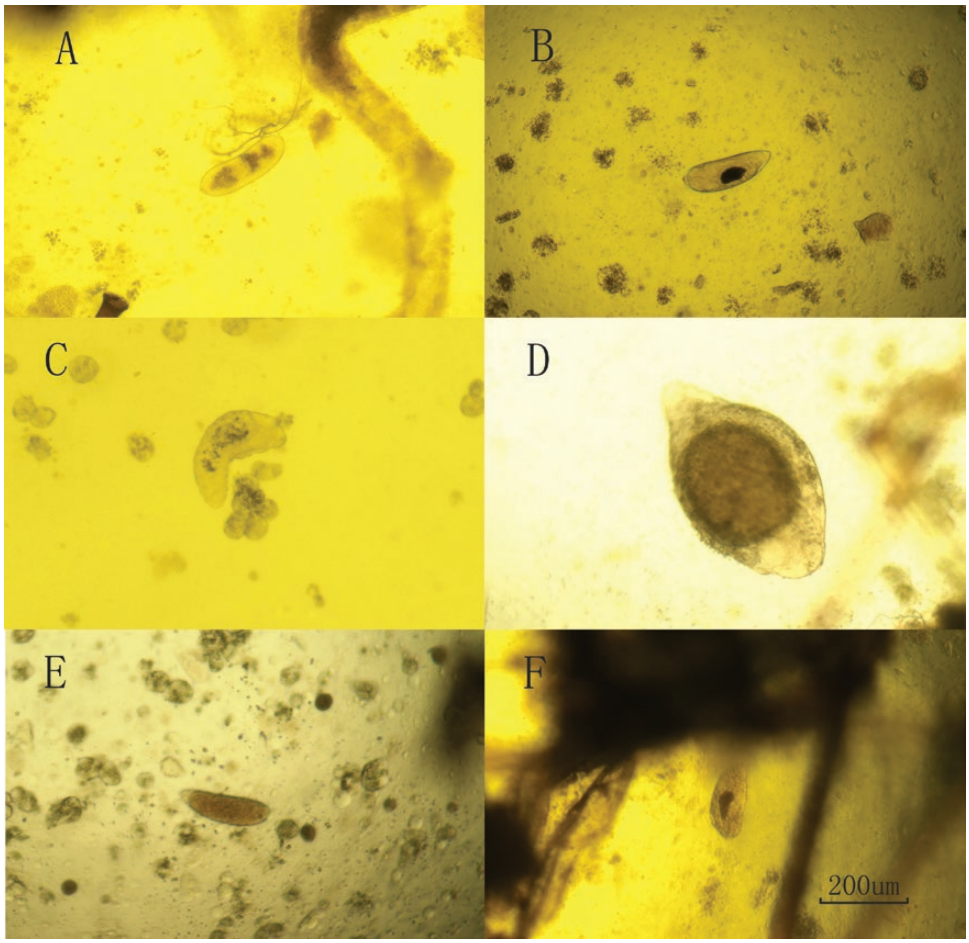

Fig. 1. Eggs and larvae of *Aphelinus asychis*. (A) Healthy egg 24 h after parasitism. (B) Healthy egg 48 h after parasitism. (C) Healthy larva 72 h after parasitism. (D) Healthy larva 120 h after parasitism. (E) Dead egg in a dead aphid. (F) Dead larva in a dead aphid. Images were photographed using Nikon 80 i microscope.

$$\sum_{x=0}^{\infty} e^{-r(x+1)} 1_x m_x = 1$$

The finite rate of increase (λ) was calculated as follows:

$$\lambda = e^r$$

The mean generation time (T) was calculated as follows:

$$T = \frac{ln(R_0)}{r}$$

The variances and standard errors of the population parameters were run using bootstrap techniques with 100,000 replications to generate more precise and stable results (Efron and Tibshirani 1993, Huang and Chi 2012). Using the TWOSEX-MSChart program, the paired bootstrap test was used to compare the differences among different stung instars and unparasitized groups. The bootstrap test relies on random sampling with replacement and it does not make any assumptions about the distribution of the sample statistics (Efron and Tibshirani 1993). Paired bootstrap test can deal with unequal sample sizes since the differences between bootstrapped samples are normally distributed (Chi 2017).

Statistical Analysis

The data of ovipositor probing time were not normally distributed, so the Mann–Whitney U test was used to analyze the variation of duration in parasitism and unsuccessful host stinging of the same instar group. Proportions of oviposition, unsuccessful host stinging, and host feeding between different instars were analyzed using a generalized linear mixed model (GLMM) with binomial logistic distribution, and parasitoid individual was involved as a random factor (Bolker et al. 2009). However, as there is no any significant variation in the data for the effect of parasitoid individual, we used a generalized linear model (GLM) with binomial logistic distribution in the final analysis. Stinging time was involved as a covariate in the model, and least significant difference (LSD) tests were used to compare differences across all groups. Data analysis was conducted using the SPSS 22.0 statistical programs (SPSS, Inc., Chicago, IL).

Results

Ovipositor Insertion Duration and Frequency of Unsuccessful Host Stinging

The female of *A. asychis* needs longer time to lay an egg in the first, third, and fourth instars of aphids than to unsuccessfully attack them (oviposition versus unsuccessful host stinging: 70.76 ± 4.233 versus 56.94 ± 3.328, Mann–Whitney U test, P = 0.023; 71.67 ± 10.89 versus 46.87 ± 3.302, Mann–Whitney U test, P = 0.011; 109 ± 20 versus 40.76 ± 2.198, Mann–Whitney U test, P = 0.003). Moreover, the time spent by a parasitoid female to parasitize a second instar host is similar to the time to unsuccessfully attack such a host (oviposition versus unsuccessful host stinging: 60.69 ± 3.619 versus

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56.45 ± 5.665, Mann–Whitney U test, P = 0.165). Overall, 496 stung aphids were observed, and the proportions of oviposition, host feeding, and unsuccessful host stinging are shown in Table 1. The proportions of unsuccessful host stinging appeared to be significantly different among different host instars (Wald χ^2 = 92.966, df = 3, P < 0.001). The proportions of unsuccessful host stinging in the third and fourth instars were nearly twice those in the first and second instars. Host instars significantly influenced the oviposition rate of *A. asychis* (Wald χ^2 = 55.678, df = 3, P < 0.001). Oviposition in the first and second instars was far more frequent than in the third and fourth instars. However, the percentage of hosts used for host feeding did not differ significantly among the four nymphal instars (Wald χ^2 = 6.425, df = 3, P = 0.093).

Age Stage, Two-Sex Life Table Parameters

Details regarding population growth parameters of *A. pisum* unsuccessfully attacked by *A. asychis* are shown in Table 2. There was a significant difference in lifespans among nonattacked hosts and those hosts unsuccessfully attacked at the first, third, and fourth instars. However, the lifespan of nonattacked aphids was not significantly differ from that of the aphids unsuccessfully attacked at the second instars. Furthermore, the value of fecundity (F) in nonattacked hosts was almost twice as many as in other unsuccessful host stinging groups. The smallest value of adult preoviposition period of female adult (APOP) was recorded in nonattacked hosts among all groups, and the differences between nonattacked and unsuccessful host stinging groups were significant. Total preoviposition periods of females counted from birth (TPOP) did not differ significantly among all groups. The preadult survival rate (S_a) of the aphids unsuccessfully attacked at the first instar was significantly lower than other groups, and the values of oviposition days in nonattacked aphids was longer than in aphids unsuccessfully attacked at the first, second and fourth instars (Table 2).

For population parameters calculated by the age stage, two-sex life tables, the intrinsic rate of increase (r), the finite rate of increase (λ), and the net reproductive rate (R_0) were found to be significantly greater in nonattacked hosts than in unsuccessfully attacked hosts (Table 2). The values of population parameters in nonattacked hosts (R_0 =87.268 ± 5.059, r = 0.32 ± 0.004, the mean generation time T = 13.986 ± 0.188, λ = 1.376 ± 0.006) suggested that if the population reached a stable age-stage distribution and if there were no mortality factors other than the physiological ones, *A. pisum* population could multiply 1.376 times per day with an average of 13.986 d at an exponential rate of 0.32 d^{-1}.

The probability of a newborn aphid surviving to age x while in stage j was depicted in the age-stage-specific survival curve (s_{xj}) (Fig. 2). The curves of s_{xj} showed the changes of aphid stage structures during their life histories. Due to variations in the developmental rate among aphid cohorts, obvious stage overlapping was observed (Fig. 2). The curve of age-specific survival rates (l_x), which was the simplified version of s_{xj}, indicated the probability of a newborn aphid surviving to age x (Fig. 3). The age-specific fecundity (m_x) and the age-specific maternity ($l_x m_x$) for different aphid treatments were also plotted in Fig. 3. Females began the production of offsprings at day 7 in all aphid groups. The m_x rose steeply and peaked before day 14 in the aphids unsuccessfully attacked at the first, second and third instars (days 13, 10, and 13), however the peak fecundity was slightly later in the aphids stung at the fourth

Table 1. Proportions of parasitism, unsuccessful host stinging, and host feeding by *Aphelinus asychis* in different host instars of *Acyrthosiphon pisum*

Type of sting	N1	N2	N3	N4
No. of stung aphids (n)	141	102	105	148
Oviposition (%)	43.97 ± 4.19a	35.29 ± 4.76a	8.57 ± 2.74b	1.35 ± 0.95c
Host feeding (%)	19.86 ± 3.37a	21.57 ± 4.09a	13.33 ± 3.33ab	11.49 ± 2.63b
Unsuccessful host stinging (%)	36.17 ± 4.06a	43.14 ± 4.92a	78.10 ± 4.06b	87.16 ± 2.76b

Data are presented as means ± SE. N1, N2, N3, and N4—first, second, third, and fourth instars of *A. pisum* stung by *A. asychis*.
Common letters show the means that did not differ between groups based on LSD comparisons at 0.05 level.

Table 2. Life table parameters of *Acyrthosiphon pisum* unsuccessfully attacked by *Aphelinus asychis* at different instars

Stinging stage	N1	N2	N3	N4	UN
N (Cohort size)	51	44	82	129	41
N_f	36	40	74	118	40
R_0	33.686 ± 4.41a	43.636 ± 4.329ab	52.585 ± 4.361b	35.256 ± 3.739a	87.268 ± 5.059c
T (days)	13.846 ± 0.272a	13.133 ± 0.236b	13.664 ± 0.155ab	13.583 ± 0.245ab	13.986 ± 0.188a
λ	1.289 ± 0.013a	1.333 ± 0.01b	1.336 ± 0.008b	1.3 ± 0.009a	1.376 ± 0.006c
r	0.254 ± 0.01a	0.288 ± 0.007c	0.29 ± 0.06c	0.262 ± 0.007b	0.32 ± 0.004d
F	47.72 ± 4.571a	48 ± 2.873a	58.27 ± 4.33b	38.54 ± 3.954a	89.45 ± 4.69c
Lifespan (days)	18.29 ± 1.52ab	22.91 ± 1.343cd	21.79 ± 1.142bc	18.03 ± 0.919a	26.54 ± 1.575d
S_a	0.706 ± 0.064a	0.909 ± 0.043b	0.902 ± 0.033b	0.915 ± 0.025b	0.976 ± 0.024b
APOP	0.912 ± 0.106ab	1.102 ± 0.102a	0.956 ± 0.09ab	0.852 ± 0.067b	0.45 ± 0.1c
TPOP	8.794 ± 0.144ab	8.487 ± 0.174abc	8.913 ± 0.137a	8.34 ± 0.113c	8.425 ± 0.132bc
Oviposition days	12.794 ± 1.046ab	13.62 ± 0.764bc	12.942 ± 0.778ab	11.148 ± 0.868a	15.525 ± 0.972c

Standard errors were estimated using bootstrap techniques with 100,000 replications. Data are presented as means ± SE. N1, N2, N3, and N4—first, second, third, and fourth instars of *A. pisum* unsuccessfully attacked by *A. asychis*. UN refers to nonattacked *A. pisum* as control.

r, intrinsic rate of increase; λ, finite rate of increase; R_0, net reproductive rate; T, mean generation time; F, mean fecundity of female; N_f, number of female adult; S_a, preadult survival rate; APOP, adult preoviposition period of female adult; TPOP, total preoviposition period of female counted from birth.

Common letters show the means that did not differ between groups based on a paired bootstrap test at 0.05 level.

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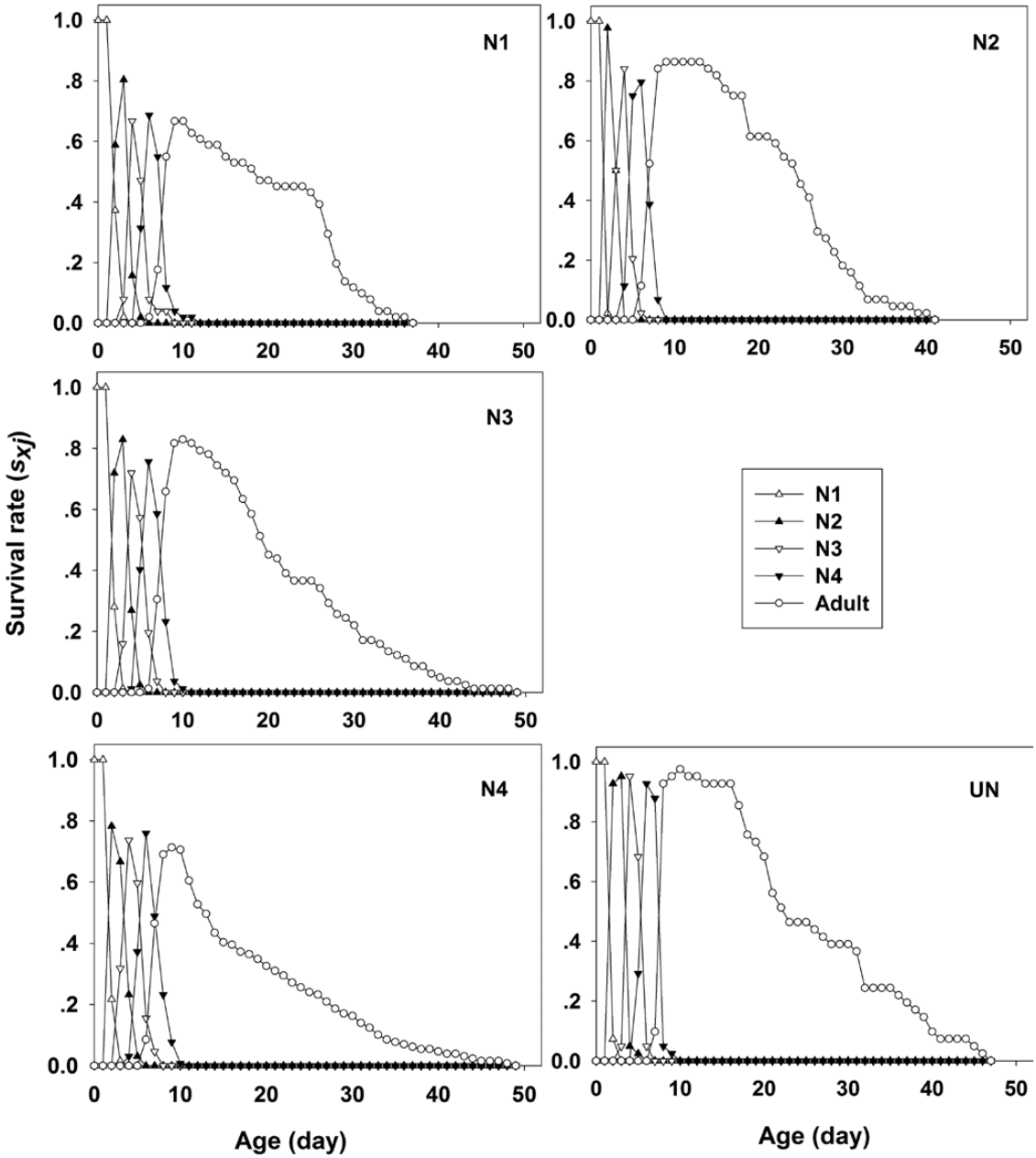

Fig. 2. Age-stage-specific survival rates (s_{xj}) of *Acyrthosiphon pisum* unsuccessfully attacked *Aphelinus asychis* at different instars and nonattacked by the parasitoid *A. asychis*. (N1, N2, N3, and N4 refer to the first, second, third, and fourth instars of aphid nymphs unsuccessfully attacked by *A. asychis*. UN refers to nonattacked aphids as control).

instars (day 18) and nonattacked aphids (day 17) (Fig. 3). The curves of age-stage-specific life expectancy (e_{xj}) and age-stage-specific reproductive value (v_{xj}) illustrated the unsuccessful host stinging impact on aphid populations of different stung instars compared to nonattacked aphid populations (Figs. 4 and 5). The parameter of e_{xj} showed that the lifespan of an individual of age x and stage j was expected to live. For example, a nonattacked adult aphid of age 21 d was expected to live for another 11.28 d, whereas the life expectancy of the 24-d-old increased to 12.84 d (Fig. 4). The v_{xj} of *A. pisum* estimated the contribution of an individual of age x and stage j to the future population, and the reproductive value of a newborn aphid (v_{01}) was exactly the finite rate of increase (Fig. 5). The v_{xj} increased sharply when the reproduction began, and the reproductive value will drop to 0 and remain thereafter (after 40 d in all groups) if an old female stops producing offspring (Fig. 5).

Discussion

In general, the results of this study showed that unsuccessful host stinging of *A. pisum* by *A. asychis* was frequent in the laboratory, and that its frequency was significantly influenced by the host's instar. In particular, the frequency of host stinging was greatest in third- and fourth-instar *A. pisum*.

Identification of unsuccessful host stinging is difficult, especially in endoparasitoids. In this study, we were unable to determine whether the egg had been deposited or not depending on the parasitoid stinging behavior. According to the results of dissection, *A. asychis* female needs at least 30 s to lay an egg in its host aphid, but there was a large proportion of unsuccessful parasitism when the stinging time lasted for more than 30 s. With *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), the mortality

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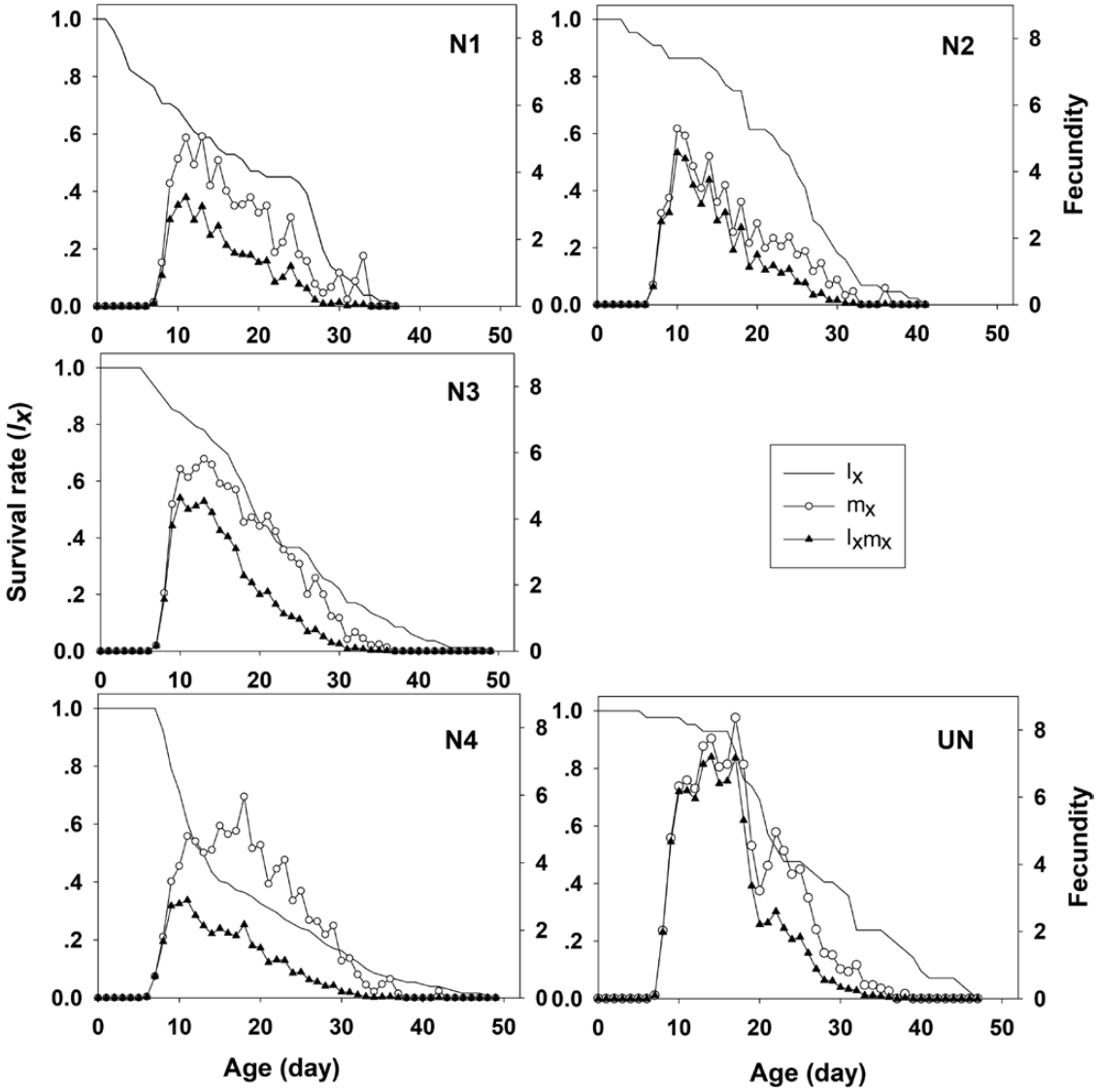

Fig. 3. Age-specific survival rates (l_x), age-specific fecundity (m_x), and net maternity (l_xm_x) of *Acyrthosiphon pisum* unsuccessfully attacked at different instars and nonattacked by the parasitoid *A. asychis*. (N1, N2, N3, and N4 refer to the first, second, third and fourth instars of aphid nymphs unsuccessfully attacked by *A. asychis*. UN refers to nonattacked aphids as control).

of rejected sting was estimated as the total number of host deaths minus the number of parasitism and host-feeding events (Liu et al. 2015). Egg laying in *A. melinus* and *A. chrysomphali* was identified by ovipositor vibration during stinging (Cebolla et al. 2018). In the present study, all the nonmummified stung aphids were carefully dissected after they had died. In order to eliminate the effects of parasitoid offspring, all aphids containing parasitoid eggs or larvae were excluded in our life table analysis. There were eggs or larvae in some dead nymphal aphids, and these eggs or larvae were still alive for a short time after their host's death. Live eggs were distinguished from deceased eggs by the time-related characteristic changes in appearance (Fig. 1). In addition, live larvae were identified by the visible body movement or peristalsis of the gut (Hartley 1922). Only in aphids parasitized at the fourth instars were dead parasitoid larvae found in unmummified aphids that survived to adulthood and only twice, indicating that the immunity defense to parasitoids was scarce in this pea aphid strain. A previous study on the interaction between *A. asychis* and some aphid species reported that encapsulation phenomenon was not detected in the pea aphids, which consistent with our observations (Carver and Woolcock 1985, Bai 1991).

The oviposition behavior of the parasitoid was usually interrupted by the host's defensive behavior, such as body shaking, sudden limb movement, or escape (Schmidt 1974, Gross 1993, Villagra et al. 2002). In the present study, the stinging behavior was often interrupted by host defensive behavior especially in the third and fourth instars of the aphids. Proportions of unsuccessful host stinging were significantly higher in the third and fourth aphid instars than in the first and second instars (Table 1), which suggested that failure in oviposition was correlated to host defensive behavior, although still some of unsuccessful host stings were not disturbed by the aphids. Oviposition success significantly declined with increasing host age, and host-feeding proportions appeared not to be affected by host instars (Table 1). These results were consistent with those in other studies, which indicated that younger hosts were more susceptible to parasitoids, whereas older hosts exhibited a more robust

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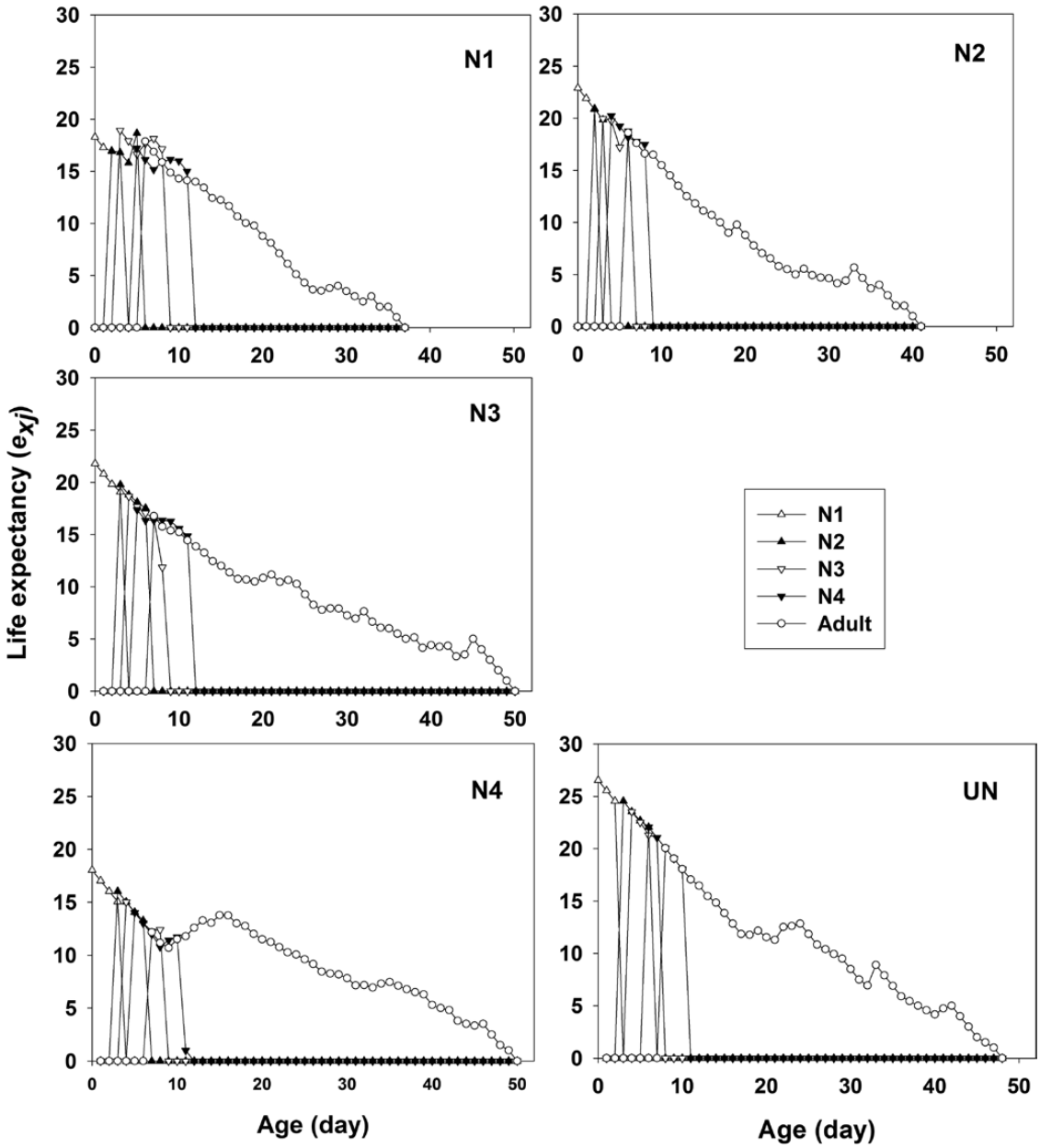

Fig. 4. Age-stage-specific life expectancy (e_{xj}) of *Acyrthosiphon pisum* unsuccessfully attacked at different instars and nonattacked by the parasitoid *Aphelinus asychis*. (N1, N2, N3 and N4 refer to the first, second, third and fourth instars of aphid nymphs unsuccessfully attacked by *A. asychis*. UN refers to nonattacked aphids as control)

behavioral defense against parasitoids (Allen 1990, Gerling et al. 1990, Mehrnejad and Copland 2006, Barrette et al. 2009).

The population growth of *A. pisum* varied considerably between the unsuccessful host stinging and nonattacked groups. The lower intrinsic rate of increase (r) among unsuccessfully attacked aphids was an indication that unsuccessful host stinging by *A. asychis* had an adverse impact on aphid populations and caused a decrease in their survival and reproduction (Table 2). The feeding behavior of aphids was probably interrupted by the attack of parasitoids as aphids may stop feeding in order to defend themselves. Indeed, the defensive behavior extended beyond the parasitoid's departure, and ceased feeding during this period. Overall, the unsuccessfully attacked second and third instar hosts, which were less affected by stinging than the aphids unsuccessfully attacked at the first and fourth instars even though the variations were not always statistically significant (Table 2). Compared to the aphids unsuccessfully attacked at the second and third instars, the s_{xj} and l_{xj} values of the first- and fourth-instar groups decrease steeply just after stinging (Figs. 2 and 3). Additionally, the curves of e_{xj} showed that there was a distinct slump in life expectancy of the aphids unsuccessfully attacked at the first and fourth instars (Fig. 4). The physical injury as well as possible chemical changes induced by unsuccessful host stinging may lead to a decrease in survival of aphids (Abram et al. 2019). The results from the current study showed that these factors may be greater in the first and fourth instars.

In this study, the aphids which had died before reaching specified instars were not included in the life table analysis, which could result in undervaluation of the unsuccessful host stinging. However, relatively few aphids died prior in that condition, so this influence could be ignored. In the system involving two *Aphytis* species and

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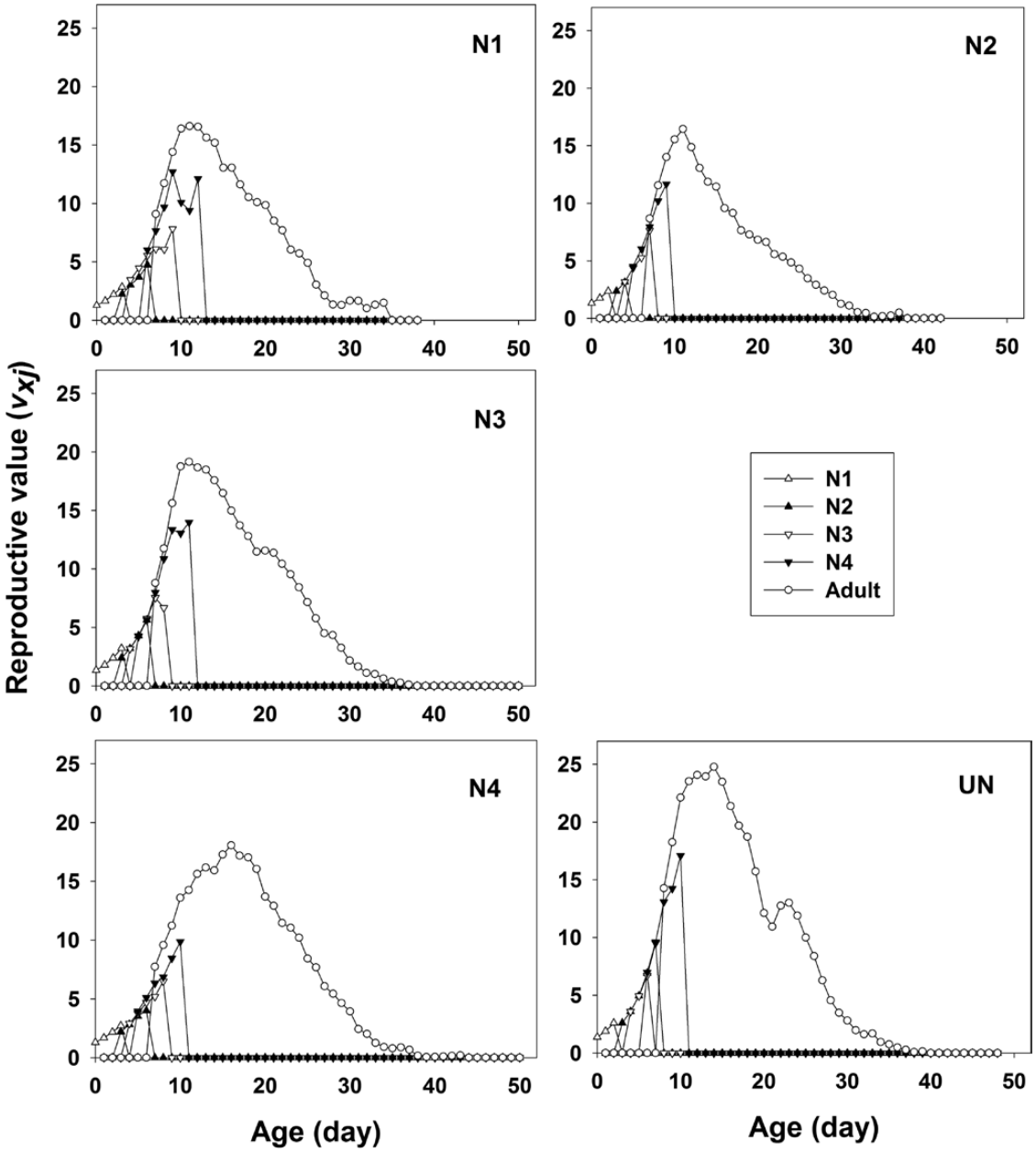

Fig. 5. Age-stage-specific reproductive value (v_{xj}) of *Acyrthosiphon pisum* unsuccessfully attacked at different instars and nonattacked by the parasitoid *Aphelinus asychis*. (N1, N2, N3, and N4 refer to the first, second, third, and fourth instars of aphid nymphs unsuccessfully attacked by *A. asychis*. UN refers to nonattacked aphids as control).

their common host *A. aurantii*, overstinging caused higher mortality in younger instars than in adults (Cebolla et al. 2018). Similar results have been reported in parasitoids of mealybugs and leafminers (Neuenschwander et al. 1986, Barrett and Brunner, 1990). In the present experiment, since unsuccessful host stinging by parasitoids does not immediately kill the host, using simplistic parameters such as mortality could lead to the effects of unsuccessful host stinging being underestimated (Ingerslew and Finke 2017). Although life table results indicate long-term and overall effects (Carey 1993), they have not yet been used by researchers to assess the influence of unsuccessful host stinging on hosts. The results of the life table parameters in our experiment were similar to those of other studies in aphids, even if the aphid species and environmental factors as well as analytical methods could influence such results (Chi and Su 2006, Hu et al. 2014, Akca et al. 2015, Akköprü et al. 2015).

While unsuccessful host stinging did not contribute directly to parasitoid reproduction, and may have reduced the host quality for reproduction, the prevalence of this behavior may still be valuable for parasitoids. Unsuccessful host stinging may be an adaptive behavior in the field, where other species of parasitoids exist. Bai and Mackauer (1991) suggested that aphids stung and rejected by *A. asychis* were more likely to be rejected by *Aphidius ervi* Haliday (Hymenoptera: Braconidae) as if they had been actually parasitized, whereas *A. asychis* accepted these aphids as if they were unparasitized. Moreover, a female of *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) could kill a host by stinging without laying an egg, and it would be selectively advantageous if these hosts were previously parasitized by conspecific individuals (Vereijssen et al. 2011). Therefore, unsuccessful host stinging may benefit parasitoids by reducing host suitability for other conspecific or interspecific parasitoids.

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Although unsuccessful host stinging may affect mass production of parasitoids, e.g., by decreasing host quality for parasitism (Abram et al. 2019), the results in this study indicated that unsuccessful host stinging of *A. asychis* may affect biological control of the pea aphid. Further investigations any molecular and biochemical changes associated with unsuccessful host stinging, including the function of any substances injected into hosts. Considering the plausible universality of this phenomenon in parasitoids, it may be important to consider the effects of unsuccessful host stinging when selecting parasitoids in biological control programs. Especially, research is needed to assess the effects of unsuccessful host stinging in the field, if any, and to consider factors that influence the frequency and impacts of unsuccessful host stinging (Cebolla et al. 2018, Abram et al. 2019).

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