Effect of precocene II on circadian rhythms of feeding and mating behavior in the milkweed bug, *Oncopeltus fasciatus*[1]

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Summary. Oncopeltus fasciatus adults were treated with the antiallatotropin, precocene II and the circadian rhythms of feeding and mating behavior were monitored at 2-h intervals from lights on to lights off under 2 photoperiod regimes. Females ovariectomized as 5th instars were monitored for feeding and mating behavior at 2–3-h intervals from lights on to lights off as well. Neither precocene treatment nor ovariectomy was found to affect the mating or feeding behavioral rhythms under any photoperiod regime.

For the most part, evidence indicating the involvement of hormones in insect circadian rhythms has been inconclusive or circumstantial at best. Truman and Riddiford's[2] evidence of a diffusible brain hormone in the gating of silkmoth circadian eclosion behavior is the only clear demonstration of this phenomenon in insects. If Harker's work with the cockroach[3,4] represents the nadir of this genre and Truman and Riddiford's work the zenith, there is little else between these 2 points. Hormonal involvement in other insect circadian rhythms remains a fascinating possibility, however, and interesting work is continuing in this area. Thus W.R. Walker's 1977 paper in Experientia[5] was greeted with some excitement as it seemed to indicate the involvement of juvenile hormone in the feeding rhythm of female *Oncopeltus fasciatus*. Walker stated that treatment of female *Oncopeltus* with the antiallatotropin precocene II, which presumably inhibited the allatal production of JH[6], resulted in a damping of the feeding rhythm due to an increase in feeding during mid-day by precocene treated animals. In preparation for a series of experiments based upon Walker's findings[5] we decided to re-examine the effect of precocene II on feeding and mating behavior in this species.

Methods. 60 pairs of adult male and female *Oncopeltus fasciatus* were individually housed in 12-cm petri dishes under a 14 h light/10 h dark 23 °C regime with food, water, and cotton for oviposition continuously available. 30 pairs of bugs were treated 3 times at 3-day intervals with 20 µg of precocene II applied topically in 1 µl acetone to the abdominal sternites. Precocene II was purchased from Eco-control Intermediates, Cambridge Mass. 30 pairs of control animals received 1 µl acetone at corresponding times (3 times at 3-day intervals). For 9 days after precocene treatment 3 behaviors, mating, feeding, and oviposition were monitored for each individual insect at 2-h intervals beginning at lights on and ending at lights off. This experiment was replicated with 58 pairs of bugs similarly maintained, reared under a 12 h light/12 h dark 23 °C regime. Half of these animals received 3 precocene II treatments (20 µg/µl in acetone, applied topically at 5-day intervals) and half received only acetone. This group was observed at 3-h intervals during the lights on portion of the day for 18 days after precocene treatment.

A group of 32 females were ovariectomized in mid-fifth instar. After adult emergence these females were paired with normal males, maintained at 16 h light/8 h dark, 23 °C, and observed for feeding and mating behavior at 2- or 3-h intervals from lights on to lights off for 6 weeks.

Individuals were recorded as displaying feeding behavior only if the stylet was anchored in a milkweed seed at the time of observation. Mating behavior was recorded only if the mating pair was coupled in copulation and oviposition behavior was recorded if the female was seen probing the cotton with the ovipositor, ovipositing, or if eggs were discovered at the subsequent observation period. Oviposition behavior was rapidly eliminated in precocene treated animals so that comparison of this behavioral rhythm with the control population was not possible.

Results and discussion. The autopsies provided evidence of the effectiveness of precocene II treatment. In the treated groups the fat bodies were hypertrophied and the ovaries atrophied. Eggs with yolk were either absent or in the process of resorption in all treated females. The control

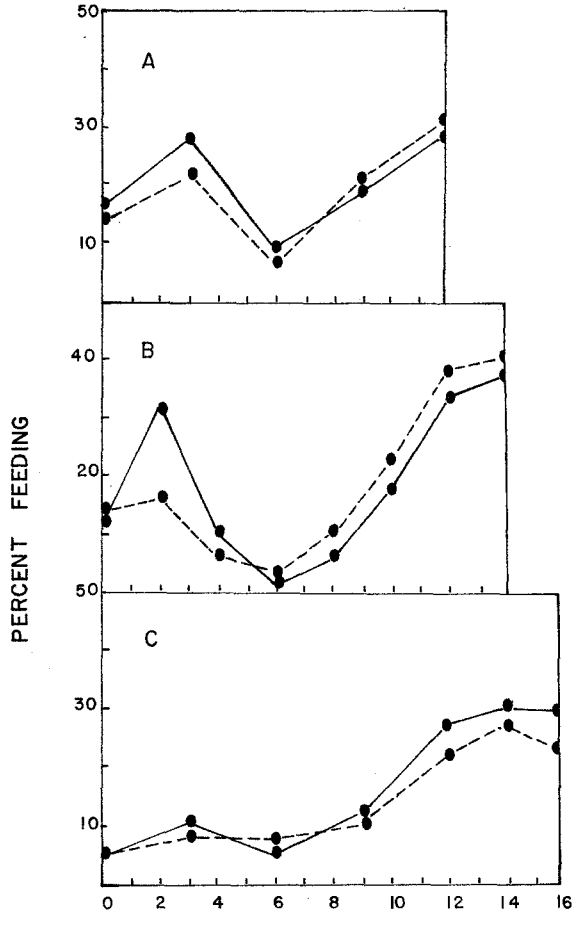

Fig. 1. Daily feeding behavior of *Oncopeltus fasciatus* after precocene treatment or ovariectomy. *A* Influence of precocene II application on animals reared in a 12 h light/12 h dark, 23 °C regime. ●——● = precocene II-treated animals, n = 58, ●– –● = acetone-treated animals, n = 58. *B* Influence of precocene II application on animals reared in a 14 h light/10 h dark, 23 °C regime. ●——● = precocene II-treated animals, n = 120, ●– –● = precocene II-treated animals, n = 60. *C* Influence of ovariectomy on daily feeding rhythms (16 h light/8 h dark, 23 °C regime). ●——● = ovariectomized females, n = 32, ●– – –● = intact females, n = 50.

group showed exactly opposite characteristics, including fully developed oocytes and oocytes in the process of vitellogenesis. The ovaries were absent in all ovariectomized animals and the fat bodies were hypertrophied.

After the adult molt, the feeding rhythm develops as a gradual depression of feeding behavior during midday[7]. Walker's results[5] showed a damping of the feeding rhythm expressed as an increase in feeding during the midday, so the population exhibited equivalent amounts of feeding behavior at all observation times. In our experiments the feeding and mating rhythms of the precocene treated animals were almost identical to those of the controls. They were most certainly rhythmic (figures 1 and 2) and were almost identical with the behavioral rhythms reported by Caldwell and Rankin[7] for this species. Thus our results are not in agreement with Walker's report[5] of a damped feeding rhythm among precocene treated animals.

One difference in experimental design which might have relevance to the difference in results between Walker[5] and ourselves is that Walker began precocene treatment at 6 days after adult emergence. He may therefore have inhibited ovarian development before it had proceded very far. We treated reproductively mature animals and therefore many of the treated females in this experiment retained partially developed (resorbing) ovaries for at least a portion of the experimental period. The daily rhythm of oviposition behavior in *Oncopeltus* occurs from 4–10 h after lights on and almost exactly corresponds to the daily decline in feeding which occurs at midday[7]. We thought it possible that in the absence of ovarian development and any stimulus to oviposit, feeding behavior during midday might increase. Assuming Walker's treated females to have been essentially chemically ovariectomized, we decided to look at feeding and mating behavior in surgically ovariectomized females.

We monitored feeding and mating behavior in a group of 32 females ovariectomized as 5th instar nymphs. As figures 1,C, and 2,C, show, we observed no significant deviation in either feeding or mating behavior from the control levels in our ovariectomized females. In the complete absence of ovaries, adult feeding and mating behavior show the same diel periodicity as intact, untreated controls. Thus we must conclude that the absence of ovarian development in Walker's bugs cannot explain his observed increase in feeding at midday among precocene treated females.

The salient feature of the feeding rhythm is the midday depression (maximal at 6 h after lights on), and one would expect any damping of the rhythm to occur during that time. However, Walker chose to examine feeding behavior at only 4 time points throughout the 16-h-day with only 4 animals per treatment group. A change in the relative feeding levels, though not in the midday depression, might have been misinterpreted using his sampling methods. For instance, in our data (figure 1,B) there is a significant increase in feeding 2 h after lights on for the precocene-treated group, actually enhancing the bimodality of the rhythm, yet the midday depression at 13.00 h is equal to that of the control group (figure 1,A and B). If sampling had been done at 3 and 8 h after lights on, the rhythm of the treated group might have appeared to have been damped (i.e., relative changes in the levels of feeding outside the depression could mimic a damped rhythm if data sampling were insufficient). Also there is a significant amount of individual variation in these behavioral rhythms and a sample size of only 4 per treatment group might have been too small to distinguish individual variance within a group from variance between groups due to drug treatment. Although the experiments described above were not exact replicas of Walker's work[5], we feel that they represent a reasonable test of his conclusions. It seems clear from our data that precocene II treatment intense enough to inhibit JH stimulation of oogenesis affected neither the feeding nor the mating rhythm under either photoperiod regime. Thus, we see no evidence from these experiments of JH involvement in the behavioral rhythms of feeding or mating in *Oncopeltus fasciatus*.

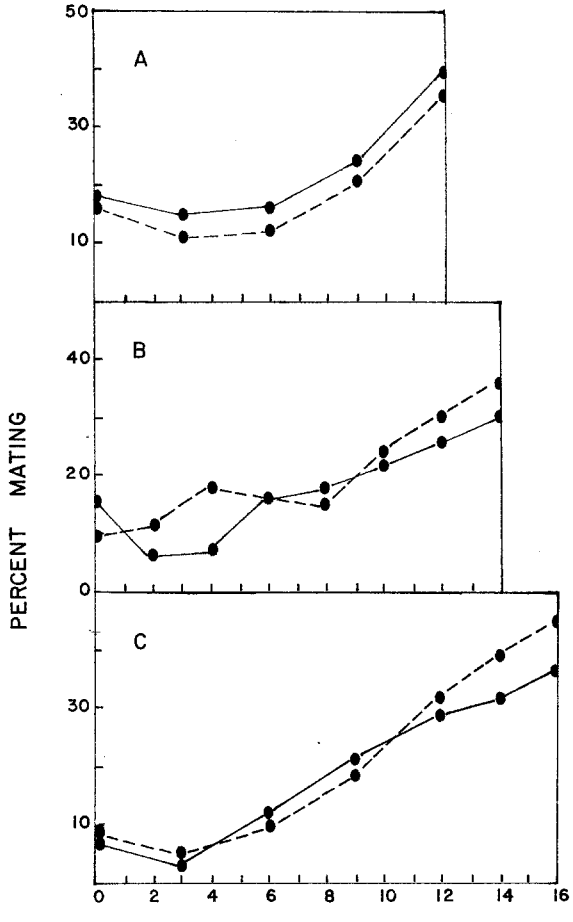

Fig. 2. Daily mating behavior of *Oncopeltus fasciatus* after precocene treatment or ovariectomy. *A* Influence of precocene II application on animals reared in a 12 h light/12 h dark, 23 °C regime. ●——● = precocene II-treated animals, n = 58, ●---● = acetone-treated animals, n = 58. *B* Influence of precocene II application on animals reared in a 14 h light/10 h dark, 23 °C regime. ●——● = precocene II-treated animals, n = 120, ●---● = acetone-treated animals, n = 60. *C* Influence of ovariectomy on daily mating rhythms (16 h light/8 h dark, 23 °C) ●——● = ovariectomized females, n = 32, ●---● = intact females, n = 50.

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