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The hormonal control of migratory flight behaviour in the convergent ladybird beetle, *Hippodamia convergens*

SUSAN M. RANKIN and MARY ANN RANKIN Department of Zoology, University of Texas, Austin, Texas, U.S.A.

ABSTRACT. Topical application of the juvenile hormone mimic, Altosid, to *Hippodamia convergens* (Guérin-Méneville) (Coccinellidae) stimulated a significant increase in long-term flight behaviour in both males and females. Altosid treatment also stimulated ovarian development in females. Topical application of precocene II to *H.convergens* inhibited flight activity in treated animals of both sexes for about 10 days. Altosid treatment to precocene-treated beetles significantly increased their migratory behaviour over that of precocene-treated or acetone-treated controls. These results indicate that juvenile hormone stimulates migratory flight behaviour in this species along with reproductive development. It is likely that the hormone serves to coordinate migration with reproduction in the young adult.

Introduction

Insect migratory behaviour is characterized by specific behavioural, ecological and physiological features (Dingle, 1972; Johnson, 1969). Behaviourally, migratory flight can be clearly distinguished from 'trivial' or 'appetitive' flight because it is typically prolonged and not arrested by stimuli such as food, oviposition site or mate, which would elicit settling behaviour during trivial flight (Kennedy, 1961). Ecologically, insect migration may be either an escape in space from unfavourable habitats or an 'investment' of a portion of a population in colonizing and exploiting resources in a habitat some distance from the one in which adult emergence of the migrants occurred. Migratory behaviour is often displayed by only a portion of a population in response to appropriate environmental cues such as photoperiod, temperature, food quality, population density, moisture, etc. Migrants are often denizens of temporary or early successional habitats, have a high reproductive potential, and hence a great capacity for rapid exploitation of a newly invaded habitat. Physiologically, migration is thus typically a pre-reproductive phenomenon, at least in females, and oogenesis or the presence of fully developed ovaries has often been shown to inhibit female migratory flight (Rankin, 1978).

Environmental cues seem to exert their effects on migration by way of the neuroendocrine system. Juvenile hormone (JH), the corpora cardiaca adipokinetic hormone and possibly ecdysone have been implicated in the control of migratory behaviour in some species (see Rankin, 1978, for review). We are interested in whether any general trends can be seen in the physiology of migratory control in insects with different life histories. To that end we have studied the effects of JH and the 'anti-allatotropin', precocene II (Bowers, 1976), on long flight behaviour in *Hippodamia convergens*, a predatory, entomophagous migrant with a somewhat different life history from those species previously investigated.

Correspondence: Dr M. A. Rankin, Department of Zoology, University of Texas, Austin, Texas 78712, U.S.A.

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H.convergens is one of the most widespread and mobile of the American coccinellids. It occurs throughout western, central and southern U.S. and is an economically important predator of aphids and mites. Adults emerge from 'hibernation' sites in the spring and migrate up to hundreds of kilometres to areas of aphid infestation (Hagen, 1962) where they may develop high densities (Dickson *et al.*, 1955). As aphid populations decrease, the coccinellids move to neighbouring habitats. Such movements may occur several times in a season and, depending on food abundance, the species may be uni- or multivoltine. If prey is unavailable or scarce, young adults enter reproductive diapause and an extended migratory phase during which they move to mountaintop aggregation sites. Diapausing beetles may remain at the hibernation sites from 6 to 9 months until they migrate back to lower altitudes. Some type of diapause development, possibly regeneration of partially degenerated flight muscle, seems to be involved in the return of the migratory response at this time (Rankin & Rankin, 1980). The commercial usefulness of *Hippodamia convergens* as a natural control agent for crop pests is limited by its migratory tendencies. Understanding its migratory physiology should thus be useful, not only theoretically, but also economically.

Materials and Methods

Adult ladybird beetles, *Hippodamia convergens* (Guérin-Méneville: Coccinellidae), were purchased from the Bio-Control Company, Auburn, California. They were kept in an unilluminated refrigerated chamber at 7°C until needed for experiment. One day before flight testing the animals were removed from the low temperature incubator, placed in 9-cm disposable Petri dishes, and kept thereafter at 24°C in a 16 h light : 8 h dark regime, five beetles of the same sex per dish. Each beetle was colour marked with a spot of enamel paint on the elytron for identification of individuals. In preliminary flight tests of beetles before and after painting of the elytra, we could see no effect of the enamel on flight activity. The beetles had constant access to water from vials placed in the dishes and were fed daily on frozen potato aphids, *Microsiphon euphobiae*, immediately following flight testing. The beetles would not eat aphids until 3 days after removal from the low temperature incubator, so none were provided until that time.

H.convergens was tested for long flight behaviour in the laboratory using the method of Dingle (1966) with slight modifications. Each animal was suspended from its pronotum by a wooden toothpick tipped with melted beeswax, and flight was stimulated by moving the animal by hand rapidly through the air in a figure-of-eight circuit until it began to fly. Each animal was given five full circuits to begin flight in each of five flight trials. Flying animals were then placed in front of a low-speed fan and continuous flight duration was timed. *H.convergens* displays a marked dichotomy of flight behaviour (Rankin & Rankin, 1980) in that beetles typically fly either for less than 2 min or for much longer, and virtually all animals that fly for 30 min fly for several hours (12 h or more) if allowed to do so. Thus flights of over 30 min were taken to indicate willingness to make a migratory flight. All flight tests were performed between 5½ and 9½ h after lights on.

To determine the degree of ovarian development after treatment, females were fixed in Heidenhain's susa (Galigher & Kozloff, 1964) for 48 h, washed repeatedly with 70% ethanol and refrigerated therein for at least 48 h before dissection. The entire female reproductive tract was removed, cuticle was carefully teased from the base, the tract blotted dry with paper tissue and weighed on a Cahn electrobalance, Model M-10. The tract included ovaries, oviducts, vagina, spermatheca and accessory glands.

Chemicals used

6,7-Dimethoxy-2,2-dimethyl chromene (precocene II, purchased from Eco-chemical Intermediates, Cambridge, Mass.) was dissolved in reagent grade acetone at a concentration of 2.0% w/v. The juvenile hormone mimic (JHM) used was Altosid^{R} (Tech. 92.990, no. 062054, kindly supplied by Zoecon Corp., Palo Alto, Calif.). It was dissolved in acetone at a concentration of 0.5% w/v. Both JHM and precocene were applied topically to the abdominal sternites in 0.5, 1.0 or 2.0 µl of solution.

JHM was used in preference to any of the natural juvenile hormones because we discovered in preliminary experiments that JHM effects were much longer lasting, possibly because, as Kramer & de Kort (1976) showed for *Leptinotarsa*, the mimic was not metabolized as readily as the natural hormones.

Results

Effect of JHM on flight behaviour

The first group of experiments was designed to examine the effect (if any) of JHM on flight behaviour. Three experiments were performed. (1) After 2 days of flight testing, three groups of fifteen females were treated: fifteen with 5 μg JHM/1 μl acetone each, fifteen with 10 μg JHM/2 μl acetone each, and fifteen with 1 or 2 μl acetone only. Flight tests were administered daily. (2) Two groups of fifteen females were flight tested on day 1; then on days 2, 4, 6 and 8: fifteen females received 10 μg JHM/1 μl acetone, and the other fifteen received 1 μl acetone. Flight tests were administered to both groups on days 3, 5, 7 and 9. (3) A group of thirty males received 10 μg JHM on day 2 and was flight tested daily thereafter for 7 days; thirty control males received 1 μl acetone only.

Topical application of 5 μg of JHM to female *H.convergens* resulted in a significant increase in the number of insects making long flights over acetone controls for the entire duration of the experiment ($P < 0.01$, Mann-Whitney U test). The maximum difference in flight between the two groups occurred on day 3, 1 day after treatment, when 33% of the JHM-treated females exhibited long flights while only 13% of the acetone treated controls did so (Fig. 1A). A much greater increase in flight activity was observed among females receiving 10 μg JHM ($P < 0.005$). One day after treatment, 40% of the JHM-treated females made a long flight, and 2 days after treatment 58% did so. By day 8, however, the percentage of such females making long flights had dropped to 13%.

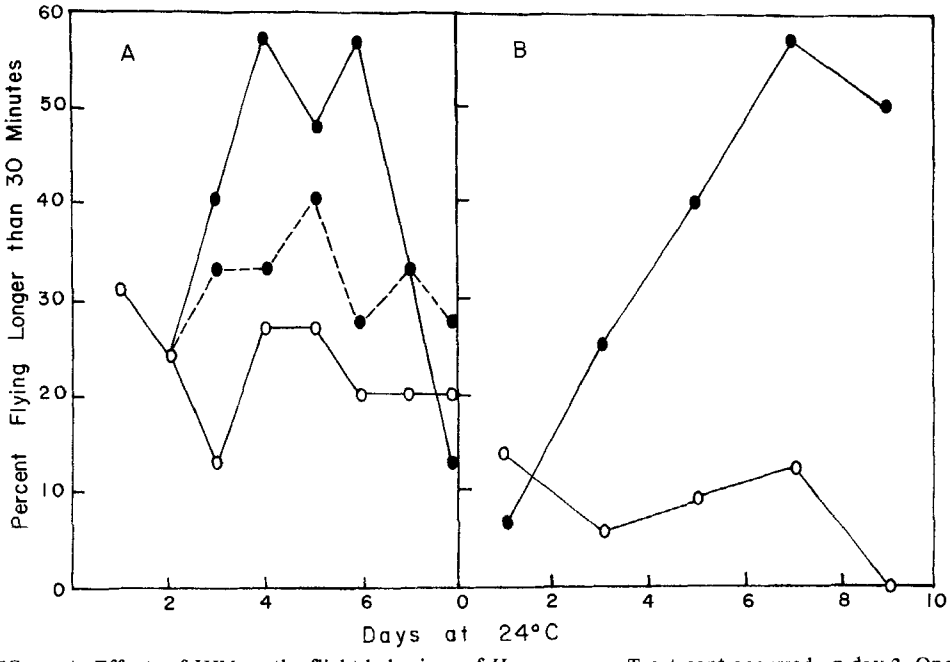

FIG. 1. A. Effects of JHM on the flight behaviour of *H.convergens*. Treatment occurred on day 2. Open circles indicate untreated (day 1) or acetone-treated females; solid circles, broken line, females treated with 5 μg JHM; solid circles, solid line, females treated with 10 μg JHM. B. Effect of repeated applications of JHM on the flight behaviour of *H.convergens*. Solid circles indicate females treated with 10 μg JHM on days 2, 4, 6 and 8; open circles, females treated with 1 μg acetone on the same days.

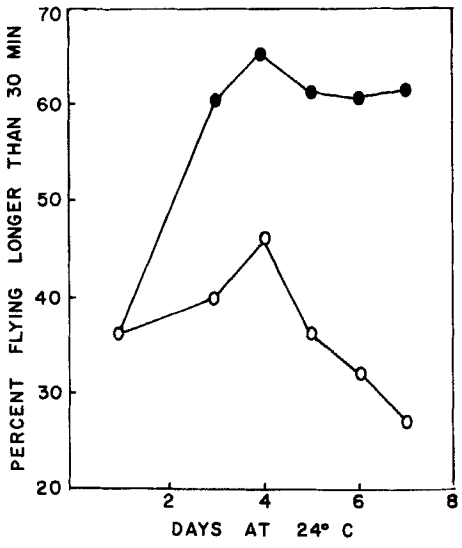

FIG. 2. Effect of JHM on the flight behaviour of *H.convergens* males treated on day 2. Open circles indicate acetone-treated animals; solid circles, animals receiving 10 μg JHM.

In the second experiment, a group of females which showed little flight activity among the controls displayed a dramatic increase in flight activity with repeated JHM applications ($P = 0.008$; Fig. 1B) and no marked decline in long flights towards the end of the experimental period was observed.

Male *H.convergens* receiving 10 μg of JHM displayed significantly more long flights than did the controls (Fig. 2; $P < 0.005$). Again, the large percentage of long flights in the JHM-treated groups was maintained throughout the experimental period.

Effect of precocene II on flight behaviour

To determine the effect of an artificially induced decrease in circulating JH on flight behaviour, a fourth experiment was performed in which animals were flight tested for 2 days and then treated at 2-day intervals: with 10 μg precocene in 0.5 μl acetone, or with 1 μl acetone, or with 10 μg precocene II in 0.5 μl acetone on day 2 followed by 10 μg JHM in 1 μl acetone as replacement therapy on day 4. Treatments were administered after the flight test on treatment days ($n = 30$ each group).

Precocene treatment resulted in a significant decrease in flight activity below that of untreated controls in both sexes. In most instances where precocene II was applied or re-applied, an immediate drop in flight activity occurred in the subsequent flight test even when flight activity was already quite low

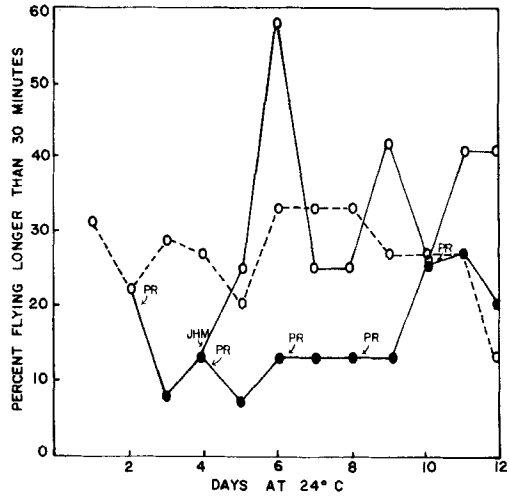

FIG. 3. Effects on flight behaviour of *H.convergens* females of 10-μg applications of precocene II every 2 days, or precocene II followed by JHM treatment. Open circles with broken line indicate untreated (day 1) or acetone-treated animals; solid circles, animals treated with 10 μg precocene II only; open circles with solid line, females treated with 10 μg precocene II on day 2 and JHM on day 4.

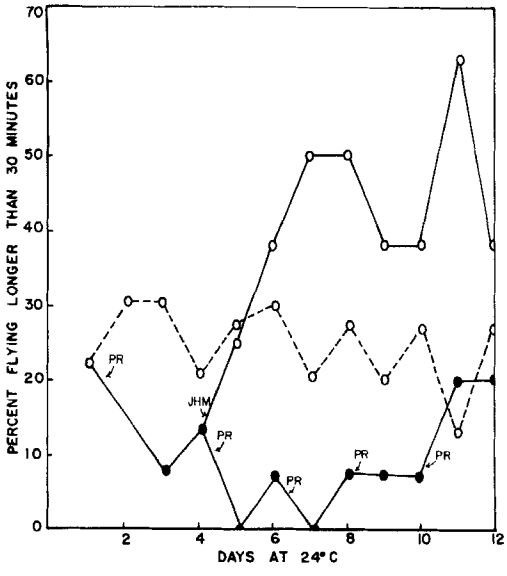

FIG. 4. Effects of 10-μg applications of precocene on flight behaviour of *H.convergens* males; details as for Fig. 3.

(Figs. 3 and 4). Flight behaviour of males was depressed after precocene II treatment to somewhat lower levels than it was in females, possibly because males, being approximately 20% lighter than females, actually received a slightly higher dose per unit body weight. After about 8–10 days of treatment, repeated precocene applications had no further effect on flight.

Precocene treatment followed after 2 days by 10 μg JHM resulted in a dramatic increase in the number of beetles exhibiting long flights (Figs. 3 and 4). The increase was more prolonged in males than in females but was significant in both groups ($P < 0.05$, χ^2). Among females given JHM on day 4 after precocene II treatment on day 2, the increase in flight activity was transient, possibly due to rapid stimulation of ovarian development by JHM. Ovarian development was observed in most of the JHM-treated females, but the females who continued to make long flights throughout the experimental period did not show as much ovarian development as their companions who did not make long flights (mean ovarian weights 0.74 mg *v.* 2.6 mg, respectively).

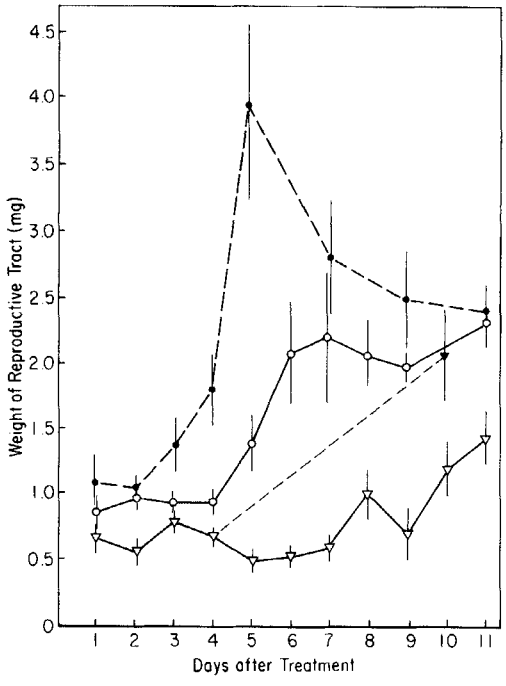

FIG. 5. Effects of 10-μg applications of precocene II and JHM on the weight of the entire fixed reproductive tract of female *H.convergens*. Closed circles indicate JHM-treated animals; open circles, acetone-treated controls; open triangles, precocene-treated animals; the solid triangle connected to the precocene treatment group indicates precocene-treated animals given JHM replacement therapy on day 4. Bars, SEM.

Effect of JHM and precocene II on reproduction

We wished to determine the effect of JHM and precocene II on ovarian development, both to confirm the anti-allatotropic effect of precocene in adult *H.convergens* and to assess the possible dual effect of JHM in adult females.

Three groups of female beetles were taken from the low temperature incubator and placed at 24°C. The first group ($n = 24$) received 10 μg of JHM on day 1 (topically on the abdominal sternites in 1 μl acetone); the second group ($n = 42$) received 10 μg of precocene II in 1 μl acetone; and the third group ($n = 32$) received 1 μl of acetone only. Treatment was repeated at 2-day intervals (a total of five times) for groups 2 and 3 except for five individuals in group 2 that received 10 μg JHM 8 days after the initial treatment instead of the last two precocene treatments. At 1–2-day intervals, three to five animals from each group were killed and the weight of the entire reproductive tract was determined for each female (Methods).

By 5 days after treatment JHM significantly ($P < 0.001$; Mann-Whitney U test) increased ovarian development over that of controls and precocene-treated females (Fig. 5). Similarly, precocene II-treated females showed significantly less ovarian development than untreated controls ($P < 0.001$). Again the efficacy of repeated precocene treatment was somewhat reduced after 8–10 days. Precocene-treated females given JH replacement therapy on day 4 showed a significant increase in ovarian development over precocene-treated controls when they were examined 6 days after JHM treatment ($P < 0.01$, χ^2). It would appear that precocene II does inhibit oogenesis via an inhibitory effect on the corpora allata as it has been shown to do in other insect species (Bowers, 1976). Similarly, JHM application

TABLE 1. Effects of JHM treatment on the mean weight of the reproductive tract in *H.convergens* (collected and tested in 1979)

Treatment	Weight of tract (mg ± SD)
Tested in March; treated on day 2 only	
5 μg JHM/♀	2.16 ± 0.11
10 μg JHM/♀	2.40 ± 0.18
1 μl acetone/♀	0.82 ± 0.09
Tested in July; treated on days 2, 4, 6 and 8	
10 μg JHM/♀	1.25 ± 0.24
1 μl acetone/♀	0.65 ± 0.01

clearly stimulates ovarian development in *H.convergens*.

Autopsies were performed on females that had been flight tested after single and multiple applications of JHM to determine the relative effects of those treatments on ovarian development in each group. These results are summarized in Table 1. All JHM-treated groups showed significantly more ovarian development than acetone-treated controls and a single 10-μg application of JHM was somewhat more effective than a single 5-μg dose ($P < 0.05$, t test). A single 10-μg JHM treatment to females at the end of their diapause period (March) was significantly ($P < 0.01$) more effective in stimulating ovarian development than multiple applications to animals newly arrived at the aggregation sites in July.

Discussion

Our results suggest that juvenile hormone plays an important role in stimulating migratory activity in *Hippodamia convergens*. Administration of JHM to both males and females resulted in an increase in long flight behaviour, as it has been shown to do in *Oncopeltus fasciatus* (Caldwell & Rankin, 1972; Rankin, 1974). Higher doses of JHM (10 μg/animal) seemed to have a dual effect in females, increasing flight and stimulating ovarian development. JH is, as expected, necessary for ovarian development in *H.convergens* and, as in *Oncopeltus*, there is an inverse relationship between ovarian development and migratory flight (Rankin & Rankin, 1980). The sharp decline in flight on day 8 among females given 10 μg JHM may have been due to such an ovarian-based inhibitory effect.

It is interesting to note that the group of females which was treated every 2 days with JHM showed a sustained increase in flight activity. Some ovarian development was induced but apparently not enough to overcome the flight-stimulating effect of the hormone. These females were collected by Bio-Control in early summer from hibernation sites in California only shortly before they were shipped to us and flight tested in the laboratory. They had not been at the aggregation sites long and were held at low temperatures for only 8 days prior to flight testing. In contrast, all other animals used had been stored at low temperature in diapause for several months prior to testing, and the two groups were significantly different in their flight activities ($P < 0.001$, Mann-Whitney U test). We have found that among animals collected from aggregation sites in Texas, flight activity is very low when beetles are newly arrived at the aggregation sites. There seems to be a gradual increase of the migratory response during diapause. Perhaps this increase in responsiveness to migratory stimuli may be due to a gradual build-up of JH. The relatively moderate increase in ovarian weight among summer females in response to high and repeated applications of JHM (Table 1) tends to support this suggestion.

The increased flight behaviour of JHM-treated males occurred without a subsequent depression in flight activity, suggesting that in the absence of ovaries, flight stimulation by JH may be more prolonged. Again this is similar to the effect of JH in male and ovariectomized female *Oncopeltus fasciatus* (Caldwell & Rankin, 1972; Rankin, 1974).

It was important to investigate the effects of allatectomy and of replacement therapy on flight behaviour. We were, however, unable to flight test surgically allatectomized animals reliably, hence the use of the anti-allatotropin, precocene (Bowers, 1976; Bowers *et al.*, 1976). Precocene appears to inhibit oogenesis, probably via the corpora allata, for at least a few days in *H.convergens*. Thus, if migratory behaviour does depend upon the presence of JH, one would expect JH to inhibit flight

activity as well, and this is exactly what was observed. The inhibitory effects of a single precocene treatment disappeared after about 4 days (S. M. Rankin, unpublished observations), but repeated applications every 2 days resulted in a sustained decrease in flight activity (Figs. 3 and 4) as well as a significant decrease in ovarian development (Fig. 5). After 8–10 days of treatment, the effect of subsequent applications on flight or on ovarian development was diminished, suggesting some escape of the corpora allata from the effects of the drug. JH replacement therapy given to precocene-treated animals resulted in a dramatic increase in flight activity, suggesting that it was, in fact, the anti-allatal activity of the precocene that caused the observed decline in flight behaviour after precocene treatment. JH therapy also stimulated ovarian development in precocene-treated females. These results are similar to those reported by Rankin (1980), who showed that treatment of adult *Oncopeltus fasciatus* with precocene results in the inhibition of migratory flight behaviour which is restored by treatment with JH III.

In *Oncopeltus fasciatus,* relatively high titres of JH are correlated with ovarian development, while migratory behaviour occurs at times of intermediate titre; and ovarian development ultimately inhibits long flights (Rankin, 1974; Rankin & Riddiford, 1977, 1978). Although further work investigating the effects of JH titre changes and of ovariectomy on flight will be necessary, our results imply that JH plays the same sort of role in coordinating ovarian development and migration in female *H.convergens.* We have not, however, eliminated the possibility of neuroendocrine involvement in flight and/or reproduction in *Hippodamia.* Indeed, migratory flight in orthopterans (Mayer & Candy, 1969; Goldsworthy *et al.,* 1972, 1973) and possibly in *Danaus plexippus* (Dahlmann & Herman, 1978) requires an adipokinetic hormone from the CC, while oogenesis control in many insects has been shown to involve brain or corpora cardiaca (Engelmann, 1968).

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