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AN ANALYSIS OF A CIRCADIAN RHYTHM OF OVIPOSITION IN *ONCOPELTUS FASCIATUS*

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INTRODUCTION

Several behavioural circadian rhythms have been described in *Oncopeltus fasciatus*, including daily cycles of mating, feeding, oviposition, flight, and general locomotor activity (Caldwell & Dingle, 1967; Dingle, 1968; Caldwell & Rankin, 1971). This insect is known to be migrant, and the temporal segregation of daily activities brought about by this temporal organization is adaptive in relation to its migratory strategy (Caldwell & Rankin, 1971). Mating and feeding occur primarily in the evening, beginning about 10 h after light onset, while in pre-reproductive individuals flight takes place in the afternoon between 5 and 10 h after light onset. When egg production begins, further flight is inhibited in the female, and oviposition occurs at this time (Dingle, 1968).

Oviposition seemed most suitable for studying the physiological mechanisms underlying these rhythms because it is easily monitored and because one component, egg development, can be separated from another, the actual oviposition behaviour.

MATERIALS AND METHODS

Laboratory stocks of *Oncopeltus fasciatus* originally collected in Johnson County, Iowa, were used in these experiments. All experimental animals were maintained as adults in small plastic refrigerator boxes, one male and one female per container. They were fed on a diet of dried milkweek seeds and furnished with a water source and cotton wool which served as an oviposition site.

The behavioural rhythms of feeding, mating, oviposition, substrate preference and general locomotor activity were observed in 41 single pairs. Observations were made at 3 h intervals during the light phase of a 16 L/8D, 23 °C, regime from the time of the imaginal moult until death. Previous observations had indicated that *O. fasciatus* is relatively inactive during the dark phase of the photoperiod. Oviposition, the primary concern of this paper, was monitored by first observing whether or not the female was in the act of ovipositing. If so, she was not disturbed and the eggs were collected at the next observation. If she was not ovipositing, the cotton wool was checked for the presence of eggs which indicated that the female had oviposited during the previous 3 h.

Similar observations were made in constant light on 23 pairs of *O. fasciatus* at 23 °C

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and on 25 pairs at 28 °C. Both groups were derived from stocks that had been in constant light for at least three generations.

A group of 32 females were ovariectomized in the mid-5th instar. These females were paired with normal males and maintained at 16 L/8 D, 23 °C, and were observed throughout their lives as described above.

RESULTS

Females, when ovipositing, first move on to the cotton-wool substrate, sample the cotton repeatedly with the proboscis and then, if the substrate is suitable, take a few steps forward, insert the ovipositor into the site sampled and begin to lay the eggs.

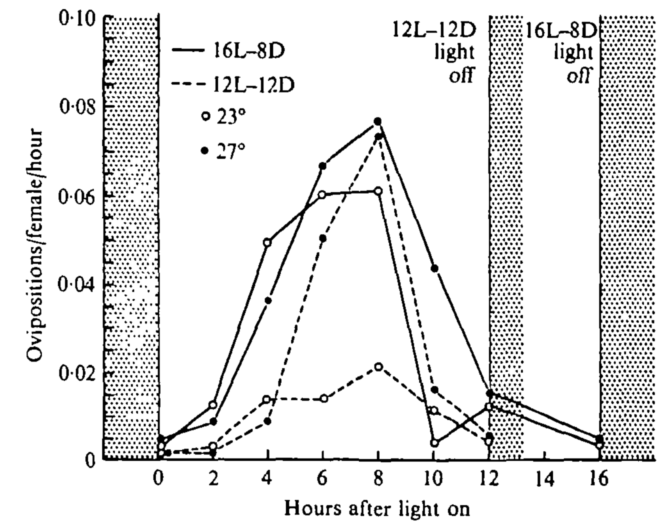

Fig. 1. Ovipositional activity observed under four temperature-photoperiod regimens in 40 pairs of *Oncopeltus* per regimen. Points represent means of values taken over a 10-day period. Points at light onset represent mean hourly ovipositions during the dark phase of the photoperiod.

While ovipositing, the female assumes a characteristic stance, head and thorax elevated and wings slightly spread. A normal female lays from a few to over 50 eggs at one time and generally oviposits each day. The entire process takes from 15 to 20 min.

Oviposition cycles for normal females maintained under two different photoperiods, 12L/12D and 16L/8D, and two different temperatures, 23 °C and 27 °C, show that under all four regimes peak activity occurs approximately 4–10 h after light onset, although oviposition does not begin until a later age under a short day and is less frequent in the 12L/12D, 23 °C, regime (see Fig. 1). Previous observations had shown that very few ovipositions occur during the dark phase of the photoperiod. Those which do occur are collected at the first morning observation and are recorded as a point at light onset representing a mean hourly value for the previous night.

Data from four individual females showing the frequencies in the percentage of the total ovipositions at each observation during the day demonstrate that individual differences exist with respect to the time of day during which ovipositions are most likely to occur (see Fig. 2). Thus female 14 was most likely to oviposit in the first

3 h after light onset whereas female 26 displayed an oviposition curve more typical of the population sample.

If a female does not oviposit on a given day, she is more likely, as Fig. 3 shows, to oviposit earlier the following day, though not before 4 h after light onset.

Inter-ovipositional intervals for a population of females maintained at 16L/8D, 23 °C, had a modal value of approximately 24 h with a second small peak at 48 h (see Fig. 4A). Fig. 4B presents the inter-ovipositional intervals for females five generations after being placed in constant light. Again the modal value for ovipositions is 21–24 h, but the variance is greatly increased.

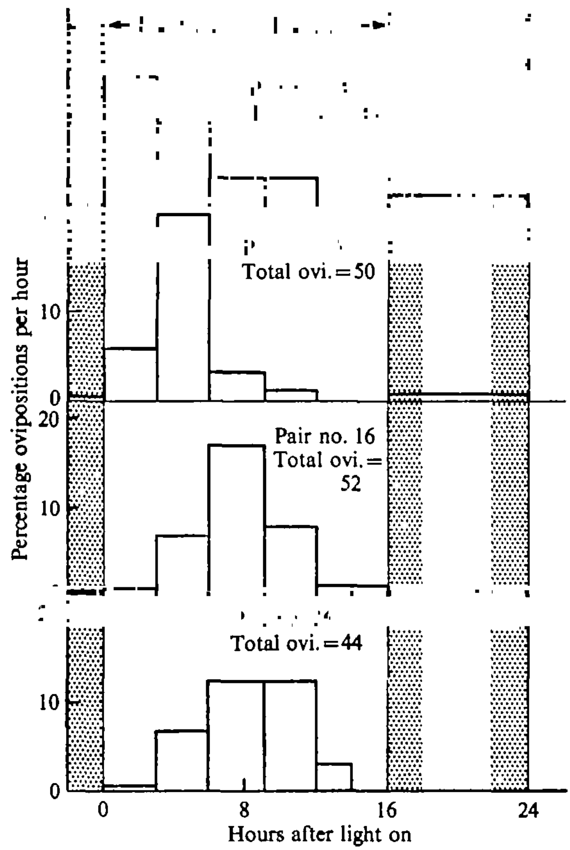

Fig. 2. Individual differences in the phase relationship of peak ovipositional activity to light onset. Data are drawn from all ovipositions made during an individual females life. Females were maintained at 16 L/8D, 23 °C.

In groups of females reared under constant light at 23 and 28 °C for at least three generations, females at 28 °C produced eggs at significantly shorter intervals than did females at 23 °C, with modes of 9–12 and 27–30 h respectively (Mann–Whitney *U*-test, $Z = 5{\cdot}11$, $P < 0{\cdot}001$). Since females with shorter inter-ovipositional intervals would, over a period of time, contribute more observations, the mean inter-ovipositional interval was calculated for each female and the percentage of total females falling within each interval was plotted (see Fig. 5).

Ovariectomized females showed normal mating and feeding behaviours. However, with the ovaries removed, the fat bodies became greatly hypertrophied, causing the abdomens of these females to gradually become distended. At about day 25–30 these females began to show behaviour characteristic of normal ovipositing females. The ovipositor was extended into the substrate and the typical ovipositional stance was

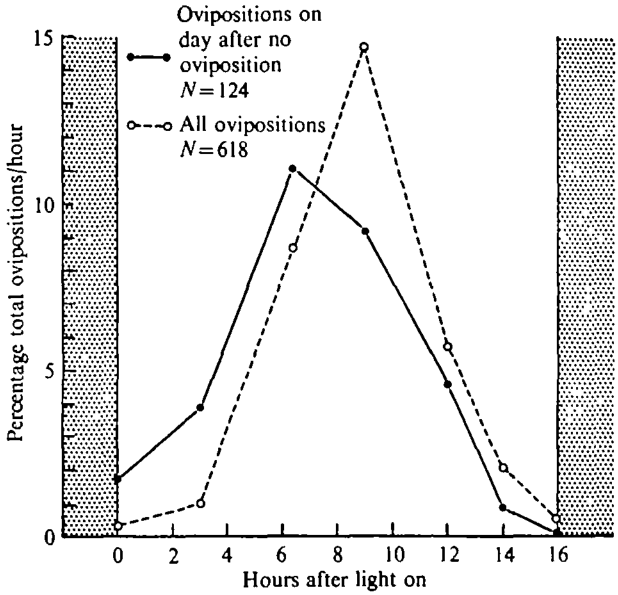

Fig. 3. Ovipositions per hour on a day following no ovipositions compared to ovipositions per hour, regardless of ovipositional history. Females were maintained at 16L/8D, 23 °C.

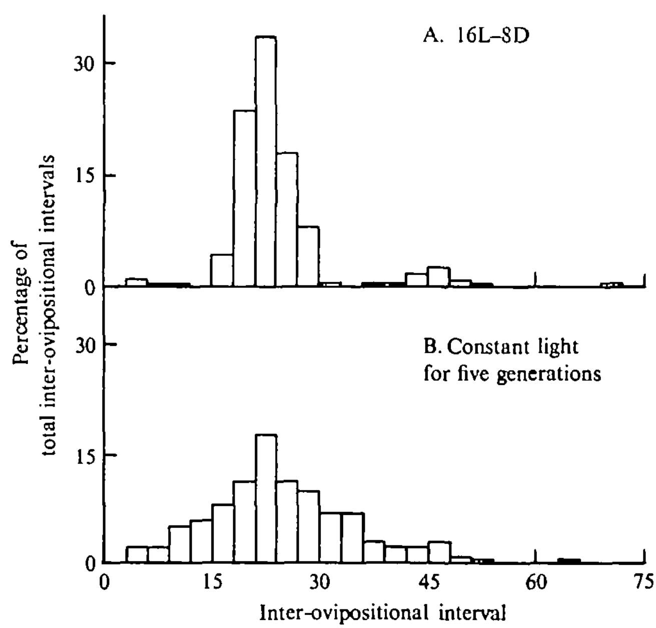

Fig. 4. Distribution of inter-ovipositional intervals from populations maintained one (A) and five (B) generations under constant illumination at 23 °C.

assumed. This behaviour occurred usually 4–10 h after light onset, just as for normal ovipositing females (see Fig. 6A).

During the course of these observations the presence of females on the cotton wool, which served as an ovipositional substrate, was noted. As is seen in Fig. 6B, both intact and ovariectomized females moved on to the cotton about 4 h after light onset and usually moved off again by early evening.

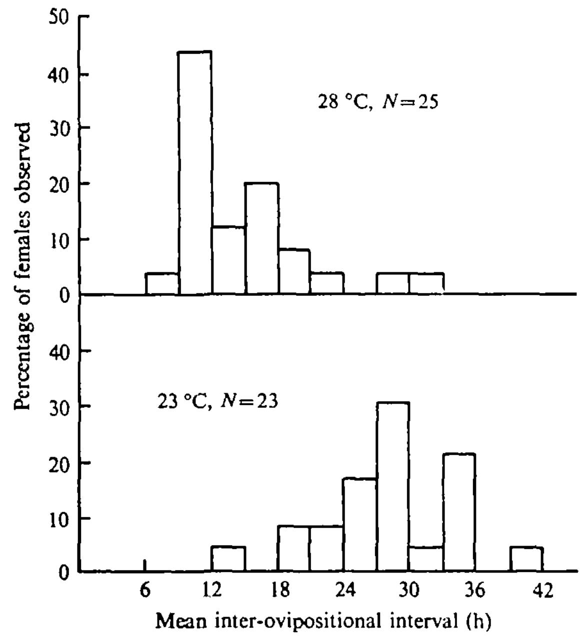

Fig. 5. Mean inter-ovipositional intervals of females maintained under constant illumination at 28 and 23 °C. A mean value was calculated for each female and the population distribution was plotted.

DISCUSSION

Our data indicate that the oviposition rhythm in *O. fasciatus* is made up of two components: the oviposition behaviour and an ovarian cycle which is highly temperature-dependent. It would appear that the oviposition rhythm is set by light onset, since, as Fig. 1 shows, the time of peak activity comes 4–10 h after light onset in either a 12L/12D or a 16L/8D photoperiod regardless of temperature. These curves are population averages and the precise phase relationship between photoperiod and oviposition varies among individuals.

When a female did not oviposit at the usual time on a given day, even though she very likely had ripe eggs ready for oviposition later that same day, she would not lay them until 4 h after light onset the following day. Thus it would appear that there is a period of lowered threshold 4–10 h after light onset, during which time a female is most likely to oviposit. This system is perhaps analogous to the eclosion rhythm in *Drosophila* described by Pittendrigh and Brice (1959) in which the concept of a 'gate' was introduced. Flies were most likely to emerge during a 4-h period just before dawn. Pupae maturing after this period would not emerge until the next pre-dawn gate.

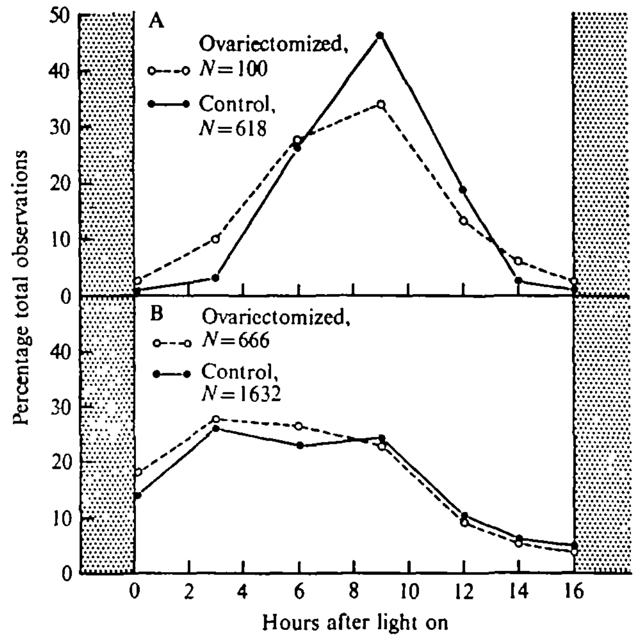

Fig. 6. (A) Time of attempted oviposition by ovariectomized females compared to that by intact control females. (B) Time of day during which ovariectomized and intact females were found on the cottonwool substrate.

When *Oncopeltus* females are reared under constant illumination at various temperatures, the intrinsic egg-production cycle of the ovary is revealed. At 28 °C the inter-ovipositional interval is only about 12 h, yet on either a 16 L/8 D or a 12 L/12 D photoperiod at this temperature eggs are laid at approximately 24 h intervals with oviposition coming during the afternoon gate. The only difference from ovipositions at lower temperatures is that more eggs are laid. At 23 °C in constant light the inter-ovipositional interval is somewhat longer than 24 h (about 27 h) and females at this temperature sometimes skip a day at regular intervals because eggs are not fully mature during the gate period. When this occurs, the female will characteristically lay very early in the gate period of the following day.

Ovariectomy removes the internal ovarian cycle of egg production. However, ovariectomized females display the characteristic ovipositional behaviour caused by abdominal distension after hypertrophy of the fat body and this makes it possible to separate the ovipositional behaviour from the ovarian cycle. Attempted ovipositions again occurred from 4 to 10 h after light onset even though the presumed stimulus of a distended abdomen was constantly present. This suggests that the ovipositional gate is independent of the ovarian cycle and is based rather on some other mechanism, perhaps the CNS or neuro-endocrine systems. An obvious experiment would be to observe ovariectomized females in constant light at various temperatures to determine whether this rhythm is temperature independent. This has been attempted. However, problems with survival and data collection have led to ambiguous results and confirmation will have to wait until an automated data-collecting system is available.

SUMMARY

1. Oviposition in *Oncopeltus fasciatus* shows a daily periodicity, eggs being laid 4–10 h after light onset.

2. In constant light the inter-ovipositional interval is temperature dependent, being about 12 h at 28 °C and 27 h at 23 °C.

3. Ovariectomized females began to display normal ovipositional behaviour and attempt oviposition after hypertrophy of the fat body.

4. Ovariectomized females attempt to oviposit 4–10 h after light onset, just as do normal intact females, indicating that control of the time of oviposition lies, at least in part, outside the ovary.

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REFERENCES

CALDWELL, R. L. & DINGLE, H. (1967). The regulation of cyclic reproduction and feeding activity in the milkweed bug, *Oncopeltus* by temperature and photoperiod. *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 510–25.

CALDWELL, R. L. & RANKIN, M. A. (1971). Segregation of flight, feeding and reproductive activities in the milkweed bug, *Oncopeltus fasciatus*. *J. Insect Physiol.* (in the Press).

DINGLE, H. (1968). The influence of environment and heredity on flight activity in the milkweed bug, *Oncopeltus fasciatus*. *J. exp. biol.* **48**, 175–84.

PITTENDRIGH, C. S. & BRUCE, V. G. (1959). Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism. *Am. Assoc. Advanc. Soc. Publ.* **55**, 475–505.