

EMERGENCE, SEXUAL MATURATION AND OVIPOSITION OF *APHIDIUS ERVI* (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

Aphidius ervi Haliday is an important parasitoid of several aphid species, and information is needed for the development of mass-rearing techniques and better understanding of biological control ecology. The emergence, sexual maturation and oviposition of *A. ervi* on pea aphid, *Acyrtosiphon pisum* (Harris), was studied in the laboratory at 20±1°C and 60-70% RH with 16:8 h light:dark. About 95% of parasitoids emerged during the photophase. Females needed a significantly longer time than males to complete their life cycle. Newly emerged males were able to perform their courtship display but failed to mate until they were 4 h old; newly emerged females were able to respond to males' courtship display and mate. Females attacked aphids in both light and dark conditions. The number of eggs laid and parasitism (number of aphids parasitised) per oviposition bout (2 h oviposition period) were significantly greater in the photophase than in the scotophase.

Keywords: Hymenoptera, *Aphidius ervi*, emergence, mating, oviposition.

INTRODUCTION

Insect emergence events are usually rhythmic (Saunders 1982). In the parasitic hymenopterans, such rhythmicity is often synchronised with mating (Gordh & DeBach 1976; Nadel & Luck 1985) and oviposition (Armstrong et al. 1996; Couch et al. 1997) activities for an optimal reproductive fitness.

Daily activity patterns have been studied in detail in some parasitic hymenopterans (Vogt & Nechols 1991; Armstrong et al. 1996; Couch et al. 1997). Quicke (1997) suggested that for many parasitoid species, most oviposition occurs in the morning, for example, the squash bug egg parasitoid, *Gryon pennsylvanicum* (Vogt & Nechols 1991). However, in some other species, such as the *Sitona* weevil parasitoid, *Microctonus aethiopoidea* Loan, oviposition may occur during light and dark with the circadian oviposition activity corresponding with its host activity (i.e. feeding and oviposition) (Armstrong et al. 1996; Couch et al. 1997). Knowledge of parasitoids' emergence, mating and oviposition patterns is vital to an understanding of the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation of biological control programs.

Aphidius ervi Haliday is a solitary endophagous parasitoid of several pest aphid species on economically important crops, such as legumes and cereals (Star 1978; Powell 1982). It was imported into New Zealand from California to control *Acyrtosiphon pisum* (Harris) and *Ac. kondoi* Shinji and has successfully established (Cameron & Walker 1989). Michaud & Mackauer (1994) reported that *A. ervi* could successfully oviposit in *Ac. pisum* in both photophase and scotophase, but the circadian emergence and oviposition patterns were still unknown prior to this study. To provide information for the development of mass-rearing and field releasing techniques and better understanding of biological control ecology of *A. ervi*, the circadian patterns of emergence and oviposition, and sexual maturation in *A. ervi* were investigated.

MATERIALS AND METHODS

Breeding colony and experimental conditions

A breeding colony of *Aphidius ervi* was established from individuals emerged from blue-green lucerne aphids, *Acyrtosiphon kondoi* Shinji, which were collected from lucerne on an AgResearch farm at Aorangi near Palmerston North in late December 2002. Prior to the experiments, the colony was reared on pea aphid, *Acyrtosiphon pisum* (Harris), feeding on potted broad bean, *Vicia faba* L. cv. Pride, for five generations. The colony was held and all experiments were carried out at 20±1°C and 60-70% RH with 16:8 h light:dark.

Experimental parasitoids and hosts

All parasitoids used for experiments emerged from mummies that were parasitised in third instar (3 days old), and third instar pea aphids were used as hosts in all experiments.

Emergence

To observe the 24-h emergence patterns of *A. ervi*, two bioassay rooms were set up. The photophase in one room was set from 0800-2400 h (normal-light regime) and in the other room the photophase was between 1800-1000 h (reverse-light regime). To obtain parasitised aphids, a mated female parasitoid (<12 h old) was introduced into a Petri dish (5.5 cm in diameter, 1.3 cm in height) containing 25 third instar aphids for a period of 5 h. Fifty-eight females in individual Petri dishes were used.

Fifty parasitised aphids were reared on the bean plant in a transparent plastic cylinder (8.5 cm in diameter, 12 cm in height) with 3 gauze-covered holes, one (5 cm in diameter) in the top and two (2 cm in diameter) in the opposite sides of the container for ventilation. Fifteen and nine cylinders were maintained in the normal- and reverse-light regimes, respectively. Parasitoid emergence was observed from 675 mummies in the photophase in the normal-light regime and 378 in the scotophase in the reverse-light regime. The emergence incidence was recorded hourly and emerged adults were sexed. Developmental time from eggs to adults of both sexes was also recorded.

Sexual maturation

Because most matings occurred during the photophase (X.Z. He, unpubl. data), all experiments on sexual maturation were carried out during this period. To detect the sexual maturation period of adult parasitoids, two experiments were set up, each with seven treatments. In the first experiment 12-h-old virgin females were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin males, and in the second experiment 12-h-old virgin males were paired with 0 (newly emerged), 2, 4, 6, 8, 10 and 12-h-old virgin females.

A virgin male was paired with a virgin female in a clear glass vial (1.5 cm in diameter, 5.0 cm in height) with a 0.5 cm mesh covered hole in lids. Twenty pairs were established for each treatment. The sexual behaviour of both sexes in a 30 min period was observed and the number of courtship displays (male wing fanning) and matings (insemination) was recorded.

Oviposition

To determine the oviposition patterns of *A. ervi* on a 24 h basis, an experiment was carried out with 20 *A. ervi* females in the normal-light regime and another 20 *A. ervi* females in the reverse-light regime. Each mated female (<12 h old) was introduced into a Petri dish containing 20 healthy aphids and allowed to stay for 2 h (first oviposition bout). She was then transferred to another Petri dish with 20 healthy aphids and allowed to stay for 2 h (second oviposition bout). This was repeated until 8 and 4 oviposition bouts were completed in the photophase and scotophase, respectively.

The parasitised aphids from each oviposition bout (in a single Petri dish) were transferred to and reared in an above-mentioned plastic cylinder. To determine the number of eggs laid by *A. ervi* in oviposition bouts, 10 aphids were dissected in each oviposition bout 4 days after parasitisation and the number of larvae in each aphid was counted under the stereomicroscope (Leica MZ12, German). The remaining parasitised aphids were reared until mummification. The number of parasitisms in each oviposition bout was recorded as the number of aphids parasitised.

Statistical analysis

A chi-square test was used to determine the difference in emergence incidence between the photophase and scotophase. The rejection level was when $\chi^2 < \chi^2_{1,0.05} = 3.8415$. The Marascuilo procedure of the nonparametric analysis (Daniel 1990) was used to assess the sexual maturation. The rejection level was when $U_0^1 < \chi^2_{6,0.05} = 12.59$. All other data were analysed using an ANOVA. When significant differences in variables occurred, means were separated using a Tukey’s studentised range (HSD) test ($P < 0.05$). The proportion of female offspring data was subject to arcsine transformation before ANOVA, but untransformed means are presented in the paper. All analyses were conducted using SAS.

RESULTS

Emergence

The developmental time from oviposition to emergence was significantly shorter for males (mean±SE, 13.94±0.03 days) than that for females (14.31±0.03 days) ($P < 0.0001$).

The hourly emergence rate (mean±SE) was significantly higher in the photophase (5.99±0.94%) than in the scotophase (0.74 ± 0.32%) ($P < 0.001$). About 95% of parasitoids emerged in the photophase in both light regimes. On a 24 h basis, the male emergence peaked 2 h after light-on and the female emergence peaked between 3-6 h after light-on (Fig. 1).

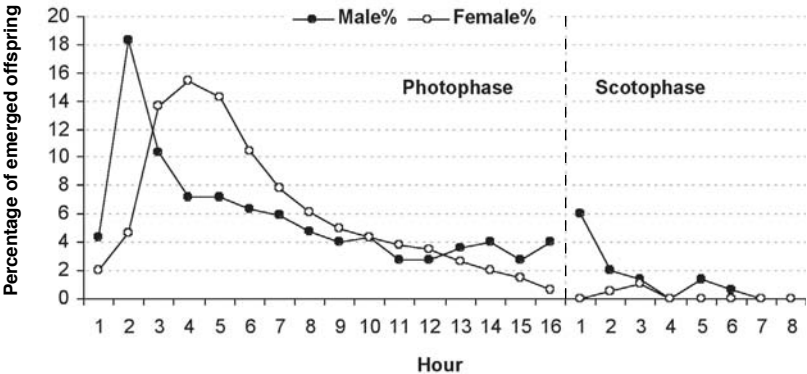


FIGURE 1: The percentage of female or male *A. ervi* offspring emerging throughout photophase or scotophase.

Sexual maturation

Newly emerged males were able to perform their courtship display to 12-h-old females but failed to mate until they were 4 h old (Fig. 2). However, 10- and 12-h-old males were significantly more likely to court females than ≤4-h-old males ($U_0^1 = 105.22$, $P < 0.0001$); mating success of 12-h-old males was also significantly higher than that of ≤4-h-old males ($U_0^1 = 80.01$, $P < 0.0001$) (Fig. 2).

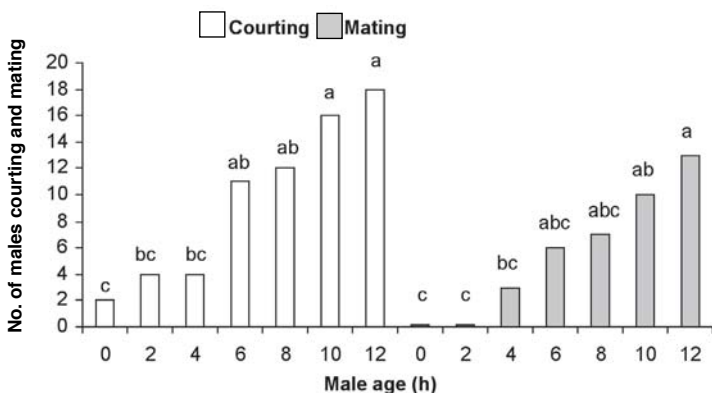


FIGURE 2: The number of *A. ervi* males courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Bars with the same letters are not significantly different ($P>0.05$).

Newly emerged females were able to entice the courtship display by 12-h-old males and successfully mate (Fig. 3). Twelve-h-old females were significantly more likely to respond to males' courtship display than of ≤ 4 -h-old females ($U_0^1 = 32.35$, $P<0.0001$); mating success of 12-h-old females was significantly higher than that of ≤ 2 -h-old females ($U_0^1 = 14.97$, $P<0.0001$).

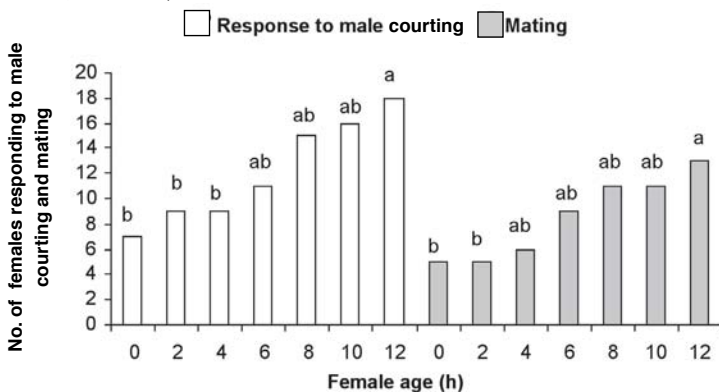


FIGURE 3: The number of *A. ervi* females responding to male courting or mating at 0, 2, 4, 6, 8, 10 or 12 h after emergence. Bars with the same letters are not significantly different ($P>0.05$).

Oviposition

Females oviposited in both photophase and scotophase. In the photophase, the mean (\pm SE) number of eggs laid (11.73 ± 2.73) and parasitism (8.6 ± 1.03) per oviposition bout was significantly greater than that in the scotophase (4.18 ± 0.88 eggs and 3.43 ± 0.69 parasitism, respectively) ($P<0.01$). In the photophase, the numbers of eggs laid and parasitism were significantly higher in the first oviposition bout ($P<0.0001$) (Fig. 4). However, no significant difference in number of eggs laid or parasitism between oviposition bouts was detected in the scotophase ($P>0.05$) (Fig. 4).

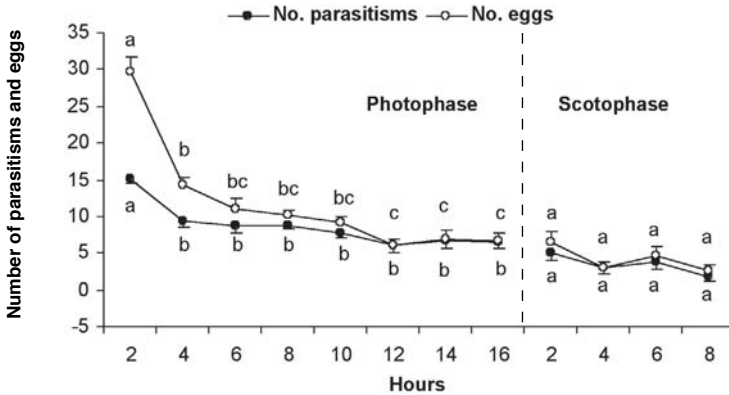


FIGURE 4: The number of parasitisms and eggs laid by *A. ervi* females throughout photophase or scotophase. Means (\pm SE) followed by the same letters within each line are not significantly different ($P>0.05$). Data from the photophase and scotophase were analysed separately.

DISCUSSION

Among parasitoid insects, adult emergence restricted to certain times of the day is known in several species, including *Trichogramma minutum* Riley (Corrigan et al. 1995), *Telenomus busseolae* Gahan (Fantinou et al. 1998) and *Encarsia formosa* Gahan (Lenteren et al. 1992). The results of the present study demonstrate that *A. ervi* males and females have an emergence peak during the first few hours of the photophase (Fig. 1). This suggests that the onset of light may act as a signal that synchronises the rhythmic function of *A. ervi* adult emergence.

This study has shown that *A. ervi* is a protandrous species with males emerging ca 9 h earlier than females. Singer (1982) argued that selection for protandry can only occur when generations are discrete. *Aphidius ervi* does not appear to match to that hypothesis because generations usually overlap in the field and females are readily available. However, *A. ervi* females mate only once during their lifespan, limiting the chance for males to encounter virgin females. Quicke (1997) suggested that the protandry is common in many parasitoids because a late-emerging male is likely to encounter already mated females and he is genetically doomed, as he cannot get any matings.

It has been reported that for some parasitic hymenopterans, a period of sexual maturation is necessary, for example, the bean weevil parasitoid, *Chryseida bennetti* Burks (Perez-Lachaud & Campan 1994) and the tortrix moth parasitoid, *Ascogaster reticulatus* Watanabe (Kainoh 1986). In the present study, newly emerged *A. ervi* females were able to mate with males but males need at least 4 h to become sexually mature. Therefore, the early emergence of *A. ervi* males may be a selective strategy for higher reproductive fitness. For example, early emerged males have a better chance to encounter virgin females (Nadel & Luck 1985, 1992), inseminate a greater number of the females they encounter (Waage & Ng 1984), and reduce the risk of females' death before oviposition (Fagerström & Wiklund 1982). Moreover, since virgin females start to lay eggs within 30 min after emergence (X.Z. He, unpubl. data), early emerged males are able to mate with females before their oviposition.

Diel periodicities of insect activity are often determined by a combination of endogenous and exogenous rhythms (Beck 1980). The decreasing oviposition and parasitism of *A. ervi* during the photophase may be the result of a decreasing load of mature eggs, as reported in the sycamore aphid parasitoid, *Monoctonus pseudoplatani*

(Marshall) (Collins & Dixon 1986). Furthermore, visual cues play an important role in host finding and attacking by *A. ervi* (Michaud & Mackauer 1994). In this study, *A. ervi* females laid fewer eggs and attacked fewer aphids during the scotophase even though they had sufficient eggs. This suggests that the oviposition pattern of *A. ervi* is determined by an exogenous factor, the light regime.

In conclusion, light appears to entrain movement, oviposition and emergence rhythms of *A. ervi*. Emergence of the adults in the morning probably coincides with more favourable conditions, such as the light, in which parasitoids may increase the chance for host habitat location, searching for mates and hosts, and oviposition. The findings of this study have implications for laboratory mass rearing and field release of *A. ervi*. For example, newly emerged parasitoids should be held for 12 h for copulation to occur before release, while the parasitoids should be released the following morning to achieve the higher reproductive output.

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