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Benefits of remating of a hyperparasitoid acting as a biocontrol agent

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HIGHLIGHTS

- *Encarsia sophia* is a heteronomous hyperparasitoid, reducing competition and maximizing biocontrol by producing fertilized female offspring.
- Multiple matings extend the oviposition period of females and significantly increase parasitism on whiteflies.
- In mass rearing, male provisioning time can be shortened to within 2 h, with each male capable of engaging in 2–3 mating sessions.
- For field releases, maintain a balanced male–female ratio on pupae cards and supplement parasitoids 15 days after the initial release.

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ABSTRACT

Encarsia sophia is the dominant parasitoid of invasive whitefly pest such as *Bemisia tabaci*. This heteronomous hyperparasitoid species lays fertilised diploid eggs in whitefly nymphs, which serve as primary hosts. Larvae develop as female progeny by consuming whitefly hosts, providing direct biocontrol benefits. However, male progeny originate from unfertilised eggs laid on secondary hosts (primary parasitoids within whitefly nymphs) and develop by consuming primary parasitoid larvae rather than whitefly hosts. Therefore, it is crucial for indoor rearing and field release of the hyperparasitoid to determine whether a female with single-mating experience can parasitise primary hosts and produce female progeny for a whole lifetime and, if not, whether it is remating events and prolong the period of female production, thereby increasing the amount of parasitism on whiteflies. Our fecundity experiments of females with single-mating experience, equally provided with primary and secondary hosts, showed that single mating was insufficient for *E. sophia* females to lay fertilised eggs in whitefly nymphs for their whole lifetime. In multiple mating experiments, 76.7 % of male adults completed their second mating 2 h after the first, whereas female adults accepted the second mating only if primary hosts were available thereafter. Remating was beneficial for female adults as it prolonged the ovipositional period of fertilised eggs (from 13.3 ± 0.6 days for single to 17.1 ± 0.7 days for twice mating) and increased parasitism on *B. tabaci* primary host (from 53.1 ± 3.8 for single to 76.4 ± 5.9 for twice mating respectively). The mating experience of males increased their copulation success, whereas that of females decreased it. In conclusion, recommendations are provided for large-scale indoor rearing and field release of *E. sophia*. Firstly, sufficient time for providing males can be shortened to within 2 h, and a single male can engage in 2–3 mating sessions. Following the 15-day peak oviposition period, the addition of newly emerged males is advised. Secondly, for field release of adult parasitoids, it is recommended to concentrate on releasing females after completing mating indoors. For the release of pupae cards, ensure a balanced ratio of males and females on each card to facilitate mating upon emergence. Additionally, release extra males and females 2 weeks after the initial batch of releases.

1. Introduction

Heteronomous hyperparasitoids are a unique type of biocontrol agent characterized by the distinct host relationships of their male and female offspring. Fertilised female eggs are laid in primary hosts (target pests), while unfertilised male eggs are laid in secondary hosts

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(previously parasitised target pests, either conspecific or heterospecific), developing into male offspring (Hunter & Woolley, 2001). This reproductive strategy offers evolutionary advantages. The high adaptability of the species suggests that this method not only self-regulates population density and maintains host-parasitoid population stability but also enables coexistence with competitors under natural conditions (Avilla & Copland, 1987). In the presence of competing species within the same habitat, they can produce more male offspring, aiding in their population expansion. In the absence of competitors, males can develop on their own species' females as a strategy to sustain population longevity (Zang et al., 2011). Due to the special reproductive mode, biological control benefits can only be achieved when they parasitise primary hosts and produce female progeny. It remains unclear whether they can reproduce females for a whole lifetime with single-mating experience and whether the females can mate multiple times. Therefore, clarifying the effect of rematings of heteronomous hyperparasitoids on female reproduction will help to evaluate their biocontrol function. *Bemisia tabaci*(Gennadius) Middle East-Asia Minor 1 (MEAM1, formerly known as the 'B biotype') is globally distributed and has been recorded in more than 70 countries across Europe, Africa, Asia, South America and Oceania (Oliveira et al., 2001). It is recognized by the Food and Agriculture Organization of the United Nations (FAO) as a 'Super Pest' (Xia et al., 2021) and is listed as one of the 100 World's Worst Invasive Alien Species (https://iucn.org). With a host plant range exceeding 600 species, it causes significant plant damage through direct feeding on plant tissue, excreting honeydew that fosters sooty mold, and by transmitting hundreds of plant viruses (Wan & Yang, 2016). *Encarsia sophia*(Girault & Dodd), previously known as *E. transvena*(Timberlake), attacks over 25 species of whiteflies, including *B. tabaci* and *Trialeurodes vaporariorum* (Polaszek et al., 1992; Luo and Liu, 2011). It is a solitary, arrhenotokous, heteronomous autoparasitoid. Fertilised eggs are laid in whitefly nymphs, where the larvae develop as primary parasitoids, resulting in female offspring. Unfertilised eggs are laid on secondary hosts, either conspecific immature females or heterospecific primary parasitoids, and develop as hyperparasitoids, resulting in male offspring (Walter, 1983; Yang et al., 2012; Xu et al., 2013). *E. sophia* has a worldwide distribution in the Old World and is considered an introduced species in the New World (Heraty & Polaszek, 2000). It was initially discovered in California as part of the classical biological control program for the tobacco whitefly in the southern United States and other parts of the world (De Barro, 1995; Gerling, 1996). It has established populations in Texas, Arizona, and the southern United States and is now globally distributed (Hoelmer & Goolsby, 2002; GBIF, 2017).

Multiple matings are widespread among various insect species (Liu et al., 2010; Nason and Kelly, 2020). In most cases, males often mate with multiple females, whereas it is less common for females to accept multiple males (Ridley, 1993). Multiple matings are important for maintaining insect populations. For males, mating with more females can lead to more offspring and their reproductive benefits are closely related to mating time (Sun et al., 2013). Maynard-Smith (1978) observed that multiple matings in males can accelerate the evolution of a population. Males of *Prunella collaris* (Alpine Accentor) increased survival rate of their offspring through multiple matings (Nakamura, 1998). Females also gain various potential benefits from multiple matings. Arnqvist and Nilsson (2000) reviewed 122 insect species from 58 genera, 38 families, and 10 orders and found that multiple mating was beneficial because it increased lifetime offspring production. There are two hypotheses related to the benefits of multiple matings in females: material (direct) and genetic (indirect) benefits (Jennions et al., 2000; Fedorka et al., 2002; Hosken et al., 2003; Zeh et al., 2010). Observations of insect mating behaviour, such as in *Panorpa cognata*(Rambur), *Protohermes grandis*(Thunberg), *Drosophila subobscura*(Collin), and *Ephippiger ephippiger* (Fiebig), indicate that males provide substantial nutritional nuptial gifts to induce females to mate, while females improve reproductive success by obtaining more nutrients through multiple matings (Hayashi, 1998; Wedell et al., 2004; Engqvist, 2007; Vahed, 2007). Studies on multiple mating in *Tribolium castaneum* (Herbst), *Coelopa frigida*(Fabricius), *Dinarmus basalis*(Rond), *Diadromus pulchellus*(Wesmael), and *Drosophila melanogaster*(Meigen) showed that females increase their number of offspring and enhance genetic diversity through sperm supplementation and competition (Boomsma, 1996; Simmons, 2001; Chevrier, 2002; Pai et al., 2005).

Therefore, understanding the multiple mating behaviours and benefits of parasitoids is essential for improving large-scale rearing techniques and evaluating their biocontrol values. In this study, we observed the mating behaviour of *E. sophia* and clarified the criteria for successful mating of both sexes, which served as the basis for assessing mating rates in subsequent experiments. As *E. sophia* is a heteronomous hyperparasitoid, only immature female offspring will parasitise whitefly nymphs, leading to biocontrol benefits. Therefore, we tested whether *E. sophia* females with single-mating experience after emergence could parasitise primary hosts for their whole lifetime. If one mating experience is insufficient for female adults to lay fertilised eggs that develop into female offspring, it is essential to identify whether adults can mate multiple times and what restrictions exist for both females and males. By observing the multiple mating behaviour of males and females and assessing their reproductive capacity under various mating experiences, measures such as the frequency of female/male utilization and replacement timing are implemented to ensure the quality control of both indoor rearing and field release of parasitoids. Additionally, the study delved into the fecundity benefits derived from multiple matings, exploring their effects on adult longevity, ovipositional period, and parasitism numbers of *B. tabaci*. The ensuing discussion examined their implications for biocontrol efficiency, thus furnishing valuable guidance and recommendations for the field release of *E. sophia*.

2. Materials and Methods

2.1. Insects and plants

The *E. sophia* population was provided by the Vegetable Integrated Pest Management Laboratory, Texas Agricultural Experiment Station, Weslaco, Texas, USA. Laboratory colonies of this parasitoid were established using *B. tabaci* as host insect and cotton plants as host plants. The *B. tabaci* MEAM1 population was collected from greenhouses at the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (CAAS) in Beijing, China, and was maintained under glasshouse with insecticide free conditions. All host plants and insect colonies were kept at 26 ± 2 °C, 65 % ± 5 % relative humidity, and a photoperiod of 14 h light and 10 h darkperiod (14:10 L:D) at Langfang Experimental Station at CAAS.

2.2. Primary (Bemisia tabaci nymph) and secondary hosts (Encarsia sophia larvae and pupae)

50 pairs of *B. tabaci* adults were introduced into microcage (4.0 cm in diameter × 3.5 cm in height, covered with 120-mesh gauze) positioned on cotton leaves and were subsequently removed after 24 h. Following a 6-day interval, an additional 30 pairs of *B. tabaci* adults were introduced using the same microcage, and the adults were again removed after 24 h. Approximately 6–8 days after the second removal of adult *B. tabaci*, when the eggs laid by the initially inserted adults had developed into 3rd instar nymphs, 15–20 mated *E. sophia* females were introduced into the microcage (Mated females were obtained by introducing both male and female wasps into petri dishes in advance, where mating behaviour was observed). After 24 h, the females were removed, and a mesh bag (10 cm × 10 cm, 120-mesh gauze) was placed on the leaves to prevent contamination. After 6 days, primary hosts (3rd-4th instar *B. tabaci* nymphs) and secondary hosts (from 3rd instar to prepupal stage *E. sophia*) of appropriate age were distributed on the leaves (Yang & Wan 2011, Xu et al. 2018).

2.3. Collection of newly emergence unmated *Encarsia sophia* females and males

Two potted cotton plants containing 3rd to 4th instar *B. tabaci* nymphs were placed in an insect cage (60 cm × 60 cm × 60 cm). Using a fluke tube, 35 mated *E. sophia* females were introduced into the cage. After 24 h, the plants were moved to a clean cage without insects. After 11 days, when the parasitised *E. sophia* females develop to the black pupae stage, carefully remove the black pupae from the leaves using an insect pin and place them into separate centrifuge tubes (1.5 mL). Newly emerged *E. sophia* females (<12 h) were collected daily for subsequent experiments.

Similarly, place two potted cotton plants with 3rd to 4th instar *B. tabaci* nymphs into an insect cage (60 cm × 60 cm × 60 cm). Using a fluke tube, 35 mated *E. sophia* adult females were introduced into the cage. Mated females were abstained as mentioned in 2.2. After 24 h, the plants were moved to a clean cage without insects. After 6 days, when the *E. sophia* female larvae have developed to the 3rd instar to prepupal stage (to be used as secondary hosts for rearing *E. sophia* males), 35 unmated *E. sophia* adult females were introduced (Unmated females were obtained by keeping them in rearing cages without males, with the age of the females being unspecified.). After 24 h, the plants were moved to a clean cage without parasitoid adults. After 11 days, when the *E. sophia* males have developed to the black pupae stage, carefully remove the black pupae from the leaves using an insect pin, and place each pupa individually into a centrifuge tube (1.5 mL). Check daily to collect newly emerged *E. sophia* males (<12 h) for subsequent experiments.

2.4. Single mating behaviour and ability to produce females and males after mating of *Encarsia sophia*

2.4.1. Single mating behaviour of *Encarsia sophia*

To elucidate the precise behavioural process of *E. sophia*'s mating and establish criteria for successful mating, we conducted observations on their mating behaviour. Newly emerged male and female adults were individually collected and introduced into culture dishes (d = 3.5 cm, with lid cover). A 5 % honey solution was provided as a nutritional supplement for parasitoids. Once adults were introduced, their behaviour was observed and photographed (VHX-2000). When mating was completed, the female was removed, and the completion time point of the first mating and the duration of the mating were recorded. The mating time point is used to confirm the interval before the next mating, and the mating duration is measured from the male mounting the female to the completion of copulation and separation. For each mating stage, the report by King and Kuban (2012) was referred. Precopulatory was defined as the stage from male mounting female to the opening of the female genital aperture. Copulation was the stage from female genital aperture opening to male genital separation. Postcopulatory refers to the stage from the end of copulation to male dismounting.

2.4.2. Ability to produce females and males after single mating of *Encarsia sophia*

To elucidate *E. sophia*'s capacity to produce females after a single mating, specifically in terms of parasitising the nymphs of *B. tabaci*, we calculated the parasitism rate of females on both the primary host (*B. tabaci* nymph) and the secondary host (primary parasitoid within the whitefly nymph) following a single mating. Cotton leaf discs (d = 3.5 cm) with 15 primary hosts (3rd instar whitefly nymphs) and 15 secondary hosts (whitefly nymphs that have been parasitised, with larvae of the parasitoid developed to the stage between 3rd instar and prepupae) were placed in Petri dishes (d = 3.5 cm, with 1 % agar solution, allowing for the distinction between primary and secondary hosts based on the internal shape and external fullness). Once mated *E. sophia* females were then introduced into each Petri dish (d = 3.5 cm, 1 % agar solution) and covered with two layers of cling wrap. Holes were made in the cling wrap using an insect pin to ensure air circulation (Petri dishes were kept in a climatic chamber at 26 ± 1 °C, 65 % ± 5 % RH, and 14:10 L:D). Females were transferred to new leaf discs every 24 h until they died or stopped laying eggs. The number of parasitised primary and secondary hosts per female was recorded daily under a dissecting microscope (BX61 Olympus).

2.5. Remating behaviour and Variations in fecundity under different mating times of *Encarsia sophia*

2.5.1. Remating behaviour of *Encarsia sophia*

To evaluate the potential for rematings in *E. sophia*, we investigated the remating behaviour of both males and females after a single mating. Building upon preliminary experiments that examined the active remating status of males and females, our observations focused on their remating behaviour at various time intervals. Criteria for successful mating, established in Section 2.4.1, included observing copulatory behaviour lasting for more than 5 s and female remaining stationary during copulation without refusing to move forward. After the first successful mating, males and females were separated from the arena. Males were provided with a 5 % honey and water solution, while females were randomly assigned to two groups. One group was provided with a honey and water solution, and the other one was provided with suitable primary hosts (3rd instar whitefly nymphs) on cotton leaf discs (d = 3.5 cm, 1 % agar solution). Each previously mated male or female was introduced to a newly emerged opposite-sex adult at different intervals. The time intervals were 0.5, 2, and 4 h for single-mated males, whereas they were 2, 6, and 12 h for single-mated females. Mating status and duration were recorded.

2.5.2. Comparison of fecundity of *Encarsia sophia* under different mating times

To elucidate the influence of rematings on fecundity, daily observations and counts were conducted on their survival and the number of parasitised hosts. Given that *E. sophia* yields biocontrol benefits through parasitising *B. tabaci* and producing females, primary host *B. tabaci* nymphs were provided for comparing reproductive benefits. When the mating behaviour observations were complete, males and females with different mating experiences were individually introduced into Petri dishes. Males were provided with a 5 % honey solution daily. Females were provided with leaf discs containing 3rd instar whitefly nymphs for oviposition and nutrition, with new leaf discs of the same age whiteflies replaced daily. The Petri dishes were kept in a climatic chamber at 26 ± 1 °C, 65 % ± 5 % RH, and 14:10 L:D. The longevity of both male and female adults and the daily parasitism per female were recorded. Parasitism was defined as the number of parasitised hosts, determined by counting the number of parasitised pupae 10 days after exposure to female adults.

2.5.3. Effect of mating experience on *Encarsia sophia* copulation success

To ascertain the influence of mating experience on copulation success, we observed the mating selection behaviour of unmated parasitoids toward males and females with and without mating experience. Two adult females, one without and the other with mating experience, were placed in a Petri dish (d = 3.5 cm, with lid cover). A newly emerged adult male without mating experience was introduced to assess female mating experience upon copulation. To assess the effect of male mating experience on the copulation of females, two male adults, one with and one without mating experience, were introduced into the arena along with a newly emerged virgin female adult. Copulation behaviour was recorded using a Canon camera (EOS 60D) for 30 min under a stereo dissecting microscope (BX61 Olympus) to determine the copulation success of adults with mates with different mating experiences.

2.6. Statistical analysis

The normality of all data related to the daily parasitism rates of primary and secondary hosts, the durations of the first and second matings, and the effects of multiple matings on female longevity and fecundity were analyzed using the Shapiro-Wilk test. Data that followed a normal distribution were analyzed using t-tests, while data that did not follow a normal distribution were analyzed using Generalized Linear Models (GLM) with a Poisson distribution. The copulation success rates of females and males with and without mating experience were calculated using chi-square tests. Data were analyzed using the SPSS 22.0 statistical program (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Single mating behaviour and ability to produce females and males after mating of Encarsia sophia

3.1.1. Single mating behaviour of Encarsia sophia

The mating process of *E. sophia* included three stages (Table 1): precopulatory, copulation and postcopulatory. The main mating behaviours observed were mounting and copulation (Fig. 1). Successful mating was defined as copulatory behaviour lasting more than 5 s, with the female remaining stationary and not refusing to move forward during copulation. These criteria were employed in subsequent experiments to assess mating success.

3.1.2. Ability to produce females and males after single mating of Encarsia sophia

After a single mating, female *E. sophia* can produce both female and male offspring in the corresponding hosts during the same period. Throughout their lifetime, they can lay unfertilised male eggs in secondary hosts, but only lay fertilised female eggs in primary hosts for a duration of 15.0 ± 1.2 days Fig. 2 Although there was no significant difference between the total numbers of primary (45.7 ± 5.2) and secondary hosts (36.5 ± 3.3) that were parasitised in whole lifetime (t = 1.498, df = 28, P=0.145), significantly more secondary hosts were parasitised by females older than 15 days than were primary hosts (GLM, *Deviance Resid* = 148.519, df = 1, P<0.001).

3.2. Multiple mating behaviour and fecundity differences under different mating times of Encarsia sophia

3.2.1. Multiple mating behaviour of Encarsia sophia

Encarsia sophia females can conditionally accept remating. After the first mating, they can accept remating when provided with available hosts, with a remating rate of 72.8 % after 12 h Table 2. The duration of the two matings shows no significant difference (t = 0.180, df = 30, P=0.861). However, if no hosts are available after the first mating, they do not accept remating, possibly due to the storage capacity for eggs within the female. Males can remate within a short period, achieving a remating rate of 76.7 % within 2 h, with the duration of the second mating being significantly longer than that of the first (t = 2.260, df = 48, P=0.029) Table 3.

Table 1
Mating behaviour of *Encarsia sophia*.

Stage	Behaviour	Description
Precopulatory	Approach	Male moves slowly in the direction of the female.
	Mount	Male gets on the female in a riding position beginning with the front legs climbing from the posterior or sides of the female.
	Antennal touch	Male antennae whip female's head or thorax.
Copulation	Hold	Male grasps the female posterior abdomen with the middle and posterior limbs.
	Copulation	Male inserts the aedeagus into female's genital cavity, with the middle and hind legs holding the female's rear end of the abdomen. The wings of the male remain upright.
Postcopulatory	Mount again	Male climbs on the female's back again.
	Resting	Both male and female remain immobile.
	Dismount	Male gets off the female.
	Walk (Immobile)	Male leaves quickly, and female still remains.

3.2.2. Comparison of fecundity of Encarsia sophia under different mating times

Fecundity of remating *E. sophia* females was superior to that of single mating females. The oviposition period for twice-mated females reached 17.1 days, significantly longer than the 13.3 days observed for single mating females (t = 4.31, df = 35, P<0.001). The whole lifetime parasitism of twice-mated females was 76.4 ± 5.9, also significantly higher than that of single-mating females (53.1 ± 3.8, t = 3.43, df = 35, P=0.002). Remating did not affect the lifespan of females (t = 1.89, df = 35, P=0.066; Table 4). From day 12 after mating, the daily average parasitism of twice-mated females was significantly higher than that of single-mating females (t = 2.21–4.69, df = 18, P=0.001–0.040; Fig. 3).

Remating by *E. sophia* males had no significant effect on their lifespan (t = 0.70, df = 38, P=0.508). Importantly, the adult longevity, oviposition period, and whole lifetime parasitism of female partners were not adversely impacted after mating with remating males (t = 1.76, df = 38, P=0.087; t = 1.93, df = 38, P=0.061; t = 1.40, df = 38, P=0.170; Table 5), Furthermore, there was no significant difference observed with increasing age (t = -0.108–1.798, df = 38, P=0.080–0.914; Fig. 4).

3.2.3. Effect of mating experience on e. Sophia copulation success

When males with and without mating experience had an equal opportunity to engage with female adults, the copulation rate for males with mating experience reached 75 %, while that for males without mating experience was 25 % (χ^2 = 5.00, df = 1, P<0.05). Similarly,when females with and without mating experience had an equal opportunity to engage with male adults, the copulation rate for virgin females reached 80 %, whereas that for females with mating experience was 20 % (χ^2 = 7.20, df = 1, P<0.05) Fig. 5.

4. Discussion

In bisexual reproductive insects, the amount of sperm transmitted to females during mating plays a key role in the reproduction of their offspring (Duplouy et al., 2018). Some primary parasitoid males provide most or even all of their sperm during mating to ensure greater fertilization of eggs (Passera et al., 1992). For example, *Eupelmus orientalis* (Crawford) can produce female offspring throughout the entire oviposition period after a single mating (Bressac et al., 1998). In contrast, other parasitoid males, such as *Dinarmus basalis*(Rond), provide only parts of their sperm during mating (Simmons et al., 2001), and females stop laying eggs on the 21st day after a single mating (Chevrier et al., 2002). In this study, *E. sophia* females with mating experience produced both female and male offspring depending on the type of host. Females with a single mating experience can parasitise secondary hosts(*E. sophia* larvae and pupae) for their whole lives to produce male offspring. However, the production of female offspring by parasitising the primary host *B. tabaci* nymphs is limited and cannot continue throughout their lifetime. Considering the heteronomous reproductive mode of *E. sophia*, females only laid fertilised eggs in whitefly nymphs when producing female progeny, and unfertilised eggs were laid on their own or other parasitic wasp larvae when producing male progeny. The biological control benefits of heteronomous hyperparasitoids for target pests are determined by the fertilization success of female adults (Hunter & Woolley, 2001; Yang et al., 2012). Therefore, whether *E. sophia* females can accept rematings for sperm supplementation to increase their ability

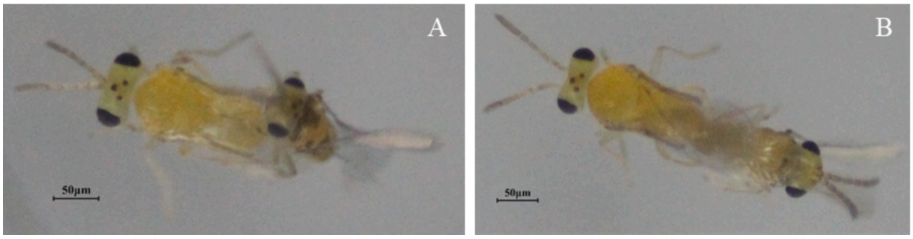

Fig. 1. Main mating behaviour of *Encarsia sophia.* A: Mount (male climbs on the female's back); B: Copulation (male inserts the aedeagus into female's genital cavity).

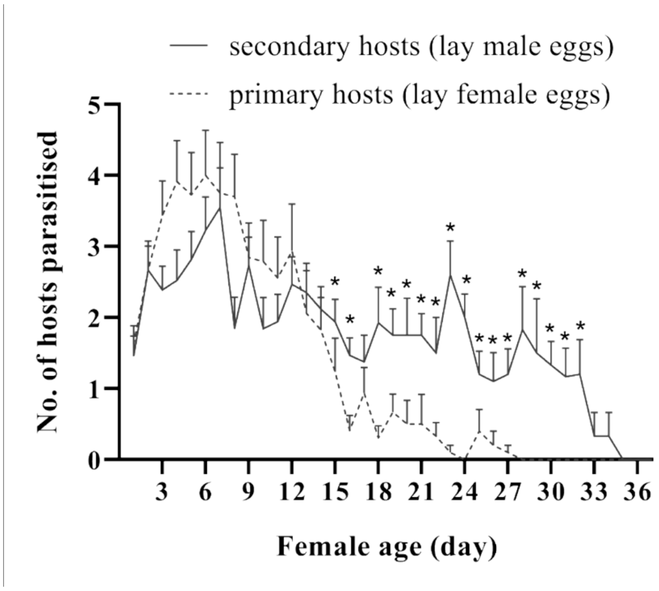

Fig. 2. Number (mean + standard error) of primary and secondary hosts parasitised daily by *Encarsia sophia* females after single mating. Note: Data with an asterisk indicate significant differences ($P<0.05$) between numbers of primary (*B. tabaci* nymph) and secondary hosts (*E. sophia* larvae and pupae) parasitised each day.

Table 2
Second mating rate of *Encarsia sophia* female adults with different host availability.

Sex	Host availability after the first mating	Accumulated 2nd mating rate (%) with a certain time interval after the first mating		
		0.5 h	2 h	> 2h
Female	Unavailable (5 % honey solution)	0	0	0
	Available (*B. tabaci* nymphs)	/	27.3	72.8
Male	Unavailable (5 % honey solution)	36.7	76.7	76.7

Table 3
Duration of multiple matings of female/male adults of *Encarsia sophia* (mean ± SE).

Sex	Duration (s)	
	First mating	Second mating
Male	20.1 ± 0.9b	25.5 ± 1.9 a
Female	21.8 ± 2.1 a	21.3 ± 1.9 a

Note: Mean values followed by the same letter within the same line are significantly different (*t*-test, $P<0.05$).

Table 4
Comparison of the number of primary hosts parasitised by *Encarsia sophia* females at different mating times (mean ± SE).

Mating times	Adult longevity	Ovipositional period	Parasitism numbers of primary hosts
1st mating	17.2 ± 0.6 a	13.3 ± 0.6b	53.1 ± 3.8b
2nd mating	18.8 ± 0.6 a	17.1 ± 0.7 a	76.4 ± 5.9 a

Note: Mean values followed by the same letter within the same column are not significantly different (*t*-test, $P<0.05$).

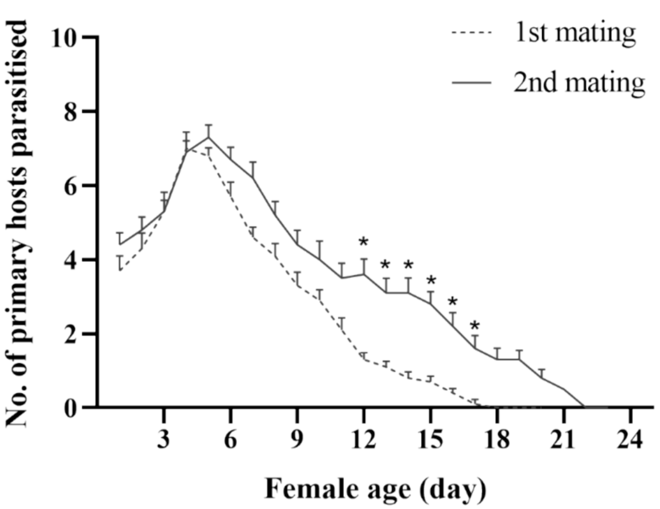

Fig. 3. Daily primary hosts (*B. tabaci* nymphs) parasitism numbers under different mating times of female *Encarsia sophia.* Note: Data with an asterisk indicate significant differences (*t*-test, $P<0.05$) between different mating times for each day.

to produce females in whiteflies is of great significance to their biological control value.

Because of the differences in reproductive benefits, the mating strategies adopted by female and male parasitoids often differ. Most females only accept a single mating, whereas males can mate multiple times (Cloutier et al., 2010). This is common in solitary parasitoids, whereas gregarious parasitoids generally accept multiple matings to avoid intrinsic competition between offspring (Liu et al., 2017). For example, regardless of whether the mating partner provides sufficient sperm, *Spalangia endius*(Walker) females can complete only a single mating (King, 2010). Most *Lariophagus distinguendus*(Foerster) females can only mate once in their whole lifetime, but remating behaviours have occasionally been observed, which is related to sperm count reserved in females (Steiner et al., 2010). Similarly, in a study of the mating patterns of *Drosophila bifurca*(Patterson & Wheeler), it was observed that newly emerged females refuse to mate again after the first mating until five days later when most of the transferred sperm is exhausted (Mery & Joly, 2002). The results from the analysis of *E. sophia*

Table 5
Comparison of the number of primary hosts parasitised by *Encarsia sophia* females after mating with males at different times (mean ± SE).

Mating times	Male adult longevity	Female adult longevity	Female ovipositional period	Female parasitism numbers of primary hosts
1st mating	5.9 ± 0.4 a	12.9 ± 0.4 a	10.9 ± 1.4 a	31.3 ± 2.2 a
2nd mating	5.5 ± 0.4 a	12.0 ± 0.3 a	9.8 ± 0.7 a	26.9 ± 4.3 a

Note: Mean values followed by the same letter within the same column are not significantly different (*t*-test, $P<0.05$).

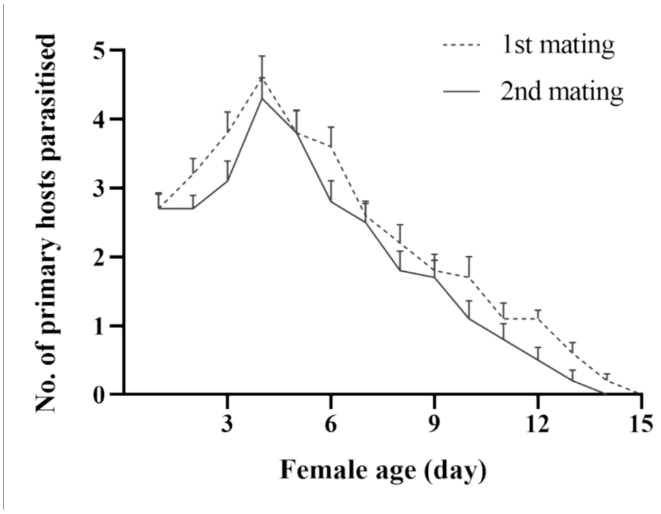

Fig. 4. Daily primary hosts (*B. tabaci* nymphs) parasitism numbers of *Encarsia sophia* females after mating with males with different mating experiences.

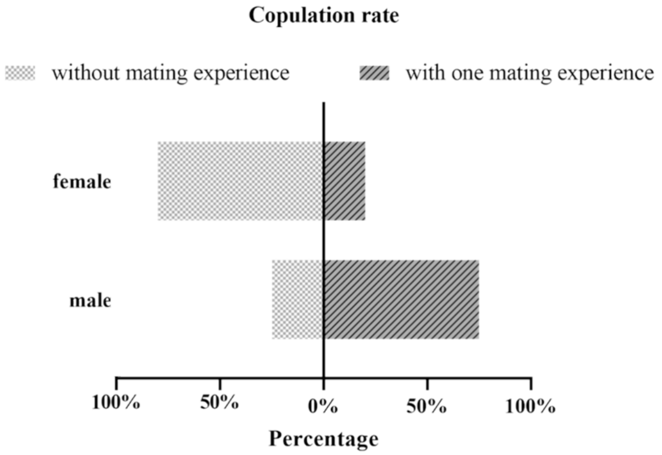

Fig. 5. Copulation rate of both sexes of *Encarsia sophia* without or with one mating experiences.

showed that both males and females can mate multiple times, whereas the egg-laying experience was indispensable for females. *E. sophia* males can engage in rematings within a short period after the initial mating, whereas females accept remating only if suitable hosts are available for parasitisation. Females lacking egg-laying experience are unable to undergo a second mating, highlighting the association between multiple matings in females, parasitic experience, and insufficient sperm reserves. This suggests that *E. sophia* females can accommodate sperm supplementation when faced with a shortage. Considering the characteristics of multiple matings in *E. sophia*, guidance can be provided for quality control in indoor rearing. During the rearing process, the time for providing males to females can be reduced to within 2 h, allowing the same male to engage in 2–3 mating sessions. Notably, the duration of the *E. sophia* male's second mating was significantly longer than that of the first. The copulation duration may be related to the size of male spermatophores and the amount of ejaculation (Zou et al., 2022). Smaller seminal vesicles and reduced ejaculation during remating increase mating duration (Wiklund, 1988; Chang & Kang, 2002).

Male and female individuals often have different mating tendencies, leading to sex conflicts (Snook 2001). Male fitness is primarily reflected in the ability to mate with high-quality females, and males have evolved strategies to control the female remating ability, which may affect the longevity of females and the number of offspring (Chapman et al., 1995). However, multiple studies shown that multiple matings of males do not affect their longevity or the total number of female offspring per couple. A study on the effect of the male mating time on the production of females in *Pachycrepoideus vindemmiae*(Rondani) and *Trichopria drosophilaede*(Perkins) showed that the mating frequency of males does not significantly affect the longevity, oviposition duration, or the total number of offspring of their female partners (Liu et al., 2017). The present study also showed that multiple matings of *E. sophia* males did not affect their longevity or their partners' reproduction. However, sexual selection is influenced by the differences in investment in gametes and offspring, with males competing for mating opportunities and females choosing partners to optimize reproductive success (Jian, 2004). The success of sexual selection directly determines the success rate of biological reproduction (Zhang et al., 2009). We observed that virgin *E. sophia* females tend to mate with males with mating experience, whereas males without mating experience prefer virgin females. Therefore, compared to males without mating experience, males with mating experience have a greater chance of mating. This phenomenon may be linked to the mating learning experience of parasitoids (He and Wang, 2008; Gabriela, 2010). Consequently, in the large-scale rearing of *E. sophia*, it is imperative to supplement honey water for males timely to optimize their utilization of multiple mating opportunities.

Different perspectives exist regarding the impact of multiple female matings on fecundity. In some species, such as *Plutella xylostella*(L.), multiple matings by females do not affect the number of ovipositions or eggs hatched (Zou et al., 2022). Conversely, in species like *Dinarmus basalis* Rondani, multiple matings by females directly influence longevity and the total number of progeny, enhancing reproductive fitness by 30 %–70 % (Arnqvist et al., 2000). Our study reveals that following multiple matings, *E. sophia* females exhibit a significantly extended oviposition period and greater total parasitism on the primary host *B. tabaci* compared to females with a single mating. As the post-mating period increases, their daily parasitism on *B. tabaci* also significantly rises. This enhancement in control efficacy against *B. tabaci* by *E. sophia* females with multiple matings may be attributed to sperm supplementation (Wedell and Tregenza, 1988). Multiple-mating females, compared to their single-mating counterparts, can produce female offspring over a more extended period due to the acquisition of more sperm (Chapman et al., 2003; Wang et al., 2021). The findings of this study offer a crucial reference for the utilization of *E. sophia* in biocontrol. Considering that one mating of *E. sophia* lasts approximately 15 days to produce female offspring, both indoor breeding and field release can adhere to this timeframe for male supplementation over about two weeks. After females engage in subsequent matings, they can continue parasitising whiteflies and exert biocontrol effects. Moreover, for field release, if adults are released, females can be released intensively after mating indoors; if pupa cards are released, ensure there are both males and females on one card to facilitate mating after emergence.

CRediT authorship contribution statement

Xiao-Ming Man: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Li-Ying Sun:** Methodology, Investigation. **Frédéric Francis:** Writing – review & editing, Supervision. **Nian-Wan Yang:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition. **Wan-Xue Liu:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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