EMERGENCE AND REPRODUCTIVE RHYTHMS OF *EPHESTIA KUEHNIELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Mediterranean flour moth, *Ephestia kuehniella*, is a cosmopolitan pest of stored products, and its eggs are widely used to rear parasitoids and predators for biological control programmes. The laboratory investigations of circadian rhythms and lifespan patterns described in this paper have shown that females had significantly shorter developmental duration from egg to adult than males. Emergence occurred throughout the 24 h cycle in both sexes with a peak at dusk. Mating mainly occurred during scotophase and peaked on the emergence day, while oviposition peaked 1 day after emergence. Calling, courtship and mating peaked in the second half of scotophase, and oviposition peaked at the start of scotophase. Permanently paired insects mated up to 3 times, with an average of 2 ± 0.13 matings. Dissection of dead females showed the number of spermatophores in the bursa copulatrix equalled the number of observed matings.
Keywords: *Ephestia kuehniella*, Mediterranean flour moth, emergence, mating, oviposition, circadian rhythms.

INTRODUCTION

Circadian rhythms influence many aspects of insect biology, fine-tuning life functions to the temperature and light cycles associated with the solar day (Giebultowicz 2000). Moreover, variations in circadian rhythmicity can reduce direct competition between species that share the same resources, and synchronise mating activities to ensure genetic isolation of sibling species (Saunders 1982).

The Mediterranean flour moth (*Ephestia kuehniella* Zeller) is a major pest of stored grain products, particularly flour. More importantly, its eggs and larvae are widely utilised to rear parasitoids and predators for biological control of a number of pests and for research into the behaviour, biochemistry and molecular biology of parasitoids and predators (Corbet 1973; Rahman et al. 2004). The female sex pheromone has been identified for *E. kuehniella* and has been suggested for control applications (Trematerra 1994). Ayvaz et al. (2007) recently developed a sterile insect technique (SIT) for the control of this pest. However, adult circadian and daily activity patterns of *E. kuehniella* have not been thoroughly investigated, knowledge of which is important for the control of this pest using pheromones or SIT and improvement of natural enemy production using this pest as food. Only a few papers have briefly described the emergence (Bremer 1926; Moeiaety 1959) and reproductive behaviour (Calvert & Corbet 1973) of this pest.

In the present study, the details of emergence and subsequent adult reproductive activity patterns of *E. kuehniella* were investigated. This information will enhance our ability to better manipulate the population of *E. kuehniella* and provide vital information for further investigations on reproductive behaviour, particularly sexual selection and sperm competition in this species.

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MATERIALS AND METHODS

Insects

Insects were maintained in plastic cylinders (8 cm diameter × 10 cm height), each filled with 50 g of a standard diet (43.5% wholemeal wheat flour, 43.5% maize meal, 3.0% yeast and 10% glycerine) (Lima et al. 2001), in the Entomology and IPM Laboratory of Massey University, Palmerston North. Cylinders were covered with two layers of nylon mesh. To start the colonies, 116 newly laid eggs (< 24 h old) (Xu et al. 2007) were introduced into each cylinder with the standard diet. Two crumpled paper towels (25×25 cm) were placed in each cylinder for pupation. Adults were given neither food nor water because this was unnecessary (Norris 1934). All experiments were carried out at 25±1°C and 70±10% RH, with a photoperiod of 14:10 h light:dark.

Adult emergence

To determine daily adult emergence rates and circadian emergence rhythm of this insect, *E. kuehniella* eggs were set up as outlined above in separate rearing rooms set with either: (1) normal photoperiod – lights on from 10:00 to 24:00 and off from 24:00 to 10:00 or (2) reversed photoperiod room – lights on from 22:00 to 12:00 and off from 12:00 to 22:00. For each room, 10 cylinders of 116 insects were set up and the number of emerged adults was recorded daily.

To detect the circadian emergence on an hourly basis, the number of emerged adults was recorded hourly during the photopase in the normal photoperiod room and the scotophase in the reversed photoperiod room on the 8th and 11th days after the first emergence. Data were pooled and presented. The number of adults emerged during the photophase and scotophase was compared using the paired-sample t test (Zar 1999). The number of adults emerged from the normal photoperiod room was recorded daily at 10:00 to determine the daily emergence pattern. A Mann-Whitney two-sample two-tailed rank test (Zar 1999) was used to determine whether daily emergence differed between sexes.

Adult activity patterns

Late pupae (when they turned dark, Karalius & Buda 1995) were collected from the crumpled paper towels and kept individually in glass vials (2 cm in diameter × 7.5 cm in height) until adult emergence to ensure virginity. The emerged moths were sexed and kept in the same glass vials before being used for experiments.

To observe adult activity patterns on a 24 h basis, 20 male and 20 female adults (< 12 h old, virgin) randomly selected from each of the above-mentioned two groups were paired in above-mentioned cylinders and maintained in their original rearing room for their lifespan. Each plastic cylinder with a pair of moths was lined with a multipore plastic film (Wicket bag plain perforated, 15 µm, Cryovac™ ; W.R. Grace Ltd, Auckland) as an oviposition substrate and was covered with the same plastic film secured with a rubber band. Behaviour observations were made during the photophase in the normal photoperiod room and scotophase in the reversed photoperiod room, and illumination during the scotophase was provided by a red light tube. Activity of both sexes was observed every 10 min by quickly scanning all pairs and recording the following: courtship – the male jumping and fanning his wings over or around the female or if the male exposed his genitalia trying to engage the female's genitalia; calling – the female protruding her abdomen between the wings with the tip everted (Dickins 1936); mating – the two insects engaged by the tip of the abdomen; oviposition – the female protruding her ovipositor to find oviposition site or to lay eggs. The total number of times each activity was recorded in a particular hour in the first four days after emergence is presented. Most *E. kuehniella* mating and oviposition occurs during the first 4 days after emergence (J. Xu, unpubl. data). The remating events of the insects in the reversed photoperiod room were recorded hourly during the scotophase and 1 hour into the photophase for their lifespan (the mating duration of this species is about 2 h). Dead females were dissected and the number of spermatophores in their bursa copulatrix was counted under a dissecting microscope (Olympus SZ2, Japan).

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RESULTS

Emergence

The Mann-Whitney rank test indicated that adult females emerged significantly earlier than males (P<0.001) (Fig. 1). About 60% of females had emerged during the first 8 days of emergence while only 44% of males emerged during this period. Adult emergence occurred throughout the 24 h cycle in both sexes and peaked 3 hours before lights off (Fig. 2). However, analysis showed that the number of adults that emerged during the scotophase (25.1 ± 1.0%, mean ± SE) was significantly lower than that during the photophase (74.9 ± 1.0%) (P<0.001).

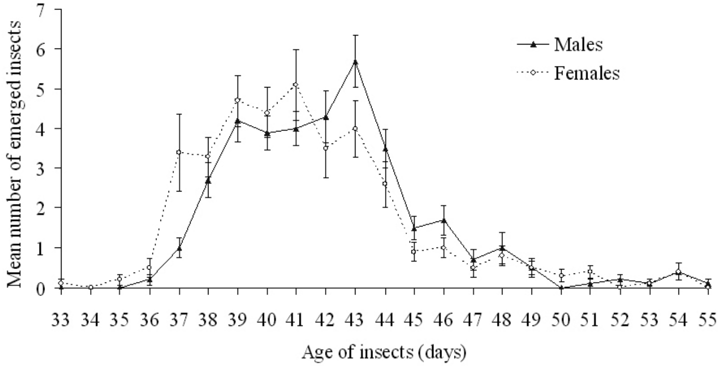

FIGURE 1: Daily emergence of females and males *Ephestia kuehniella* adults. Bars are SE.

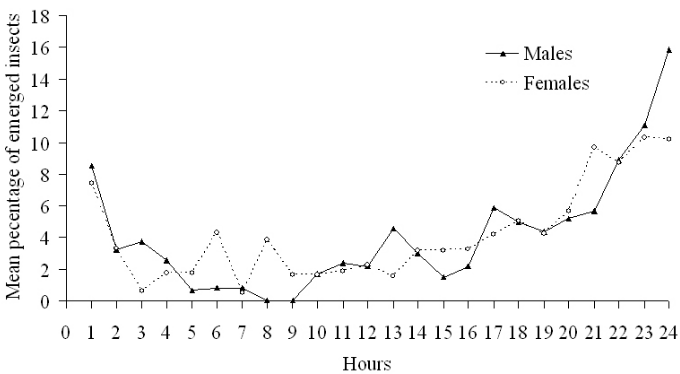

FIGURE 2: Circadian adult emergence rhythms of *Ephestia kuehniella* (lights on at 10:00 and off at 24:00).

Activity patterns

Circadian rhythms of adult activities during the first 4 days after emergence are shown in Figure 3. Adult activity took place mostly during the scotophase, calling, courtship and mating continued to the early hours of the photophase (Figs 3a-c). Two days after emergence, both calling and courtship showed an obvious peak in the last

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hour of the scotophase. All paired insects performed the first mating within the first day after emergence and peaked at midnight but remating peaked at dawn on the subsequent 3 days (Fig. 3c). Paired insects could mate up to three times, with an average of 2 ± 0.13 matings (Fig. 4). Females started to lay eggs 1 day after the first mating, during the night, and oviposition activity peaked during the first few hours of the scotophase and declined sharply afterwards (Fig. 3d). The number of spermatophores in female bursa copulatrix was equal to the number of matings observed.

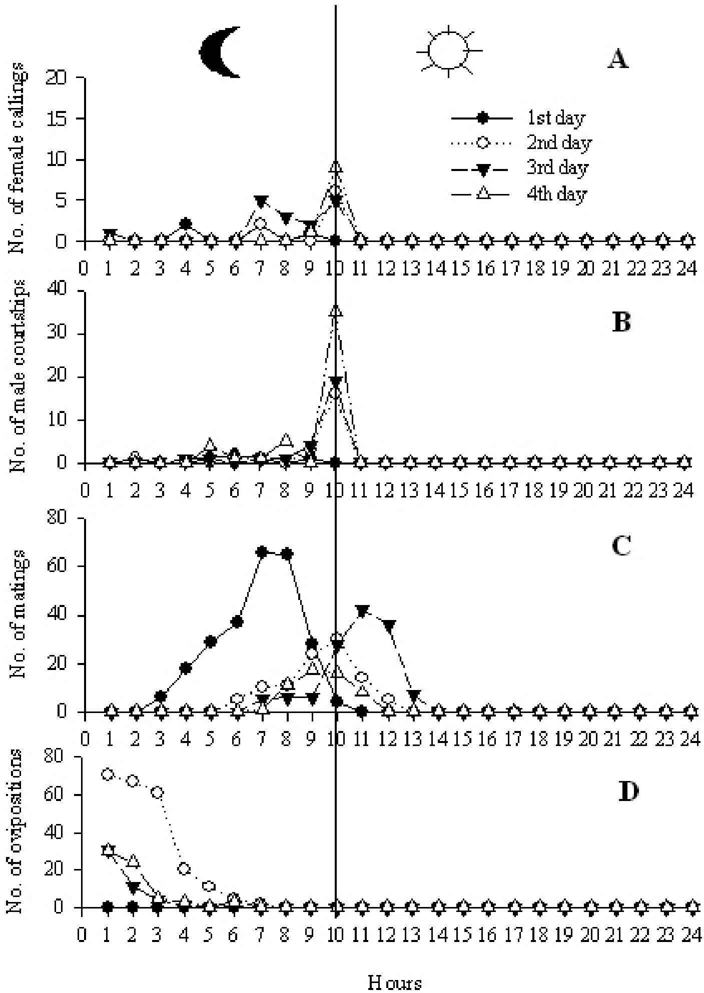

FIGURE 3: Circadian reproductive rhythms of *Ephestia kuehniella*, (a) female calling, (b) male courtship, (c) mating and (d) oviposition. The lights went on at 10:00 and off at 24:00.

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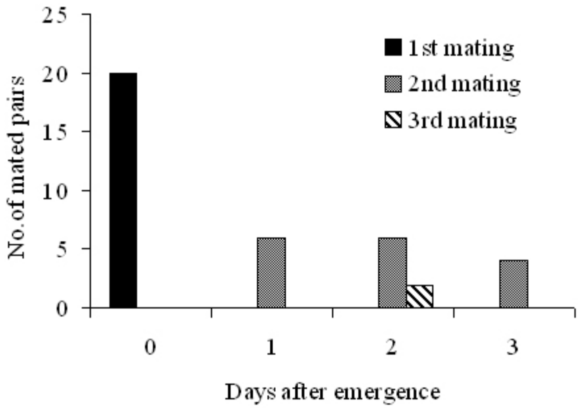

FIGURE 4: Daily mating rhythms of *Ephestia kuehniella*.

DISCUSSION

These results suggest that *E. kuehniella* is a protogynous species, since females emerged significantly earlier than males and all paired females and males mated on the emergence day. Protogyny may be a mechanism that has evolved to reduce inbreeding (Rhainds et al. 1999) because early emerged females are less likely to mate with their brothers. Norris (1934) found that some mature eggs were already present at the time of adult emergence in *E. kuehniella*, and Calvert & Corbet (1973) showed that the male and female reproductive systems of this insect became mature soon after emergence. Therefore, pairing *E. kuehniella* on the emergence day may result in highest fecundity and fertility.

Calling and courtship peaks were always followed by the mating peak, suggesting that female calling and male courtship are essential for successful matings in this species. Therefore, using sex pheromone for mating disruption or mass trapping in *E. kuehniella* appear to be control tactics worth investigating.

Oviposition did not occur until the second night after emergence. This may be because the movement of the sperm from the bursa copulatrix to vestibulum where fertilisation is achieved (Benz 1991) needs a few hours, and the time between mating and the end of scotophase in the first night is too short for females to initiate oviposition at the same night.

Other work from this study shows that *E. kuehniella* is a polyandrous species (J. Xu, unpubl. data). Female remating increases her chance of mating with a wild, fertile male and thus decreases the effectiveness of the sterile insect technique (SIT) (Kraaijeveld et al. 2005). However, the negative effects of remating on SIT may be ameliorated by increasing the overflooding rate (the ratio of sterile males to wild females), or releasing sterile males after mass trapping using female sex pheromones to remove wild males.

On the circadian basis, both emergence and reproductive activities of *E. kuehniella* were highly rhythmic. It is suggested that the end of photophase (emergence peak) and the start of scotophase (oviposition peak) are optimal time to collect fresh moths and eggs, respectively, for research or natural enemy rearing.

This study has provided the foundation for the better manipulation of *E. kuehniella* and future studies of reproductive behaviour of this pest, particularly sexual selection and sperm competition.

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