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BEHAVIORAL RHYTHMS OF DROSOPHILA SUZUKII AND DROSOPHILA MELANOGASTER

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Abstract

Drosophila suzukii and *Drosophila melanogaster* feed on various fruits, causing great economic losses. In order to find the optimum time for controlling *D. suzukii* and *D. melanogaster*, the daily rhythms of oviposition, egg hatch, pupation, adult eclosion, copulation, and feeding of these two pests were studied. We found the circadian rhythm of *D. suzukii* oviposition to have a single pattern with a peak from 20:00-24:00, while the peak oviposition of *D. melanogaster* was from 16:00-4:00 (the next day). Neither *D. suzukii* nor *D. melanogaster* showed a daily pattern of egg hatch; the single peak of egg hatch for *D. suzukii* occurred 24-32 h after oviposition, while that for *D. melanogaster* followed a bimodal pattern, with the first peak of egg hatch from 0-4 h after oviposition and the second from 32-36 h after oviposition. Pupation in *D. suzukii* showed a single peak from 8:00~16:00, while in *D. melanogaster* pupation followed a bimodal pattern, with peaks from 4:00-8:00 and 12:00-20:00. Eclosion of of *D. suzukii* adults followed a unimodal pattern, and generally took place from 0:00-8:00, while that of *D. melanogaster* also showed a single peak, generally from 0:00-12:00. Meanwhile copulation of *D. suzukii*, which showed a bimodal pattern, was concentrated from 0:00-12:00 and 20:00-24:00 (the next day), while copulation of *D. melanogaster* showed a single peak, generally from 0:00-12:00. Both *D. suzukii* and *D. melanogaster* had a preference for feeding in light, and in a 24 h photoperiod the percentages of feeding insects were 80.8and 81.1, respectively.

Key Words: circadian rhythm, egg hatch, pupation, adult eclosion, feeding, copulation, oviposition

Resumen

Drosophila suzukii y *Drosophila melanogaster* se alimentan de varias especies de frutas, causando grandes pérdidas económicas. Con el fin de encontrar el momento óptimo para el control de *D. suzukii* y *D. melanogaster*, los ritmos diarios de la oviposición, la eclosión de los huevos, la fase de pupa, la eclosión de adultos, la cópula, y la alimentación de estas dos plagas fueron estudiados. Encontramos que el ritmo circadiano del la oviposición de *D. suzukii* tiene un patrón único con un pico de 20:00 a 24:00, mientras que el pico de oviposición de *D. melanogaster* fue de 16:00-4:00 (al día siguiente). Ni *D. suzukii* ni *D. melanogaster* mostraron un patrón diario de eclosión de los huevos; el único pico de eclosión de los huevos de *D. suzukii* ocurrió 24-32 horas (h) después de la oviposición, mientras que la de *D. melanogaster* siguieron un patrón bimodal, con el primer pico de eclosión de los huevos de 0-4 h después de la oviposición y el segundo 32-36 h después de la oviposición. La pupación en *D. suzukii* mostró un solo pico de 8:00 a 16: 00, mientras que la pupación en *D. melanogaster* siguió un patrón bimodal, con picos de 4:00-8:00 y 12:00-20:00. La eclosión de los adultos de *D. suzukii* siguió un patrón unimodal y en general se realizó entre 0:00-8:00, mientras que la de *D. melanogaster* también mostró un solo pico, generalmente entre 0:00-12:00. Mientras tanto, la cópula de *D. suzukii*, que mostró un patrón bimodal, se concentró entre 0:00-12:00 y 20:00-24:00 (al día siguiente), mientras que la copulación de *D. melanogaster* mostró un solo pico, generalmente entre 0:00-12:00. Tanto *D. suzukii* y *D. melanogaster* tenían una preferencia para alimentarse en la luz y en un fotoperiodo de 24 h el porcentaje de insectos alimentandose fue 80.8 y 81.1, respectivamente.

Palabras Clave: ritmo circadiano, eclosión de los huevos, fase de pupa, eclosión de adultos, alimentación, cópula, oviposición

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) and *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) are both important fruit pests. *Drosophila suzukii* is especially damaging to ripe cherries (Van der Linde et al. 2006). It's hard, serrated ovipositor can easily pierce the fruit skin, inserting eggs within intact fruits with little visible damage from oviposition on the fruit surface. The larvae hatching from eggs feed on the fruit, and cause it to soften, brown and completely rot. Many fruits are damaged by *D. suzukii*, including blueberry, blackberry, cherry, strawberry, plums, peaches, grapes, figs, kiwi fruit and pears (Dreves et al. 2009). In the United States, losses of strawberry, blueberry and raspberry caused by *D. suzukii* reached 80, 40, and 70%, respectively (Bolda et al. 2011). With increased planting of fruit trees in China, *D. melanogaster* has increased in importance as a pest of Chinese bayberry and cherries. In Tianshui, Gansu Province, some late-maturing varieties of cherries are especially susceptible to damage by *D. melanogaster*, and they suffer losses generally above 35%, and as high as 80% on some cultivars (Guo et al. 2007).

Daily biological rhythms are common in insects (Pittendrigh 1993; Takahashi 1995). Many life activities of insects, such as phototaxis, body color change, migration, feeding, hatching, eclosion, mating and oviposition exhibit a rhythm (Saunders 2002). It is helpful to determine the activity rhythms of populations of both beneficial and harmful insects as a basis for developing and improving methods for preventing or controlling the latter (Tu & Chen 2013). Applications of insect pheromones in pest control developed in recent decades, are based to some extent on studies of the timing of eclosion and rhythms of sexual activities insect pest species, and it may be possible to further improve pheromone-based control technology through better understanding of the circadian rhythms of pest species (Ran et al. 2013). There have been many studies on the behavioral rhythms of *D. melanogaster,* but few on those of *D. suzukii*. This study examines the rhythms

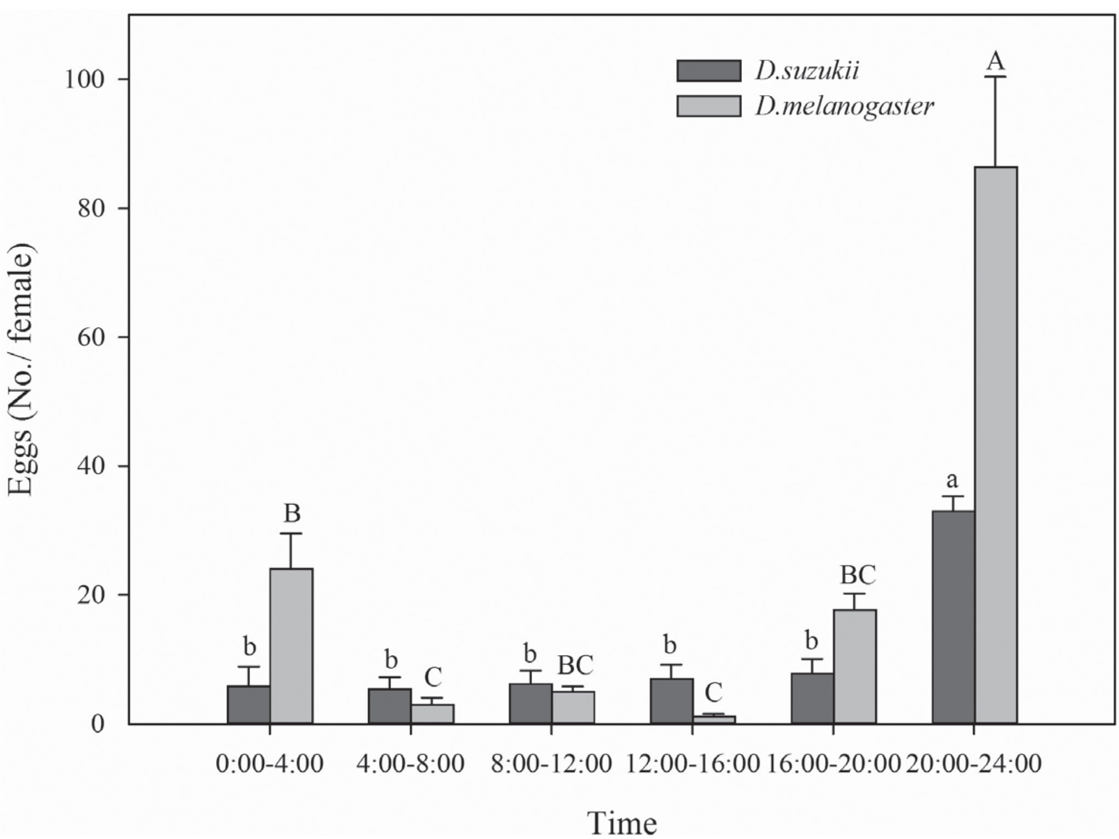

Fig. 1. The ovipositional rhythms of *Drosophila suzukii* and *Drosophila melanogaster*. Data represent the mean values ± SEM (n = 4) for 4-h periods, and histograms with different letters indicate a significant difference (P = 0.05, Duncan's multiple range test), i.e., lower case letters show a significant difference for *D. suzukii*, and upper case letters C show a significant difference for *D. melanogaster.* Fecundity of *D. melanogaster* was much greater than that of *D. suzukii.* Lights were turned on at 4:30 am and off at 20:30 pm.

of both species with respect to oviposition, egg hatch, pupation, adult eclosion, feeding and copulation. This information further understanding of the biological characteristics of *D. suzukii* and *D. melanogaster* these species, and may provide an important basis and technical guidance for their integrated control.

Materials and Methods

Experimental Insects

Both *D. suzukii* and *D. melanogaster* were obtained as larvae from infested fruit from cherry orchards in Tai'an, Shandong Province in May 2012, and flies were then raised in an insectary for about 11 generations at 25 ± 1 °C, 70 ± 5% RH, and 16:8 h (L:D) with the lights were turned on at 4:30 am and off at 20:30 pm. This light regime approximated the local natural photoperiod. The off light intensity was 10,000 lux. Table grapes ('Kyoho' grape cultivar, *Vitis vinifera* L.; Vitales: Vitaceae), purchased from the market, were rinsed 3 times in distilled water and dried and cut into halves for adult oviposition and larval development. The average weight of each grape was 20.38 g.

Oviposition Rhythm

Females 4-6 days after emergence were used for quantifying the oviposition rhythm. These females had mated and could have laid eggs for 2-3 days. Each group had 5 individual females with 4 replicates of either *D. suzukii* or *D. melanogaster*. They were placed into circular insectary bottles (2000 mL flat drum glass bottles) containing one fresh grape cut into halves. The grapes were replaced every four hours for 48 h, and the number of eggs laid was determined. The experiment was replicated 4 times.

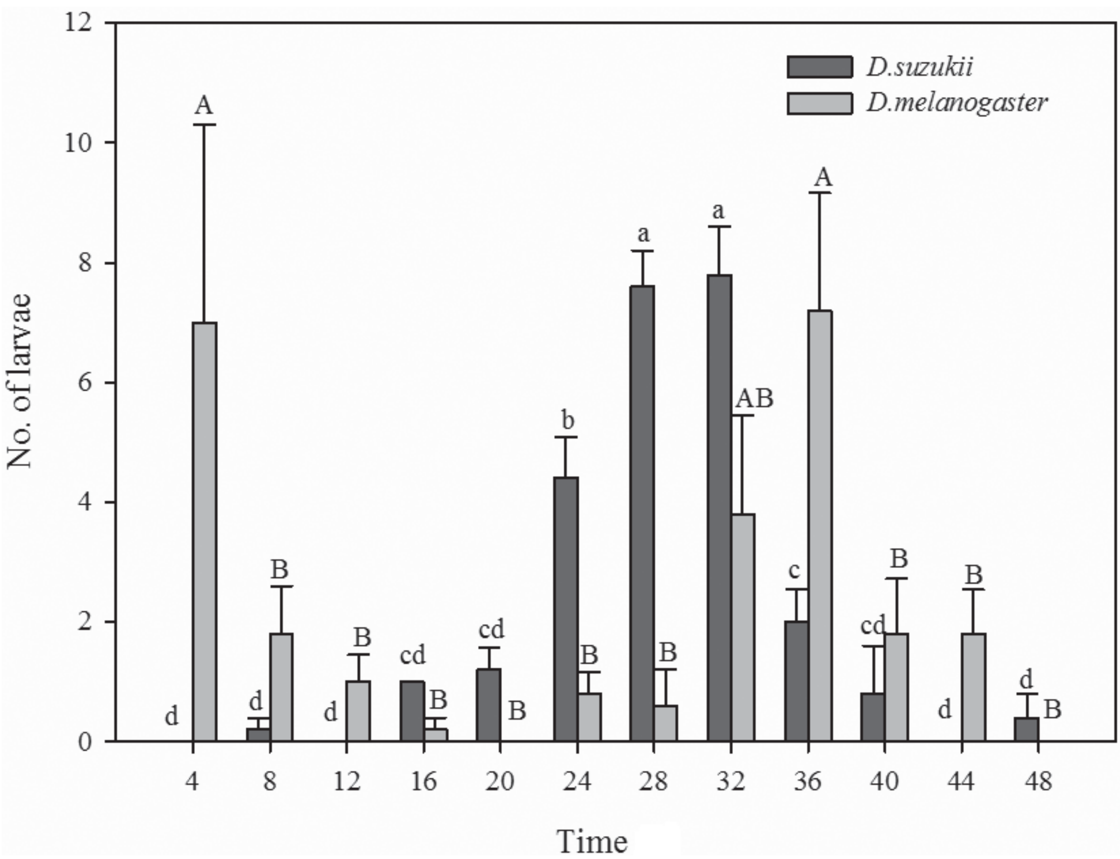

Fig. 2. The incubation rhythms of *Drosophila suzukii* and *Drosophila melanogaster.* Data represent the mean values ± SEM (n = 5) at 4 h, and significant differences at P = 0. 05 by Duncan multiple range test. Most *D. suzukii* eggs hatched with a single peak at 24-32 h after the eggs were laid, but *D. melanogaster* eggs hatched with two peaks, one at 4 h and the second at 32-36 h after eggs were laid. Error bars topped with different letters are significantly different.Lights were turned on at 4:30 am and off at 20:30 pm.

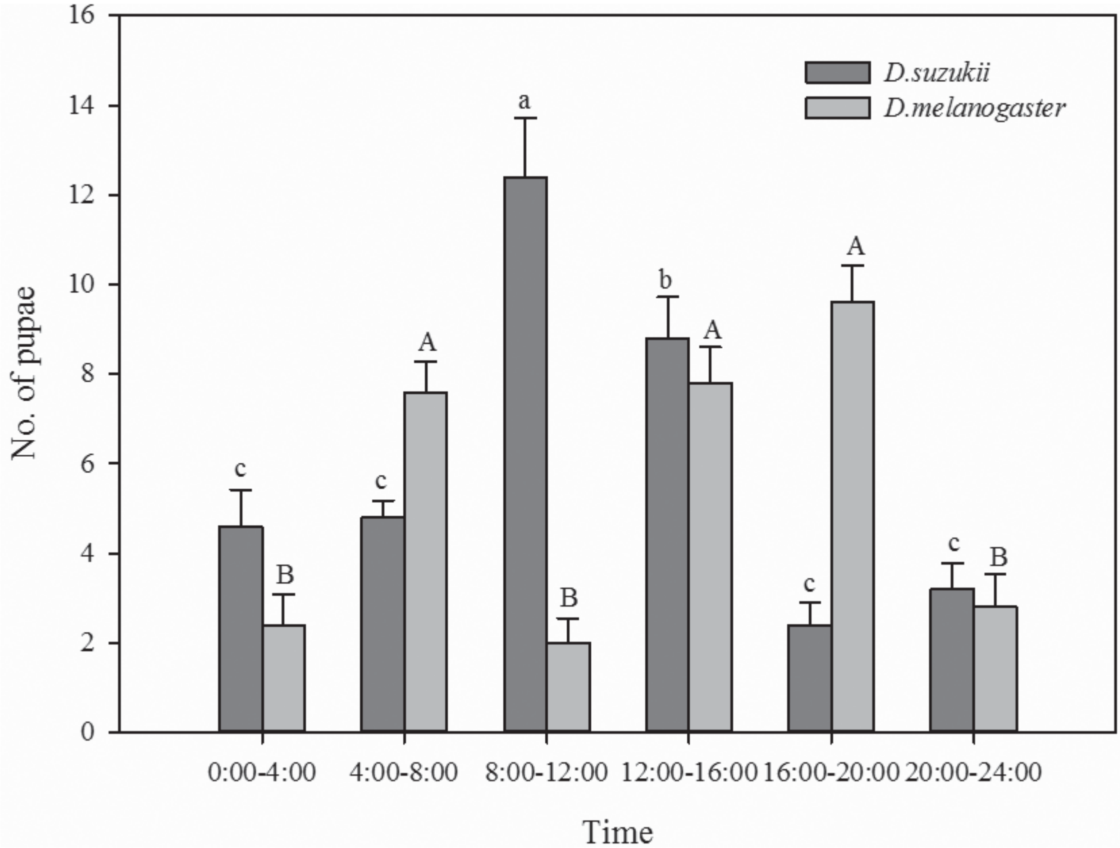

Fig. 3. The pupation rhythms of *Drosophila suzukii* and *Drosophila melanogaster*. Data represent values ± SEM (n = 5) in 4 h observation periods, at 4 h, and histograms with different letters indicate a significant difference (P = 0.05, Duncan's multiple range test). Most *D. suzukii* larvae pupated with a single peak between 8:00 to 16:00, but most *D. melanogaster* larvae pupated with two peaks, one at 4:00-8:00 and the second at 12:00-20:00. Error bars topped with different letters are significantly different. Lights were turned on at 4:30 am and off at 20:30 pm.

Hatching Rhythm

Grapes were cut into halves and placed in the circular bottle described above with the cut surface upward to provide food for the fruit flies. A certain number of either mated *D. suzukii* or *D. melanogaster* females were selected and each cohort was held in a bottle usually for 4 h to lay eggs. The number of new larvae from hatched eggs was counted every 4 h after oviposition for a continuous 48 h observation period. The experiment was replicated 5 times for each species.

Pupation Rhythm

In this experiment, each group had 40 individual 3rd instar larvae of either *D. suzukii* or *D. melanogaster*. The larvae were reared in petri dishes (12 cm dia., 1.5 cm high), and fed mashed grapes. The number of pupae was counted every 4 h over 48 h. The experiment was replicated 5 times.

Adult Eclosion Rhythm

In this experiment, each group had 30 pupae of either *D. suzukii* or *D. melanogaster*. The pupae were placed in petri dishes (12 cm dia, 1.5 cm high) with cotton soaked in distilled water to stabilize the relative humidity. Total emergence and the numbers of eclosed males and females were calculated every 4 h over a 48 h observation period. The experiment was replicated 5 times.

Copulation Rhythm

Two hundred unmated male and 200 female *D. suzukii* flies were selected and held separately by sex in 2,000 mL flat drum bottles. After 12 h, the males and females were divided into 4 groups each with 50 of each gender, and the number of pairs of mated flies was counted every 4 h over 48 h. The identical experiment was conducted with *D. melanogaster*.

Feeding Rhythm

Two hundred healthy adults of either *D. suzukii* or *D. melanogaster* were released into a closed screened cage (1.0 × 0.8 × 0.8 m). A transparent plastic bottle filled with a 10 mL honey-water mixed with 1 mL emamectin benzoate (2.2%) solution was hung in the cage and the numbers of flies feeding from the bottle every 4 h over 48 h period were counted. The experiment was replicated 4 times.

Statistical Analysis

The means were assessed with one-way ANOVA, significant differences ($P < 0.05$) were tested with Duncan's test. All statistics were analyzed in SPSS 17.0.

Results

Oviposition Rhythm

In this experiment, *Drosophila suzukii* females laid a total of 1,297 eggs, with the egg-laying peak extending from 20:00-24:00. In this peak each female laid an average of 33 eggs, which accounted for 50.9% of the eggs one female laid in a single light cycle. Likewise *D. melanogaster* females laid a total of 2,743 eggs, with the egg-laying peak extending across 16:00-4:00 (the next day). During this protracted peak each female laid and average of 128.06 eggs, which accounted for 93.4% of the total per female in a single circadian period (Fig. 1).

Egg Hatching Rhythm

In total, 132 *D. suzukii* eggs and 130 *D. melanogaster* eggs were observed to hatch per 5-female replicate; i.e., an average of 26.4 and 26.0 per female, respectively. Per replicate an average 19.8 *D. suzukii* eggs of hatched 24~32 h after the eggs were laid; i.e., 75% of the eggs that hatched. Hatching of *D. melanogaster* occurred in 2 peaks, the first at 0~4 h and the second 32~36 h after oviposition. In the first peak 7.0 eggs hatched, and in the second peak, 11.0 eggs hatched. These two peaks accounted for 26.9% and 42.3% of the total eggs that hatched, respectively (Fig. 2). No

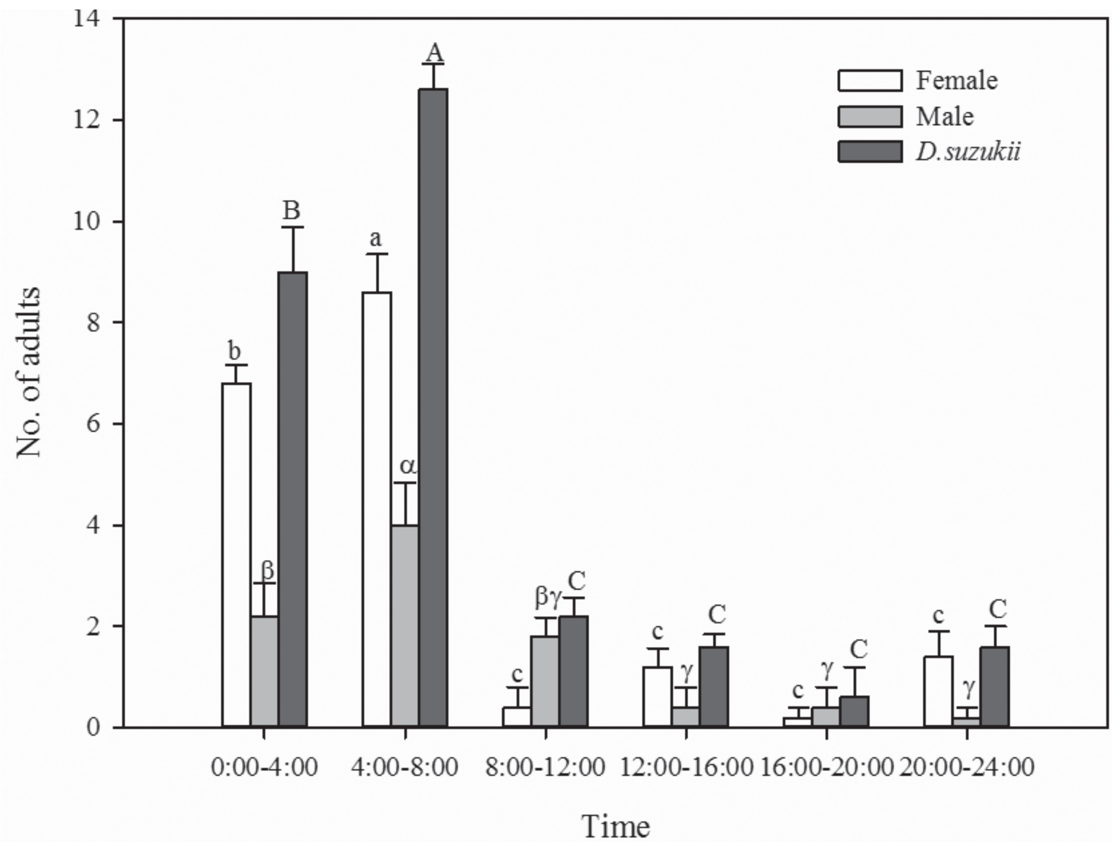

Fig. 4. The eclosion rhythms of *Drosophila suzukii* males and females. Most adults emerged during the hours that correspond to dawn. The number of females was larger than the number of males. Error bars topped with different letters are significantly different. Lights were turned on at 4:30 am and off at 20:30 pm.

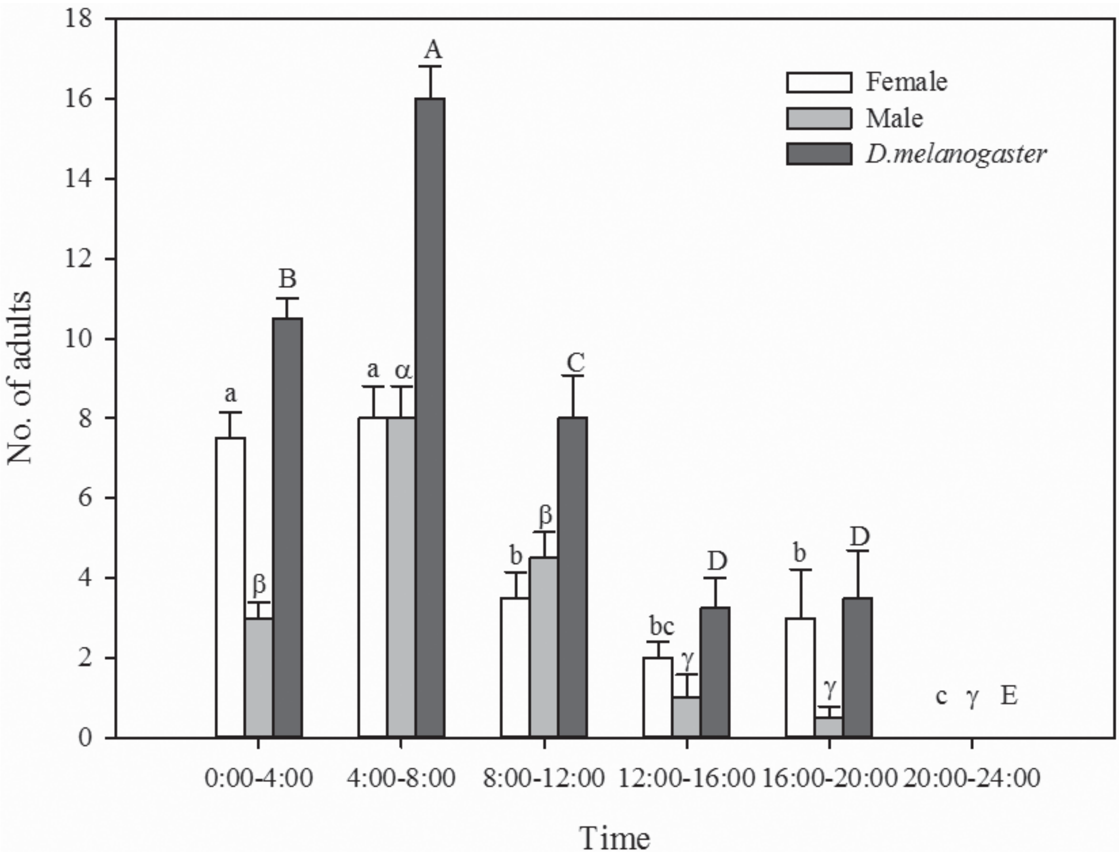

Fig. 5. The eclosion rhythms of *Drosophila melanogaster* males and females. Most adults emerged during the hours that correspond to dawn. The number of females was larger than the number of males. Error bars topped with different letters are significantly different. Lights were turned on at 4:30 am and off at 20:30 pm.

photoperiodic rhythm of egg hatching was found in either species.

Pupation Rhythm

A total of 181 third-instar *D. suzukii* larvae pupated, i.e., an average of 36.2 pupae per 5-female replicate. Pupation occurred in a single major peak from 8:00~16:00 during which 21.2 pupae were formed, accounting for 58.6% of the pupae. In contrast, 161 *D. melanogaster* larvae pupated, i.e., an average of 32.2 pupae per 5-female replicate. Most pupation occurred in 2 peaks from 4:00-8:00 and 12:00-20:00. During the first peak 7.6 pupae were formed and during the second peak 17.4 pupae were formed. These peaks accounting for 23.6% and 54.0% of the pupae, respectively (Fig. 3).

Adult Eclosion Rhythm

Adults emerged from 138 *D. suzukii* pupae and from 206 *D. melanogaster* pupae. Both species exhibited a unimodal pattern of emergence with a peak during the 0:00~8:00 period. The eclosion rates for *D. suzukii* and D. melanogaster were 78.3% and 83.6%, respectively (Figs. 4 and 5).

Copulation Rhythm

Drosophila suzukii adults were observed to mate 192 times. Matings were distributed in a bimodal pattern and both two peaks occurred from 20:00~12:00 (the next day). These two peaks accounted for 89.6% of the total matings. In contrast, the 217 matings of *D. melanogaster* were generally concentrated in the period 0:00~12:00, and this peak accounted for 96.8% of the total copulations (Fig. 6).

Feeding Rhythm

A total of 638 *D. suzukii* and 752 *D. melanogaster* adults were trapped during the observation period. Both species were found to favor feeding during the photophase. During 4:00-20:00, 128.8

D. suzukii and 152.5 *D. melanogaster* adults were found to feed, i.e., on average 80.8% and 81.1%, respectively. The study revealed that slightly more flies of both species tended to feed during the forenoon than in the afternoon, i.e., 42.5% of *D. suzukii* and 45.0% of *D. melanogaster* fed during the forenoon (Fig. 7).

Discussion

Reports on the ovipositional rhythms of insects began to appear in the 1950s and have gradually increased in recent years (Haddow & Gillett 1957; Pittendrigh & Minis 1964; Minis 1965). These reports included research on *Ostrinia nubilalis* and *Rhodnius prolixus* (Skopik & Takeda 1980; Ampleford & Davey 1989). Several studies on the ovipositional rhythms of fruit flies have involved *D. melanogaster*, other Drosophila spp. and *Zaprionus* spp. (Rensing & Hardeland 1967; Gruwez et al. 1972; David & Fouillet 1973; Allemand 1974, 1976a, 1976b, 1976c, 1977). Fleugel (1978) studied the egg production of *D. melanogaster* individuals in weak light of the light-dark cycle, and found an oviposition rhythm under 12:12 h L:D conditions, which he held to be the hourglass timing mechanism, not an endogenous rhythm. Many recent studies, however, have found that wild and mutant varieties of fruit flies also display rhythmic oviposition (McCabe & Birley 1998; Sheeba et al. 2001). This study found the oviposition of both *D. suzukii* and *D. melanogaster* followed a circadian rhythm, with the effects of light having a greater effect on *D. suzukii,* which showed two oviposition peaks compared to one peak by *D. melanogaster*.

A previous study on *Dacus tryoni* found that the rhythm of egg hatching played a certain role in total egg development (Bateman 1955). Meanwhile, our study found that neither *Drosophila* species displayed a photoperiodic rhythm for egg hatch, nor while both species' eggs may contain a timing mechanism, it is not related to the circadian system.

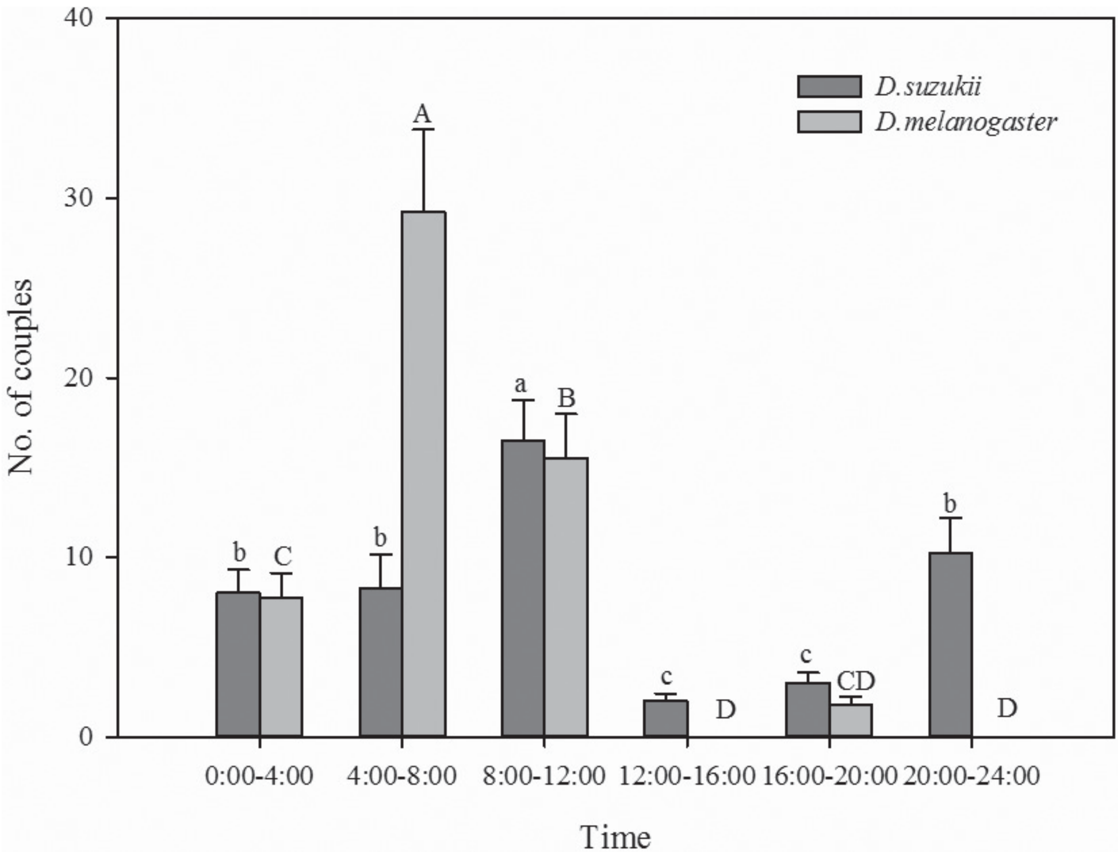

Fig. 6. The copulation rhythms of *Drosophila suzukii* and *Drosophila melanogaster*. Data represent the mean values ± SEM (n = 4) in 4 h observation periods. Few matings of either species were observed between 12:00 and 20:00. Error bars topped with different letters are significantly different. Lights were turned on at 4:30 am and off at 20:30 pm.

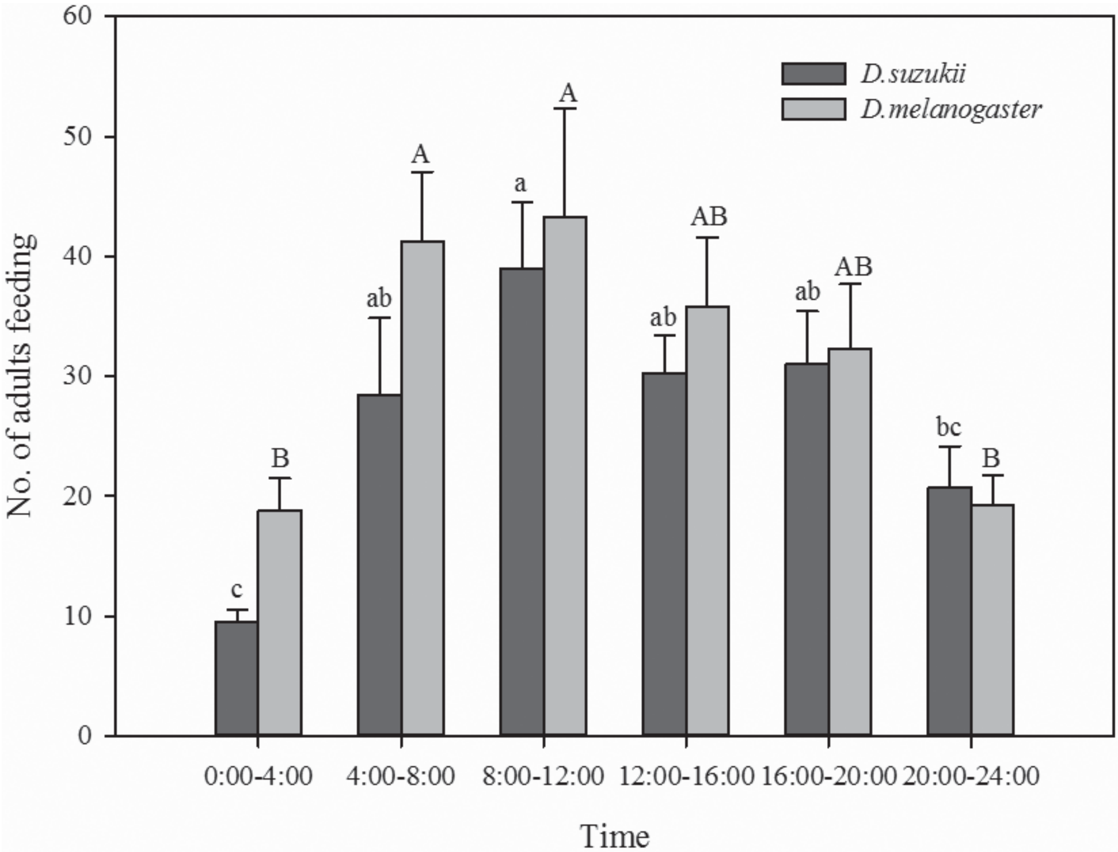

Fig. 7. The feeding rhythms of *Drosophila suzukii* and *Drosophila melanogaster*. Data represent the mean values ± SEM ($n = 4$) in 4 h observation periods. Both species fed more extensively during the photophase than during the scotophase. Error bars topped with different letters are significantly different. Lights were turned on at 4:30 am and off at 20:30 pm.

While the behavior of Sarcophagidae larvae before pupation and adult eclosion were found to be rhythmic (Saunders 1986), pupation itself was not rhythmic (Richard et al. 1986), possibly because pupation occurred underground. The two *Drosophila* species in this study, however, both showed different pupation rhythms and the process occurred aboveground. As peak pupation of *D. suzukii* was in the morning, it seems likely that light promotes the formation of the puparium. The two peaks of *D. melanogaster* occurred in the morning and afternoon, and such a rhythm may serve to protect the newly emerged adults from the effects of intense sunlight.

Emergence rhythms of a variety of insects have been studied, but those of *Drosophila* spp. have been reported on in the most detail. The circadian rhythm period of *Drosophila* emergence is about 24 h, and the eclosion rhythms are endogenous. The emergence of *Drosophila pseudoobscura*, for instance, is usually concentrated at dawn, and the peak shifts with changing photoperiod (Pittendrigh 1965). For example, the emergence peak occurred before dawn under short illumination conditions (light periods shorter than 6-7 h), after dawn under long photoperiod conditions, and from 2~3 h after the start of the photoperiod under 12:12 h L:D conditions (Bünning 1935). This study found that both *D. suzukii* and *D. melanogaster* had a single peak, generally concentrated from 0:00~8:00 and 0:00~12:00, respectively. One of the possible explanations is that relative humidity and cool air favor the expansion of wings in newly emerged adults, and high temperatures disturb the process of wing expansion significantly (Tanaka & Watari 2009; Shereen & Shakunthala 2012). In addition, among the newly emerged adults of the two species, females were found to outnumber males, and this may be related to the carbon/nitrogen ratio in the diet.

While a mating rhythm controlled by an endogenous biological clock has been confirmed in some insects (Smith 1979), the mating behavior of insects is affected to some extent by photoperiod.

The mating rhythms of *Anastrepha ludens* (Flitters 1964) and *Dacus tryoni* (Tychsen & Fletcher 1971) both showed a certain light-based cycle. Our study found that *D. suzukii* mating had a bimodal pattern, with the peak concentrating in 20:00 ~ 12:00 (the next day), indicating that individuals' mating ability might be affected by light duration in one photoperiod. The mating of *D. melanogaster* was unimodal, with more than 50% concentrated in the 4 h immediately after the dark period and more than 80% in the 8 h following the dark period, showing an extremely significant effect of photoperiod.

No research on the feeding rhythm of fruit flies has been found, but other studies found that cockroaches are the most active at night and usually feed in the dark. The American cockroach, *Periplaneta americana* feeds in the early to mid-dark period, exhibiting an endogenous circadian rhythm (Lipton & Sutherland 1970). Nymphs and adults of the cricket *Acheta domesticus* also showed a feeding rhythm (Nowosielski & Patton 1963). In laboratory experiments, bumble bee foragers showed free-running circadian rhythms in both LL and DD, with mean free-running periods significantly shorter in LL than DD (Stelzer et al. 2010). In addition, Xiao et al. (2009) found that a wild variety of *D. melanogaster* exhibited an obvious bimodal feeding pattern with peaks in the morning and evening. In our study, both *D. suzukii* and *D. melanogaster* individuals preferred to feed in the light, with the percentage of feeding individuals in the morning being slightly larger than that in the afternoon.

The damage caused by *Drosophila* larvae feeding inside fruit is imperceptible at first, and as the systemic use of insecticides increases the risk of residues in fruit, adult trapping techniques are an important tool in the prevention and control of fruit flies (Sun et al. 2005). Behavioral rhythms factor into the control of *D. suzukii* and *D. melanogaster*, and an understanding of these patterns should be helpful in the forecasting populations and further improving the control of these pest species.

Acknowledgments

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