# CHAPTER 5

# CALLING BEHAVIOUR IN *M. CONVECTA* FEMALES UNDER DIFFERENT TEMPERATURE AND PHOTOPERIODIC CONDITIONS

## 5.1. Introduction

The expression of calling behaviour in female moths is influenced by both endogenous and exogenous factors (Chapter 2.2.1). Temperature and photoperiod are two important environmental factors that can modify calling behaviour. Temperature can influence the length of pre-calling period, the onset of calling and the duration of calling (Sanders & Lucuik, 1972; Turgeon & McNeil, 1983; Delisle & McNeil, 1987a; Gerber & Howlader, 1987; Webster, 1988). Temperature effects on pheromone release can be physiological, such as changing the rate of metabolism, or physical, such as changing the rate of evaporation and dispersion of pheromone substances from the pheromone gland surface (Alford & Hammond, 1982). Temperature also influences the rate of maturation in insects, including their reproductive maturation (Smith, 1984; Berger, 1985; Gerber & Howlader, 1987; Hill & Gatehouse, 1992).

The endogenous periodicity of calling behaviour may be entrained by photoperiod (Sower *et al.*, 1970; Cardé & Roelofs, 1973). The timing of the diel periodicity of calling can depend on either the "lights-off" or the "lights-on" signals (e.g., Sower *et al.*, 1971; Sanders & Lucuik, 1972; Cardé & Roelofs, 1973; Delisle & McNeil, 1986; Webster & Conner, 1986; Gerber & Howlader, 1987). Like temperature, photoperiod can also influence the age at first calling, onset of calling and the length of the calling period (Delisle & McNeil, 1986; Noldus & Potting, 1990). The expression of calling behaviour can be modified when the entraining light:dark cycle is altered (Sanders & Lucuik, 1972; Delisle & McNeil, 1987a).

Nocturnal behaviour, particularly calling behaviour, in *M. convecta* females at 20°C and 16:8 light:dark (LD) conditions has been previously described (Chapter 4). It was shown that calling behaviour in this species varied with age and time of the scotophase. The highest number of females calling for the first time was during the 4th scotophase and calling duration peaked during the 7th hour of the scotophase. In this chapter, the effects of different temperature and photoperiodic conditions on calling behaviour are discussed.

## 5.2. Materials and methods

The laboratory conditions for moth rearing and methodology for behavioural observations, as well as recording of observations, were described in Chapters 3.1 and 3.2. Upon emergence moths, held in individual clear plastic containers, were transferred to the desired temperature and photoperiodic conditions. Behavioural observations were done at 30-min intervals during the scotophase. Parameters measured were the age at first calling, mean time of onset of calling, and mean duration of calling at different ages and at different hours of the scotophase.

Three experiments were conducted. Experiment 1 investigated calling behaviour under five constant temperatures at 16:8 LD period. In Experiment 2, female calling was studied under three constant photoperiodic conditions at 20°C. The possibility of a circadian rhythm in calling behaviour was examined in Experiment 3.

## 5.3. Experiment 1 - Calling behaviour at different temperatures

### 5.3.1. Materials and methods

Five groups of females were observed under five constant temperatures - 10, 15, 20, 25 and 30 ± 1°C at 16:8 LD regime, with the scotophase during 1000-1800 h AEST. The number (N) of females for each group was 9 except at 30°C which had 14. All five groups were observed during one experimental period. Due to time and labour constraints, it was only possible to observe a relatively small number of moths per group. The alternatives would have been to have observed more moths in fewer temperature regimes, or to have split the experiment between two or more batches of larvae. The latter procedure might have introduced extraneous sources of variation.

At 10°C, no female started calling even after 10 scotophases. To investigate whether females from this temperature would eventually call when subjected to warmer conditions, 5 of the 9 females in this group were transferred to 20°C after 10 scotophases and were observed for a further 13 scotophases. This group of 5 females will be referred to as the "ex-10°C" group. The remaining 4 females were kept at 10°C and observed for a further 21 days.

Data on the various calling parameters were analysed using one-way analysis of variance, regression analyses and the generalised linear model (GLM) routine in MINITAB (Ryan *et al.*, 1992) as described in Chapter 3.2.1.

Raw data for calling at the different temperatures are provided in the sub-directory A:\CHAP5 of the floppy disk.

### 5.3.2. Results and discussion

The numbers of moths surviving and calling at different ages under the different temperatures are presented in Table 5.1.

| | Number of moths surviving and calling at different ages under various temperatures | | | | | |
|---|---|---|---|---|---|---|
| Age (S) | 10°C | 15°C | 20°C | 25°C | 30°C | ex-10°C |
| 1 | 9 (0) | 9 (0) | 9 (0) | 9 (0) | 14 (0) | - |
| 2 | 9 (0) | 9 (0) | 9 (0) | 9 (0) | 14 (0) | - |
| 3 | 9 (0) | 9 (0) | 9 (0) | 9 (2) | 14 (1) | - |
| 4 | 9 (0) | 9 (0) | 9 (0) | 9 (4) | 14 (0) | - |
| 5 | 9 (0) | 9 (0) | 9 (2) | 9 (4) | 12 (0) | - |
| 6 | 9 (0) | 9 (0) | 9 (2) | 9 (3) | 6 (0) | - |
| 7 | 9 (0) | 9 (1) | 9 (2) | 6 (5) | 6 (2) | - |
| 8 | 9 (0) | 9 (6) | 8 (3) | 6 (6) | 6 (2) | - |
| 9 | 9 (0) | 9 (3) | 8 (3) | 6 (4) | 5 (4) | - |
| 10 | 9 (0) | 9 (4) | 8 (5) | 6 (4) | 5 (4) | - |
| 11 | 4 (0) | 9 (7) | 8 (4) | 6 (4) | 5 (4) | 5 (0) |
| 12 | 4 (0) | 9 (4) | 5 (2) | 6 (3) | 3 (2) | 5 (0) |
| 13 | 4 (0) | 9 (4) | 3 (1) | 4 (2) | 3 (1) | 1 (0) |
| 14 | 4 (0) | 9 (3) | 3 (1) | 4 (2) | 2 (0) | 5 (0) |
| 15 | 3 (0) | 9 (2) | 2 (0) | 4 (4) | - | 5 (1) |
| 16 | 3 (0) | 9 (4) | 2 (0) | 3 (3) | - | 5 (2) |
| 17 | 3 (0) | 9 (4) | 1 (0) | 3 (1) | - | 4 (4) |
| 18 | 3 (0) | 9 (5) | - | 3 (1) | - | 4 (2) |
| 19 | 3 (0) | 9 (4) | - | 2 (2) | - | 4 (1) |
| 20 | 3 (0) | 8 (3) | - | 1 (0) | - | 3 (1) |
| 21 | 3 (0) | 1 (0) | - | - | - | 2 (0) |
| 22 | 3 (0) | - | - | - | - | 1 (1) |
| 23 | 3 (0) | - | - | - | - | 1 (1) |
| 24 | 3 (0) | - | - | - | - | 1 (0) |
| 25 | 3 (0) | - | - | - | - | - |
| 26 | 3 (0) | - | - | - | - | - |
| 27 | 3 (0) | - | - | - | - | - |
| ..... | ..... | - | - | - | - | - |
| 31 | 3 (0) | - | - | - | - | - |
| ..... | ..... | - | - | - | - | - |
| 50 | 3 | - | - | - | - | - |
| ..... | ..... | - | - | - | - | - |
| 57 | 1 | - | - | - | - | - |

**Table 5.1**. Experiment 1. Number of *M. convecta* females surviving and calling (in parenthesis) at different ages (number of scotophases) under various temperatures. After age 31, no further observations of calling behaviour were made on the 10°C group. At age 10, 5 females were transferred from 10°C to 20°C, to become the "ex-10°C" group.

Moth longevity, expressed as the number of scotophases moths survived, ranged from 15 to 57 at 10°C; 18 to 31 at 15°C; 8 to 17 at 20°C and 4 to 15 scotophases at 30°C. The longevity of ex-10°C females ranged from a further 5 to 13 scotophases at 20°C.

*Age at first calling*

The number of moths that called for the first time at different ages for each temperature group is shown on Table 5.2.

| Age (S) | 15°C | 20°C | 25°C | 30°C | ex-10°C |
|---|---|---|---|---|---|
| 3 | 0 | 0 | 2 | 1 | 0 |
| 4 | 0 | 0 | 2 | 0 | 1 |
| 5 | 0 | 2 | 1 | 0 | 1 |
| 6 | 0 | 0 | 2 | 0 | 2 |
| 7 | 1 | 1 | 0 | 2 | 0 |
| 8 | 5 | 2 | 1 | 1 | 0 |
| 9 | 0 | 2 | 0 | 1 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 |
| 11 | 2 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 |
| 14 | 1 | 0 | 0 | 0 | 0 |
| Total | 9 | 7 | 8 | 5 | 4 |

**Table 5.2.** Experiment 1. Number of *M. convecta* females calling for the first time at different ages under various temperatures. The number of females that did not call were: 20°C - 2/9, 25°C -1/9, 30°C - 9/14 and ex-10°C - 1/5. Four females that remained at 10°C did not call. Ages for ex-10°C females were the number of scotophases following transfer to 20°C.

At 15°C, first calling was observed between the 7th and 14th scotophases, at 20°C, between the 5th and 9th scotophases, at 25°C, between the 3rd and 8th scotophases and at 30°C, between the 3rd and the 9th scotophases. There was a significant linear regression ($F_{1,27} = 9.53$, $p = 0.005$, $R^2 = 0.26$) between the age at first calling and temperature. The regression equation was:

$$A = 12.2 - 0.235t$$

where A = age at first calling

t = temperature

The regression has a negative slope indicating that at lower temperatures, *M. convecta* moths generally called for the first time when they were older. The significance derived mainly from differences occurring between moths kept at 15, 20 and 25°C. When the 30°C group was excluded, regression analysis yielded the equation:

$$A = 15.8 - 0.434t \qquad (F_{1,22} = 21.91, p < 0.001, R^2 = 0.5)$$

There was no significant difference between moths kept at 25 and 30°C.

Because of the non-normal distribution of age at first calling found earlier (Chapters 4.3.2.2 and 4.5.3.), non-parametric tests were also applied to these data. Table 5.3 shows the results of pair-wise Mann-Whitney tests between all the groups for which calling females were recorded. There were significant differences in the age at first calling between moths kept at 15 and 25°C and those between 20 and 25°C, but not between those at 30°C and any other temperature.

| | 20°C (N=7) | 25°C (N=8) | 30°C (N=5) |
|---|---|---|---|
| 15°C (N=9) | 0.202 | 0.002*** | 0.108 |
| 20°C (N=7) | - | 0.03** | 0.740 |
| 25°C (N=8) | - | - | 0.139 |

**Table 5.3.** Experiment 1. P values obtained from Mann-Whitney tests to determine differences in the age at first calling between temperature groups. N refers to the number of moths that called for each group.

The four females kept continuously at 10°C were observed at 30-min intervals for 31 scotophases. They never exhibited calling behaviour during this period. These moths were maintained for another 25 scotophases and spot checking was done at the end of each scotophase to monitor their survival. Three females survived up to the 50th scotophase and one up to the 57th scotophase (Table 5.1). Of the five ex-10°C females, four initiated calling between the 4th and 6th scotophases following transfer to 20°C and one that did not call died after 6 scotophases at 20°C. Mann-Whitney tests were conducted to determine differences in the age at first calling between ex-10°C females and those at the different temperature groups. The age at first calling in ex-10°C females at 20°C were significantly different from those at 15°C ($p = 0.005$) but not from those at 20, 25 and 30°C.

*Onset time of calling*

The mean onset calling time at different ages under various temperatures is shown in Fig. 5.1. A one-way analysis of variance showed that onset calling time significantly varied with temperature ($F_{3,148} = 13.93$, $p < 0.001$). Average onset calling times were earlier at 15 and 20°C than at 25 and 30°C (Table 5.4).

| Temperature (°C) | Mean onset calling time ± s.e. |
|---|---|
| 15 | 340.1 ± 6.1 |
| 20 | 294.8 ± 17.0 |
| 25 | 385.6 ± 9.4 |
| 30 | 377.3 ± 10.2 |

**Table 5.4.** Average onset time of calling per scotophase (minutes after lights-off) at different temperatures.

Further analyses were done using the generalised linear model (GLM) in MINITAB, with temperature as the factor and age as the covariate. The model was:

GLM OT = T A T*A;
COVARIATE A.

where OT = onset calling time
T = temperature
A = age (chronological or calling)

With chronological age as the covariate, both the factor (temperature) and the covariate (chronological age) were significant ($p < 0.001$ and $p < 0.05$, respectively). When calling age was used as the covariate, only the factor was highly significant ($p < 0.001$). In both models, there was no significant interaction between the factor and the covariate and the order of fitting the terms was not important. These results indicate that *M. convecta* females generally started calling earlier in the night at lower temperatures.

Regression analyses of onset calling time against chronological or calling age were conducted. Onset calling time was not significantly correlated with calling age but was significantly correlated with chronological age (although the $R^2$ value was very low).

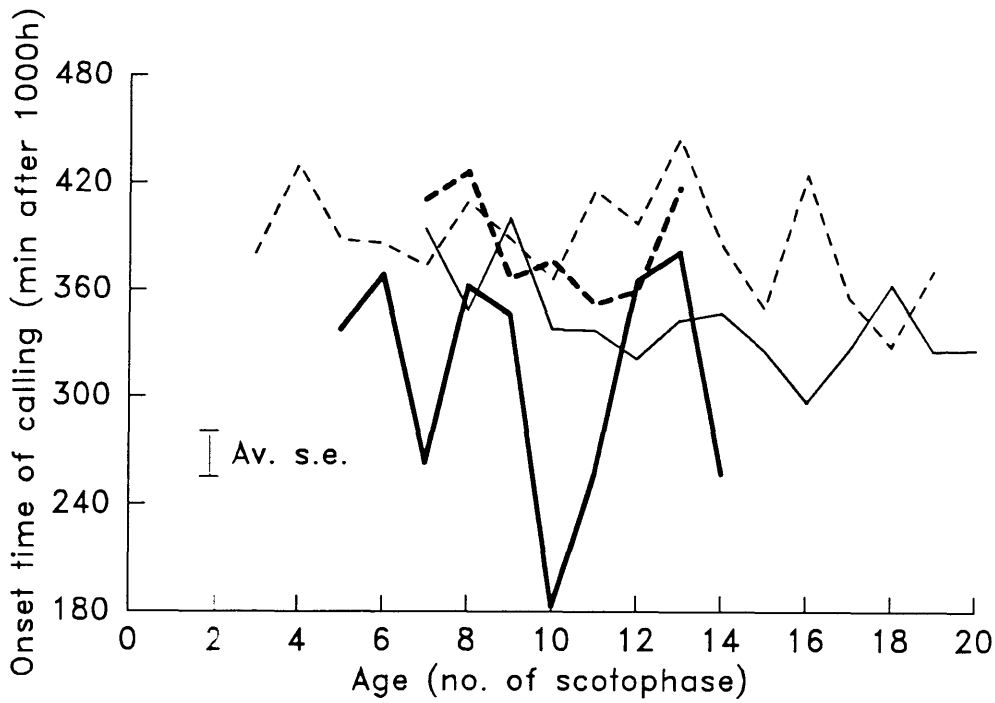


**Fig. 5.1.** Experiment 1. Mean onset time of calling in *M. convecta* females at different ages under different temperatures at 16:8 LD regime: 15°C (——), 20°C (▬▬), 25°C ( – – – ) and 30°C (▬ ▬ ▬). N is shown in Table 5.1.

The regression equation was:

$$OT = 386 - 2.89a \qquad (F_{1,150} = 4.42, p = 0.037, R^2 = 0.03)$$

where OT = onset calling time
a = chronological age

Separate regression analyses of onset calling time against age, were also done for each temperature group. Onset calling time generally advanced with age in all the groups, but these trends were not statistically significant, probably due to smaller sample sizes for these groups.

*Duration of calling at different ages*

Calling duration was very variable at different ages (Fig. 5.2). A one-way analysis of variance showed that calling duration did not significantly vary with temperature ($F_{3,251} = 0.75$, $p = 0.523$). GLM analyses of duration of calling, using temperature as the factor and either chronological or calling age as the covariate, yielded non-significant results. Similarly, regression analyses on calling duration against age for each temperature group also yielded non-significant results indicating that the amount of calling was not significantly influenced by either temperature or current age.

*Duration of calling at different hours of the scotophase*

Temperature did not seem to modify the period when most calling occurred during the scotophase. At all temperatures including ex-10°C, most calling was in the 2nd half of the scotophase, with the mean duration longest in the 7th hour (Fig. 5.3). Calling in the 1st half of the scotophase was never observed at any other temperature except at 20°C. At this temperature, 4 females started calling during the 1st half of the scotophase. One female started to call as early as the 2nd hour, two in the 3rd hour and one in the 4th hour of the scotophase.

In Experiments 1 and 2 (Chapter 4), maximal calling during the latter half of the scotophase was also observed at 20°C and calling in some moths started as early as the 2nd hour of the scotophase (see Figs. 4.16 and 4.20). It might be that 20°C is about the optimum temperature for calling in *M. convecta*. On the other hand, due to smaller sample sizes in the other temperature groups and less frequent observation interval (i.e., 30 minutes), calling during the 1st half of the scotophase in these groups might have been overlooked. Further investigation of calling behaviour under different temperatures using bigger sample sizes and possibly a more frequent observation interval would help elucidate this aspect.

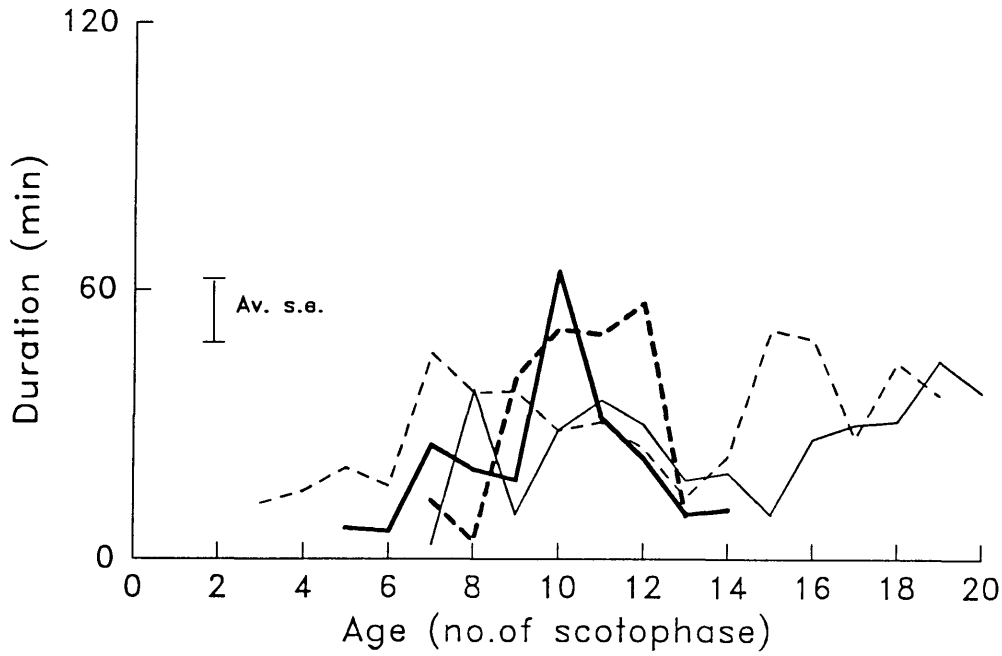


**Fig. 5.2.** Experiment 1. Mean duration of calling in *M. convecta* females at different ages under different temperatures at 16:8 LD regime: 15°C (——), 20°C (▬▬), 25°C ( – – – ) and 30°C (▬ ▬ ▬). N is shown in Table 5.1.

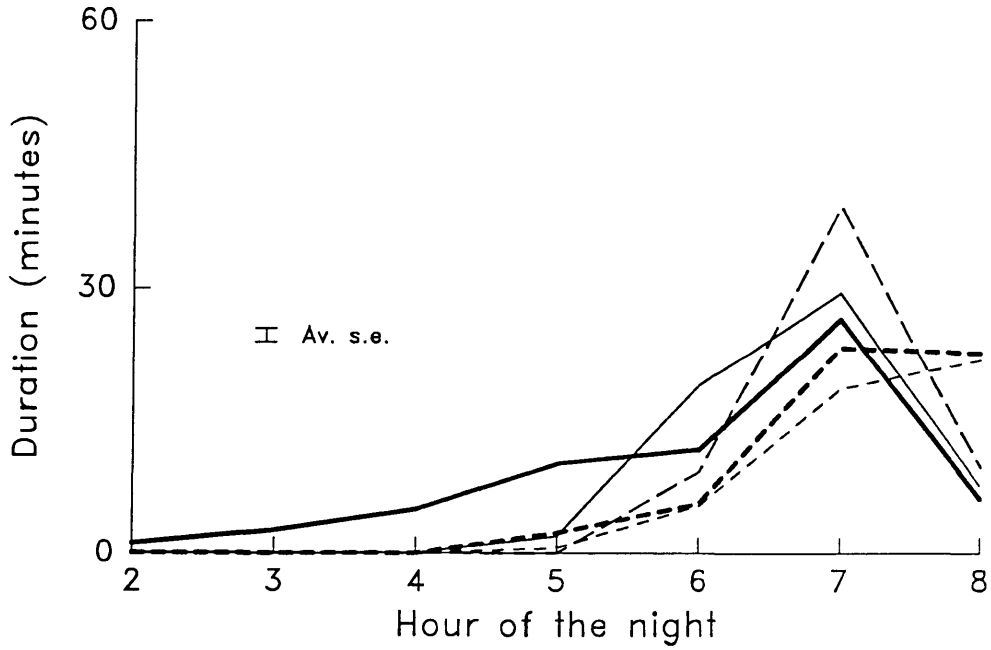


**Fig. 5.3.** Experiment 1. Mean duration of calling in *M. convecta* females during each hour of the scotophase under different temperatures at 16:8 LD regime: 15°C (——), 20°C (▬▬), 25°C ( – – – – ) and 30°C (▬ ▬ ▬ ▬). Data from all females of all ages for each temperature group are combined.

## 5.4. Experiment 2 - Calling behaviour at different photoperiods

### 5.4.1. Materials and methods

Three groups of *M. convecta* females were tested at three constant light:dark (LD) periods - 16:8, 14:10 and 12:12 at 20°C, with the scotophase during 0900-1700 h, 0900-1900 h and 0900-2100 h, respectively. Sample sizes for each group were: 16:8 LD = 10, 14:10 LD = 20 and 12:12 LD = 20. As in Experiment 1, moths were obtained from the same batch of larvae and were observed during one experimental period at 30-min intervals throughout the scotophase.

Data for the different calling parameters were analysed by one-way analysis of variance, regression analyses and the GLM analysis in the MINITAB package as described in Chapter 3.2.1.

Raw data for calling under the different photoperiodic conditions are provided in the sub-directory A:\CHAP5 of the floppy disk.

### 5.4.2. Results and discussion

*Age at first calling*

The numbers of *M. convecta* females surviving and calling at different ages for each LD group are given in Table 5.5. The numbers calling for the first time at different ages are given in Table 5.6.

A regression analysis showed that the age at which *M. convecta* females called for the first time was positively correlated with the length of the scotophase, that is, the longer the scotophase, the older the moths were when they started calling for the first time. At 16:8 LD, first calling occurred between the 3rd and 7th scotophases, at 14:10 LD, between the 3rd and 11th scotophases and at 12:12 LD, between the 5th scotophase and 13th scotophases.

The regression equation was:

$$A = 3.99 + 1.07s \qquad (F_{1,48} = 23.26,\ p < 0.001,\ R^2 = 0.33)$$

where A = age at first calling
s = length of scotophase

| Age (S) | 16:8 LD | 14:10 LD | 12:12 LD |
|---|---|---|---|
| 1 | 10 (0) | 20 (0) | 20 (0) |
| 2 | 10 (0) | 20 (0) | 20 (0) |
| 3 | 10 (2) | 20 (3) | 20 (0) |
| 4 | 10 (5) | 20 (3) | 20 (0) |
| 5 | 10 (5) | 20 (8) | 20 (2) |
| 6 | 10 (9) | 20 (8) | 20 (5) |
| 7 | 10 (8) | 20 (9) | 20 (5) |
| 8 | 10 (8) | 20 (10) | 20 (8) |
| 9 | 10 (6) | 20 (11) | 20 (7) |
| 10 | 10 (8) | 20 (12) | 20 (8) |
| 11 | 10 (9) | 20 (16) | 20 (14) |
| 12 | 10 (8) | 20 (15) | 20 (11) |
| 13 | 10 (8) | 20 (15) | 20 (11) |
| 14 | 10 (7) | 20 (16) | 20 (10) |
| 15 | 10 (9) | 20 (15) | 20 (15) |
| 16 | 10 (7) | 20 (19) | 20 (19) |
| 17 | 9 (9) | 20 (17) | 19 (17) |
| 18 | 8 (6) | 20 (20) | 19 (16) |
| 19 | 7 (4) | 9 (9) | 12 (9) |
| 20 | - | - | 4 (4) |

**Table 5.5.** Experiment 2. Number of *M. convecta* females surviving and calling (in parenthesis) at different ages (number of scotophases) under various photoperiodic conditions.

| Age (S) | 16:8 LD | 14:10 LD | 12:12 LD |
|---|---|---|---|
| 3 | 2 | 3 | - |
| 4 | 3 | 2 | - |
| 5 | 1 | 3 | 2 |
| 6 | 3 | 1 | 3 |
| 7 | 1 | 5 | 2 |
| 8 | - | 2 | 2 |
| 9 | - | 2 | 1 |
| 10 | - | 0 | 3 |
| 11 | - | 2 | 4 |
| 12 | - | - | 1 |
| 13 | - | - | 2 |
| Total | 10 | 20 | 20 |

**Table 5.6.** Experiment 2. Number of *M. convecta* females calling for the first time at different ages under different photoperiodic conditions.

Results from pair-wise Mann-Whitney tests to examine the differences in the age at first calling in a non-parametric way are shown in Table 5.7. Significant differences were obtained between moths held at 16:8 and 12:12 LD and between 14:10 and 12:12 LD.

| | 16:8 LD (N=10) | 14:10 LD (N=20) | 12:12 LD (N=20) |
|---|---|---|---|
| 16:8 LD | - | 0.068 n.s. | 0.0003*** |
| 14:10 LD | - | - | 0.008** |

**Table 5.7.** Experiment 2. P values obtained from Mann-Whitney tests to determine differences in the age at first calling between LD groups. N refers to the number of moths that called for each group.

*Onset time of calling*

Mean onset calling times on each night for the 3 LD groups are shown in Fig. 5.4. A one-way analysis of variance showed that onset calling time significantly varied with the length of the scotophase ($F_{2,484} = 40.99$, $p < 0.001$). Average onset calling time per scotophase for each LD group is given in Table 5.8.

| L:D group | Mean onset calling time ± s.e. |
|---|---|
| 16:8 | 351.2 ± 7.3 |
| 14:10 | 428.8 ± 6.3 |
| 12:12 | 456.1 ± 9.3 |

**Table 5.8.** Experiment 2. Average onset time of calling per scotophase (minutes after lights-off) at different light:dark regimes.

GLM analyses across all LD groups were conducted using the length of scotophase as the factor and age as the covariate, with the model:

GLM OT = S A S*A;
COVARIATE A.

where OT = onset calling time
S = length of scotophase
A = age (chronological or calling)

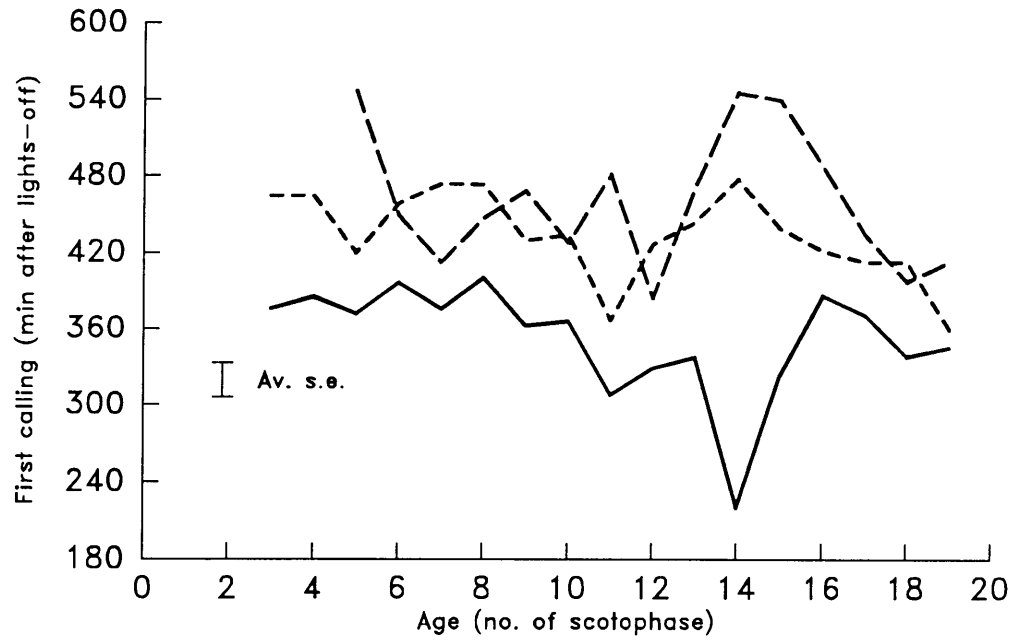


**Fig. 5.4.** Experiment 2. Mean onset time of calling in *M. convecta* females at different ages under different light:dark (LD) regimes at 20°C: 16:8 LD (———), 14:10 LD ( ▬ ▬ ▬ ) and 12:12 LD (▬ ▬). N is shown in Table 5.5.

With either chronological or calling age as the covariate, both the factor (length of scotophase) and the covariates (chronological and calling ages) were highly significant ($p < 0.001$). In both models, there were no significant interactions between the factor and the covariate. With the model using calling age as the covariate, the order of fitting the factor or the covariate was not important but with the model using chronological age as the covariate, the order of fitting the terms did matter. The factor (length of scotophase) remained significant whichever order was used but the significance of the covariate (chronological age) depended on the order of fitting the terms. This indicates that the effects of chronological age and length of scotophase on onset calling time were confounded. The reasons for this can be seen in Table 5.6. Since age at first calling varied with the length of the scotophase, the number of data available on the onset of calling at different ages also varied between the scotophase treatments.

The major difference in onset calling times was between 16:8 LD and the other two regimes (Fig. 5.4). Further analysis with the GLM method was done using data for 16:8 LD and data combined for 14:10 and 12:12 LD, using the same model described above. As with the previous GLM analysis, when chronological age was used as the covariate, both the factor (length of scotophase, at two levels only) and the covariate (chronological age) were significant ($p < 0.001$ and $p < 0.01$, respectively). There was no significant interaction between the factor and the covariate but the order of fitting the terms was important, for the same reasons as in the analysis where the three scotophase treatments were considered separately.

Regression analyses on onset calling time against age for each LD group were also conducted. Although the $R^2$ values were generally low, significant negative correlations were obtained for all groups except at 12:12 LD where regression with chronological age was non-significant (Table 5.9).

| LD group | Regression equation | F ratio | p value | $R^2$ |
|---|---|---|---|---|
| 16:8 | $396 - 3.95a^1$ | 5.94 | 0.016 | 0.048 |
| | $383 - 4.04a^2$ | 6.32 | 0.013 | 0.051 |
| 14:10 | $470 - 3.24a^1$ | 4.90 | 0.028 | 0.023 |
| | $455 - 3.30a^2$ | 4.95 | 0.027 | 0.024 |
| 12:12 | $489 - 2.40a^1$ | 0.98 | 0.324 | 0.006 |
| | $501 - 7.05a^2$ | 8.77 | 0.004 | 0.052 |

**Table 5.9.** Experiment 2. Results from regression analyses for each LD group of onset calling time against moth age ($a^1$ = chronological age; $a^2$ = calling age).

The onset time of calling was significantly influenced by the length of the scotophase. Females were more likely to start calling earlier when the nights were shorter. Moth age also influenced their onset calling time, although its significance depended on what pathway in the GLM analyses was followed. The models yielded negative coefficients of the covariates (chronological and calling ages) indicating that onset calling times under the different photoperiodic conditions advanced as the moths became older. In Experiment 1 as well as in previous experiments (Chapter 4), onset calling time also advanced with moth age, suggesting that generally, older *M. convecta* females tend to start calling earlier in the scotophase than younger ones, no matter what the temperature or photoperiodic conditions.

*Duration of calling at different ages*

Mean duration of calling at different ages for each LD group is shown in Fig. 5.5. A one-way analysis of variance showed that the mean duration of calling did not significantly vary with the length of the scotophase ($F_{2,615} = 1.66$, $p = 0.19$).

GLM analyses on calling duration were conducted using the length of the scotophase as the factor and age as the covariate, with the model:

GLM D = S A S*A;
COVARIATE A.

where D = calling duration
S = length of scotophase
A = age (chronological or calling)

With either chronological or calling age as the covariate, the factor (length of scotophase) was non-significant but the covariate (age) was highly significant ($p < 0.001$). In both models, there was a significant interaction between the factor and the covariate ($p < 0.001$). Thus, the amount of calling in *M. convecta* females was not significantly influenced by the length of scotophase alone but by their current ages and by the interaction of this effect with the length of the scotophase. Moths tended to call more as they got older, and the strength of this effect depended on the length of the scotophase.

Regression analyses on calling duration against age were also conducted for each LD group. There appeared to be a separate regression line for each LD group (Fig. 5.5). Significant positive correlations were obtained for 14:10 and 12:12 LD but not for 16:8 LD (Table 5.10).

| LD group | Regression equation | F-ratio | p value | $R^2$ |
|---|---|---|---|---|
| 16:8 | $41.4 + 1.24a^1$ | 2.31 | 0.130 | 0.016 |
| | $43.3 + 1.57a^2$ | 3.85 | 0.052 | 0.026 |
| 14:10 | $10.1 + 4.20a^1$ | 32.91 | <0.001 | 0.113 |
| | $19.0 + 5.86a^2$ | 73.18 | <0.001 | 0.220 |
| 12:12 | $-15.2 + 6.04a^1$ | 27.43 | <0.001 | 0.116 |
| | $20.7 + 7.43a^2$ | 45.11 | <0.001 | 0.177 |

**Table 5.10.** Experiment 2. Results from regression analyses for each LD group on calling duration against moth age ($a^1$ = chronological age; $a^2$ = calling age).

*Duration of calling at different hours of the scotophase*

At all photoperiodic conditions, calling occurred mostly during the 2nd half of the scotophase (Fig. 5.6). These results were consistent with those in previous experiments (Chapters 4 and 5.3). Peak calling time was chronologically earlier at shorter scotophases, but was later in relation to the length of the scotophase. At 16:8 and 14:10 LD periods, peak calling occurred an hour before lights-on, i.e., in the 7th and 9th hours, respectively, while at 12:12 LD period, calling peaked in the 10th hour of the scotophase or two hours before lights-on.

## 5.5. Experiment 3 - Calling behaviour after an entrainment period

### 5.5.1. Materials and methods

Experiment 3 aimed to investigate the possibility of a circadian rhythm in the calling behaviour in *M. convecta* females. Four groups of females (N=10/group) were tested at 20°C. Group 1 females were held at constant 16:8 LD period during the first 72 hours following emergence (i.e., 3-day entrainment) and were at constant darkness for the following 288 hours (12 days). Group 2 females were entrained at 16:8 LD regime for the first 192 hours following emergence (i.e., 8-day entrainment) and were at constant darkness for the following 192 hours (8 days). Group 3 females were held under constant light for 288 hours (12 days) following emergence. Group 4 females were kept at constant darkness for 264 hours (11 days) following emergence. Pupae of Groups 1, 2 and 3 females were kept under the entraining 16:8 LD regime while those of Group 4 females were in constant darkness.

All groups were observed at 30-min intervals during 0900-1700 h when normal scotophase would have occurred under the entraining photoperiodic condition of 16:8 LD regime. Under either constant light or constant darkness, moth age corresponded to a 24-hour period commencing at 0900 h, i.e., the "lights-off" time or the time when normal scotophase in the reverse-cycle would have started.

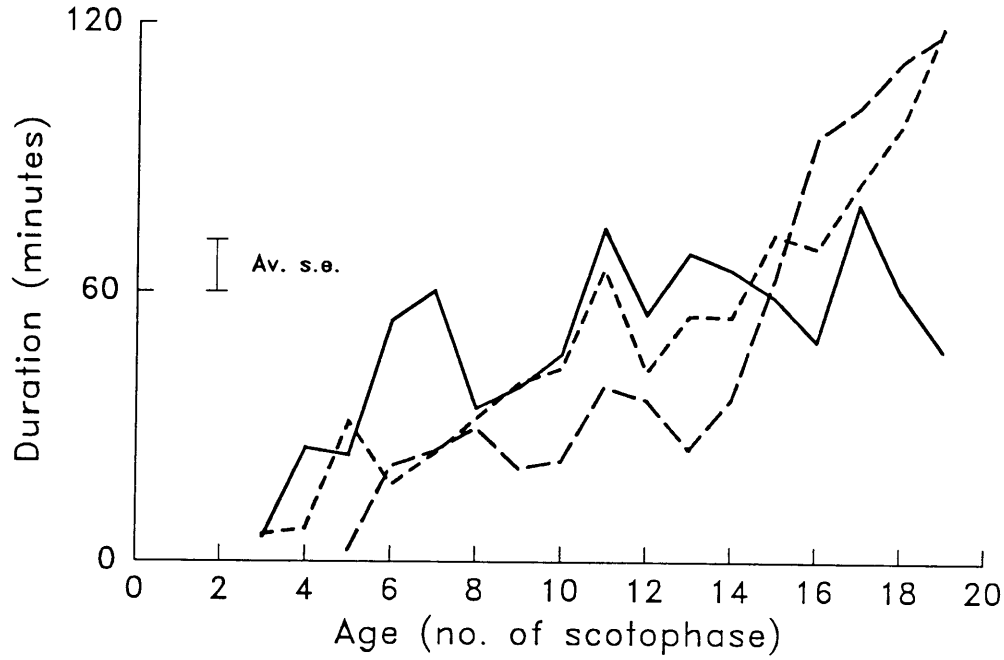


**Fig. 5.5.** Experiment 2. Mean duration of calling in *M. convecta* females at different ages under different light:dark (LD) regimes at 20°C: 16:8 LD (———), 14:10 LD (▬ ▬ ▬) and 12:12 LD (▬ ▬). N is shown in Table 5.5.

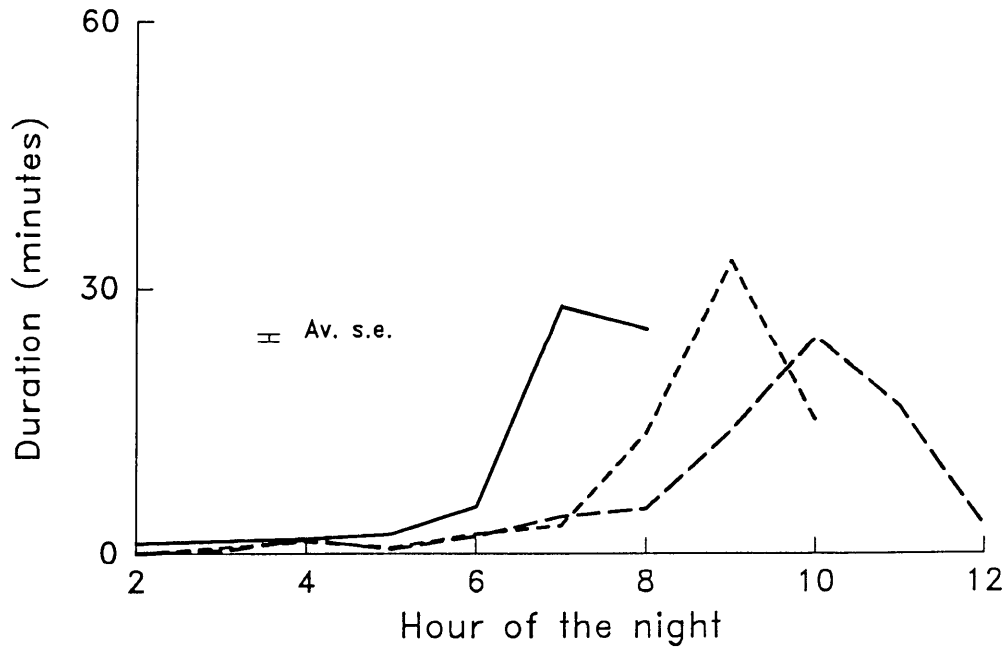


**Fig. 5.6.** Experiment 2. Mean duration of calling in *M. convecta* females during each hour of the scotophase under different light:dark (LD) regimes at 20°C: 16:8 LD (———), 14:10 LD ( ▬ ▬ ▬ ) and 12:12 LD ( ▬ ▬ ). Data from all females of all ages for each LD group combined.

Ideally, it would have been best to conduct observations at 30-min intervals constantly throughout the 288 hours of the maximum post-entrainment period, but time and labour constraints prevented this. Instead, on several occasions, observations at 30-min intervals were conducted throughout a 36-hour period to investigate if calling occurred at times other than 0900-1700 h. These observation periods for the different groups were: Group 1 - at 0-36, 48-84, 96-132, 144-180, 192-228 and 240-276 h following transfer to constant darkness; Group 2 - at 0-36, 48-84, 96-132 and 144-180 h following transfer to constant darkness; Group 3 - at 0-36, 48-84, 96-132, 144-180, 192-228 and 240-276 h following emergence, and Group 4 - at 0-36, 48-84, 96-132, 144-180 and 192-228 h following emergence.

#### 5.5.1.1. Statistical analyses

Means and standard errors of calling duration at different ages under the entraining condition (16:8 LD) and at different times following transfer to constant darkness (Groups 1 and 2) or at different times following emergence under constant light or darkness (Groups 3 and 4) were calculated using the MINITAB statistical package.

The generalised linear interactive model (GLIM) package (Payne, 1985) was used to compare the calling patterns in Groups 1 and 2 females under the entraining 16:8 LD regime (i.e., pre-transfer period to darkness) with that under constant darkness (i.e., post-transfer period). The same analysis was also used to compare the calling pattern in entrained moths with that in moths of similar chronological ages held under the normal 16:8 LD regime in reverse-cycle (Experiment 2, section 5.4.2). The technique used involved comparison of models based on separate or combined cubic regression equations, testing for significant differences in the intercept and for significant non-parallelism, as was done in Experiment 2 (Chapter 4).

Raw data for calling of moths under 3-day and 8-day entrainment, and constant darkness are provided in the sub-directory A:\CHAP5 of the floppy disk.

### 5.5.2. Results and discussion

The numbers of moths surviving and calling in the different groups are given in Table 5.11.

Under the entraining 16:8 LD regime, only one female in Group 1 initiated calling during the 3rd scotophase while all Group 2 females started calling between the 3rd and 5th scotophases. No calling was observed in Group 3 females which were under con-

tinuous light. First calling in Group 4 females (constant darkness) occurred between ages 4 and 9 (i.e., between 73 and 216 h following emergence).

| Age | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| 1 | 10 (0) | 10 (0) | 10 | 10 (0) |
| 2 | 10 (0) | 10 (0) | 10 | 10 (0) |
| 3 | 10 (1) | 10 (2) | 10 | 10 (0) |
| 4 | 10 (3)* | 10 (7) | 10 | 10 (1) |
| 5 | 10 (3) | 10 (9) | 10 | 10 (3) |
| 6 | 10 (3) | 10 (9) | 10 | 10 (1) |
| 7 | 10 (0) | 10 (10) | 10 | 10 (0) |
| 8 | 10 (1) | 10 (5) | 10 | 10 (0) |
| 9 | 10 (0) | 10 (5)* | 10 | 5 (3) |
| 10 | 10 (2) | 10 (8) | 10 | 5 (0) |
| 11 | 10 (2) | 10 (6) | 10 | 1 (0) |
| 12 | 10 (7) | 10 (6) | 10 | - |
| 13 | 10 (3) | 10 (7) | - | - |
| 14 | 10 (5) | 10 (5) | - | - |
| 15 | 10 (2) | 10 (6) | - | - |
| 16 | - | 10 (1) | - | - |

**Table 5.11.** Experiment 3. Number of *M. convecta* females surviving and calling (in parentheses) in the different groups at different ages. Age with * is the age when moths were transferred to constant darkness. Group 1 females were entrained at 16:8 LD for the first 3 days following emergence then transferred to constant darkness. Group 2 females were entrained at 16:8 LD for the first 8 days following emergence then transferred to constant darkness. Group 3 females were kept at constant light and Group 4 females at constant darkness following emergence.

Group 1 females (entrained for 3 days) called between 6 and 56 h after transfer to constant darkness (Fig. 5.7). In this time, the periodicity of calling seemed to be similar to that which normally occurred in a 16:8 LD regime (Experiment 2, section 5.4.2). After this, however, there was a break of 102 hours in which no calling was seen (although for two 12-hour periods within this break, the moths were not being observed). When calling resumed from 158 h on, the periodicity of calling was quite different. It appeared to be centred not around the scotophase experienced by the adults during the entrainment period, but around the scotophase which the larvae had received in the stock cultures, before their transfer as pupae to the reverse-cycle regime. This larval scotophase (2300-0700 h AEST) was also approximately correlated with the natural scotophase being experienced in the Armidale region at the time of the experiments, which was about 1900 to 0700 h.

Group 2 females (entrained for 8 days) called between 5 and 169 h after transfer to darkness (Fig. 5.8). As with Group 1 females, there seemed to be an initial period when the periodicity of calling correlated with the scotophase of the entrainment period. This period lasted for 80 hours, compared with 56 hours in the Group 1 females. It was then followed by a second period, from 96 to 169 h, when some calling seemed better correlated with the larval scotophase rather than the entraining scotophase. However, unlike the Group 1 females, there was not a long period between these two phases when no calling occurred. Instead, there was a period when calling became irregular, with some moths calling around the time of larval scotophase, others around the time of the entraining adult scotophase, and still others at intermediate periodicities.

Six out of 10 females in Group 4 exhibited calling behaviour in constant darkness between 81 and 208 h following emergence. There was no clear pattern in the calling behaviour in this group (Fig. 5.9). Some moths called within the adult scotophase and photophase of the entraining 16:8 LD regime while others called within the larval scotophase.

The possibility of a circadian rhythm in the calling behaviour in Group 1 and Group 2 females was first examined by comparing their mean calling duration at different times of the scotophase during the entraining period (pre-transfer) with that during the first 72 hours in constant darkness (post-transfer). In Group 1, the pre-transfer period (during which only one moth called) was ages 1-3 at 16:8 LD while post-transfer period was ages 4-6 in constant darkness (Fig. 5.7). In Group 2, the pre-transfer period was ages 1-8 at 16:8 LD while post-transfer period was ages 9-11 in constant darkness (Fig. 5.8).

The calling duration in Group 1 during the pre-transfer and post-transfer periods to darkness is shown in Fig. 5.10. Peak calling under constant darkness coincided with the 7th hour of the scotophase under the entraining period. A GLIM analysis showed that both the intercept and non-parallelism between the two lines were not significantly different. The lack of significance may be due in part to the fact that pre-transfer calling patterns were based on data from only one moth. However, these patterns were very similar to those previously found with larger samples in previous experiments (Chapter 4).

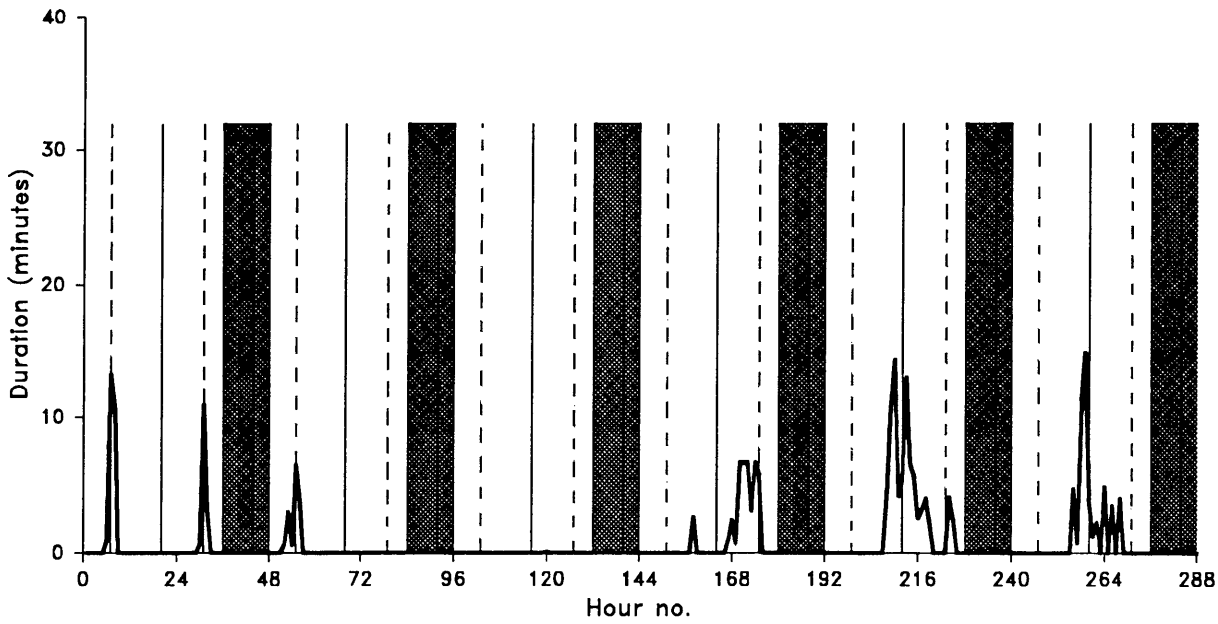


**Fig. 5.7.** Experiment 3. Calling periodicities under constant darkness in *M. convecta* females that were entrained at 16:8 LD regime at ages 1-3 (Group 1). Hour number is the period after transfer to constant darkness. Moths were observed at 30-min intervals over a 36-hour period. Shaded areas were the 12-hour periods when the moths were not observed; 7th hour of adult scotophase (– – –) was at 1600 h AEST and 7th hour of larval scotophase (———) was at 0600 h AEST. Temperature during entraining and constant darkness was 20°C.

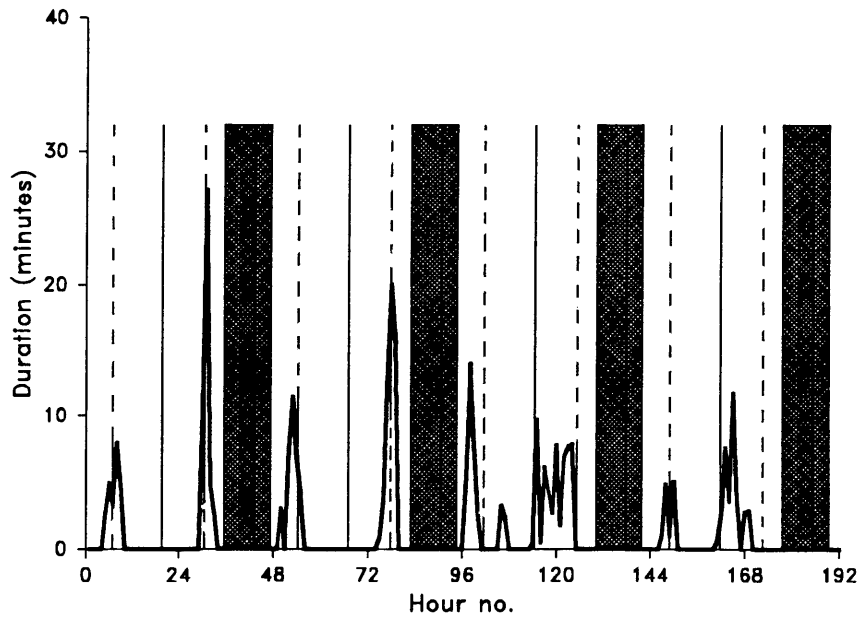


**Fig. 5.8.** Experiment 3. Calling periodicities under constant darkness in *M. convecta* females that were entrained at 16:8 LD regime at ages 1-8 (Group 2). Hour number is the period after transfer to constant darkness. Moths were observed at 30-min intervals over a 36-hour period. Symbols are as for Fig. 5.7.

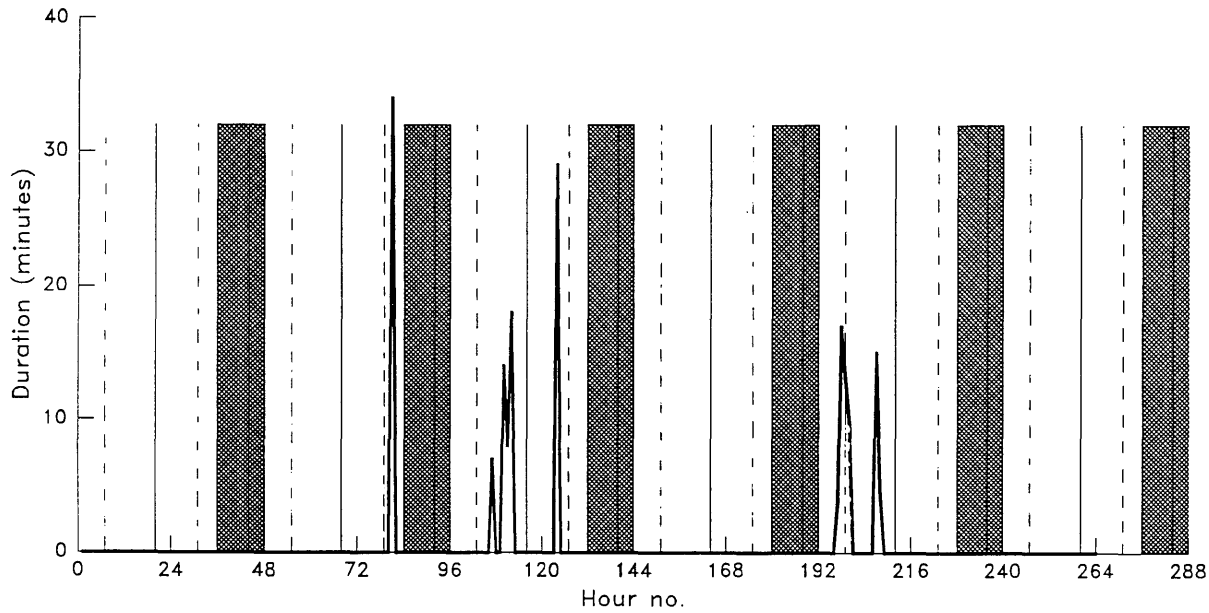


**Fig. 5.9.** Experiment 3. Calling periodicities in *M. convecta* females that were maintained at constant darkness at 20°C during the pupal and adult stages (Group 4). Hour number is the period after emergence. Moths were observed at 30-min intervals over a 36-hour period. Symbols are as for Fig. 5.7.

In Group 2, similar patterns were shown during pre-transfer and post-transfer periods (Fig. 5.11). However, while the intercept between the two lines was non-significant, the non-parallelism was highly significant ($p < 0.001$). This suggests that although the calling pattern under constant darkness was similar to that under the entraining period, the timing was slightly different. The main difference between these periods was in the amount of calling during the 8th hour of the scotophase. It was significantly greater during pre-transfer than during the post-transfer period.

The above analyses involved comparison of the calling patterns of the same moths, but at different ages. It has already been shown that age affects calling pattern (Chapter 4.3.2.2). To minimise moth age as a source of variation in the analysis, calling duration in constant darkness at 0900-1700 h (i.e., when normal adult scotophase would have occurred) was compared with the calling duration in moths of similar chronological ages, that had been normally at 16:8 LD regime in Experiment 2. GLIM analyses were used to compare calling patterns under these two conditions. In Group 1, calling during the first 72 hours following transfer to darkness (ages 4-6) was compared with that in 4- to 6-day-old moths at 16:8 LD. In Group 2, calling during the first 72 hours in darkness (ages 9-11) was compared with that in 9- to 11-day-old moths held at 16:8 LD.

There were no significant differences in both the intercept and the non-parallelism between Group 1 and the 16:8 LD group (Fig. 5.12), indicating that the calling pattern in Group 1 females during the first 72 hours in constant darkness was similar to those held at the normal 16:8 LD regime. Peak calling in both groups was in the 7th hour after lights-off. Similar results were obtained in Group 2. The intercept and the non-parallelism between Group 2 and the 16:8 LD group were also not significantly different (Fig. 5.13).

To test the hypothesis that the shift in the calling pattern which occurred in both Groups 1 and 2 from about 4 days after transfer to constant darkness (Figs. 5.7 and 5.8), represented some kind of reversion to the larval scotophase, calling duration in the entrained females under constant darkness (i.e., after 4 days) during the period corresponding to the larval scotophase was compared with those in females of similar ages that were normally held at 16:8 LD regime (Experiment 2). The scotophase of *M. convecta* larvae in the insectary was between 2300 and 0700 h.

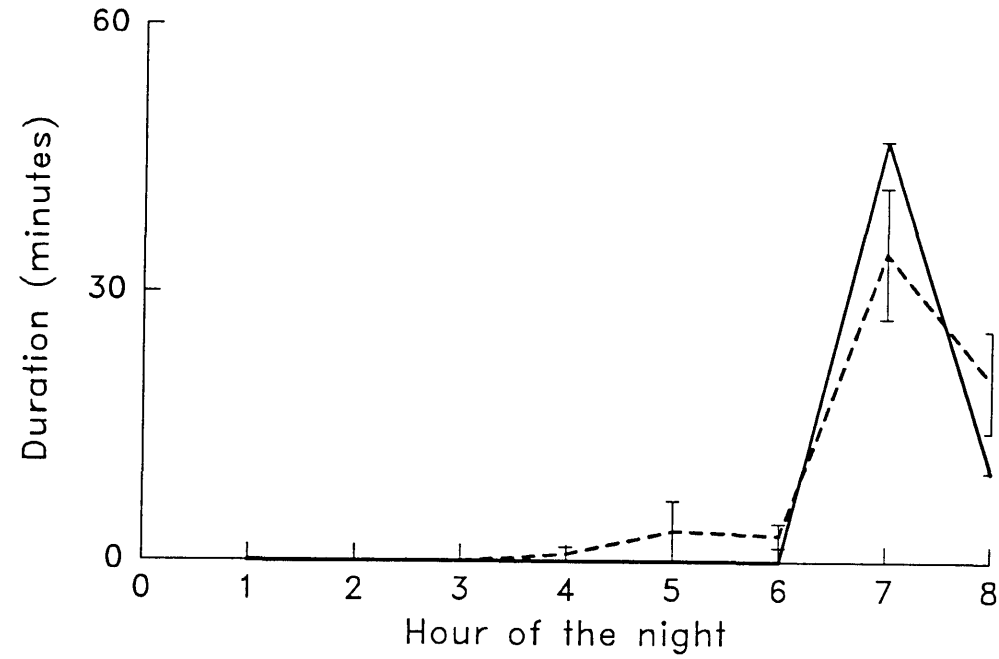


**Fig. 5.10.** Experiment 3. Mean duration of calling during each hour of the adult scotophase in *M. convecta* females under the entraining 3-day-period at 16:8 LD regime, i.e., pre-transfer (———) and after transfer (– – – –) to constant darkness. Data were from only one moth that called at age 3 before transfer and from females that called at 0-72 h (ages 4-6) after transfer to constant darkness. Temperature at 16:8 LD and constant darkness was 20°C.

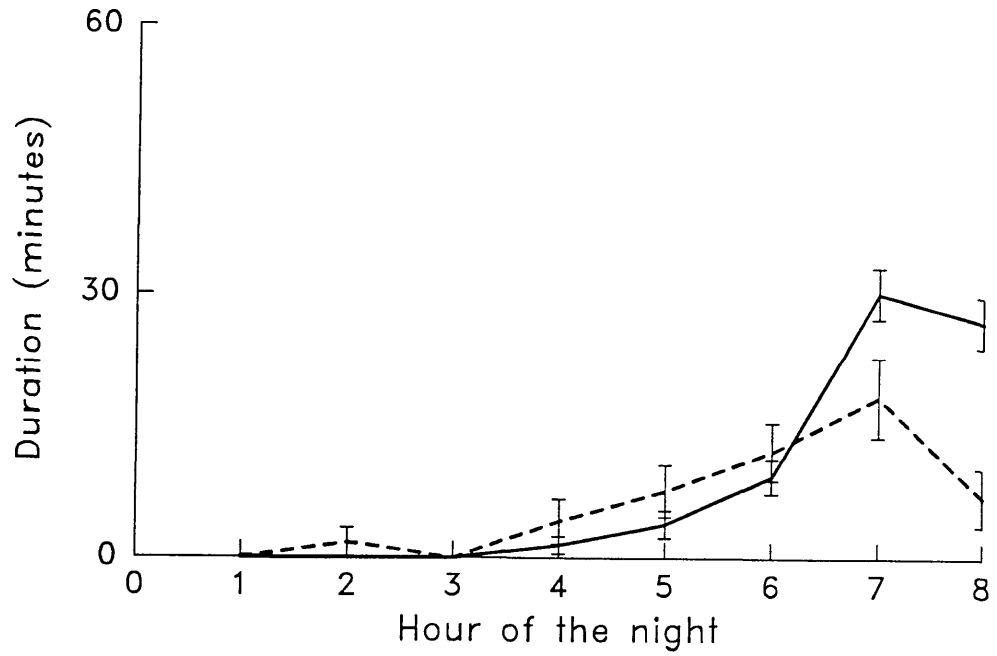


**Fig. 5.11.** Experiment 3. Mean duration of calling during each hour of the adult scotophase in *M. convecta* females under the entraining 8-day-period at 16:8 LD regime, i.e., pre-transfer (———) and after transfer (– – – –) to constant darkness. Data were from females that called at ages 1-8 before transfer and from females that called at 0-72 h (ages 9-11) after transfer to constant darkness. Temperature at 16:8 LD and constant darkness was 20°C.

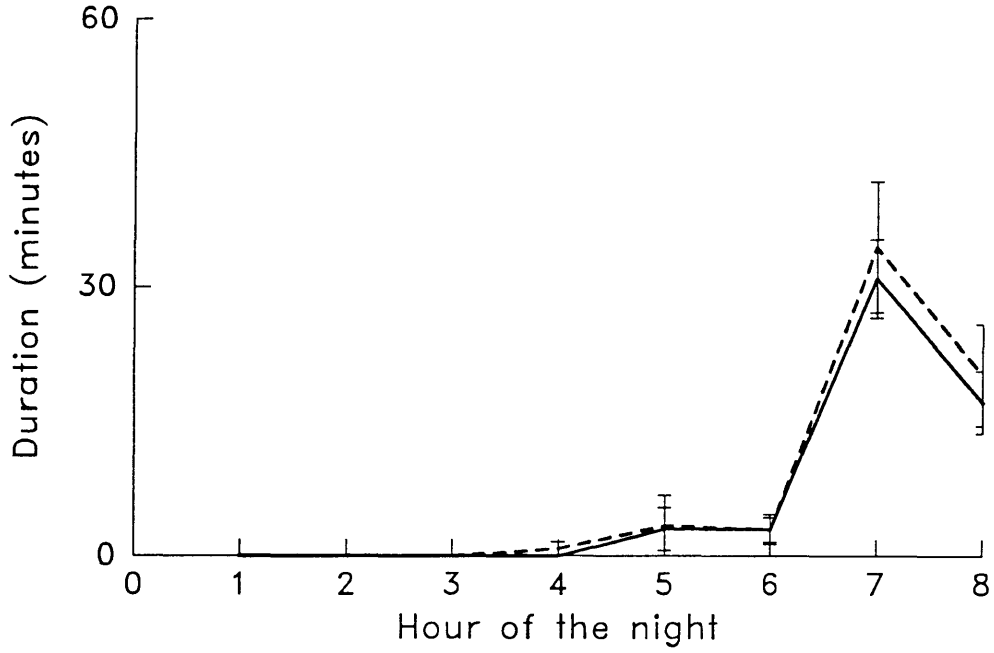


**Fig. 5.12.** Experiment 3. Mean duration of calling during each hour of the adult scotophase in *M. convecta* females that were entrained for 3 days at 16:8 LD regime (- - - -) and females that were normally held at 16:8 LD (———) in Experiment 2. Data were from entrained females that called in constant darkness at 0-72 h (ages 4-6) and from females held at 16:8 LD in Experiment 2 that called at ages 4-6, respectively. Temperature for both groups of females was 20°C.

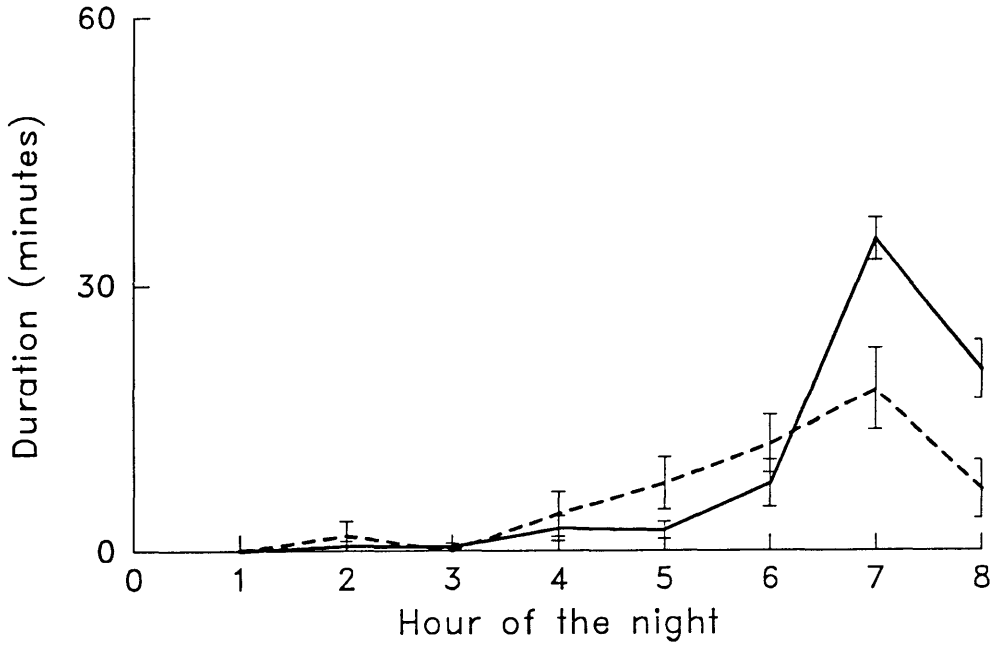


**Fig. 5.13.** Experiment 3. Mean duration of calling during each hour of the adult scotophase in *M. convecta* females that were entrained for 8 days at 16:8 LD regime (- - - -) and females that were normally held at 16:8 LD (———) in Experiment 2. Data were from entrained females that called in constant darkness at 0-72 h (ages 9-11) and from females held at 16:8 LD in Experiment 2 that called at ages 9-11, respectively. Temperature for both groups of females was 20°C.

In Group 1, reversion to the larval scotophase appeared to occur at between 192 and 216 h (age 12) and between 240 and 264 h (age 14). For these ages, calling duration was calculated for the periods that corresponded to the larval scotophase which were at 206-214 h and at 254-262 h, respectively. These data were then compared with those from females of ages 12 and 14, held at 16:8 LD regime in Experiment 2. In Group 2, similar reversion seemed to be between 96 and 120 h (age 13) and between 144 and 168 h (age 15). For these ages, calling duration was calculated for the periods corresponding to the larval scotophase which were at 110-118 h and at 158-166 h, respectively. These data were then compared with those in females of ages 13 and 15 under 16:8 LD regime in Experiment 2. For females that were held at 16:8 LD regime in Experiment 2, data for 0900-1700 h were transposed to 2300-0700 h.

A similar GLIM analysis was used for the comparison. The intercept between Group 1 and the 16:8 LD group was not significant but the non-parallelism was highly significant ($p < 0.001$) (Fig. 5.14). A similar result was obtained between Group 2 and the 16:8 LD group. The intercept was also non-significant but the non-parallelism was significant ($p < 0.05$) (Fig. 5.15). These results indicate that, although the broad pattern of calling during the second period was similar to that which might have been expected under the larval scotophase, there were significant differences. The pattern was thus not simply the usual one of a peak in the 7th hour, but transposed to the larval scotophase.

## 5.6. General discussion

The age at which *M. convecta* females called for the first time significantly varied with temperature. The initiation of calling was delayed at lower temperatures. This result is similar to those found in other armyworm species. First calling in *P. unipuncta* was 11 days later at 10°C than at 25°C (Delisle & McNeil, 1987a). *M. configurata* females called for the first time during the 5th-6th scotophases at 10°C, 3rd-4th scotophases at 15°C, and in the 2nd-3rd scotophases at 20 and 30°C (Gerber & Howlader, 1987).

Variability in the initiation of calling may be related to the reproductive maturation rate under varying temperatures. Gerber & Howlader (1987) reported that at temperatures ranging from 10 and 35°C, the time of first calling in *M. configurata* was closely synchronised with the appearance of the first chorionated eggs. At 10°C, the first chorionated eggs were produced at the end of the 4th scotophase and calling started in the 5th scotophase; at 20 and 30°C, the first chorionated eggs were produced at the beginning of the 2nd scotophase and calling started in the same scotophase and at 35°C, the first chorionated eggs were produced at the end of the 3rd scotophase and calling started in the 4th scotophase.

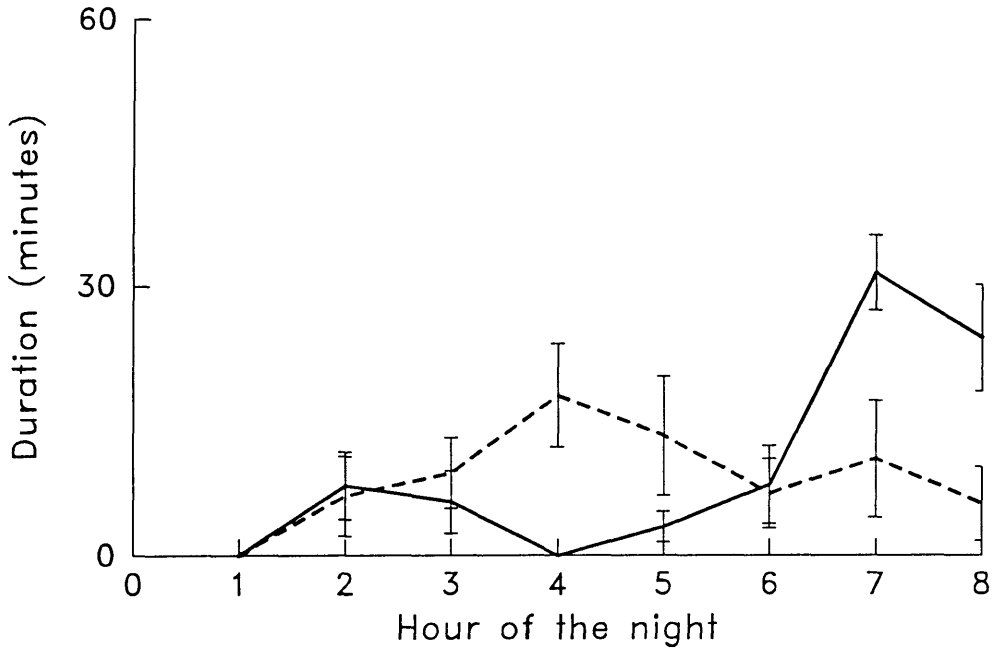


**Fig. 5.14.** Experiment 3. Mean duration of calling during each hour of the larval scotophase in *M. convecta* females that were entrained for 3 days at 16:8 LD regime (- - - -) and females that were normally held at 16:8 LD (———) in Experiment 2. Larval scotophase was at 2300-0700 h AEST. Data were from entrained females that called in constant darkness at 206-214 h (ages 12) and at 254-262 h (age 14), and from females held at 16:8 LD in Experiment 2 that called at ages 12 and 14. In the latter, 0900-1700 h data were transposed to 2300-0700 h. Temperature for both groups of females was 20°C.

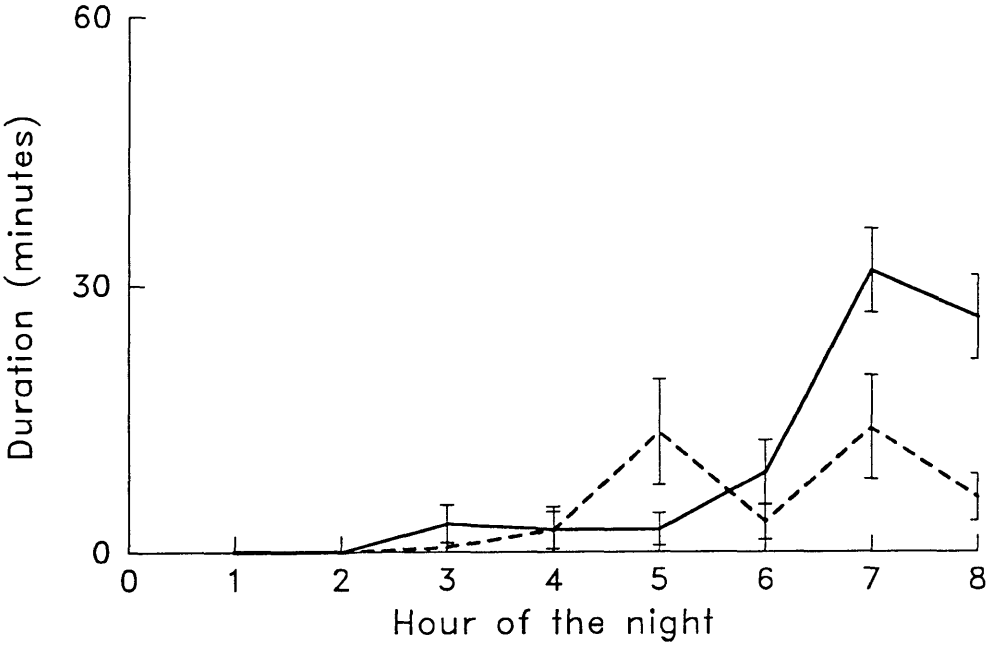


**Fig. 5.15.** Experiment 3. Mean duration of calling during each hour of the larval scotophase in *M. convecta* females that were entrained for 8 days at 16:8 LD regime (- - - -) and females that were normally held at 16:8 LD (———) in Experiment 2. Larval scotophase was at 2300-0700 h AEST. Data were from entrained females that called in constant darkness at 110-118 h (ages 13) and at 158-166 h (age 15), and from females held at 16:8 LD in Experiment 2 that called at ages 13 and 15. In the latter, 0900-1700 h data were transposed to 2300-0700 h. Temperature for both groups of females was 20°C.

Ovarian development in *M. convecta* under different temperatures has not been investigated but if a similar pattern to that in *M. configurata* exists in this species, this explains why females held at lower temperatures began calling later than those at warmer conditions. Delayed egg development is to be expected at lower temperatures.

The temperature limits for calling vary in different species. At 10°C, about 50 and 70% of *P. unipuncta* females exhibited calling behaviour within 35 days under 12:12 and 16:8 LD regimes, respectively (Delisle & McNeil, 1987a). In *M. configurata,* the optimal temperature range for calling was about 10-25°C, with the upper limit near 35°C and the threshold at < 5°C (Gerber & Howlader, 1987). The temperature range for sex pheromone release in *T. ni* was reported to be between 12 and 36°C (Sower *et al.*, 1971) while in *P. stultana,* the lower limit was about 13°C and the upper limit within a few degrees above 35°C (Webster, 1988). The optimal temperature for reproduction in *M. separata* ranged between 20 and 25°C (Sinchaisri & Sogawa, 1969). *M. convecta* females held continuously at 10°C were not seen to call even though they were observed for 31 scotophases. At this temperature, females were mainly resting with occasional feeding and walking in the containers. At 15°C, all females exhibited calling behaviour while at 30°C, only 5 out of 14 females called. It is possible that the lower limit for calling in this species may be a few degrees above 10°C and the upper limit above 30°C. These results suggest that, although *M. convecta* can survive for long periods at 10°C, the threshold for calling might be a few degrees above this temperature.

Hill & Gatehouse (1992) suggested that the relationship between temperature and the pre-reproductive period in *A. gamma* is due to the direct effects of temperature on its metabolic processes. In this species, the pre-oviposition period of females at 13-19°C (ranging from 3-11 days, with median of 6 days) was significantly longer than those at 22-25°C (ranging from 3-9 days, with median of 4-5 days). In Experiment 1, *M. convecta* females that were maintained at 10°C never called within 31 scotophases suggesting that reproductive development might have been absent, or very slow at this temperature. Ex-10°C females did not call at 10°C within 10 scotophases, but when transferred to warmer conditions at 20°C, they started to call between the 4th and 6th scotophases following transfer, i.e., they required 3-5 days to attain reproductive maturity at 20°C. This is a similar period to that of moths held continuously at 20°C from emergence. The capacity for calling in *M. convecta* thus appears to be predominantly a consequence of reproductive maturation, which is temperature dependent.

Temperature conditions experienced during the pupal or adult stages can modify the pre-reproductive period in female moths. Turgeon & McNeil (1983) reported that at 25°C, *P. unipuncta* females that were held at 15°C during pupal development were significantly older when they started calling than those held at 20 or 25°C as pupae. Females with pupal development at 25°C called significantly earlier when held as adults at 20 or 25°C than those at 10 or 15°C as adults. When both the pupal and adult stages were at the same temperature, females at 15°C were significantly older when they started calling than those held at 20 or 25°C. The pre-calling periods in *M. separata* reared as larvae at 23°C then transferred to three temperatures during pupal and adult stages were significantly different (Han & Gatehouse, 1991b). Females held at lower temperatures had longer pre-calling periods. At 18°C, females had a median pre-calling period of 19.5 days, at 23°C, 9 days and at 28°C, 7 days. In Experiment 1, *M. convecta* pupae were held at 20°C and 16:8 LD regime and the adults were kept at the desired temperature conditions. The effects of different pupal temperatures on adult calling were not specifically tested in this thesis.

Webster (1988) showed that, in *P. stultana,* the initiation and termination of calling depended on an interaction between temperature and photoperiod, yielding a "calling window" which could extend from 2.5 to 5 hours, between about 7 hours prior to lights-off and about 7 hours after. Calling could be initiated by a rise or fall in temperature into the window, or terminated by a rise or fall out of the window, as well as by the passage of time. He proposed an ingenious hypothesis involving sensitising and desensitising substances, and temperature-sensitive thresholds for responses to them to explain these results. In contrast with *P. stultana*, *M. convecta* is a strictly nocturnal species. At no temperature regime was any calling observed during the photophase. Moths which were calling at lights-on invariably ceased within seconds. Even in the constant light regime of Experiment 3, no calling occurred in the light. The scope for a temperature-dependent calling window in *M. convecta* is therefore limited to the hours of the scotophase. At all temperatures, the bulk of calling occurred in the 2nd half of the scotophase, as observed in many nocturnal species (see Chapter 4 for references).

Temperature was significantly correlated with onset calling time. *M. convecta* females generally started calling earlier in the scotophase at lower temperatures. Similar results were found in other species like *P. unipuncta, M. configurata, P. stultana, C. fumiferana* and *C. rosaceana* (Sanders & Lucuik, 1972; Delisle & McNeil, 1987a; Gerber & Howlader, 1987; Webster, 1988; Delisle, 1992). Under natural conditions, earlier calling when the temperatures are low would have adaptive advantages. Maximum

pheromone release to enable mating success would occur before temperature falls to levels too low to allow normal reproductive activity.

The seasonal adaptations of insects depend on important environmental cues which allow them to modify their physiology, behaviour or morphology prior to the onset of unfavourable conditions (Tauber *et al.*, 1986). In nocturnal species, photoperiod might provide the primary cue, although their responses might be regulated by other factors such as temperature or biotic factors (Beck, 1980). Migration is a major component in the life history strategy of many insects. It allows them to escape unfavourable environments and colonise suitable habitats (Dingle, 1982). The length of the pre-reproductive period as an index of the migratory potential has been suggested for several noctuids (e.g., Han & Gatehouse, 1991a; Colvin & Gatehouse, 1993a; Wilson & Gatehouse, 1993; Hill & Gatehouse, 1993). In Experiment 2, it was shown that the pre-reproductive period in *M. convecta* was significantly correlated with the length of the scotophase, i.e, the shorter the scotophase, the shorter the pre-calling period. Similar results were reported for the armyworms, *P. unipuncta* and *M. separata* (Delisle & McNeil, 1986; Delisle & McNeil, 1987a; Han & Gatehouse, 1991b).

The relationship between the pre-calling period and the migratory potential of *M. convecta* has not been examined. McDonald & Cole (1991) investigated the effects of photoperiod and crowding on its reproductive development, as judged by the size of the oocytes. They reported that oocyte development in females reared under a short scotophase (8 h) was slower than that in females reared under a longer scotophase (12 h) in the laboratory, and the proportion of immature females caught in field traps was higher in summer than in spring. Results from their experiments are contrary to what might be expected in view of the results of Experiment 2, if both oocyte development and calling behaviour are measures of reproductive maturity. Experiment 2 showed that females kept at shorter scotophases had shorter pre-calling periods. McDonald & Cole (1991) subjected the larvae and pupae to the experimental photoperiodic conditions (16:8 and 12:12 LD) and the adults to 12:12 LD, whereas in Experiment 2, the larvae and pupae were reared under the normal rearing condition of 16:8 LD and the adults were observed at 16:8, 14:10 and 12:12 LD regimes. The variation between these two studies may be due to the difference in the developmental stages when the scotophase treatments were applied (i.e., larval/pupal vs adult stages).

McNeil (1987) suggested that delayed calling in response to long nights would be an advantageous adaptation for migratory species like *P. unipuncta,* which is regarded as a seasonal migrant in North America. Under fall conditions when the nights are longer

and temperatures are lower, females have longer pre-calling periods. McNeil (1987) argued that delayed calling would increase the window for migration, enabling them to move to more suitable overwintering sites in the lower latitudes. Similarly, in *M. separata* where the pre-calling period is influenced by X-linked genes (Han & Gatehouse, 1991a), longer nights during autumn may induce longer pre-calling periods allowing southward migration prior to the onset of unfavourable winter conditions (Han & Gatehouse, 1991b). In these two species, photoperiod appears to be the primary environmental cue for movement to more favourable habitats to escape the harsh conditions in winter. The influence of photoperiod on the reproductive development in male moths is less clear.

*M. convecta* is a highly migratory species in Australia (Farrow & McDonald, 1987; McDonald, 1991; Gregg *et al.*, 1993). Seasonal variation in temperature is probably not so important for this species as it is for *P. unipuncta* and *M. separata.* However, the erratic distribution of rainfall is critical (McDonald, 1994, in press). Coombs *et al.* (1993) found that *M. convecta* females caught in a tower-mounted light trap were predominantly unmated (97.1%), suggesting that migration in this species occurs pre-reproductively (Johnson, 1969). Del Socorro (1991) also reported that large numbers of unmated females were caught in traps during winter/spring in the north-eastern area of New South Wales, when local emergence was not expected on the basis of phenological modelling. Back-tracking studies suggest that spring migrants were generally from the north or north-western regions. *M. convecta* can breed on several grass hosts in southern Queensland or north-western New South Wales during winter-autumn (McDonald, 1989). In summer, probable sources of *M. convecta* migrants are from the coastal regions of northern NSW.

McDonald (1994, in press) proposed three phases of migration in *M. convecta*: movement out of the inland in spring and summer, return movement to the inland in autumn, and gradual movement to the south in winter and spring. In the first phase, the absence of suitable food or breeding sources, due to the drying up of grassland habitats in the inland, provides the environmental cue for movement out of this area. Appropriate weather systems assist their migratory movement, particularly in spring, (Drake & Farrow, 1988) to locate favourable habitats in the south or east coast. In the second phase, the presence of autumn rainfall is critical for the re-invasion of the inland habitats by moths from the coastal or highland areas. Habitat quality would depend on rainfall to allow the growth of patchy and ephemeral grasslands in the inland areas. Easterly winds or sea-breezes that extend to the inland (Drake, 1982) provide opportunities for migration from the coastal areas. During the third phase, the deterioration of sub-tropical grass-

lands force *M. convecta* to move to southern areas where suitable hosts such as winter pastures or cereals are available. These winter migrations, however, are likely to be less extensive than those in spring, summer or autumn.

As in *P. unipuncta* and *M. separata,* long scotophases might induce migratory movement in *M. convecta.* The results obtained in Experiments 1 and 2 show that under low temperatures and long scotophases (i.e., autumn-early winter-spring conditions), the pre-calling period was longer than that at warmer temperatures and longer photoperiods (i.e., summer). Whether the pre-calling period is much more delayed under scotophases longer than those which were observed in the laboratory (i.e., > 12 h) was not investigated. The scotophases when delayed calling was observed in the laboratory correspond to autumn and spring in southern Queensland and northern NSW, that is, to the conditions of the first and second phases of McDonald's model of the seasonal movement of *M. convecta.* If migration occurs pre-reproductively in this species, then delayed calling would provide more time available for their migratory movement. Longer pre-reproductive periods would therefore favour return migrations to the inland as well as migration from it. It should be noted, however, that, in the laboratory experiments, daylengths were constant, thus spring and autumn daylengths are assumed to be similar. In the field, daily variation occurs and the direction of this variation changes in autumn and spring (i.e., autumn daylength declines towards winter and spring daylength increases towards summer). Whether the direction of this change can affect the pre-calling period is less clear.

In general, the effect of photoperiod on the pre-calling period is consistent with the known patterns of *M. convecta* migration. Sappington & Showers (1992), however, argued that pre-calling period might not be a good index of migratory potential, as in the case of *A. ipsilon* (see Chapter 4.7). They argued that the reproduction-flight syndrome can only be used as a "convenient descriptive term". This syndrome needs systematic study in *M. convecta,* and until this is done, interpretations such as the previous one remain speculative.

Most calling in *M. convecta* occurred during the 2nd half of the scotophase at different photoperiodic conditions, as observed in Experiment 1 and in previous experiments (Chapter 4). Onset calling time was earlier at shorter scotophases. Similar observations were reported for *P. unipuncta* and *M. configurata* (Delisle & McNeil, 1987a; Gerber & Howlader, 1987). The effects of temperature and photoperiod on the calling behaviour in *M. convecta* were examined separately. Studying the calling behaviour under different combinations of temperature and photoperiod treatments in the laboratory

and behavioural studies in the field at different seasons would be useful to further elucidate the influence of these factors on calling behaviour.

A circadian rhythm of calling behaviour is present in many Lepidopteran females. The phase of the rhythm occurs at some specific time each day in relation to the periodicity of the light:dark cycle and, in the absence of the entraining light:dark signals, the rhythm re-occurs at a 24-hour periodicity (Shorey, 1974). In Experiment 3, similar calling patterns under the entraining period and within the first 72 hours in constant darkness in the two groups of entrained females, demonstrated the presence of a circadian rhythm in the calling behaviour in *M. convecta.* Calling in these females within the first 72 hours following transfer to darkness occurred at approximately 24-hour intervals. In both groups, peak calling occurred in the 7th hour of the adult scotophase during the pre-transfer and post-transfer periods. Peak calling during this period also coincided with that in females that were under the normal 16:8 LD regime.

The periodicity of calling in constant darkness appeared to be correlated with the length of the entraining period. Moths with shorter entraining period (3 days) exhibited the circadian rhythm of calling within the first 72 hours in darkness, followed by a break of about 102 hours when no calling occurred, after which calling resumed again (Fig. 5.7). On the other hand, moths with a longer entrainment period (8 days) seemed to have maintained their calling periodicities longer in the extended scotophase (Fig. 5.8). The circadian rhythm of calling lasted for 96 hours and there was no break in calling.

Although entrained moths still exhibited calling behaviour under prolonged darkness, their calling rhythm changed as they got older. In both Groups 1 and 2, calling periodicities became de-synchronised from those observed within the first 72-96 hours after transfer to darkness. The calling patterns seemed to be roughly what might be expected during the entraining scotophase in the larval stage (although statistical analyses showed that they were not precisely the same), rather than just a case of random calling. The de-synchronisation of the daily rhythms of entrained females could be due to changes in some neural or hormonal factors that regulate pheromone production and the expression of calling behaviour in the absence of a regular light:dark cycle. The underlying physiological mechanisms for the re-synchronisation of the calling rhythm to a different clock, (i.e., larval scotophase) are not understood. It is possible that moths have the ability to "remember" the entraining larval scotophase under prolonged darkness. Some neuroendocrine substances affecting behavioural rhythm during larval development might have been carried through to adult development. The persistence of diurnal rhythms from the

larval through to the adult stage, and even from one generation to the next, have been observed in a Coleopteran and a Dipteran species (Harker, 1961).

Another possible mechanism for the apparent reversion to the larval rhythm is that moths were able to detect the time of the ambient scotophase outside the experimental cabinets (which was approximately the same as the larval scotophase). The experimental cabinet where the moths were kept remained closed most of the time except when observations were being recorded, and the room which housed this cabinet was dark all the time and at roughly constant temperature. It was therefore very unlikely that any kind of light or temperature signals from the outside would have affected these moths. That electromagnetic waves from solar radiation might act as signals for detecting the external scotophase cannot be entirely discounted. Insects are sensitive to electromagnetic radiation over a wide range of wavelengths (Callahan, 1977), and it is conceivable that some of these wavelengths might have been detectable by moths inside the cabinet. Sensitivity of bees to the earth's magnetic fields has been documented (Gould, 1980). These insects can set their circadian rhythms by daily fluctuations in the earth's magnetic fields.

Both these hypotheses ("remembrance" of the larval scotophase and detection of the ambient scotophase) are complicated by the fact that there was no clear periodicity in the calling of moths reared in complete darkness from emergence (Fig. 5.9). This suggests, that, whatever the mechanism, it requires exposure to some light:dark signals in the adult stage.

Similar re-synchronisation of calling patterns to a different clock does not seem to have been previously reported for any species, though, Harker (1961) reports two cases where insect activity entrained to abnormal light:dark cycles (i.e.,< 24 h) re-synchronised to a 24-hour cycle when the insects were placed in continuous darkness. Observations in Experiment 3 were continued for much longer under constant darkness than most other studies on circadian calling rhythms. For example, Turgeon & McNeil (1982) entrained *P. unipuncta* females at 16:8 LD regime for 7 days and observed them in darkness for 56 hours. *H. armigera* females were held at 16:8 LD regime and were in the dark for 60 hours (Kou & Chow, 1987). Itagaki & Conner (1988) observed *M. sexta* females in the dark for 96 hours after a 1-day entrainment period at 16:8 LD regime. Babilis & Mazomenos (1992) maintained the corn stalk borer, *S. nonagrioides* at 16:8 LD regime from the larval stage until 1 day after emergence and kept them in continuous darkness for 72 hours. As in *M. convecta*, some degree of de-synchronisation of calling were also observed in these species, that is, onset calling times not occurring at exactly 24-hour intervals, but whether re-synchronisation to a different clock might have occurred in these

species (if observed for long enough) is not known. Re-synchronisation might have occurred in *M. convecta* because the entraining light:dark conditions during larval and adult development were different. Pupal and adult scotophase was at 0900-1700 h while larval scotophase was at 2300-0700 h. On the other hand, the larvae and adults of the above three species were reared under similar conditions and therefore experienced similar light:dark signals throughout their development.

One species, that has been studied over an entrainment period and length of time in continuous darkness comparable to that in *M. convecta* in Experiment 3, is *M. configurata* (Howlader & Gerber, 1986a). In this case, the larval scotophase was normal, and the pupal/adult scotophase was reversed, as was the case with *M. convecta* in this experiment. *M. configurata* females were entrained at 16:8 LD regime for 3 days then observed in the dark for 192 hours. During the 192-hour darkness, de-synchronisation of calling periodicities was observed. The mean onset calling time in the first cycle in the dark was similar to that in the 3rd scotophase at 16:8 LD, whereas onset times from the 2nd to the 8th cycles shifted later by 3-5.5 hours, compared to the first cycle. However, there was no evidence of a reversion to the larval cycle.

Although calling behaviour in constant darkness is not relevant to the field situation, the unexpected finding of re-synchronisation to a different clock in this study is apparently novel, and warrants further study.

# CHAPTER 6

# BEHAVIOUR OF *M. CONVECTA* MALES IN THE PRESENCE OF CONSPECIFIC FEMALES IN A WIND TUNNEL

## 6.1. Introduction

Synthetic sex pheromones of many species are widely used for population monitoring and have the potential for use in pest management. Pheromone traps may be useful as early warning device in detecting infestations before damage occurs. Before developing pheromone-based pest management programs, however, an understanding of the pheromone biology of the emitters, usually the females, and the pheromone-mediated behaviour of the receivers, usually the males, is necessary.

Laboratory experiments on the calling behaviour or the behaviour associated with the release of the sex pheromone in *M. convecta* females (Chapter 4) provided a basic understanding of their reproductive biology. Modifications in the calling behaviour due to temperature and photoperiod have also been examined (Chapter 5). In this chapter, how conspecific males behave in a wind tunnel when the females are releasing the sex pheromone is investigated. It was first necessary to observe the males without any pheromone source to describe their behaviour in the tunnel. This was followed by observing male behaviour in the presence of the natural sex pheromone emitted by the females during calling to describe what behaviour constitutes a sexual response to the pheromone, which will then be used to describe male behaviour in the presence of different synthetic pheromone blends discussed in Chapter 11.

## 6.2. Materials and methods

Experimental larvae were reared under the normal rearing conditions in the insectary. Pupae were sexed and kept in separate reverse-cycle cabinets at 25 ± 1°C and 16:8 light:dark (LD) regime with the scotophase at 1000-1800 h AEST. Upon emergence, individual moths were held in containers provided with dental wick soaked in sucrose solution and were kept in the same conditions as the pupae (Chapter 3.1).

Males of different ages were used in experiments. They were transferred to the wind tunnel room at least an hour before the start of the experiment to acclimatise. They were tested in the wind tunnel (Chapter 3.3) for several scotophases but were released only once during a given scotophase. Males undergoing their first release in the wind tunnel are described as "fresh" while those undergoing subsequent releases in later scotophases are described as "re-used". Fresh and re-used males were released in the

wind tunnel at random during the 2nd half of the scotophase (1400-1800 h AEST) when most female calling occurs (Chapter 4.3.2.2).

Three sets of experiments were conducted. In Experiment 1, males were first tested in the wind tunnel without any pheromone source upwind. In Experiment 2, male behaviour when females were present in the wind tunnel was investigated. Their behaviour when these females were actually calling or not calling was examined separately. In Experiment 3, the behaviour of males that had been flown in flight mills before being tested in the wind tunnel was studied.

### 6.2.1. Types of behaviour

Males were allowed to respond for 5 minutes. If a male did not leave the container 3 minutes after release, it was removed from the wind tunnel. Males were scored for the following behaviours: (1) take off - initiation of flight from the release container, (2) upwind flight - directed flight to within 50 cm of the upwind wall, (3) approach - flight to within 10-15 cm from the source, usually characterised by hovering, (4) landing - contact with the cage containing the pheromone source and (5) clasper extrusion - full extrusion of the claspers, usually followed by an attempt to copulate when the male thrusts its claspers towards the source. These behaviours were compared between the different bait types - no pheromone source, females that were not calling and females that were calling.

All behaviours were recorded on a portable computer using a small program in QuickBASIC called DATA.BAS and later transcribed for statistical analyses. This program is provided in the sub-directory A:\CHAP3 of the floppy disk.

### 6.2.2. Statistical analyses

Data on the frequency of observations for each behaviour at different ages in fresh or re-used males were analysed by chi-square ($\chi^2$) tests using contingency tables (Devore & Peck, 1993). The following null hypotheses were tested for each source type: (1) that male behaviour and moth age were independent and (2) that male behaviour and male experience (i.e., either fresh or re-used) were independent. In the presence of calling females a third hypothesis was tested - that male behaviour and time of the scotophase were independent.

Comparisons of the different behaviours between source types, (i.e, blank source, non-calling females and calling females) were also done by the chi-square method. Data were pooled for both fresh and re-used males across all ages.

Raw data for the three experiments are provided in the sub-directory A:\CHAP6 of the floppy disk.

## 6.3. Experiment 1 - Male behaviour in the absence of a pheromone source

### 6.3.1. Materials and methods

Males used in the experiment were between 1 and 10 days old. They were flown in the wind tunnel using either empty female cages (Chapter 6.4.1) or a blank disc (Chapter 3.3) on a stand in the upwind end. Data presented are combined for males tested with empty cages and those tested with a blank disc as preliminary analysis indicated that they were not significantly different.

### 6.3.2. Results and discussion

The number of observations at different ages is given in Table 6.1.

| Age (S) | Fresh males | Re-used males | Total |
|---|---|---|---|
| 1 | 18 | 0 | 18 |
| 2 | 7 | 15 | 22 |
| 3 | 11 | 15 | 26 |
| 4 | 1 | 19 | 20 |
| 5 | 1 | 17 | 18 |
| 6 | 5 | 15 | 20 |
| 7 | 4 | 11 | 15 |
| 8 | 0 | 13 | 13 |
| 9 | 8 | 8 | 16 |
| 10 | 4 | 10 | 14 |
| Total | 59 | 123 | 182 |

**Table 6.1.** Experiment 1. Number of observations at different ages of *M. convecta* males flown in the wind tunnel in the absence of a pheromone source. Fresh males were those undergoing their first release while re-used males were those undergoing subsequent releases in the wind tunnel. Wind tunnel temperature was 25 ± 1°C.

Only take off and upwind flight were observed when there was no pheromone source in the wind tunnel (Fig. 6.1). When males took off from the container, they either rested, flew upwind, flew at random in the wind tunnel, or alighted on the walls or floor and then rested.

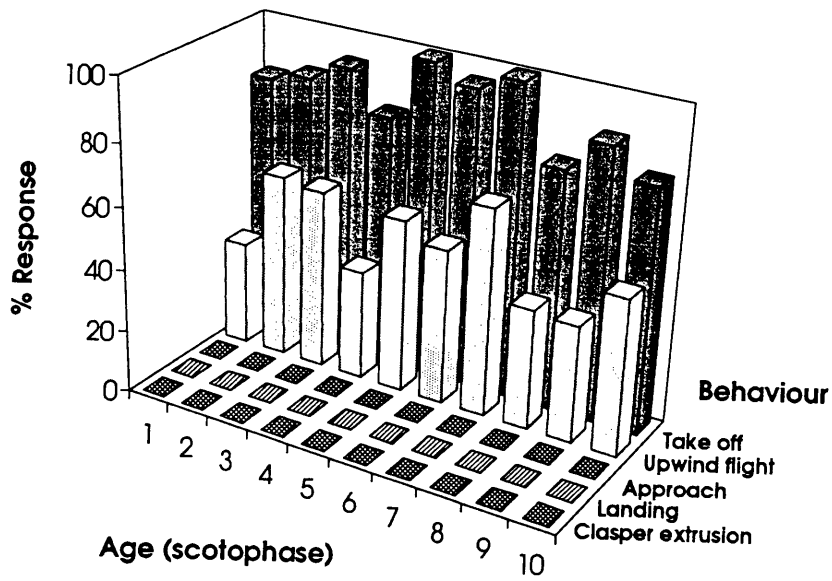


**Fig. 6.1.** Experiment 1. Percentage (%) of *M. convecta* males exhibiting the different behaviours at different ages in the absence of a pheromone source in the wind tunnel at 25 ± 1°C. Observations were done between 1400 and 1800 h AEST. N for each age group is given in Table 6.1.

The percentage of males that took off from the containers was more than 75 % for all ages (Table 6.2). A chi-square test using a 2 x 10 contingency table on the response category (whether male did or did not exhibit the behaviour) and moth age for take off was not significant ($\chi^2_9 = 10.53$), indicating that moth age did not significantly influence this behaviour.

| | Response category for take off | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 3 (17) | 15 (83) | 18 (100) |
| 2 | 3 (14) | 19 (86) | 22 (100) |
| 3 | 2 (8) | 24 (92) | 26 (100) |
| 4 | 4 (20) | 16 (80) | 20 (100) |
| 5 | 0 (0) | 18 (100) | 18 (100) |
| 6 | 1 (5) | 19 (95) | 20 (100) |
| 7 | 0 (0) | 15 (100) | 15 (100) |
| 8 | 3 (23) | 10 (77) | 13 (100) |
| 9 | 2 (12) | 14 (88) | 16 (100) |
| 10 | 3 (21) | 11 (79) | 14 (100) |
| Total | 21 (12) | 161 (88) | 182 (100) |

**Table 6.2.** Experiment 1. Number and percentage (in parentheses) of *M. convecta* males that did and did not take off from the container at different ages in the absence of a pheromone source in the wind tunnel. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for upwind flight | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 12 (67) | 6 (33) | 18 (100) |
| 2 | 9 (41) | 13 (59) | 22 (100) |
| 3 | 11 (42) | 15 (58) | 26 (100) |
| 4 | 13 (65) | 7 (35) | 20 (100) |
| 5 | 8 (44) | 10 (56) | 18 (100) |
| 6 | 10 (50) | 10 (50) | 20 (100) |
| 7 | 5 (33) | 10 (67) | 15 (100) |
| 8 | 8 (62) | 5 (38) | 13 (100) |
| 9 | 10 (62) | 6 (38) | 16 (100) |
| 10 | 7 (50) | 7 (50) | 14 (100) |
| Total | 93 (51) | 89 (49) | 182 (100) |

**Table 6.3.** Experiment 1. Number and percentage (in parentheses) of *M. convecta* males that did and did not fly upwind at different ages in the absence of a pheromone source in the wind tunnel. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

The percentage of upwind flight ranged from 33 to 67 % per scotophase (Table 6.3). A chi-square test of the response category and moth age was also not significant ($\chi^2_9 = 8.64$).

The response categories for these two behaviours were compared in fresh and re-used males. Chi-square tests using 2 x 2 contingency tables yielded non-significant results ($\chi^2_1 = 0.009$ and 0.99, for take off and upwind flight, respectively), indicating that these behaviours were not significantly different between fresh and re-used males (Tables 6.4 and 6.5).

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 7 | 14 | 21 |
| Yes | 52 | 109 | 161 |
| Total | 59 | 123 | 182 |

**Table 6.4.** Experiment 1. A 2 x 2 contingency table of response category and male experience for take off behaviour in the absence of a pheromone source.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 27 | 66 | 93 |
| Yes | 32 | 57 | 89 |
| Total | 59 | 123 | 182 |

**Table 6.5.** Experiment 1. A 2 x 2 contingency table of response category and male experience for upwind flight behaviour in the absence of a pheromone source.

## 6.4. Experiment 2 - Male behaviour in the presence of females

### 6.4.1. Materials and methods

Males used in the experiment were 1 to 14 days old. Five 3- to 6-day-old unmated females were used as the pheromone source. Each female moth was held in a 4.5 x 4.5 x 15 cm clear plastic container closed with fly screen on opposite ends to allow air flow. Female cages were held in a calling tower (Chapter 3.3) in the upwind end. Female baits were transferred to the wind tunnel before 1300 h AEST to acclimatise to tunnel conditions. Male releases began following the onset of calling in any of the five females during the 2nd half of the scotophase. Releases continued at approximately 5-min intervals, whether any females were calling or not. The number of females calling, and their location in the calling tower, was recorded prior to each release. Separate analyses were conducted for data on male behaviour when at least one female was calling, and when no females were calling.

### 6.4.2. Results and discussion

#### 6.4.2.1. Behaviour when females were not calling

In some instances when the males were released in the wind tunnel, none of the five female baits were calling or actually extruding their ovipositors. The number of male observations at different ages when the females were not calling are given in Table 6.6.

| Age (S) | Fresh males | Re-used males | Total |
|---|---|---|---|
| 1 | 10 | 0 | 10 |
| 2 | 12 | 12 | 24 |
| 3 | 6 | 9 | 15 |
| 4 | 4 | 14 | 18 |
| 5 | 2 | 19 | 21 |
| 6 | 5 | 17 | 22 |
| 7 | 0 | 10 | 10 |
| 8 | 0 | 8 | 8 |
| 9 | 0 | 3 | 3 |
| Total | 39 | 92 | 131 |

**Table 6.6.** Experiment 2. Number of observations at different ages of *M. convecta* males flown in the wind tunnel in the presence of non-calling conspecific females. Fresh males were those undergoing their first release while re-used males were those undergoing subsequent releases in the wind tunnel. Female baits were 3-6 day old. Wind tunnel temperature was 25 ± 1°C.

When the females were not calling, only take off, upwind flight and very low levels of approach were recorded (Fig. 6.2). The overall percentages of observations that exhibited these behaviours were: take off - 89%, upwind flight - 61% and approach 2%. Chi-square tests using 2 x 9 contingency tables of response category and moth age were done for these three behaviours. The incidences of take off and approach (Tables 6.7 and 6.9) were not significantly different in males of different ages ($\chi^2_8$ = 2.39 and 12.75, respectively) while the incidence of upwind flight (Table 6.8) was significantly different ($\chi^2_8$ = 19.29, $p < 0.05$). The very small percentage of approaches might be due to pheromone emitted by the females when they called being still present in the cages (possibly adsorbed to the plastic cage walls).

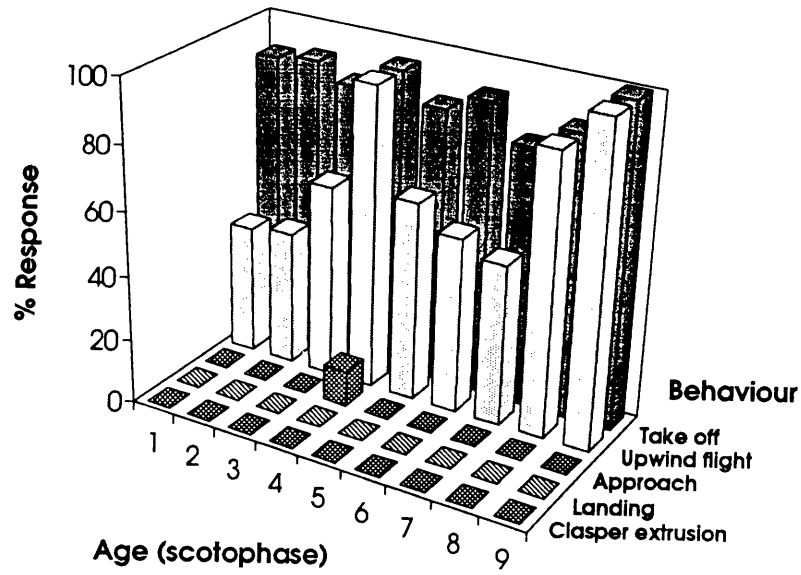


**Fig. 6.2.** Experiment 2. Percentage (%) of *M. convecta* males exhibiting the different behaviours at different ages in the presence of conspecific females that were not calling in the wind tunnel at 25 ± 1°C. Observations were done between 1400 and 1800 h AEST. N for each age group is given in Table 6.7.

| | Response category for take off | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 1 (10) | 9 (90) | 10 (100) |
| 2 | 2 (8) | 22 (92) | 24 (100) |
| 3 | 2 (13) | 13 (87) | 15 (100) |
| 4 | 1 (6) | 17 (94) | 18 (100) |
| 5 | 3 (14) | 18 (86) | 21 (100) |
| 6 | 2 ( 9) | 20 (91) | 22 (100) |
| 7 | 2 (20) | 8 (80) | 10 (100) |
| 8 | 1 (12) | 7 (88) | 8 (100) |
| 9 | 0 ( 0) | 3 (100) | 3 (100) |
| Total | 14 (11) | 117 (89) | 131 (100) |

**Table 6.7.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not take off at different ages when females were not calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for upwind flight | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 6 (60) | 4 (40) | 10 (100) |
| 2 | 14 (58) | 10 (42) | 24 (100) |
| 3 | 6 (40) | 9 (60) | 15 (100) |
| 4 | 1 (6) | 17 (94) | 18 (100) |
| 5 | 8 (38) | 13 (62) | 21 (100) |
| 6 | 10 (45) | 12 (55) | 22 (100) |
| 7 | 5 (50) | 5 (50) | 10 (100) |
| 8 | 1 (12) | 7 (88) | 8 (100) |
| 9 | 0 ( 0) | 3 (100) | 3 (100) |
| Total | 51 (39) | 80 (61) | 131 (100) |

**Table 6.8.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not fly upwind at different ages when females were not calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for approach | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 10 (100) | 0 (0) | 10 (100) |
| 2 | 24 (100) | 0 (0) | 24 (100) |
| 3 | 15 (100) | 0 (0) | 15 (100) |
| 4 | 16 (89) | 2 (11) | 18 (100) |
| 5 | 21 (100) | 0 (0) | 21 (100) |
| 6 | 22 (100) | 0 (0) | 22 (100) |
| 7 | 10 (100) | 0 (0) | 10 (100) |
| 8 | 8 (100) | 0 (0) | 8 (100) |
| 9 | 3 (100) | 0 (0) | 3 (100) |
| Total | 129 (98) | 2 (2) | 131 (100) |

**Table 6.9.** Experiment 2. Number and proportion (in parentheses) of *M. convecta* males that did and did not approach the pheromone source at different ages when females were not calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

Chi-square tests using 2 x 2 contingency tables were done to compare response category between fresh and re-used males (Tables 6.10 and 6.11). The results for both take off and upwind flight were not significant ($\chi^2_1$ = 0.01 and 2.24, respectively), indicating that these two behaviours were not significantly different between fresh and re-used males.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 4 | 10 | 14 |
| Yes | 35 | 82 | 117 |
| Total | 39 | 92 | 131 |

**Table 6.10.** Experiment 2. A 2 x 2 contingency table of response category and male experience for take off behaviour when females were not calling.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 19 | 32 | 51 |
| Yes | 20 | 60 | 80 |
| Total | 39 | 92 | 131 |

**Table 6.11.** Experiment 2. A 2 x 2 contingency table of response category and male experience for upwind flight behaviour when females were not calling.

### 6.4.2.2. Behaviour when females were calling

The number of male observations at different ages is shown in Table 6.14.

| Age (S) | Fresh males | Re-used males | Total |
|---|---|---|---|
| 1 | 8 | 0 | 8 |
| 2 | 4 | 11 | 15 |
| 3 | 9 | 27 | 36 |
| 4 | 6 | 32 | 38 |
| 5 | 8 | 34 | 42 |
| 6 | 5 | 26 | 31 |
| 7 | 4 | 26 | 30 |
| 8 | 3 | 18 | 21 |
| 9 | 0 | 19 | 19 |
| 10 | 0 | 14 | 14 |
| 11 | 0 | 11 | 11 |
| 12 | 0 | 8 | 8 |
| 13 | 0 | 6 | 6 |
| 14 | 0 | 3 | 3 |
| Total | 47 | 235 | 282 |

**Table 6.12.** Experiment 2. Number of observations at different ages of *M. convecta* males flown in the wind tunnel in the presence of calling conspecific females. Fresh males were those undergoing their first release while re-used males were those undergoing subsequent releases in the wind tunnel. Female baits were 3-6 days old. Wind tunnel temperature was 25 ±1°C.

All the five behaviours were recorded in the presence of calling females (Fig. 6.3). Take off and upwind flight were recorded in the 1st scotophase, whereas approach, landing and clasper extrusion were not observed until the 3rd scotophase. Chi-square tests using 2 x 14 contingency tables of the response category and moth age for each behaviour were conducted. The results for take off, landing and clasper extrusion (Tables 6.13, 6.16 and 6.17) were not significant ($\chi^2_{13}$ = 16.77, 17.26 and 15.36, respectively), whereas those for upwind flight and approach were significant ($\chi^2_{13}$ = 27.99, $p < 0.01$ and 26.95, $p < 0.05$, respectively (Tables 6.14 and 6.15).

| | Response category for take off | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 1 (12) | 7 (88) | 8 (100) |
| 2 | 3 (20) | 12 (80) | 15 (100) |
| 3 | 5 (14) | 31 (86) | 36 (100) |
| 4 | 2 (5) | 36 (95) | 38 (100) |
| 5 | 4 (10) | 38 (90) | 42 (100) |
| 6 | 4 (13) | 27 (87) | 31 (100) |
| 7 | 1 (3) | 29 (97) | 30 (100) |
| 8 | 4 (19) | 17 (81) | 21 (100) |
| 9 | 2 (10) | 17 (90) | 19 (100) |
| 10 | 1 (7) | 13 (93) | 14 (100) |
| 11 | 1 (9) | 10 (91) | 11 (100) |
| 12 | 1 (12) | 7 (88) | 8 (100) |
| 13 | 0 (0) | 6 (100) | 6 (100) |
| 14 | 2 (67) | 1 (33) | 3 (100) |
| Total | 31 (11) | 251 (89) | 282 (100) |

**Table 6.13.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not take off at different ages when females were calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

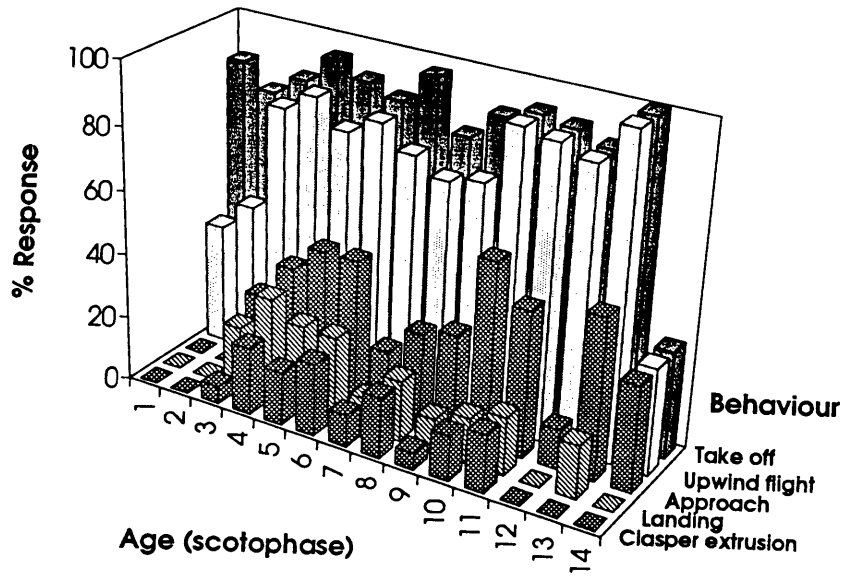


**Fig. 6.3.** Experiment 2. Percentage (%) of *M. convecta* males exhibiting the different behaviours at different ages in the presence of conspecific females that were calling in the wind tunnel at 25 ± 1°C. Observations were done between 1400 and 1800 h AEST. N for each age group is given in Table 6.12.

| | Response category for upwind flight | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 5 (62) | 3 (38) | 8 (100) |
| 2 | 8 (53) | 7 (47) | 15 (100) |
| 3 | 7 (19) | 29 (81) | 36 (100) |
| 4 | 5 (13) | 33 (87) | 38 (100) |
| 5 | 9 (21) | 33 (79) | 42 (100) |
| 6 | 5 (16) | 26 (84) | 31 (100) |
| 7 | 7 (23) | 23 (77) | 30 (100) |
| 8 | 6 (29) | 15 (71) | 21 (100) |
| 9 | 5 (26) | 14 (74) | 19 (100) |
| 10 | 1 (7) | 13 (93) | 14 (100) |
| 11 | 1 (9) | 10 (91) | 11 (100) |
| 12 | 1 (12) | 7 (88) | 8 (100) |
| 13 | 0 (0) | 6 (100) | 6 (100) |
| 14 | 2 (67) | 1 (33) | 3 (100) |
| Total | 62 (22) | 220 (78) | 282 (100) |

**Table 6.14.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not fly upwind at different ages when females were calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for approach | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 8 (100) | 0 (0) | 8 (100) |
| 2 | 15 (100) | 0 (0) | 15 (100) |
| 3 | 27 (75) | 9 (25) | 36 (100) |
| 4 | 24 (63) | 14 (37) | 38 (100) |
| 5 | 23 (55) | 19 (45) | 42 (100) |
| 6 | 17 (55) | 14 (45) | 31 (100) |
| 7 | 24 (80) | 6 (20) | 30 (100) |
| 8 | 15 (71) | 6 (29) | 21 (100) |
| 9 | 13 (68) | 6 (32) | 19 (100) |
| 10 | 6 (43) | 8 (57) | 14 (100) |
| 11 | 6 (55) | 5 (45) | 11 (100) |
| 12 | 7 (88) | 1 (12) | 8 (100) |
| 13 | 3 (50) | 3 (50) | 6 (100) |
| 14 | 2 (67) | 1 (33) | 3 (100) |
| Total | 190 (67) | 92 (33) | 282 (100) |

**Table 6.15.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not approach the pheromone source at different ages when females were calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for landing | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 8 (100) | 0 (0) | 8 (100) |
| 2 | 15 (100) | 0 (0) | 15 (100) |
| 3 | 29 (81) | 7 (19) | 36 (100) |
| 4 | 26 (68) | 12 (32) | 38 (100) |
| 5 | 31 (74) | 11 (26) | 42 (100) |
| 6 | 23 (74) | 8 (26) | 31 (100) |
| 7 | 27 (90) | 3 (10) | 30 (100) |
| 8 | 17 (81) | 4 (19) | 21 (100) |
| 9 | 17 (89) | 2 (11) | 19 (100) |
| 10 | 12 (86) | 2 (14) | 14 (100) |
| 11 | 9 (82) | 2 (18) | 11 (100) |
| 12 | 8 (100) | 0 (0) | 8 (100) |
| 13 | 5 (83) | 1 (17) | 6 (100) |
| 14 | 3 (100) | 0 (0) | 3 (100) |
| Total | 230 (82) | 52 (18) | 282 (100) |

**Table 6.16.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not land on female cages at different ages when females were calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

| | Response category for clasper extrusion | | |
|---|---|---|---|
| Age (S) | No | Yes | Total |
| 1 | 8 (100) | 0 (0) | 8 (100) |
| 2 | 15 (100) | 0 (0) | 15 (100) |
| 3 | 34 (94) | 2 (6) | 36 (100) |
| 4 | 30 (79) | 8 (21) | 38 (100) |
| 5 | 35 (83) | 7 (17) | 42 (100) |
| 6 | 24 (77) | 7 (23) | 31 (100) |
| 7 | 27 (90) | 3 (10) | 30 (100) |
| 8 | 17 (81) | 4 (19) | 21 (100) |
| 9 | 18 (95) | 1 (5) | 19 (100) |
| 10 | 12 (86) | 2 (14) | 14 (100) |
| 11 | 9 (82) | 2 (18) | 11 (100) |
| 12 | 8 (100) | 0 (0) | 8 (100) |
| 13 | 6 (100) | 0 (0) | 6 (100) |
| 14 | 3 (100) | 0 (0) | 3 (100) |
| Total | 246 (87) | 36 (13) | 282 (100) |

**Table 6.17.** Experiment 2. Number and percentage (in parentheses) of *M. convecta* males that did and did not extend claspers at different ages when females were calling. Males were observed between 1400 and 1800 h AEST at 25 ± 1°C.

Chi-square tests on response category and male experience for each behaviour were conducted using 2 x 2 contingency tables (Tables 6.18 to 6.20). The results were not significant for all behaviours. For approach, landing and clasper extrusion, days 1 and 2 were excluded because these behaviours did not occur at these ages. These results indicate that either non-exposure (fresh males) or previous exposure (re-used males) to wind tunnel conditions (including to calling females) did not strongly influence male behaviour.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 5 | 26 | 31 |
| Yes | 42 | 209 | 251 |
| Total | 47 | 235 | 282 |

**Table 6.18.** Experiment 2. A 2 x 2 contingency table of response category and male experience for take off behaviour when females were calling.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 12 | 50 | 62 |
| Yes | 35 | 185 | 220 |
| Total | 47 | 235 | 282 |

**Table 6.19.** Experiment 2. A 2 x 2 contingency table of response category and male experience for upwind flight behaviour when females were calling.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 26 | 141 | 167 |
| Yes | 9 | 83 | 92 |
| Total | 35 | 224 | 259 |

**Table 6.20.** Experiment 2. A 2 x 2 contingency table of response category and male experience for approach behaviour when females were calling. Females of ages 1 and 2 were excluded because approach did not occur in females less than 3 days old.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 26 | 181 | 207 |
| Yes | 9 | 43 | 52 |
| Total | 35 | 224 | 259 |

**Table 6.21.** Experiment 2. A 2 x 2 contingency table of response category and male experience for landing behaviour when females were calling. Females of ages 1 and 2 were excluded because landing did not occur in females less than 3 days old.

| | Male experience | | |
|---|---|---|---|
| Response category | Fresh | Re-used | Total |
| No | 27 | 196 | 223 |
| Yes | 8 | 28 | 36 |
| Total | 35 | 224 | 259 |

**Table 6.22.** Experiment 2. A 2 x 2 contingency table of response category and male experience for clasper extrusion behaviour when females were calling. Females of ages 1 and 2 were excluded because clasper extrusion did not occur in females less than 3 days old.

The percentage of males that exhibited approach during each hour in the 2nd half of the scotophase was calculated. Male responses were recorded between the 6th and 8th hours of the scotophase and peaked in the 7th hour (Fig. 6.4a). It appeared that male sexual response was synchronous with maximal calling in the females, which also peaked in the 7th hour of the scotophase (Fig. 6.4b, Fig. 4.16 of Chapter 4). The male peak cannot be simply a response to the female peak, because these data included only the trials in which females were calling. Thus, the pheromone stimulus should have been present, even when the males did not exhibit sexual responses.

The onset time of calling in females generally advanced as the females got older (Chapter 4.3.2.2). To see if a similar pattern was found in males, the time of approach by young and old males was investigated. The median age of all males that exhibited approach was 5 days. All males that approached at between 3 and 5 days were therefore classified as "young" and those approaching at between 6 and 14 days as "old". The percentage of approach behaviour at each hour in the 2nd half of the scotophase for each group was calculated. Fig. 6.5 shows that approach behaviour in the old males tend to occur earlier in the scotophase than that in the young ones.

### 6.4.2.3. Comparison between source types

Chi-square tests using contingency tables were conducted to compare the different behaviours between the three source types (Tables 6.23). Overall, the different behaviours were significantly different between source types ($\chi^2_8 = 151.72$, $p < 0.001$). The behaviours when females were calling were significantly different from those males tested in the presence of non-calling females ($\chi^2_4 = 66.84$, $p < 0.001$) and from those tested in the absence of a pheromone source ($\chi^2_4 = 95.68$, $p < 0.001$). Most of the significance was due to the presence of definitive sexual behaviour (approach, landing, clasper extrusion) when the females were calling.

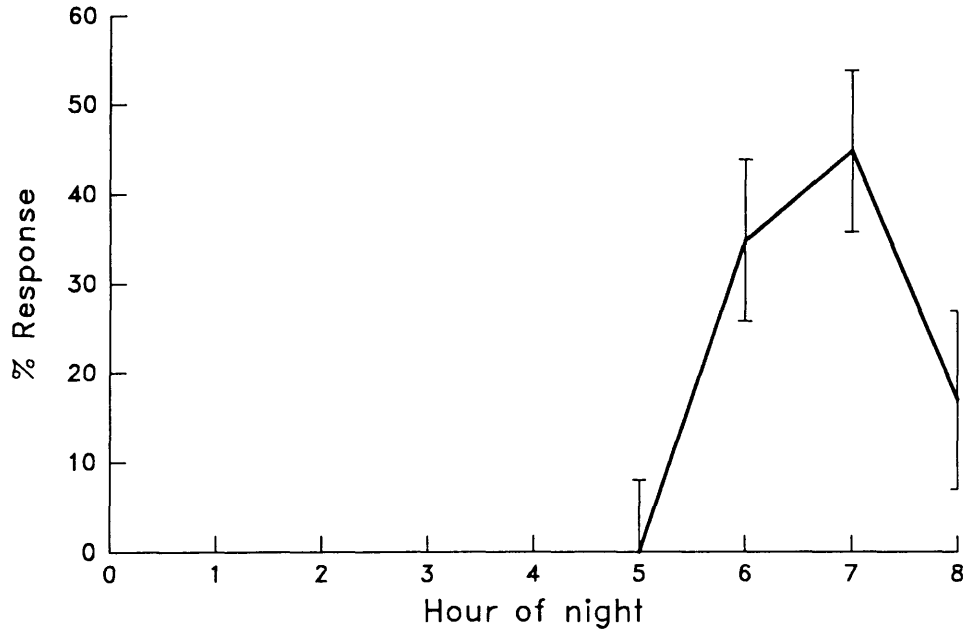


**Fig. 6.4a.** Experiment 2. Percentage (%) of *M. convecta* males exhibiting approach in the wind tunnel during the 2nd half of the scotophase (1400-1800 h AEST). Bars are 95% c.i.'s.

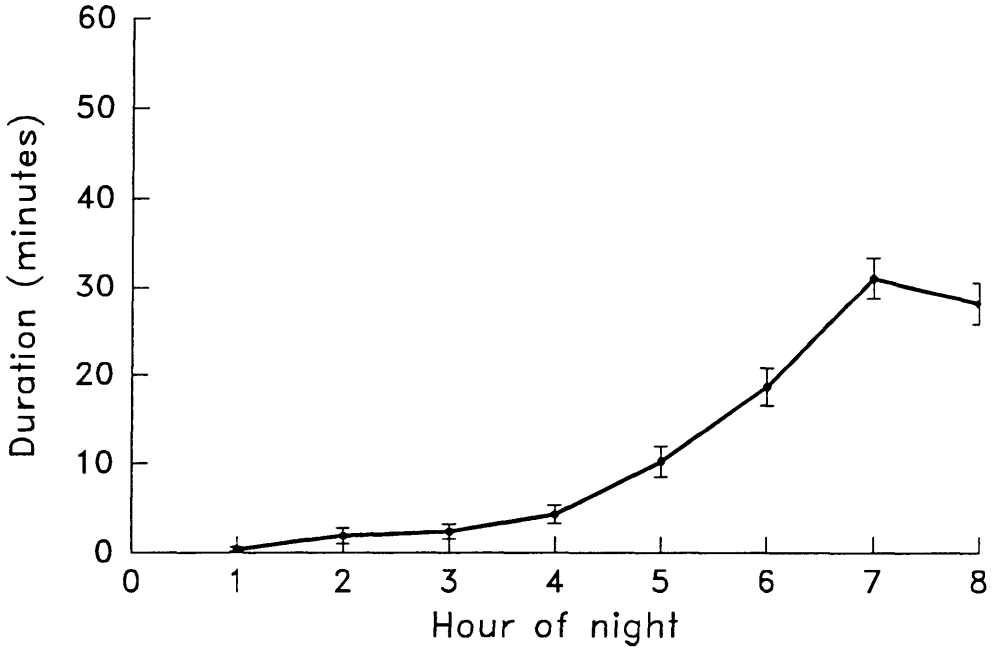


**Fig. 6.4b.** Mean duration of calling in *M. convecta* females at different hours of the scotophase. Bars are s.e.'s. Data are from Chapter 4.

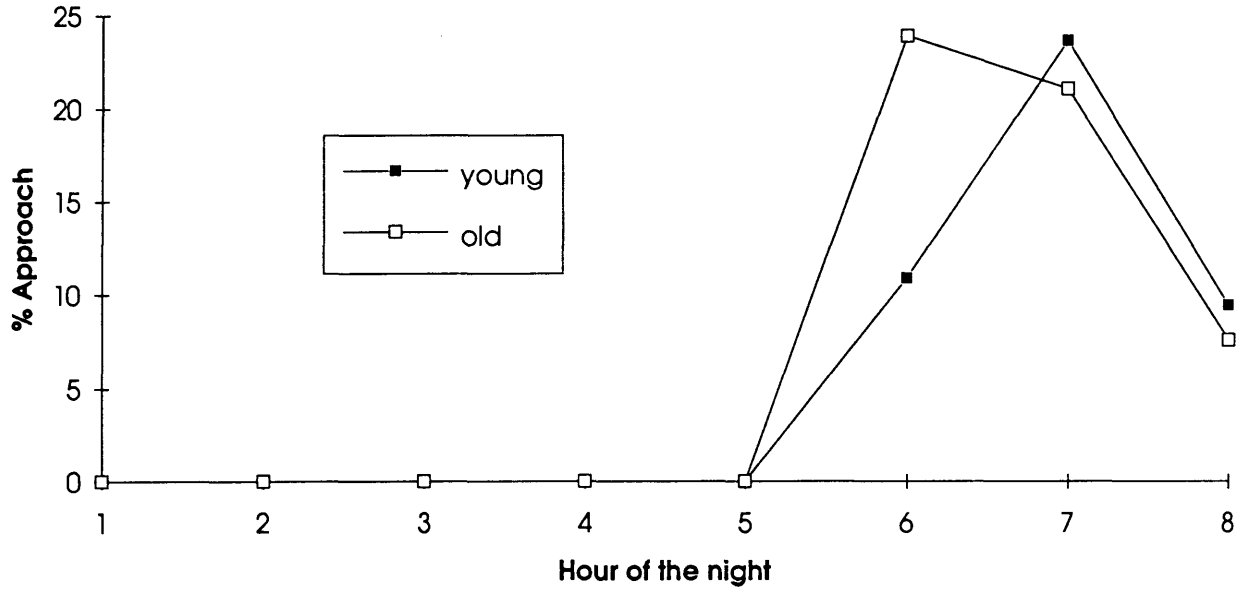


**Fig. 6.5.** Experiment 2. Percentage (%) of young and old *M. convecta* males exhibiting approach in the wind tunnel during the 2nd half of the scotophase (1400-1800 h AEST). Young males were between 3 and 5, whereas old males were between 6 and 14 days old.

| Behaviour | Source type | | |
|---|---|---|---|
| | Blank source | Non-calling females | Calling females |
| Take off | 161 | 117 | 251 |
| Upwind flight | 89 | 80 | 220 |
| Approach | 0 | 2 | 92 |
| Landing | 0 | 0 | 52 |
| Clasper extrusion | 0 | 0 | 36 |
| No. observed | 182 | 131 | 282 |

**Table 6.23.** Experiment 2. Number of observations of *M. convecta* males that exhibited the different behaviours in the presence of blank source (empty female cage or blank disc), non-calling and calling females in the wind tunnel.

## 6.5. Experiment 3 - Effects of flight on male behaviour

### 6.5.1. Materials and methods

The effects of flight on the behavioural responses of males to calling females was investigated. A total of 24 males with ages ranging from 1-4 days were tethered on flight mills for one scotophase (1000-1800 h). The flight mills were modified from a system described by Cooter & Armes (1993) except these ones did not have automatic computers or event recorders to determine the flight capacity of individual moths. Observations on when each male started and ended flight were recorded manually on a computer and later transcribed for statistical analysis.

Each moth was tethered on its own flight mill. The mill arm, which was made of copper wire, had a radius of 15 cm with the end where the moth was tethered bent. The end opposite the tethered moth was counterweighted to balance the weight of the moth. The technique for tethering moths was based on the method of Schmidt & Seabrook (1981) for *C. fumiferana*. Each moth was anaesthetised by ether and the scales on the dorsal side of the thorax carefully removed with feather forceps. The anaesthetised moth was allowed to rest on a temporary platform of the same height as the mill arm. After the scales were removed a tiny drop of glue (Bostik, Thomastown, Victoria, Australia) was touched to the bare area. A 2-mm long polyethylene tube (0.5mm ID, Dural Plastics and Engineering, Auburn, NSW, Australia) was quickly touched to the glue spot on the thorax. The bent tip of the arm was inserted into this tube to hold the moth.

Males were tethered on the flight mills for one scotophase ("flown males") and their behaviour was observed in the wind tunnel on the following scotophase, that is, when they were 2 to 5 days old. The plastic tubes were clipped from the thorax before testing them in the wind tunnel. The behaviour of another group of 2- to 5-day-old males

from the same batch of moths (N=24), which had not been tethered on flight mills ("unflown males") was also observed. To minimise variation between these two groups, plastic tubes were also glued on the thorax of unflown males then removed prior to testing in the wind tunnel. In both groups, 3- to 6-day-old females were used as the pheromone source. Data on the mean duration of flight during each hour of the scotophase and at different ages were calculated using the MINITAB statistical package.

### 6.5.2. Results and discussion

Flown males had a mean total flight duration of 189.7 ± 33.2 (s.e.) minutes per night. Flight duration significantly varied with moth age (Fig. 6.6). It was shortest in 1-day-old males and longest in 4-day-old ones. Flight duration did not significantly vary with the time of the scotophase (Fig. 6.7). The number and percentage of flown and unflown males that exhibited the different behaviours are shown in the following tables. Total N for each group was 24 but one male in each group was accidentally released when females were not calling, and observations from them were discarded.

| | Unflown males | | | Flown males | | |
|---|---|---|---|---|---|---|
| Age (S) | No | Yes | Total | No | Yes | Total |
| 2 | 2 (33) | 4 (67) | 6 (100) | 3 (50) | 3 (50) | 6 (100) |
| 3 | 2 (33) | 4 (67) | 6 (100) | 4 (67) | 2 (33) | 6 (100) |
| 4 | 1 (20) | 4 (80) | 5 (100) | 3 (60) | 2 (40) | 5 (100) |
| 5 | 1 (17) | 5 (83) | 6 (100) | 0 (0) | 6 (100) | 6 (100) |
| Total | 6 (26) | 17 (74) | 23 (100) | 10 (43) | 13 (57) | 23 (100) |

**Table 6.24.** Experiment 3. Number and percentage of unflown and flown *M. convecta* males that did and did not take off at different ages when females were calling. Flown males were tethered on flight mills for one scotophase whereas unflown ones were not.

| | Unflown males | | | Flown males | | |
|---|---|---|---|---|---|---|
| Age (S) | No | Yes | Total | No | Yes | Total |
| 2 | 4 (67) | 2 (33) | 6 (100) | 4 (67) | 2 (33) | 6 (100) |
| 3 | 3 (50) | 3 (50) | 6 (100) | 5 (83) | 1 (17) | 6 (100) |
| 4 | 3 (60) | 2 (40) | 5 (100) | 3 (60) | 2 (40) | 5 (100) |
| 5 | 1 (17) | 5 (83) | 6 (100) | 0 (0) | 6 (100) | 6 (100) |
| Total | 11 (48) | 12 (52) | 23 (100) | 12 (52) | 11 (48) | 23 (100) |

**Table 6.25.** Experiment 3. Number and percentage of unflown and flown *M. convecta* males that did and did not fly upwind at different ages when females were calling. Flown males were tethered on flight mills for one scotophase whereas unflown ones were not.


**Fig. 6.6.** Experiment 3. Mean duration of flight at different ages in *M. convecta* males that were tethered on flight mills for one scotophase. N=23. Bars are s.e.'s.

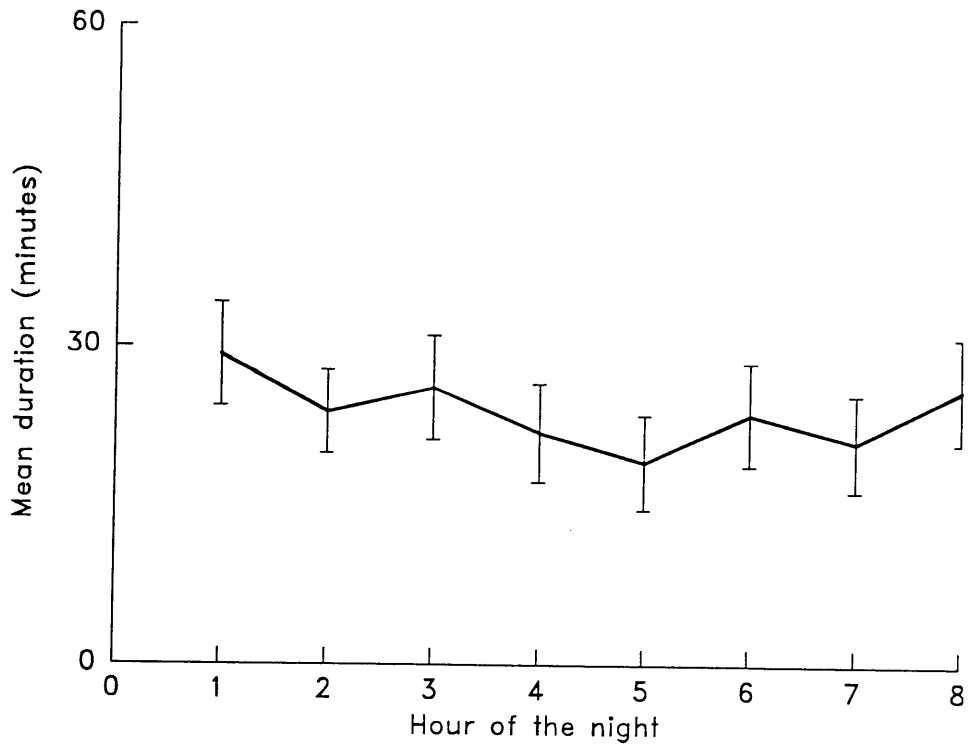


**Fig. 6.7.** Experiment 3. Mean duration of flight at different hours of the scotophase in *M. convecta* males that were tethered on flight mills for one scotophase. N=23. Bars are s.e.'s.

| | Unflown males | | | Flown males | | |
|---|---|---|---|---|---|---|
| Age (S) | No | Yes | Total | No | Yes | Total |
| 2 | 6 (100) | 0 (0) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 3 | 4 (67) | 2 (33) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 4 | 5 (100) | 0 (0) | 5 (100) | 4 (80) | 1 (20) | 5 (100) |
| 5 | 1 (17) | 5 (83) | 6 (100) | 5 (83) | 1 (17) | 6 (100) |
| Total | 16 (70) | 7 (30) | 23 (100) | 21 (91) | 2 (9) | 23 (100) |

**Table 6.26.** Experiment 3. Number and percentage of unflown and flown *M. convecta* males that did and did not approach the pheromone source at different ages when females were calling. Flown males were tethered on flight mills for one scotophase whereas unflown ones were not.

| | Unflown males | | | Flown males | | |
|---|---|---|---|---|---|---|
| Age (S) | No | Yes | Total | No | Yes | Total |
| 2 | 6 (100) | 0 (0) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 3 | 6 (100) | 0 (0) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 4 | 5 (100) | 0 (0) | 5 (100) | 4 (80) | 1 (20) | 5 (100) |
| 5 | 4 (67) | 2 (33) | 6 (100) | 5 (83) | 1 (17) | 6 (100) |
| Total | 21 (91) | 2 (9) | 23 (100) | 21 (91) | 2 (9) | 23 (100) |

**Table 6.27.** Experiment 3. Number and percentage of unflown and flown *M. convecta* males that did and did not land on female cage at different ages when females were calling. Flown males were tethered on flight mills for one scotophase whereas unflown ones were not.

| | Unflown males | | | Flown males | | |
|---|---|---|---|---|---|---|
| Age (S) | No | Yes | Total | No | Yes | Total |
| 2 | 6 (100) | 0 (0) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 3 | 6 (100) | 0 (0) | 6 (100) | 6 (100) | 0 (0) | 6 (100) |
| 4 | 5 (100) | 0 (0) | 5 (100) | 4 (80) | 1 (20) | 5 (100) |
| 5 | 4 (67) | 2 (33) | 6 (100) | 5 (83) | 1 (17) | 6 (100) |
| Total | 21 (91) | 2 (9) | 23 (100) | 21 (91) | 2 (9) | 23 (100) |

**Table 6.28.** Experiment 3. Number and percentage of unflown and flown *M. convecta* males that did and did not extend claspers at different ages when females were calling. Flown males were tethered on flight mills for one scotophase whereas unflown ones were not.

All behaviours were observed in both the unflown and flown males (Figs. 6.8 and 6.9). Chi-square tests using 2 x 4 contingency tables on the response category and moth age were conducted for both groups. In the flown males, age did not significantly influence take off, approach, landing and clasper extrusion behaviours, but did significantly influence upwind flight ($\chi^2_3 = 9.51$, $p < 0.05$). Older moths were more likely to fly upwind. In the unflown males, the results were not significant for all behaviours. These indicate that in general, moth age was not a significant factor influencing the behaviour in both the unflown and flown males. The response categories for each behaviour were then compared between the flown and unflown males. Chi-square tests yielded non-significant results indicating that behaviour of the flown and unflown males in the wind tunnel was not significantly different.

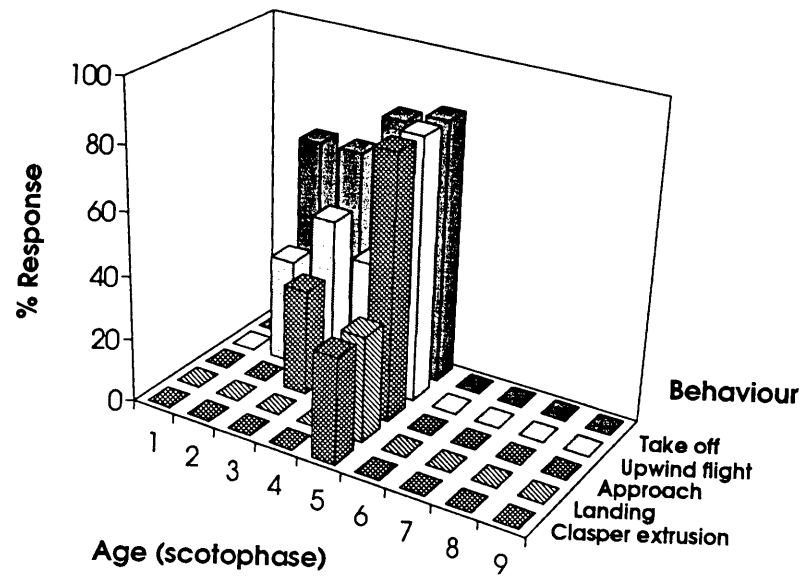


**Fig. 6.8.** Experiment 3. Percentage (%) of *M. convecta* males of different ages exhibiting the different behaviours in the presence of conspecific females that were calling in the wind tunnel at 25 ± 1°C. Males were not flown in flight mills before wind tunnel testing. Observations were done between 1400 and 1800 h AEST on scotophases 2-5. N=23.

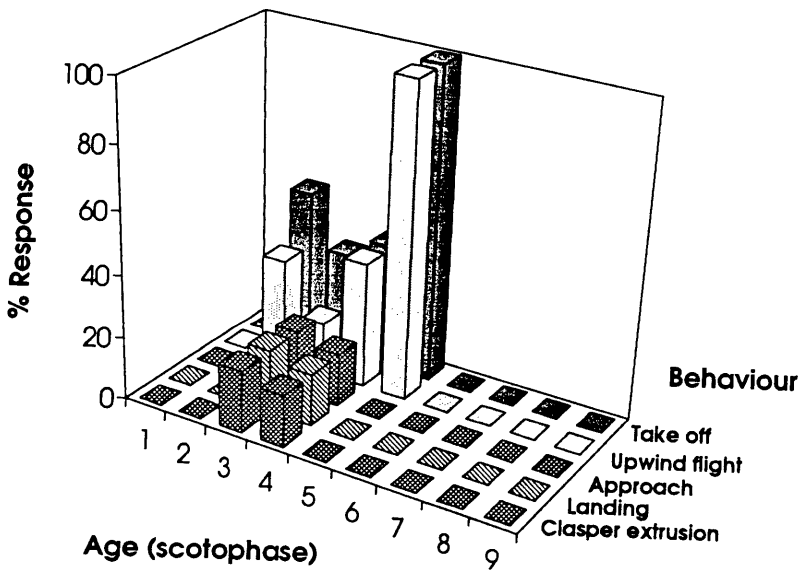


**Fig. 6.9.** Experiment 3. Percentage (%) of *M. convecta* males of different ages exhibiting the different behaviours in the presence of conspecific females that were calling in the wind tunnel at 25 ± 1°C. Males were flown in flight mills before wind tunnel testing. Observations were done between 1400 and 1800 h AEST on scotophases 2-5. N=23

## 6.6. General discussion

Experiments 1 and 2 characterised the behaviour of *M. convecta* males with and without a pheromone source in the wind tunnel. In the absence of calling females, only take off and upwind flight occurred, whereas in the presence of calling females, a behavioural repertoire from initiation of flight to copulatory attempt was observed. Behavioural responses of males flown to calling females were significantly greater than those males flown to non-calling females or empty female cages or a blank disc.

Take off and upwind flight were recorded both in the absence and in the presence of a pheromone source. Upwind flight in the presence of calling females was greater compared with that when females were not calling or when there was no pheromone source, suggesting that this behaviour might be induced by the female pheromone. Without a pheromone source, upwind flight consisted of wide horizontal or vertical sweeps throughout the entire volume of the wind tunnel followed by either resting on the wall, floor or roof of the tunnel or continued random flight in the tunnel. With calling females, upwind flight began with similar vertical or horizontal sweeps mostly in the downwind half of the tunnel, then movement of responding males become slower and usually oriented to the source as they got nearer to it. Burns & Teal (1989) described similar orientation behaviour in *H. micacea* to conspecific females.

In the presence of calling females, before males initiated flight, waving or erection of the antennae, sometimes with the forelegs touching them, was observed. Males did not exhibit these behaviours when there was no pheromone source in the wind tunnel. Antennal movement by males when responding to the sex pheromone has been observed in other species like *P. unipuncta* and *H. micacea* (Turgeon *et al.*, 1983; Burns & Teal, 1989).

The study demonstrated that a kind of male behaviour consistent with a response to pheromone release by females occurred. Males exhibited definitive sexual responses when females were calling. These behaviours, which included approach, landing and clasper extrusion with attempt to copulate, were not observed when there was no pheromone source. *M. convecta* males responding to the pheromone followed a behavioural sequence. Once a male was released in the wind tunnel, it initiated flight within a few seconds and proceeded upwind along the presumed pheromone plume. This was followed by hovering then approach to the female cage. After approach, a male often landed on the cage then fully protruded its claspers. During clasper extrusion, the male exhibited copulatory attempt with lateral curving of its abdomen and thrusting the claspers to the female cage. In most instances, once a male had completed its repertoire, it continued

oriented or mate-searching type of flight between the source and about 100 cm down-wind, then would approach and land on the source again. Repeated approaches up to 3 or 4 times were observed. It is likely that these repeated approaches were a consequence of unsuccessful copulation in the first attempt.

The periodicity of sexual responsiveness by males to the pheromone appeared to parallel maximal calling in the females of some species like *A. kühniella, S. litura, P. carduidactyla* and *H. peltigera* (Traynier, 1970; Ohbayashi *et al.*, 1973; Haynes & Birch, 1984; Dunkelblum & Kehat, 1992). On the other hand, in other species like *H. aurantiaca,* male responsiveness was found to be non-synchronous with female calling. In this species, maximal calling in the laboratory occurred within the first 3 hours of an 8-hour scotophase at 24°C while in the field where temperature ranged from 14-27°C, the highest number of males were caught in the pheromone trap 4-5 hours before sunset (Cardé, 1974).

In *M. convecta*, sexual responsiveness by males to calling females, as measured by approach, landing and clasper extrusion behaviours, seemed to be synchronous with the age when first calling in females occurred as well as with the time of the scotophase when maximal calling in females was observed. First calling in *M. convecta* females began in the 2nd scotophase, peaking in the 4th-5th scotophases (Chapter 4.6), whereas male responsiveness was not observed until the 3rd scotophase. Maximal calling in females occurred in the 2nd half of the scotophase and peaked in the 7th hour (Chapter 4.3.2.2), while male responsiveness was recorded between the 6th and 8th of the scotophase and also peaked in the 7th hour. These responses would be advantageous under natural conditions, resulting in coordinated reproductive activities between male and female moths. Haynes & Birch (1984) suggested that the synchronisation of male response and female calling might be an adaptation to ensure reproductive isolation between sympatric species utilising similar pheromone components.

The synchronisation in male responsiveness to the pheromone and female calling observed in *M. convecta* may be explained by either of the following hypotheses:

(1) *M. convecta* females exhibited some calling before the 5th hour of the scotophase (Fig. 6.4b) but males did not begin to respond until the 5th hour (Fig. 6.4a). It might be that, though females were apparently calling before this time, they were not actually releasing sufficient pheromones of sufficient quality or quantity to elicit male response. Giebultowicz *et al.* (1992) reported that calling in *L. dispar* females was not dependent on the presence of pheromone in their glands. Newly emerged *L. dispar* females exhib-

ited calling behaviour before pheromone could be detected and calling occurred at temperatures that inhibited pheromone titre (33 and 35°C). The relationship between pheromone titre and the expression of calling behaviour in *M. convecta* females was not investigated.

(2) Another possible explanation is that *M. convecta* males had a "window" of responsiveness to the female pheromone. If the females started calling before this window opened, males would not respond to the pheromone. This window of male responsiveness might vary with the passage of time. The onset of male responsiveness occurred earlier in older males than in younger ones (Fig. 6.5), a trend which is similar to the onset calling time in females (Chapter 4.3.2.2). Like calling behaviour in female Lepidoptera, the rhythm of male responsiveness to the female pheromone might be regulated by an endogenous circadian rhythm, as suggested for *G. molesta* (Baker & Cardé, 1979). Studying male response to the synthetic lure, which emits the pheromone continuously, would help elucidate whether the initiation or cessation of male responsiveness is related to the periodicity of female pheromone release, or to a response rhythm in the males. Behavioural responses of *M. convecta* males to synthetic pheromones are discussed in latter sections (Chapter 11, Volume 2 of this thesis).

In some species, previous exposure to the synthetic female pheromone reduced male responsiveness to the pheromone. For example, pre-exposure for 10 minutes of *A. velutinana* males to Z-11-tetradecenyl acetate (Z11:14 Ac) 60 minutes before bioassay inhibited sexual response (Bartell & Lawrence, 1973). In *E. postvittana*, the reduction in male responsiveness to female gland extracts was related to the length of pre-exposure time and the interval between times of pre-exposure and bioassay (Bartell & Roelofs, 1973). The percentage of source contact to the full blend of the synthetic pheromone in a wind tunnel was significantly reduced in *T. ni* males after a 3-day pre-exposure to the same blend (Liu & Haynes, 1993). The interval of pre-exposure to bioassay in these species ranged between 30 and 240 minutes. Kuenen & Baker (1981) suggested that a reduction in the responsiveness to the pheromone by males that have been previously exposed to the pheromone might be due to male habituation to the pheromone.

In Experiment 2, males were re-used in the wind tunnel on subsequent nights. Sexual responses of fresh and re-used *M. convecta* males were not significantly different, suggesting that previous exposure to calling females did not significantly influence their behaviour on subsequent nights. In this experiment, males were only tested once in the wind tunnel (i.e., for 5 minutes) during the night and were returned to the rearing cabinet at lights-on. Thus, habituation to the pheromone seemed unlikely because of the very

short exposure time to the pheromone. Moths were in clean air for the whole duration of the photophase and 475 minutes of the scotophase.

Most flight studies of moths found in literature aim to investigate migratory capacity (e.g., Gatehouse & Woodrow, 1987; Armes & Cooter, 1991; Cooter & Armes, 1993; Colvin & Gatehouse, 1993c). They usually focus on the relationship between reproduction and flight capacity or the "oogenesis-flight syndrome" in female moths (Johnson, 1969; see discussion in Chapter 4.7). This syndrome in male moths is less understood. Whether there is an equivalent male "reproduction-flight syndrome" is not known (Sappington & Showers, 1992). The effect of flight on male responsiveness to the female sex pheromone has not been documented.

In Experiment 3, the behavioural responses of males flown in flight mills to calling females in the wind tunnel was compared with those of unflown males. Two possibilities might be expected after males had been flown. First, flown males would be more ready to mate because they had finished their "migratory flight" and according to the reproduction-flight syndrome, might respond more readily to the female pheromone once exposed. Alternatively, males might be less able to mate because they were exhausted from their flight the previous night, and needed to recuperate before mating. The results obtained in Experiment 3 showed that behavioural responses between flown and unflown *M. convecta* males were not significantly different. This suggests that neither of the two possible effects occurred, or perhaps that the effects tended to cancel each other. Further studies using larger numbers of males, flown, recuperated and tested in the wind tunnel over various periods rather than the one night used in this study, might help clarify this.