CHAPTER 7

INTERACTIONS BETWEEN *M. CONVECTA* MALES AND FEMALES

7.1. Introduction

The behaviour of *M. convecta* males in the presence of calling females in a wind tunnel has been described in Chapter 6. When the females exhibited the calling posture, characteristic sexual behaviours such as approach to the source, landing on the female cage and copulatory attempt were observed. Female baits used in this experiment, however, were confined in cages, thus restricting males from contacting them.

In this chapter, the interactions between males and females, particularly their copulatory behaviour, in the laboratory and in the field are described. Under laboratory conditions, single and multiple pairs of males and females were observed in cages while in the field, males and females were observed in a walk-in cage.

7.2. Laboratory experiments

7.2.1. Materials and methods

Larvae were reared in the insectary under the normal rearing conditions. Male and female pupae were kept in separate reverse-cycle cabinets at 20 ± 1°C and 16:8 light:dark (LD) regime. Two sets of experiments were conducted. In Experiment 1, 43 pairs of *M. convecta* males and females of the same ages were observed in individual 150-ml clear plastic cages (Chapter 3.1). Male moths were distinguished by small markings on their forewings with oil paints (George Rowney & Co. Ltd., England). When either the male or female died, the pair was excluded from observation. In Experiment 2, "mass-mating cages" were used. These plastic cylindrical cages (21.5 cm diameter and 30 cm tall) were similar to those used for rearing culture moths (Chapter 3.1). Each of the two cages contained six pairs of males and females of the same ages. Male moths were distinguished by small markings on the tips of their forewings. In each cage, individual males were identified by the following markings: male 1 with blue paint on right wing (rw), male 2 with red paint on rw, male 3 with blue on left wing (lw), male 4 with red on lw, male 5 with blue on rw and red on lw and male 6 with red on rw and blue on lw. Female moths were marked at the bases of their forewings. The markings of individual females were: female 1 with blue on rw, female 2 with red on rw, female 3 with blue on lw, female 4 with red on lw, female 5 with blue on rw and red on lw and female 6 with red on rw and blue on lw.

The experiments were conducted in reverse-cycle cabinets at 20 ± 1°C at 16:8 LD regime, with the scotophase at 1000-1800 h AEST. Observations were done at 30-min intervals and recorded on a portable computer. The MINITAB statistical package (Ryan *et al.*, 1992) was used to calculate means and standard errors of the different parameters of female calling and copulation. Data were also analysed using regression analysis and one-way analysis of variance.

Raw data on calling and copulation in the two experiments are provided in the sub-directory A:\CHAP7 of the floppy disk.

7.2.2. Results and discussion

Experiment 1 - Mating behaviour in individual cages

The number of pairs observed in individual cages at different ages is given in Table 7.1.

Age (S)	No.	Age	No.
1	43	8	40
2	43	9	37
3	43	10	34
4	42	11	34
5	42	12	33
6	42	13	25
7	40	14	19

Table 7.1. Experiment 1. Number of pairs of *M. convecta* males and females observed in individual cages at different ages.

Of the 43 pairs that were observed, only 11 (26%) copulated. The ages at which these 11 females called for the first time and the ages when they first copulated are shown in Table 7.2. A regression analysis showed that the age at first copulation was not significantly correlated with the age at which females called for the first time ($p = 0.24$).

The mean duration of calling at different ages was compared between unmated and mated females (Fig. 7.1). A one-way analysis of variance showed that unmated females called significantly longer than mated ones ($F_{1,288} = 13.35$, $p < 0.001$). The mean duration of calling in unmated females was 92.3 ± 3.2 minutes whereas in mated females, it was 44.5 ± 8.6 minutes per scotophase. Calling started during the 3rd hour of the scotophase and peaked in the 7th hour (Fig. 7.2), a result which is consistent with those obtained in previous experiments (Chapters 4 and 5).

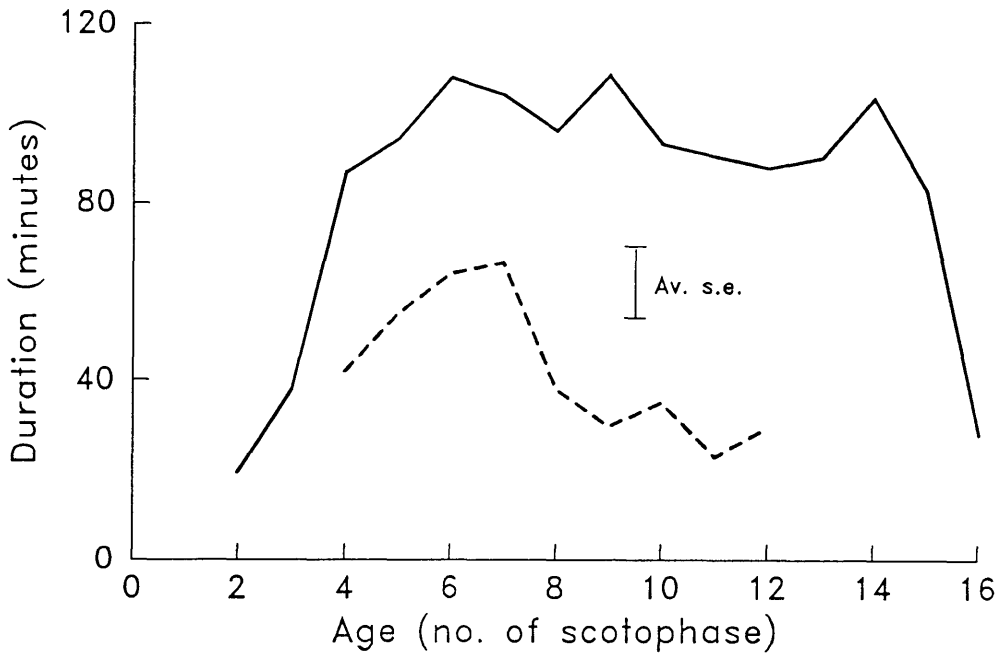

Fig. 7.1. Experiment 1. Mean duration of calling at different ages in unmated (——) and mated (- - -) *M. convecta* females. Laboratory conditions were 20°C and 16:8 light:dark (LD) regime.

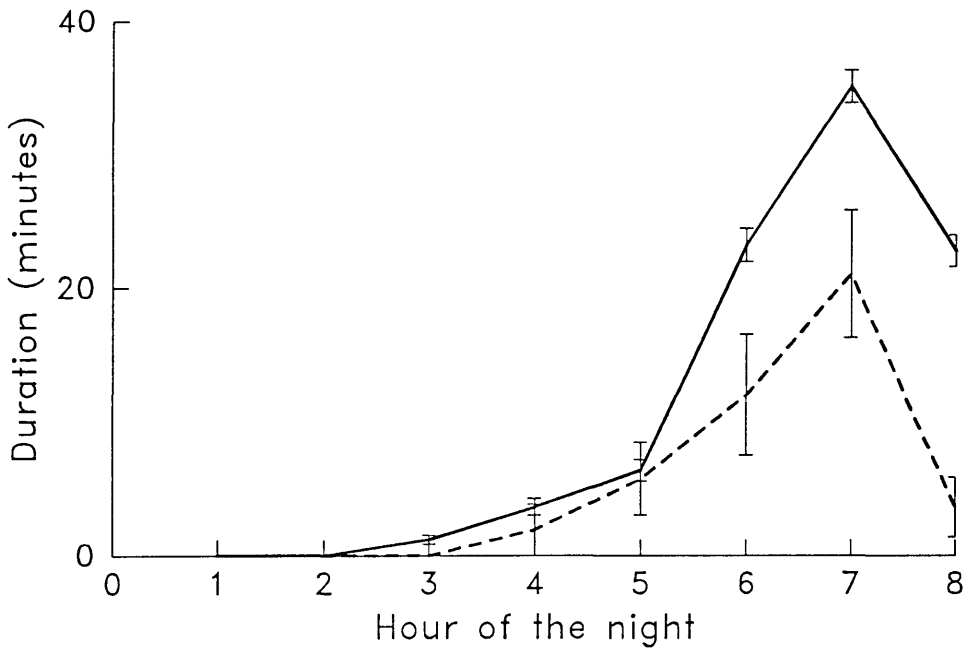

Fig. 7.2. Experiment 1. Mean duration of calling in unmated (——) and mated (- - -) *M. convecta* females in individual cages at different times of the scotophase. Laboratory conditions were 20°C and 16:8 LD regime. Bars are s.e.'s.

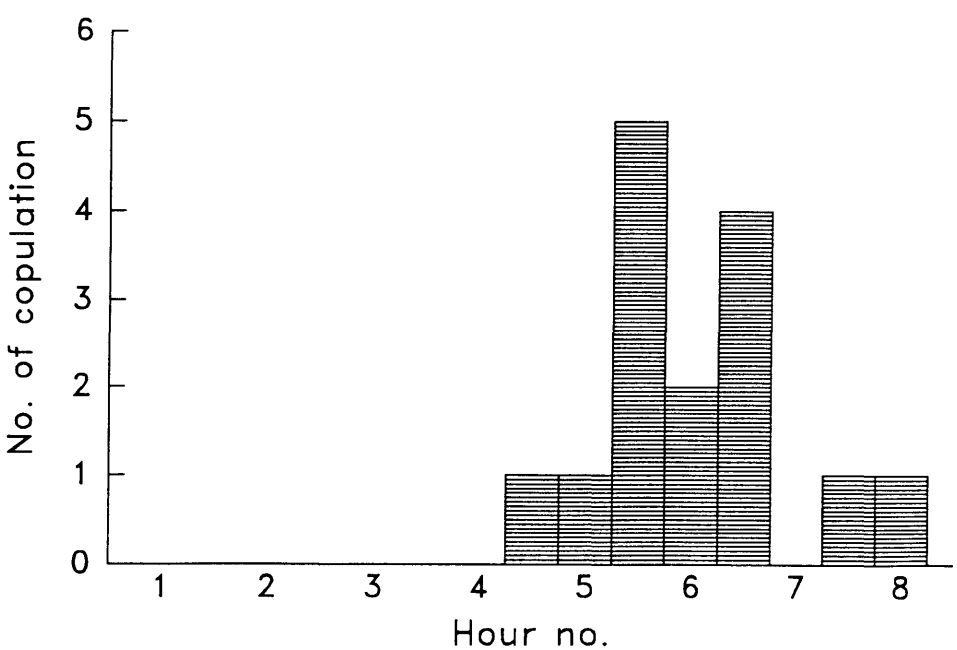

Fig. 7.3. Experiment 1. Number of copulations of *M. convecta* pairs in individual cages initiated every 30 minutes during the scotophase. Laboratory conditions were 20°C and 16:8 LD regime.

Female no.	Age at 1st calling (S)	Age at 1st mating (S)
1	3	6
2	4	3
3	3	7
4	8	5
5	8	9
6	5	7
7	6	12
8	3	4
9	6	4
10	3	3
11	3	6

Table 7.2. Experiment 1. Ages when first calling and first mating occurred in *M. convecta* females that were observed in individual cages.

Moths copulated for the first time between the 3rd and 12th scotophases (Table 7.2). Each pair copulated only once per scotophase. Two out of the 11 pairs had three copulations at different ages. One pair copulated on the 7th, 8th and 10th scotophases and the other pair copulated on the 3rd, 7th and 8th scotophases.

Like female calling, copulation occurred during the 2nd half of the scotophase (Fig. 7.3). Most copulation events were initiated between 5.5 and 6.5 hours after lights-off. The average onset time of copulation during the night was at 349 ± 13.9 (s.e.) min (6th hour) after lights-off. A regression analysis showed that onset time of copulation was not significantly correlated with age ($p = 0.83$). The average duration of copulation was 113.5 ± 12.2 minutes.

In earlier experiments, the amount of calling in unmated *M. convecta* females that were ovipositing was less than that in non-ovipositing ones (Fig. 4.14, Chapter 4). In this experiment, mated females laid more eggs than unmated ones (Fig. 7.4). The average for mated females was 119.4 ± 10.6 (s.e.) and for unmated females it was 38.7 ± 5.1 eggs per night ($F_{1,116} = 48.98$, $p < 0.001$). Mated females also spent more time in oviposition (41.5 ± 5.1 minutes) compared with unmated females (24.5 ± 3.7 minutes per scotophase) ($F_{1,208} = 7.44$, $p = 0.007$).

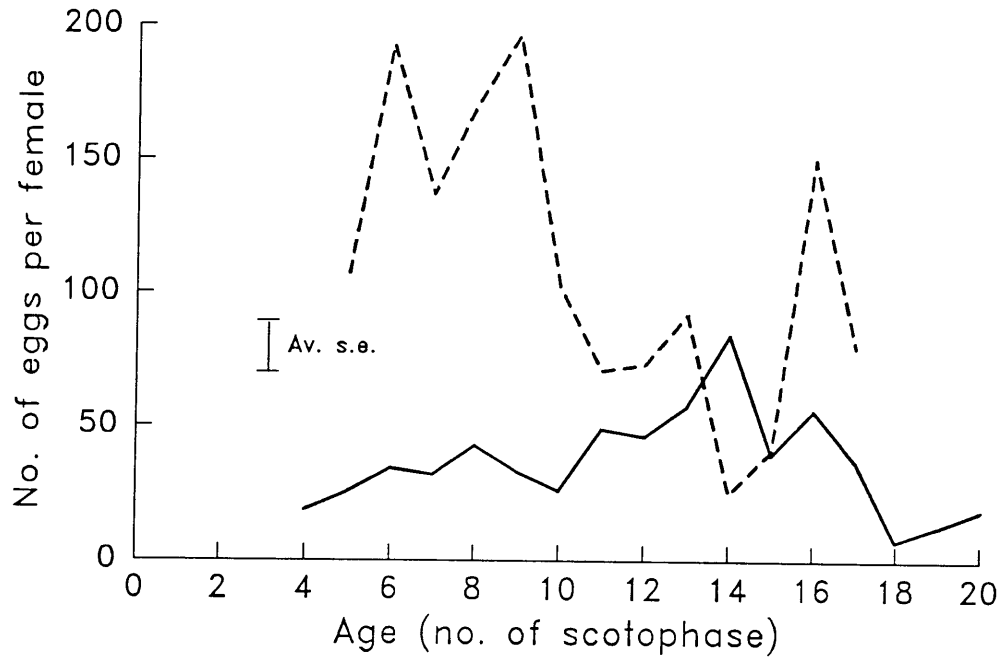

Fig. 7.4. Experiment 1. Mean number of eggs laid by unmated (——) and mated (- - -) *M. convecta* females at different ages in individual cages. Laboratory conditions were 20°C and 16:8 LD regime.

Experiment 2 - Mating behaviour in mass-mating cages

As in the individual cages, calling in the mass-mating cages also occurred during the 2nd half of the scotophase, peaking during the 7th hour (Fig. 7.5). There was no significant difference in calling duration between mated and unmated females, in contrast to the result in individual cages. Copulation started between 4.5 and 7.5 hours after lights-off (Fig. 7.6). Of the 12 males and 12 females in the two cages, 9 males and 9 females (75%) copulated. Most of them copulated only once. Two females copulated twice. One of these copulated twice to the same male and the other one copulated with a different male each time. One male copulated thrice, twice with the same female and once with a different female. The duration of copulation lasted between 120 and 156 minutes, with an average of 122.3 ± 10.8 (s.e.) minutes. Three pairs that copulated were locked, that is, their genitalia were stuck and did not disengage.

The pre-copulatory and copulatory behaviour of *M. convecta* males and females in the mass-mating cages is described below:

Pre-copulatory behaviour started when a female initiated protruding and retracting the ovipositor for a short period before assuming the calling posture or the full protrusion of the ovipositor. During calling, the female either remained stationary and continued calling or walked around the cage with the ovipositor extruded. When a female was calling, some males began to be active while others remained resting. An active male then approached the calling female, usually touching the tip of her abdomen with his legs. Sometimes, a calling female chased an active male, also touching the tip of his abdomen. The male opened the claspers and thrusted them towards the female ovipositor. When copulation was successful, the male and female immediately faced each other's opposite direction. When copulation was unsuccessful, both male and female became active again, and moved around the cage. The female immediately retracted the ovipositor while the male retracted claspers. Oftentimes, the male made a second attempt to copulate with the same calling female. If it was not successful, both became active for a while then rested.

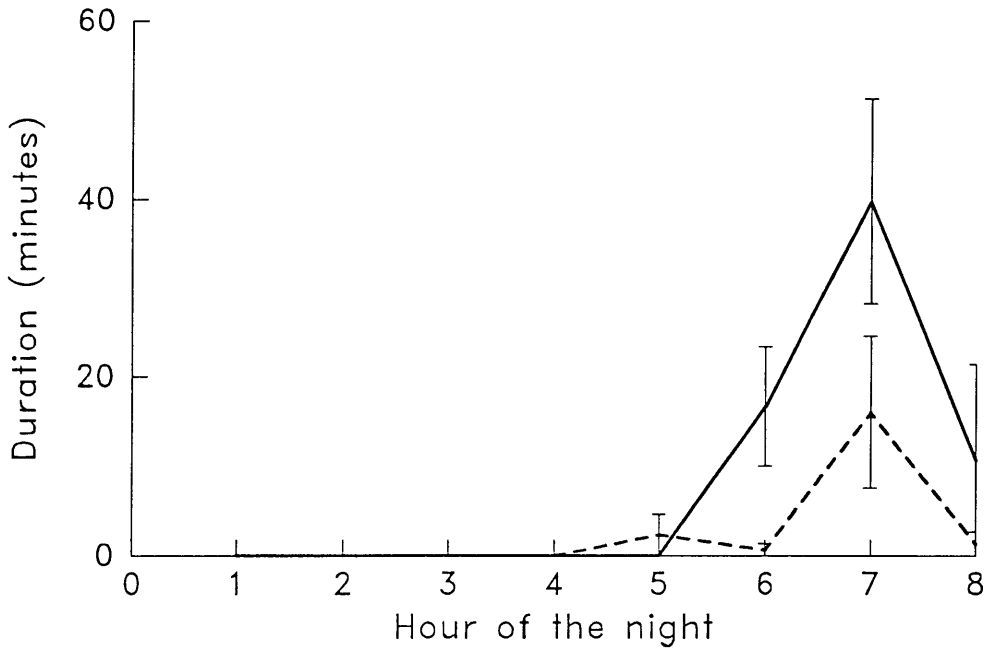

Fig. 7.5. Experiment 2. Mean duration of calling in unmated (——) and mated (- - -) *M. convecta* females in the mass-mating cages at different times of the scotophase. Laboratory conditions were 20°C and 16:8 LD regime. Bars are s.e.'s.

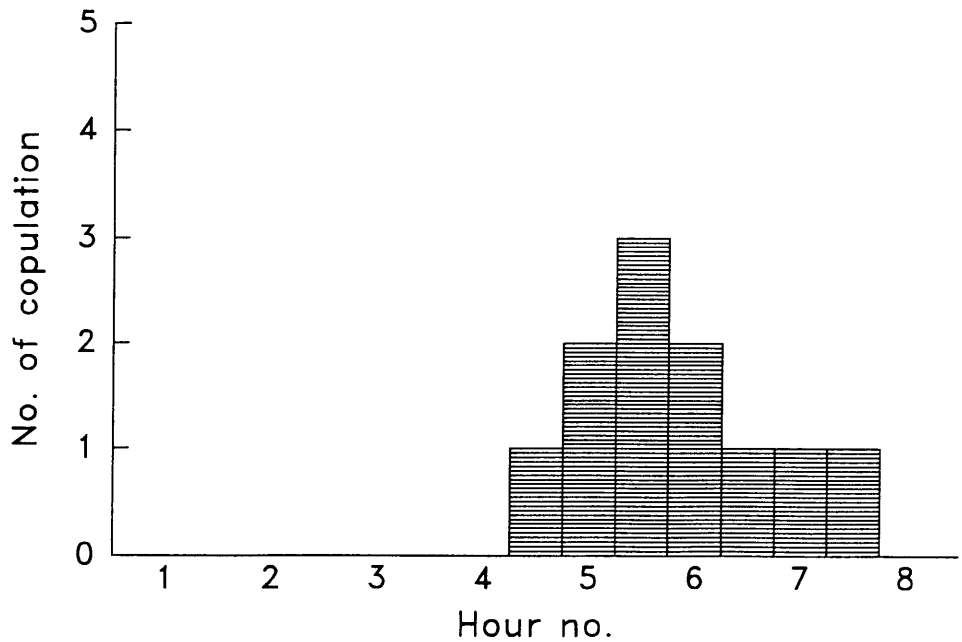

Fig. 7.6. Experiment 2. Number of copulations of *M. convecta* pairs in the mass-mating cages every 30 minutes during the scotophase. Laboratory conditions were 20°C and 16:8 LD regime.

7.3. Field experiment

7.3.1. Materials and methods

The field experiment was conducted at the University of Queensland-Gatton College campus, Gatton, Queensland (27° 32' S, 152° 18' E) for five nights on 5-10 May, 1992. This site was chosen because, at the time of the year, night temperatures in the Armidale region would have been too low. A walk-in field cage was set up on a paddock of native pastures, adjacent to a sorghum crop (see Chapter 3.3). A chart recorder (Grant Model D, Grant Instruments, Ltd., Barrington, Cambridge, UK) was set up inside the cage to record hourly temperatures during the observation period. During the experimental period the daylength averaged about 11 hours and the mean times of sunrise and sunset were at 0616 and 1712 h AEST, respectively (Department of Conservation and Land Management, Brisbane, Queensland, pers. comm., 1994).

Larvae were reared under the normal rearing conditions in the insectary. Pupae were kept in the insectary at 25 ± 1°C under a 16:8 LD regime, with the scotophase during 2100-0500 h AEST. A total of 78 males and 92 females with ages ranging from 1 to 7 scotophases, were placed in the cage. Males were marked on their right forewings to distinguish them from females, but because of the number of moths it was not possible to identify individuals.

Moths were observed by means of night vision goggles and close observations were done using a torch covered with a layer of red filter (see Chapter 3.4). Observations were done from 1700 to 0300 h at 15-min intervals except on the 1st night when recording of observations did not start until 2000 h due to delay in putting up the field cage. During each observation period, a 1 meter-wide area of the cage was searched at a time, starting from one corner of the cage till the whole cage was covered. The behaviour of each moth that was seen on the roof, walls and base of the cage was recorded. The ground was also searched for any moths that might have been resting, walking, ovipositing or mating. All observations were recorded on a cassette recorder and later transcribed for statistical analysis.

Moth behaviours were categorised into the following: take off from the ground, resting, activity (walking or flying), female calling and oviposition, male approach to calling females and copulation. Behaviours of males when females were calling were described. At the end of the experiment, all moths that could be found inside the field cage were recovered and placed in 70% alcohol. Moths were dissected to determine the mated status of females.

Male and female behaviours were analysed separately. The numbers observed for each male and female behaviour were pooled for each hour of the night. Data for a given behaviour were expressed as a percentage of the total number of observations for all behaviours. Means and standard errors for these data were calculated using the MINITAB package (Ryan *et al.*, 1992). The same data were transformed using the arc-sine method (Steel & Torrie, 1981) for one-way analysis of variance against the time of the night. Moths used in the experiment were of different ages, thus analysis of the proportion of observations with regard to moth age was not possible.

The total number of observations for male and female behaviours at different times of the night are provided in the sub-directory A:\CHAP7 of the floppy disk.

7.3.2. Results and discussion

Hourly temperature readings during the experimental period are shown in Fig. 7.7. Maximum temperatures ranged from 19 to 22°C at 1700-1800 h, and minimums ranged from 10 to 15°C at 0200-0300 h.

Male and female behaviours at different times of the night

Take off from the ground was recorded mostly during the 1st hour (Fig. 7.8). A small proportion of take off was observed in the males from the 4th hour on. Resting was observed throughout the night (Fig. 7.9). The percentage of observations of resting females gradually declined between the 6th and 8th hours then increased again in the 9th-10th hours. Male resting increased towards the 2nd hour and remained roughly constant throughout the night. Activity of females was highest in the 2nd hour then declined later in the night while male activity was recorded throughout the night with a small peak during the 8th hour which was not seen in females (Fig. 7.10).

Female calling started during the 4th hour and peaked in the 8th hour then declined during the 9th-10th hours (Fig. 7.11). Oviposition was observed as early as the first hour peaking in the 3rd hour, after which it declined (Fig. 7.12). No females were seen ovipositing during the 9th and 10th hours. Female moths usually laid eggs on the leaves of grasses on the ground. Male approach to calling females was recorded from the 4th hour on (Fig. 7.13).

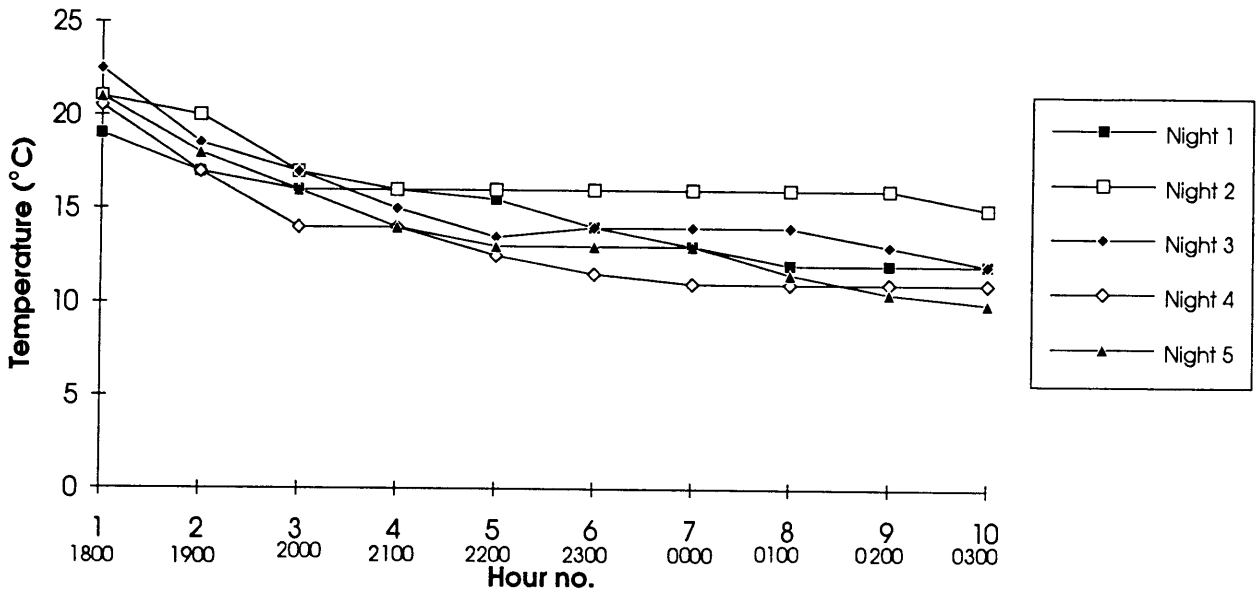

Fig. 7.7. Hourly temperature during the 10-hour observation period (1700-0300 h) on each of the five nights in the field cage.

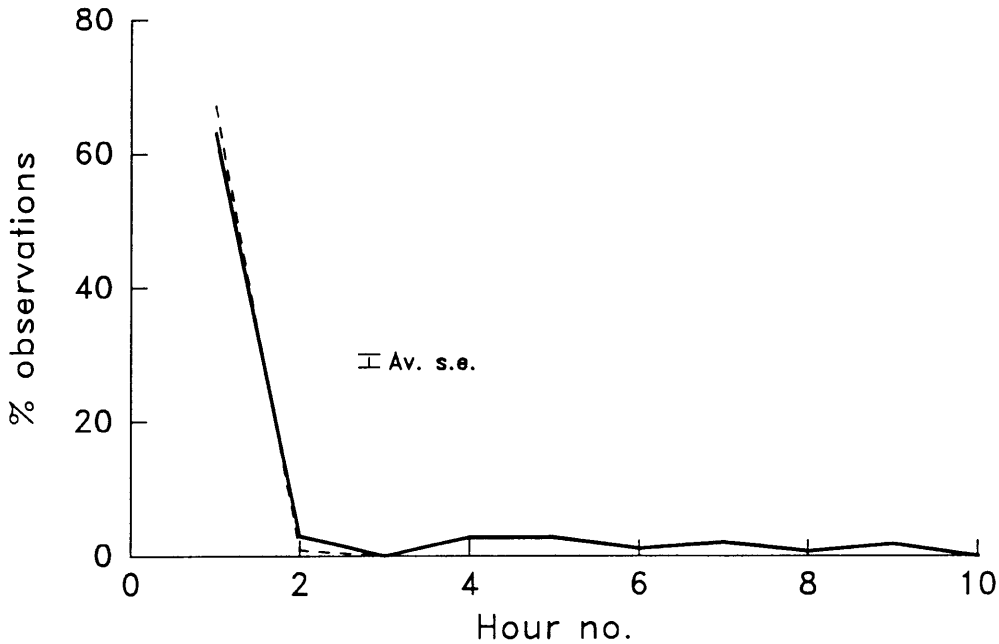

Fig. 7.8. Percentage (%) observations of *M. convecta* males (——) and females (- - -) that took off from the ground in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

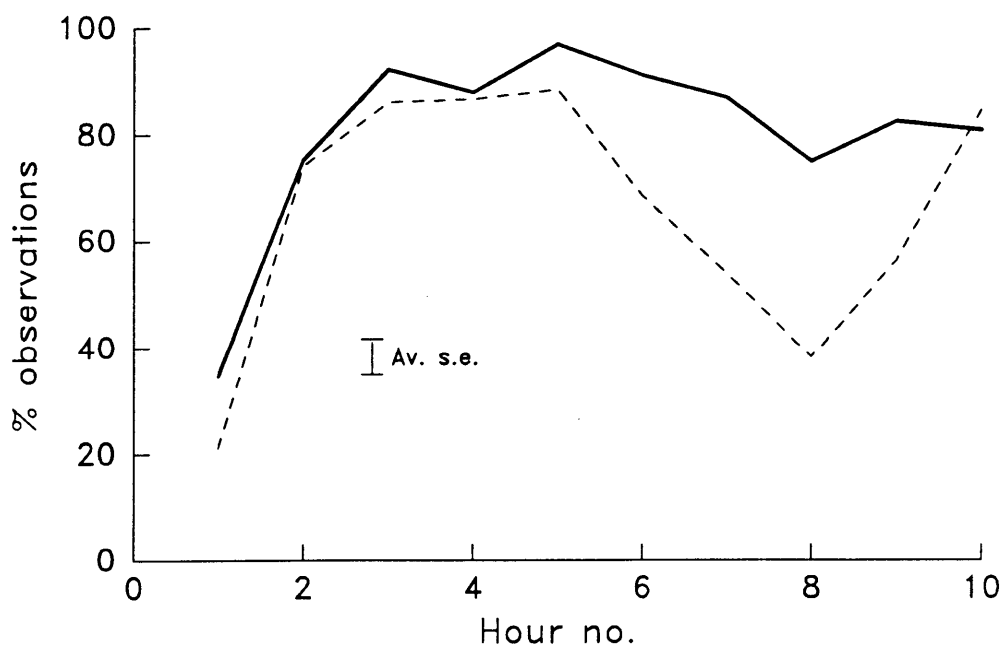

Fig. 7.9. Percentage (%) observations of *M. convecta* males (——) and females (- - -) that were resting in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

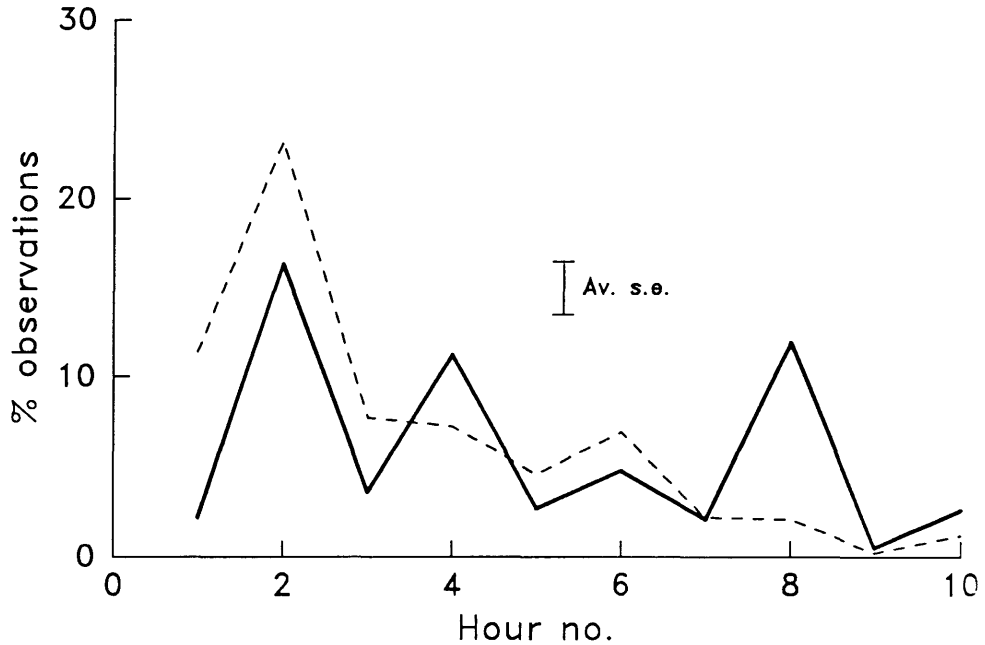

Fig. 7.10. Percentage (%) observations of *M. convecta* males (——) and females (– – –) that were active in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

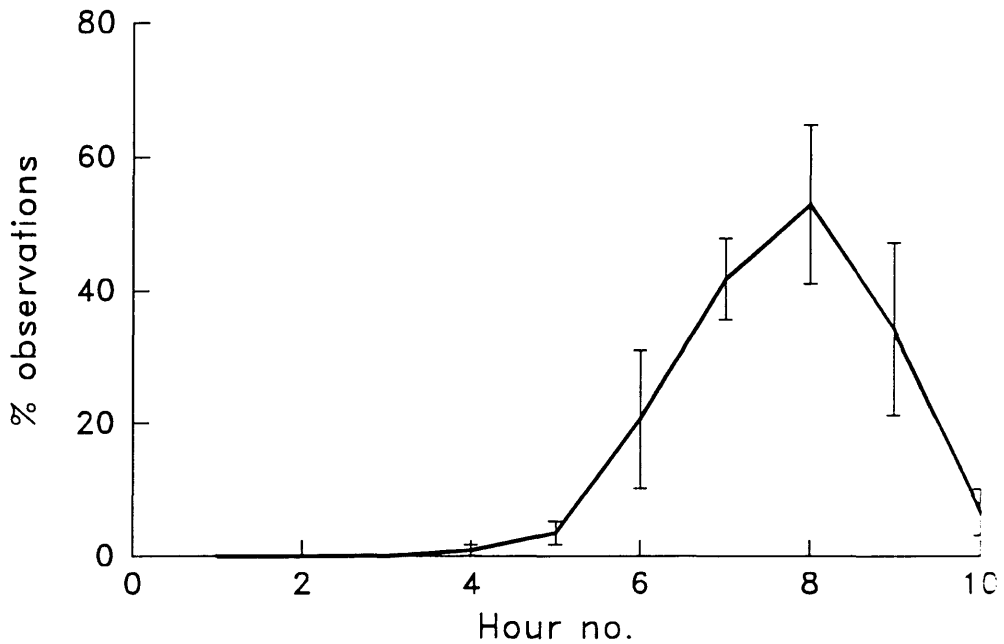

Fig. 7.11. Percentage (%) observations of *M. convecta* females that were calling in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

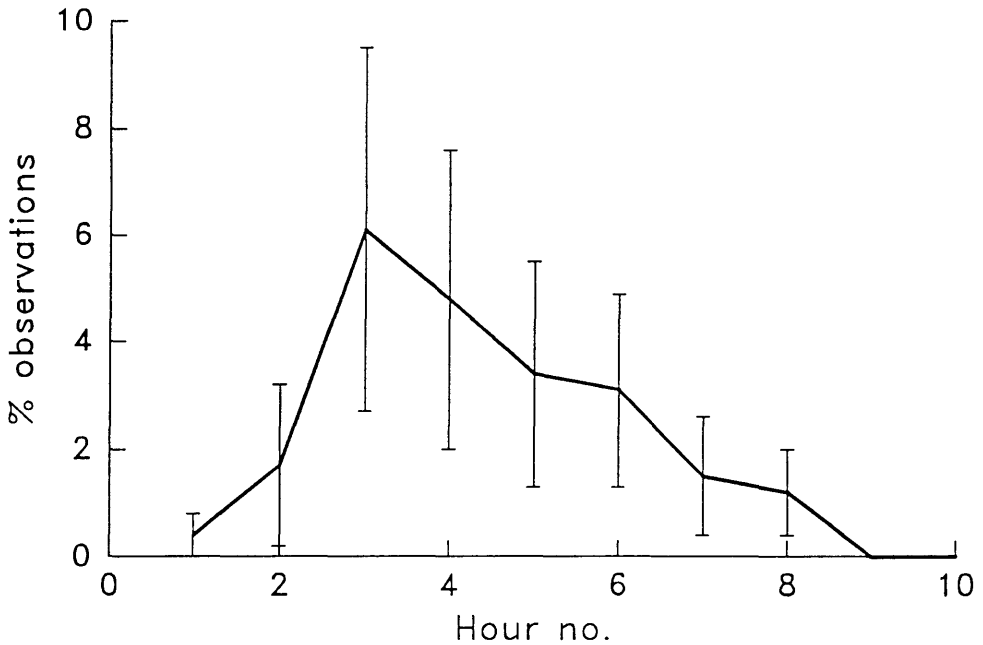

Fig. 7.12. Percentage (%) observations of *M. convecta* females that were ovipositing in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

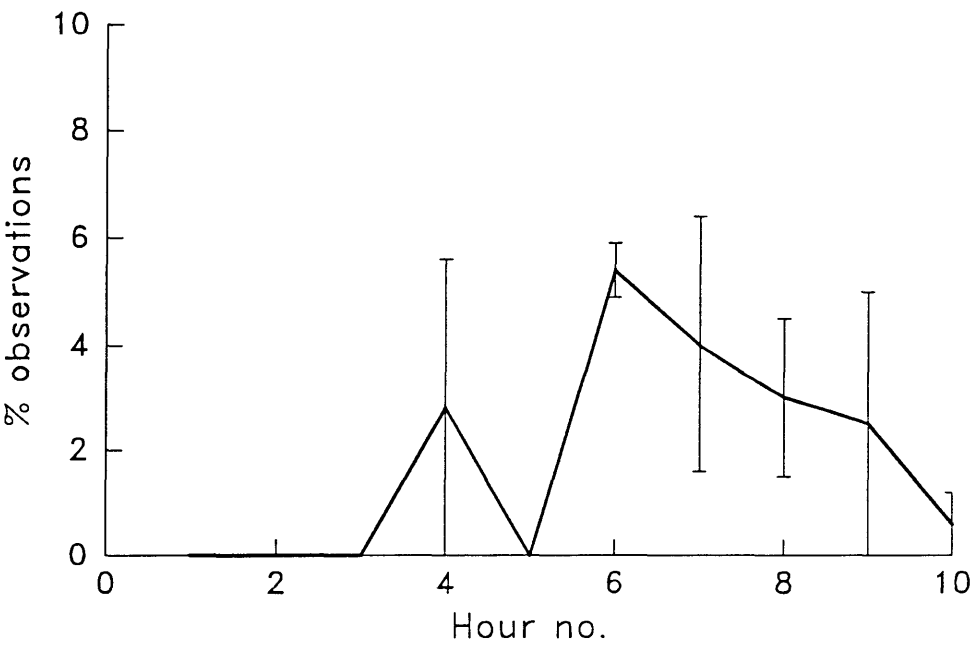

Fig. 7.13. Percentage (%) observations of *M. convecta* males that approached calling females in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

Relationship between calling and temperature

Under laboratory conditions, *M. convecta* females did not call at 10°C but did call at 15°C (Chapter 5.3.2). In the field cage, most calling was observed between the 6th and 9th hours of the night (Fig. 7.11). Temperatures at these times ranged between 11 and 16°C during the five nights of the experiment (Fig. 7.7). A regression analysis was conducted to test the relationship between calling and temperature during this period in the field cage (Fig. 7.14).

The regression equation was:

$$C = -9.1 + 3.45t$$

where C = % calling observations
t = temperature

From this regression an estimate of the threshold for calling was 9.1°C. However, the regression analysis was not significant ($p = 0.34$) and the R^2 was low (0.05), indicating that calling was not strongly related to temperature.

Mating behaviour

Different male behaviours were observed when females were calling. These are described below.

(1) Resting without movement - the male remained resting even if only 5-10 cm away from a calling female.

(2) Resting with some movement - a resting male close to a calling female fluttered wings, moved antennae touching them with the forelegs, then opened claspers but did not move towards female.

(3) Approach to calling female with clasper extrusion then resting - a male resting on the wall near the ground climbed up towards the calling female with claspers opened. When about 5 cm from the female, male rested with the claspers still slightly opened.

(4) Chasing calling female - a male chased a female that was walking or flying around the cage and had the ovipositor protruded. The male occasionally touched protruded ovipositor while chasing the female.

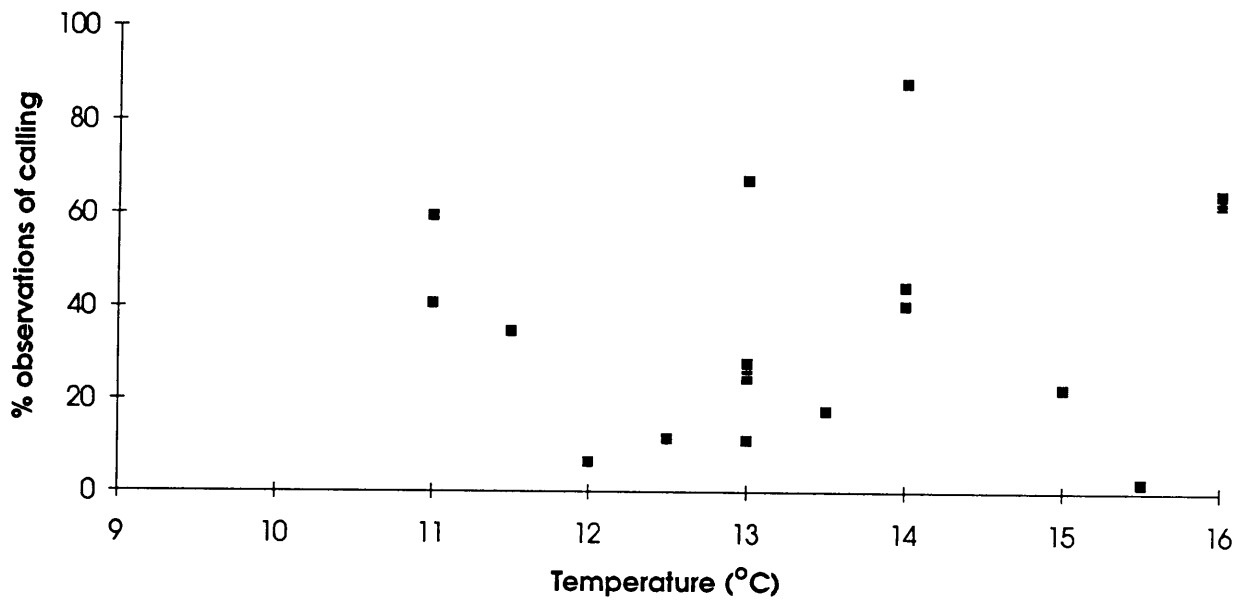

Fig. 7.14. A scatter diagram of the percentage (%) observations of *M. convecta* females that were calling in the field cage between 2300 and 0200 h plotted against temperature during these times..

(5) Attempt to copulate but unsuccessful - a male close to a calling female opened claspers, raised abdomen then thrusted claspers to female ovipositor. When copulation was unsuccessful, both male and female became active. The female retracted ovipositor and the male either retracted claspers or left them slightly opened. In some cases, the male continued to chase the active female with the claspers still extruded and tried to copulate again. After a second unsuccessful attempt, the male usually retracted claspers and rested while the female continued to fly around the cage.

(6) Successful copulation - a male approached a calling female, fluttering wings and moving antennae. The male then opened claspers when about 2 cm from the female, thrusted claspers towards the female ovipositor and successfully copulated. Once coupled, the male and female immediately faced each other's opposite direction.

Copulation usually lasted for more than an hour. Copulation started during the 6th hour of the night (Fig. 7.15). The percentage of observations of males in copulation was greater than the females during the 9th and 10th hours. This may have been because males not engaged in copulation were inconspicuously resting on the cage floor at this time, where they were less likely to be observed than the females, which were more likely to be conspicuously calling or ovipositing. The difference would then be an artifice of the observation technique.

One-way analyses of variance were conducted for the different behaviours against time of the night (Table 7.3).

Behaviour	$F_{9,34}$ value	P value
Male		
Take off	9.73	< 0.001
Activity	2.08	0.06
Resting	5.37	< 0.001
Approach	1.46	0.20
Copulation	7.27	< 0.01
Female		
Take off	13.41	< 0.001
Activity	3.93	0.002
Resting	7.73	< 0.001
Calling	6.90	< 0.001
Oviposition	1.17	0.34
Copulation	5.41	< 0.001

Table 7.3. F and P values obtained by one-way analyses of variance of the proportions of the different behaviours at different times of the night. Data for these analyses were arc-sine transformed.

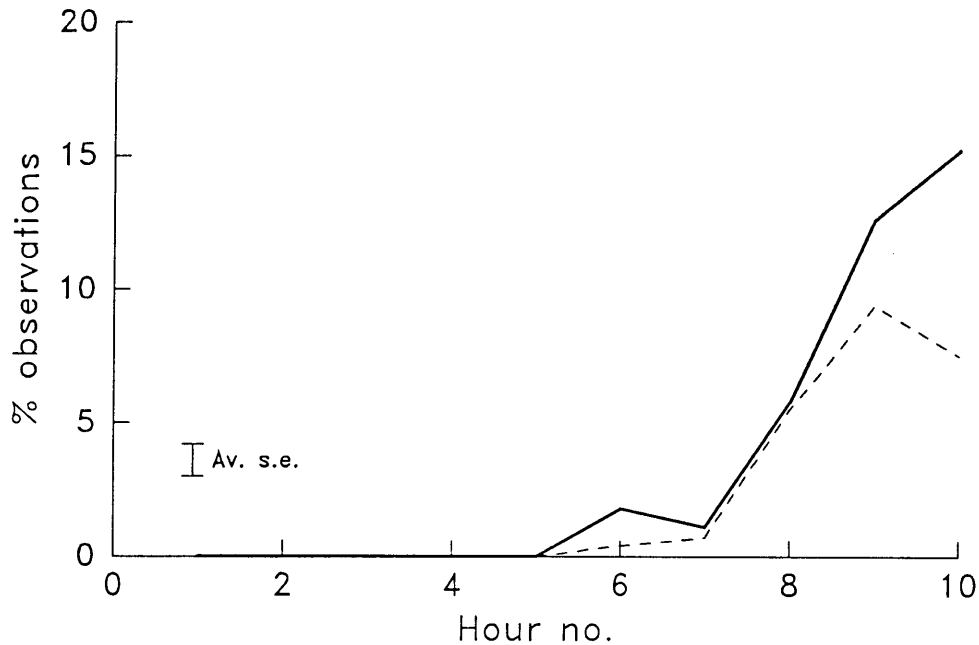

Fig. 7.15. Percentage (%) observations of *M. convecta* males (——) and females (- - -) that copulated in the field cage at different hours of the night. Hour 0 = 1700 h, hour 10 = 0300 h AEST. Data were transformed by the arc-sine transformation method. Bars are s.e.'s of the mean percentage for the five nights of observation.

The results indicate that the time of the night significantly influenced take off, resting and copulation behaviours of both males and females, and female calling. Take off from the ground was observed soon after sunset. Resting was recorded throughout the night, but was most pronounced between the initial period of take off and activity early in the night, and the onset of reproductive behaviours in the 2nd half of the night. Female calling and copulation occurred later in the night.

A total of 31 males, 43 unmated females and 5 mated females were recovered from the cage at the end of the experiment. This represents a survival rate, over the 5 nights of the experiment, of 40% for males and 52% for females, and a successful copulation rate of 10% for the surviving females. The copulation rate is lower than might be expected in view of the fact that a peak of about 10% of moths were copulating on any night, over a period of five nights. On this basis, it might be expected that at least 50% of the females would have successfully mated.

There are two possible reasons for this discrepancy. One is that, for some unknown reason, most of the observed copulations were unsuccessful, that is, they did not result in the transfer of a spermatophore. The second (more likely) is that mortality was biased towards the mated females, which were probably older on average than unmated ones, since the cage contained moths of varying ages. Unmated females tend to survive longer than mated ones (Smith, 1986).

Of the five mated females, all had only one spermatophore in the bursa copulatrix, indicating that they copulated only once. This result is consistent with those observed in the individual and mass-mating cages.

7.4. General discussion

Copulation in the individual and mass-mating cages did not start until the 3rd scotophase. This result is consistent with that found in Chapter 6, where male sexual response to calling females in a wind tunnel was not recorded until the 3rd scotophase, and with those of Chapter 4 which indicated that most females did not begin calling until at least the 3rd scotophase. It provides further evidence that *M. convecta* males and females do not reach reproductive maturity until this time.

The pre-copulatory and copulatory behaviours of *M. convecta* males and females were similar to those described in other noctuids (e.g, Agee, 1969; Lingren *et al.*, 1977). Pre-copulatory behaviour started when the female exhibited calling behaviour. A male responding to a calling female exhibited a sequence of behaviour similar to that observed

in the wind tunnel (Chapter 6.4.2.2). When the pheromone was perceived, a male moved towards the vicinity of the calling female with the claspers fully extruded. He then curved his abdomen towards the female ovipositor and attempted to copulate with the female. Once copulation was successful, the mating pair quickly assumed the position with their heads facing each other's opposite direction.

Some noctuids like *M. configurata, H. zea* and *S. frugiperda*, copulate several times, whereas others like *S. nonagrioides*, mate only once during their lifetime (Howlader & Gerber, 1986b; Raina *et al.*, 1986a; Simmons & Marti, 1992; Babilis & Mazomenos, 1992). In *M. convecta*, multiple copulation up to 3 times was observed in the individual cages as well as in the mass-mating cages. However, most individuals mated only once.

Copulation has been shown to suppress calling on subsequent nights in some noctuids like *M. configurata* and *H. zea* (Howlader & Gerber, 1986b; Raina *et al.*, 1986a). In individual cages, *M. convecta* females that had copulated called for shorter periods than those which had not. However, females which had copulated also laid more eggs and spent more time in oviposition than those which had not. It has been previously shown that oviposition suppresses calling, even in virgin females (Chapter 4.3.2.2). Thus, it is not possible to determine whether the reduction of calling in females which had copulated is due to copulation *per se*, or to the increased oviposition which followed copulation. In any case, the result was not repeated in the mass-mating cages.

Copulation did not occur in the majority of the pairs in the individual cages, although the females had been calling for several nights. Only 11 out of the 43 pairs copulated. When the female was calling, the male was either resting or moving around the cage but often did not open claspers or attempt copulation with the female. This male behaviour was also observed in the field cage. Some males which were only about 5-10 cm away from a calling female remained resting and did not exhibit any sexual response behaviour.

In the laboratory, most copulation was initiated between 5.5 and 6.5 hours after lights-off (Figs. 7.3 and 7.6). By contrast, the peak calling duration was in the 7th hour (Figs. 7.2 and 7.5). It appeared that females that called early were more receptive or more likely to attract a male for copulation than when they called later. Copulation occurred only once during the night. It seemed that if it was going to happen, it happened quickly. But often it did not happen at all. Thus, much of the calling later in the night was prolonged calling by females that did not copulate earlier.

The reasons why very few matings occurred are not clear. It may be that even if the females were extruding their ovipositors they were not receptive or not releasing the right pheromone quantity and quality to attract the males. In the case of individual and mass-mating cages, there was no wind, so pheromone plumes would not have formed. It is possible that the whole cage might have been saturated in pheromone, and this disrupted male response. Mating of *S. littoralis* in closed containers permeated with Z9,E11:14 Ac was significantly reduced (Dunkelblum *et al.*, 1987). Alternatively, when the females were calling, the males were not responding, because of the lack of the right olfactory, tactile, visual or auditory stimuli. The space in the cages may not have been sufficient to allow the full behavioural repertoire, which might cause lack of receptivity in the females or lack of sexual response in the males.

In the case of the field cage, lack of mating might sometimes be because the direction of the wind carrying the pheromone emitted by the calling female was different from where the male antennae were oriented. Alternatively, in both the field and in the smaller cages, female receptivity might sometimes have been asynchronous with the male "response window". Evidence that this might occur at certain times of the night is discussed in Chapter 6.6. The contrast between the larval and pupal scotophase (8 hours) in the insectary and the constant laboratory temperatures compared with fluctuating temperatures and longer scotophase might have also affected moth behaviour in the field.

Copulation and calling in *M. convecta* in the field cage occurred between the 6th and 10th hours (2300-0300 h). On the other hand, oviposition was observed as early as the 1st hour (1800 h) till the 8th hour (0100 h). The timing of these behaviours were similar to those of other noctuids observed in field cages. In *H. virescens* and *P. includens* which were observed in a field cage between 1900 and 0800 h, oviposition started between 2000 and 2200 h and mating occurred between 2300 and 0400 h (Lingren *et al.*, 1977). Ramaswamy (1990) found that in mated *H. virescens* females observed in a field cage between 1930 and 0600 h, most oviposition and calling occurred before 0300 h and between 0100 and 0430 h, respectively.

On the five observation nights in the field cage, the temperature during the observation period (1700-0300 h) ranged from 10 to 22°C. The temperatures when most calling occurred (between 2300 and 0200 h), ranged from 12 to 16°C (Fig. 7.14). In the laboratory, females did not call at constant 10°C but did call at 15°C (Chapter 5). These results and those from the field cage study cannot be compared directly because in the latter, moths experienced fluctuating temperatures whereas in the laboratory, they were

under constant temperatures during the photophase and scotophase. The expression of calling behaviour under constant and fluctuating temperatures may vary (Delisle, 1992). The threshold for calling in the field cage was estimated to be 9.1°C, which is not very different to what was previously suggested to be the lower limit for calling in *M. convecta*, i.e., slightly above 10°C (Chapter 5.6). However, the regression line was not significant, indicating that temperature was not a major factor influencing calling. This result suggests that the length of the night or its combined effect with temperature, might have been more important than temperature alone in the expression of calling behaviour in the field.

The percentage of observations of calling females in the field cage declined from 0100 h (Fig. 7.11). Two possible reasons might explain this. At this time, it might be getting too cold or it might be too late in the night to continue calling. As shown above, temperature was not a major factor. Observations of *M. convecta* in the field cage were not continued after 0300 h. At this time, most of the moths were quiescent, although low percentages of calling and copulation were still recorded. In the laboratory, the peak calling time was advanced (relative to the end of the scotophase) by longer scotophases (Chapter 5.4.2). Thus, the decline in calling observed from the 8th hour in the field cage was probably associated with the length of the scotophase, which at about 13 hours was longer than any tested in the laboratory.

Copulatory behaviour in many species is mediated by the sex pheromones produced by the females. However, the mating strategies of some species may also involve the presence of a sex pheromone produced by the males. Landolt & Heath (1989) reported that in a wind tunnel, *T. ni* females exhibited upwind flight, plume tracking and source contact to live males, whole-body extracts and hairpencil extracts and that females were attracted throughout the scotophase. In a later study, Lenczewski & Landolt (1991) found that mating strategies of *T. ni* involved a male-produced attractant earlier in the night and a female-produced attractant later in the night. They observed in a field cage that males were attracted to confined females between the 3rd and 8th hours, whereas females were attracted to confined males during the 1st hour of a 10-hour scotophase. Female attraction, in this case, was not associated with hairpencil display by the males. The presence of male pheromones in *M. separata* and *P. unipuncta* has also been reported (Grant *et al.*, 1972; Hirai, 1980).

Whether there is a sex pheromone in *M. convecta* males is not known. The effects of any male-produced attractants were not evident either in the laboratory or in the field experiments, although there were a few cases when a calling female chased an active male, sometimes touching the tip of his abdomen, and a calling female vibrated her wings and

erected antennae when near a male. Whether these female behaviours are characteristics of their receptivity for copulation or responses to an attractant produced by the males needs further investigation.

CHAPTER 8

SUMMARY AND CONCLUSIONS

8.1. Behaviour of *M. convecta* females

The nocturnal behaviour of *M. convecta* females was studied in the laboratory at 20 ± 1°C and 16:8 light:dark (LD) conditions. Female behaviour was classified as non-reproductive (activity, feeding and resting) and reproductive (calling, extrusion and oviposition). The patterns of these behaviours at different times of the scotophase and at different ages were described.

The non-reproductive behaviours, activity, feeding and resting, were recorded throughout the scotophase while the reproductive behaviours, calling, extrusion and oviposition occurred mostly during the 2nd half of the scotophase. The duration of activity was longest during the 1st hour of the scotophase, declining in the next hour, then gradually increased again with a broad peak between the 6th and 7th hours. Peak activity soon after lights-off might be related to dispersal to locate food sources or oviposition sites. On the other hand, activity later in the night might represent an attempt to locate more suitable sites for calling when they were unsuccessful in attracting mates, although in the field where space is not restricted, calling females are likely to be mated. Females were most active at 3 and 4 days of age. Activity declined as they became older. Feeding duration was generally constant throughout the scotophase and there were no clear patterns of feeding at different ages. Resting duration was longest during the 2nd hour declining towards the 7th hour but increased again in the last hour of the scotophase. Resting generally declined with age.

Extrusion or the partial extrusion of the ovipositor was observed to be a pre-oviposition behaviour. Extrusion and oviposition were observed throughout the scotophase with a single broad peak of duration between the 3rd and 7th hours. Calling behaviour or the behaviour associated with the release of sex pheromone to attract males, in *M. convecta* females is similar to that observed in many moth species. Calling started with a short period of protrusion and retraction of the ovipositor followed by its full protrusion, with the wings slightly raised above the abdomen.

The age at which *M. convecta* called for the first time was between the 2nd and 11th scotophases, with the peak on the 4th scotophase. The distribution of age at first calling was significantly different from the normal distribution, with evidence of bimodality. It was suggested that the early callers were the ones with earlier ovarian devel-

opment, whereas the late callers might be more adapted to migration instead of reproduction early in their lives. The frequency and duration of calling during the scotophase varied with age. Younger moths called more frequently with shorter duration, whereas older ones called less frequently but for longer. Calling, which occurred mostly during the 2nd half of the scotophase, peaked in the 7th hour.

Both chronological and calling ages were considered in the analyses of the different calling parameters on the basis that although calling age might provide homogeneity for comparison, the physiological condition of individual moths at different chronological ages might be a confounding factor. Statistical analyses of the relationships between different calling parameters or dependent variables (duration, onset time, number of bouts, duration per bout and peak calling time) and inherent factors or independent variables (age at first calling, chronological age, calling age and oviposition status) yielded consistent results. Calling duration and peak calling time were significantly correlated with age at first calling and oviposition status. Onset calling time and duration per bout were significantly influenced by chronological and calling ages. The number of calling bouts was significantly correlated with all four independent variables. These patterns suggested that there was a continuum from "dedicated callers", which began calling when younger and called for longer durations each night, with a peak later in the scotophase, and "reluctant callers", which started calling when older, called for shorter durations, and peaked earlier in the scotophase.

An experiment using young and old females in the same experimental chamber showed that their calling patterns were similar, suggesting that individual females did not influence each other's behaviour. Thus, conspecific pheromones might not be an important factor influencing calling behaviour in *M. convecta.*

8.2. Effects of temperature and photoperiodic conditions on calling behaviour

Temperature and photoperiod are two important environmental factors influencing the expression of calling behaviour in female moths. The calling behaviour in *M. convecta* females was studied at five temperatures (10, 15, 20, 25 and 30°C) and three photoperiodic conditions (16:8, 14:10 and 12:12 LD). The possibility of an endogenous circadian rhythm of calling was also examined by transferring females to constant darkness after various entraining periods.

The age at first calling was delayed at lower temperatures. Females maintained at 10°C did not call even after 31 scotophases, but those that were at this temperature for 10 scotophases exhibited calling behaviour when transferred to 20°C, after a delay comparable to that which normally occurred at 20°C. These results suggest that although *M. convecta* can survive at 10°C, the threshold for calling might be a few degrees above this temperature. Average onset calling times were earlier at 15 and 20°C than at 25 and 30°C. At all temperatures, most calling occurred during the 2nd half of the scotophase with a peak in the 7th hour.

The age at first calling was likewise delayed at longer scotophase. Mean onset time of calling was earlier at 16:8 LD than at 14:10 or 12:12 LD periods. At all photoperiods, calling occurred mostly in the 2nd half of the scotophase. Peak calling at 16:8 LD was in the 7th hour, at 14:10 LD in the 9th hour and at 12:12 LD in the 10th hour of the scotophase. Females that were entrained at a reverse-cycle 16:8 LD regime during the pupal stage and for 3 or 8 scotophases after emergence demonstrated an endogenous circadian rhythm of calling under continuous darkness. After 3-4 days, however, this rhythm became disrupted and females then adopted a calling pattern similar to that which would have been expected under either their previous larval scotophase or the ambient scotophase. The reasons for this re-synchronisation of calling are not clear. There was no obvious pattern of calling in females held at constant darkness during the pupal and adult stages, whereas females held at continuous light did not call.

The length of the pre-reproductive period as an index of the migratory potential has been suggested for some noctuids, in line with the "oogenesis-flight" syndrome. This study showed that *M. convecta* females have longer pre-reproductive periods at lower temperature and longer scotophases. This finding agrees with the seasonal migration patterns which have been documented for *M. convecta*. However, the relationship between pre-reproductive period and migratory potential in this species has not been investigated, although other studies have shown that *M. convecta* migrants caught in traps are usually pre-reproductive. Pre-calling period might not always be a good index of migratory potential, thus, the oogenesis-flight syndrome needs systematic study in this species.

8.3. Interactions between males and females

The behaviour of *M. convecta* males was studied in a wind tunnel. Observations were made during the 2nd half of the scotophase (1400-1800 h), when most female calling occurred. Males were first tested in the absence of females (i.e., blank source) then in the presence of females (calling or non-calling). Males were scored for take off, upwind flight, approach to source, landing on female cage and clasper extrusion, which was usually accompanied by copulatory attempt.

The full behavioural sequence, from take off to copulatory attempt, was recorded only with calling females. Males exhibited definitive sexual responses such as approach, landing and clasper extrusion, when flown to calling females. These behaviours did not occur in 1- and 2-day-old males, suggesting that males might not be reproductively mature before the 3rd scotophase. The pattern of male sexual response (i.e., approach to source) appeared to be synchronous with the calling pattern in females during the 2nd half of the scotophase. Two hypotheses were thought to explain this: that calling females early in the scotophase were not releasing sufficient quantity and quality of pheromone, or that males had a "window" of responsiveness to the pheromone. Investigating the relationship between pheromone titre and calling behaviour in *M. convecta* would help clarify the first hypothesis. Studying male responsiveness to a constant pheromone source using synthetic pheromones would elucidate the second hypothesis. The latter was done in Chapter 11 and the results are presented in Volume 2 of this thesis.

The effect of flight on male sexual responses in the wind tunnel was also studied. This was done by tethering males on flight mills for one scotophase, then testing them with calling females in the wind tunnel on the next scotophase. Responses of flown males were not significantly different from those of unflown males.

The interactions between male and female moths, particularly copulatory behaviour, were studied in laboratory cages and in a field cage. In the laboratory, most copulation was initiated between 5.5 and 6.5 hours after lights-off while female calling peaked in the 7th hour. Copulation occurred only once during the night. Females that called earlier were more likely to attract males than when they called later. Thus, most calling later in the night was due to prolonged calling of females that did not copulate. The duration of copulation averaged 113.5 ± 12.2 minutes in individual cages, and 122.3 ± 10.8 minutes in the mass-mating cages. Unmated females spent more time in calling and less time in oviposition compared with mated ones.

In the field, moths were observed in a walk-in cage between 1700 and 0300 h. Behaviours recorded were take off from the ground, activity, resting and copulation of both males and females, female calling and oviposition, and male approach to females. Male and female take off usually occurred during the 1st hour of the night. Female activity peaked in the 2nd hour then declined later in the night. Male activity was observed throughout the night with a small peak in the 8th hour. Calling, which started in some females during the 4th hour, peaked in the 8th hour and declining towards the 9th and 10th hours. Male approach to calling females was recorded from the 4th hour on. Oviposition on the leaves of grasses started in some females during the 1st hour, increasing towards the 3rd hour then gradually declined towards the 8th hour. Copulation, which started in some pairs in the 6th hour, increased later in the night.

Similar pre-copulatory and copulatory behaviours were observed in the laboratory and in the field. When a female exhibited the calling posture, some males became active. An active male approached the calling female, usually touching the tip of her abdomen with his legs. When the male was close to the calling female, he fully extruded his claspers and thrusted them towards the female ovipositor. When copulation was successful, they immediately faced each other's opposite direction. Copulatory behaviour in *M. convecta* was similar to that in other noctuid species.