

## CHAPTER 3

### GENERAL MATERIALS AND METHODS

This chapter deals with the general materials and methods used in various laboratory and field experiments. Detailed experimental procedures for specific experiments are discussed in the appropriate sections of later chapters.

#### 3.1. Insect rearing

A culture of *M. convecta* was established and maintained in the insectary at the University of New England using stock insects caught from fermentation traps (McDonald, 1990), as well as larvae collected from various host plants (mostly oats and pasture grasses) around Armidale, New South Wales. The culture was re-stocked with fresh insects at least twice a year. The rearing conditions in the insectary were  $25 \pm 1^\circ\text{C}$  and 16:8 light:dark (L:D) period with the dark period or scotophase during 2300-0700 h Australian Eastern Standard Time (AEST).

Culture moths were held in transparent plastic cylindrical cages (21.5 cm diameter and 30 cm tall). These cages were provided with dental wicks soaked in 5% sucrose solution as food and folded paper strips as oviposition sites (Smith, 1986). The dental wicks and paper strips were changed daily. Paper strips with eggs were kept in 500-ml plastic containers until hatching. Newly hatched larvae were transferred individually to 35-ml clear plastic containers (Solo, P101M, Urbana, Illinois, USA) provided with a 1 cm<sup>3</sup> block of artificial diet based on that of Griffith & Smith (1977) using freeze-dried ground barley leaves. The recipe for the artificial diet (DIET.DOC) can be found in the floppy disk in the back cover of this thesis, in the sub-directory A:\CHAP3. Larvae were transferred to new containers with fresh diet at least once a week. Pupae were sexed and kept in similar plastic containers with moist vermiculite to prevent desiccation.

Experimental pupae were kept in reverse-cycle controlled environment cabinets at 20°C and 16:8 L:D regime with the scotophase during 1000-1800 h. These conditions will henceforth be referred to as the normal rearing conditions, but were varied on some occasions for specific experiments. Upon emergence, experimental moths were individually placed for observation in 150-ml clear plastic containers (Polarcup (Australia) Ltd., Bankstown, NSW) with ventilation holes and provided with cotton wicks soaked in 5% sucrose solution as food. Moths were transferred to clean containers and given fresh cotton wicks daily during the photophase.

### 3.2. Studies of female behaviour in the laboratory

Behavioural observations of females were done in reverse-cycle controlled environment cabinets (Model No. E7H, Controlled Environments, Pembina, N.D., USA) under the normal experimental conditions of 20°C and 16:8 L:D period. Moths were observed during the scotophase under continuous light from red photographic safe lamps (Philips No. B22 PF712B), to which most moths are not sensitive (Webster & Cardé, 1982; Dunkelblum *et al.*, 1987). These lights were reflected from the white back of the cabinet at a distance of about 50 cm to provide a diffused red silhouetting light. Close observations were made using a small flashlight modified to provide a beam about 2 mm wide and covered by a double thickness of red photographic filters (Kodak Wratten No. 25). Light intensity measured by a lightmeter (Panlux, Gossen Electronic, Germany), ranged between 1500 and 2000 lux during the photophase, and between 0.5 to 1.5 lux during the scotophase, depending on the position within the cabinet.

Continuous observation of female behaviour was done in the first experiment while in subsequent experiments, moths were observed at 30-min intervals. All observations were recorded on a portable computer, the clock of which was accessed to provide timing to the nearest minute of each record, and were later transcribed. A small program (DATA.BAS) was written in Microsoft QuickBASIC for this purpose. A copy of this program is in the sub-directory A:\CHAP3 on the floppy disk attached to the rear cover of this volume of the thesis.

#### 3.2.1. Statistical analyses

Raw data for all the experiments described in this thesis can be found on the accompanying floppy disk, in files which are referred to in the relevant sections of each chapter.

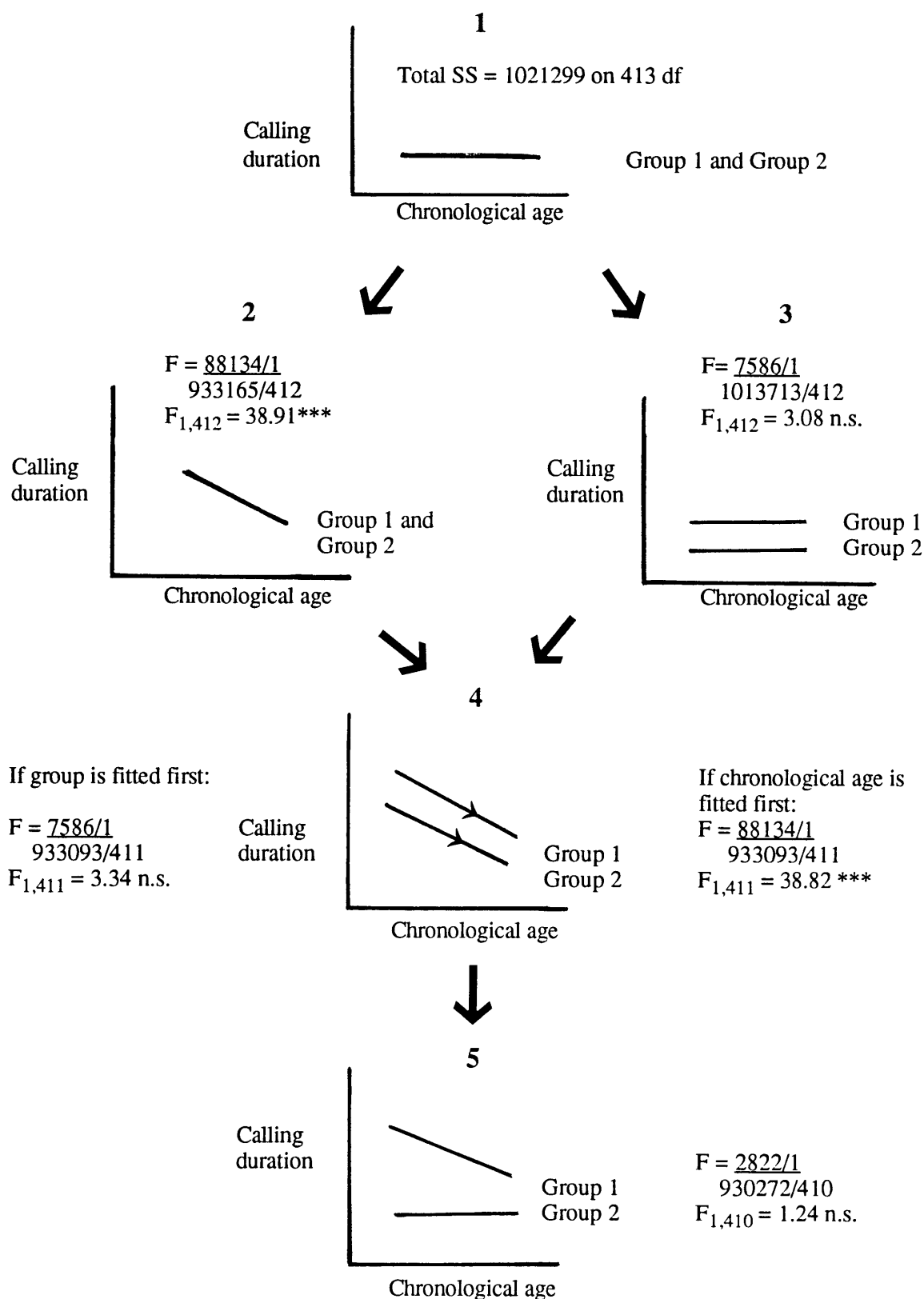
A computer program in QuickBASIC called STRAT3.BAS (Del Socorro & Gregg, 1991), which can be found on the floppy disk in the sub-directory A:\CHAP3, was used to facilitate the calculation of parameters for the different behaviours from the transcribed data. These included duration of behaviour during each hour of the scotophase, duration at different ages, number of bouts and average duration per bout. Means and standard errors of these parameters were obtained using the MINITAB statistical package (Ryan *et al.*, 1992).

For calling behaviour, several statistical analyses were conducted. These included regression analysis, one-way analysis of variance and the generalised interactive linear model (GLIM) analysis. GLIM analyses (Payne, 1985) were used routinely for

unbalanced and/or non-orthogonal data. Data from some experiments were unbalanced because the number of moths varied between different ages, and non-orthogonal because some factor combinations were unavailable due to moth mortality. For such data sets, conventional analyses of variance and covariance (e.g., Devore & Peck, 1993) were not possible. In the GLIM approach, the F-tests of the mean sum of squares due to main factors and interactions (which are the criteria of statistical significance in conventional analyses) are replaced by F-tests of the reductions in deviance obtained by fitting progressively more complex models. The generalised linear modelling (GLM) routine in the MINITAB package (Ryan *et al.*, 1992) was used for relatively simple analysis with small data sets and the GLIM statistical package (Payne, 1985) was used for more complex models.

An example to illustrate the hierarchy of models used to describe a GLIM analysis equivalent to an analysis of covariance is shown in Fig. 3.1. The data for this analysis are from Chapter 4.5.3 (*Duration of calling at different ages*) and can be found in the files EX2CALL.OLD and EX2CALL.YNG in the sub-directory A:\CHAP4 on the floppy disk. In this example, group (i.e., Group 1 and Group 2) was used as the factor and moth age as the covariate. The simplest model (Model 1) assumes that Group 1 is the same as Group 2 and that there are no effects of age. From this model, there are two possible pathways, Models 2 and 3, with the first one fitting the covariate (age) first and the second one fitting the factor (group) first. With unbalanced and non-orthogonal data, the order of fitting the factor or the covariate may be important in determining significance of these terms. Following these two pathways are two more which lead to the next most complex model, Model 4 which shows an effect of both the factor and the covariate, but no interaction between them (i.e., the lines are parallel). Finally, the most complex model is Model 5 which shows non-parallelism or interaction between the factor and the covariate.

For data where GLIM analyses were used, F values are not quoted because they varied depending on which pathway was followed, i.e., which term (factor or covariate) was fitted first in the model. Only the levels of significance (p values) are given. Unless otherwise stated, these are the same whichever pathway of analysis was taken.

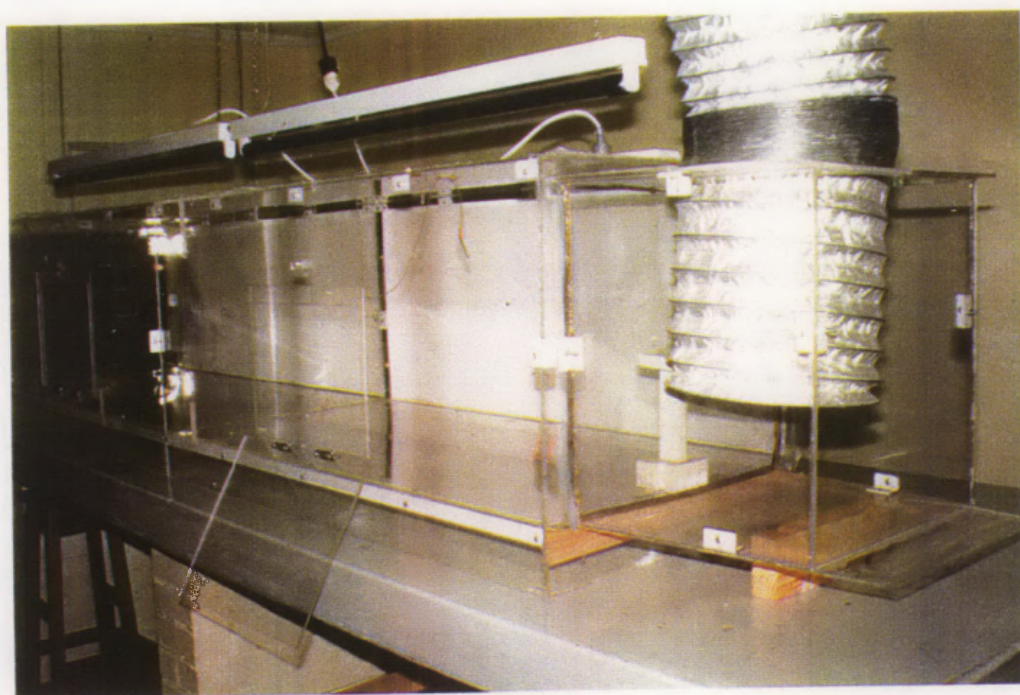


**Fig. 3.1.** An example of a hierarchy of models in the Generalised Linear Interactive Model (GLIM) analysis. The pathways of fitting the models are described on the text. At each step, an F ratio is calculated from the reduction in deviance due to fitting the new model, and the residual deviance, divided by their respective degrees of freedom.

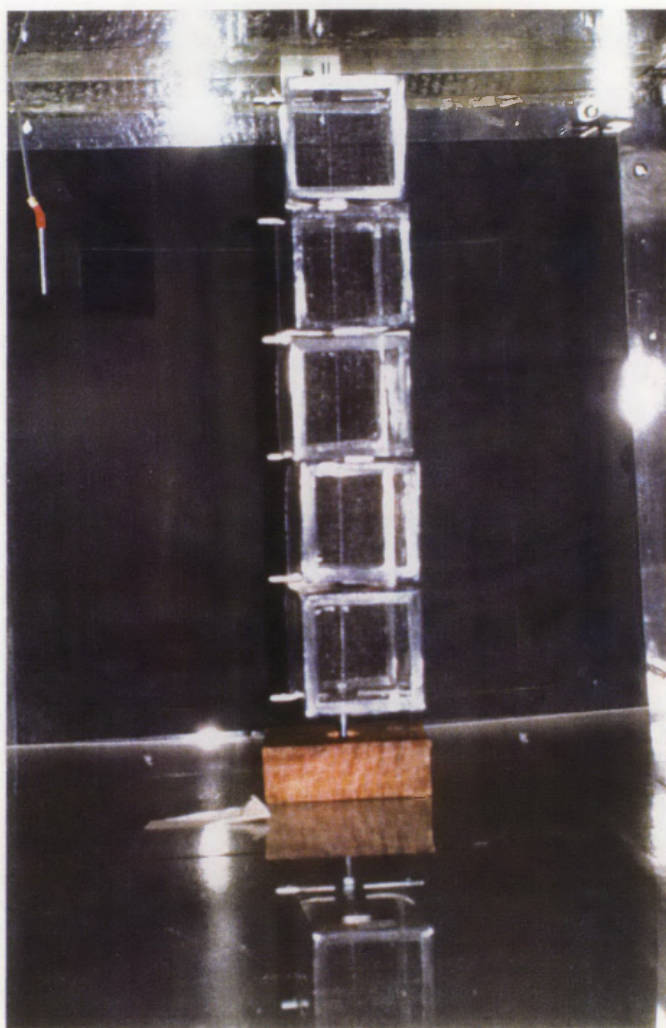
### 3.3. Studies of male behaviour in the wind tunnel

Male pupae were kept in separate environmental cabinets at  $25 \pm 1^\circ\text{C}$  and 16:8 LD period. Upon emergence, individual moths were transferred to 70-ml clear plastic containers (Polarcup (Australia) Ltd., Bankstown, NSW) provided with cotton wicks soaked in 5% sucrose. Their behaviour in the laboratory was studied in a wind tunnel housed in a room which was separate from where the experimental moths were kept. The design of the wind tunnel was based on those described by other workers (e.g., Miller & Roelofs, 1978; Burns & Teal, 1989). It was made of Plexiglas® and measured 260 cm long, 60 cm wide and 60 cm high (Plate 3.1). The upwind and downwind ends were closed with two layers of fine wire mesh. Air in the wind tunnel was pulled by a fan through a 30 cm-diameter exhaust tube leading to the outside of the building. The wind speed measured in the centre of the tunnel by a thermo-anemometer (Type 8500, Alnor Instrument Co., Chicago, USA), was about 30-40 cm/s. The direction of the pheromone plume was predetermined by means of smoke generated from titanium tetrachloride ( $\text{TiCl}_4$ ) placed on a cotton wick in the upwind end (Cardé & Hagaman, 1979; Vetter & Baker, 1983). The movement of the white smoke from  $\text{TiCl}_4$  from upwind to downwind was generally along the centre of the wind tunnel with some turbulence along the sides. The plume in the wind tunnel resembled the filamentous plume structure described by Murlis *et al.* (1992). The room temperature was maintained at  $24\text{--}25^\circ\text{C}$  during experimental work. Male moths used in the experiments were transferred to the wind tunnel room at least one hour before the observation time to acclimatise them to room conditions. After each scotophase, moths were transferred to clean containers and the wind tunnel cleaned with 4% bleach and absolute ethanol.

Males were flown to either live females or synthetic pheromone blends in the wind tunnel. Pheromone baits were placed on the upwind end at least 15-20 cm from the wind tunnel entrance or wall, while individual males were released 15 cm from the downwind end. Females used as baits were held individually in rectangular plastic cages (15 x 9 x 9 cm) with the opposite ends made of mesh material, arranged vertically on a steel rack (Plate 3.2). The desired dosage of the synthetic pheromone blend was pipetted on a 13 mm-diameter glass fibre filter disc (Type A/E, Gelman Sciences, USA) clipped by a 3-cm long copper wire. The pheromone disc rested on a 30-cm high wooden stand painted white. The discs were changed at hourly intervals. Pheromone substances were supplied by Dr. S. Voerman of the Research Institute for Plant Protection, Wageningen, The Netherlands and Mr. K. Ogura of Shin-Etsu Chemical Co. Ltd., Tokyo, Japan.



**Plate 3.1.** Wind tunnel used in experiments to observe behaviour of *M. convecta* males with conspecific females and synthetic pheromones (Chapters 6 and 11).



**Plate 3.2.** Cages of *M. convecta* females used as baits in the wind tunnel arranged vertically on a rack (Chapter 6).

As with females, behavioural observations of males were done under continuous red photographic safe lights (Encapsulite, Type R10) suspended above the wind tunnel. A sheet of plastic packaging material was placed between the fluorescent tubes and the wind tunnel to diffuse the light. Light intensity during the scotophase in the wind tunnel was 1-1.5 lux. Observations were made during the 2nd half of the scotophase (1400-1800 h) when most female calling occurred. All behavioural observations were recorded on a portable computer using the program DATA.BAS and later transcribed.

### **3.3.1. Statistical analyses**

Data on the frequency of observations for each type of behaviour at different ages were analysed using the chi-square method in the MINITAB package (Ryan *et al.*, 1992).

### **3.4. Field experiments**

A field experiment was conducted to observe the behaviour of *M. convecta* moths under natural conditions at Gatton, Queensland (27° 32' S, 152° 18' E). A walk-in cage about 1.8 m high covering an area of about 9 m<sup>2</sup> was set up on a paddock of native pasture grasses, with the rim buried 10-15 cm in the ground (Plate 3.3). Moth behaviours were observed by means of night vision goggles (Lingren *et al.*, 1978) fitted with electron photo-multiplying tubes (Litton Electron Devices, Tempe, Arizona, USA). Close observations were done using a flashlight covered by a 49-mm infrared filter disc (R72, Hoya, Japan). Behavioural observations were recorded on a tape recorder and later transcribed.

### **3.5. Analyses of female sex pheromone and field studies with pheromone traps**

Details of the methods for analysing pheromones and conducting field trapping studies are given in Volume 2 of this thesis.



**Plate 3.3.** Field cage used in experiment to observe behaviour of *M. convecta* males and females in the field (Chapter 7).

## CHAPTER 4

### GENERAL NOCTURNAL BEHAVIOUR OF *M. CONVECTA* FEMALES

#### 4.1. Introduction

A basic requisite in studying the pheromone biology of an insect is an understanding of its behaviour, particularly its reproductive behaviour. The reproductive behaviour, particularly calling and mating, of many moth species is well documented (e.g., Turgeon & McNeil, 1982; West *et al.*, 1984; Howlader & Gerber, 1986a; Kou & Chow, 1987; Itagaki & Conner, 1988; see Chapter 2). Calling behaviour, the term used to describe the characteristic posture of female moths associated with the release of sex pheromone, has been reported for the North American armyworm species, the true armyworm, *P. unipuncta* and the bertha armyworm, *M. configurata* (Turgeon & McNeil, 1982; Howlader & Gerber, 1986a) as well as in a number of other noctuids (e.g., Swier *et al.*, 1976; West *et al.*, 1984; Snir *et al.*, 1986; Dunkelblum *et al.*, 1987; Kou & Chow, 1987; Noldus & Potting, 1990; see Chapter 2).

Similar studies on Australian armyworm species have not previously been undertaken. This chapter describes laboratory experiments conducted to provide some basic information on the pattern of calling in relation to other types of nocturnal behaviour, both reproductive and non-reproductive. The experiments also provided information about potential confounding factors and their implications for experimental design and statistical techniques.

#### 4.2. Materials and methods

##### 4.2.1 Types of behaviour

The behaviour of each moth at any given time was classified as one of three non-reproductive and three reproductive types.

Non-reproductive behaviours included activity, feeding and resting: **Active moths** either walked or flew around in the containers. **Feeding moths** extended their proboscis on the dental wick. **Resting moths** had their wings in a roof-like position and their ovipositors retracted. Reproductive behaviours included calling, extrusion and oviposition. **Calling moths** fully protruded their ovipositors, and the wings were slightly raised. **Extruding moths** partially protruded their ovipositors, usually with side to side movement. **Ovipositing moths** were producing eggs at intervals of no more than a few minutes.

#### **4.2.2 Techniques for recording behaviour**

The general methods for recording of observations were described in Chapters 3.1 and 3.2. In Experiment 1, all moths were observed continuously and changes from one behaviour to another were recorded on the portable computer as soon as they were noticed. A computer program, written in QuickBASIC called STRAT3.BAS (on A:\CHAP3), was used to transform these sequential observations into hourly total durations and number of bouts of each behaviour. This program also simulated the effects of non-continuous observations at varying time intervals by using the continuous records to note behaviour at fixed times. For this purpose it was assumed that if the same behaviour was exhibited at two consecutive times no change had occurred in the interim. Any change in behaviour was assumed to have occurred midway between the times. The aim of these simulations was to determine the extent to which non-continuous observation might bias data on the frequency and duration of each type of behaviour. This information was required because, for subsequent experiments, non-continuous observation was envisaged. Experiments involving more than about 10-12 moths cannot be assessed by continuous observation because a single observer cannot effectively monitor more than this number at once. Moreover, if the experimental design calls for different environmental conditions such as temperature or photoperiod (for example, the experiments in Chapter 5), separate controlled environment cabinets are required for each regime. The observer has to move between cabinets, and this precludes continuous observation. For these reasons, non-continuous observation is frequently used in experiments on reproductive behaviour, with observation intervals usually in the range of 15-30 minutes (e.g., Itagaki & Conner, 1988; Ramaswamy *et al.*, 1988; Babilis & Mazomenos, 1992; Dunkelblum & Kehat, 1992; Simmons & Marti, 1992).

### **4.3. Experiment 1 - General nocturnal behaviour patterns**

#### **4.3.1. Material and methods**

##### **4.3.1.1. Experimental design**

Thirteen females, held in individual containers placed on a rack in a controlled environment cabinet, were observed throughout the 8-hour scotophase for a maximum of 16 scotophases. Moths which died during the experiment were not replaced, so the numbers of moths observed declined during the course of the experiment. The numbers observed for each moth age are shown in Table 4.1.

Moth Age	N	Moth Age	N
1	10	9	10
2	12	10	10
3	13	11	8
4	13	12	8
5	12	13	5
6	11	14	4
7	11	15	4
8	10	16	3

**Table 4.1.** Experiment 1. Numbers of moths observed for each moth age in the study on basic nocturnal behaviour of *M. convecta*. Age is the number of scotophases experienced by the moth after emergence (Noldus & Potting, 1990). At ages 1 and 2, not all of the 13 available moths were observed. From age 4 onwards, the number of moths observed declined because of mortality.

#### 4.3.1.2. Statistical analyses

For each type of behaviour, the mean number of bouts and duration per bout, duration at different ages and duration during each hour of the scotophase, were calculated using the STRAT3 program. For calling, the age at first calling and mean onset time of calling at different ages were also determined.

No statistical analyses were undertaken for behaviours other than calling. Means and mean standard errors for each behaviour, for each moth age and for each hour of the 8-hour scotophase (with all moth ages pooled) were calculated using the statistical package MINITAB (Ryan *et al.*, 1992). The number of bouts, their total duration and average duration per bout were calculated. Analysis was limited to visual comparisons of these data (Figs. 4.1 to 4.10).

For calling, several statistical analyses were conducted. The statistical distribution of the age at first calling was assessed to determine whether it followed a normal distribution pattern, or a skewed distribution, or neither. For this purpose, the statistical package Maximum Likelihood Program (MLP) (Ross, 1987) was used. Other parameters were analysed using regressions, one-way analysis of variance or the generalised linear interactive modelling (GLIM) analysis (Payne, 1985; Ryan *et al.*, 1992) as described in Chapter 3.2.1.

Raw data for each type of behaviour are provided in the sub-directory A:\CHAP4 of the floppy disk.

### 4.3.2. Results and discussion

#### 4.3.2.1. Behavioural patterns

##### Activity

The mean number of bouts was lowest but the mean duration per bout was longest in 1-day-old moths (Fig. 4.1a). The number of bouts was highest during the 4th scotophase and the mean duration per bout gradually declined from the 1st scotophase and remained more or less constant from the 7th scotophase onwards. Females were most active during the 3rd and 4th scotophases then activity decreased with increasing age (Fig. 4.1b). During the scotophase, females were active in the 1st hour soon after lights-off. Activity then declined in the 2nd hour, after which it gradually increased to peak again in the 6th hour of the scotophase (Fig. 4.2).

##### Feeding

Bouts were few and the average duration per bout was variable but longest during the 1st scotophase and in very old moths (Fig. 4.3a). There were no clear patterns of feeding duration at different ages (Fig. 4.3b) and throughout the scotophase the mean duration of feeding was more or less constant (Fig. 4.4).

##### Resting

The least number of bouts and the longest duration per bout was during the 1st scotophase (Fig. 4.5a). Younger moths spent more time resting than older ones (Fig. 4.5b). During the scotophase, the mean duration of resting was longer earlier in the night, gradually declining until the 7th hour, then rising again during the last hour of the scotophase (Fig. 4.6).

##### Extrusion

The mean number of events and mean duration per event generally increased with age although both were very variable (Fig. 4.7a). It started in some moths in the 4th scotophase and increased as the females became older. The longest duration was between the 11th and 16th scotophases (Fig. 4.7b). Extrusion occurred throughout the scotophase with evidence of a single broad peak between the 3rd and the 7th hours (Fig. 4.8).

Extrusion, which is characterised by the partial protrusion of the ovipositor, was considered to be a pre-oviposition behaviour rather than "weak calling" as distinguished from "strong calling" by other workers (e.g., Barrer & Hill, 1977; Swier *et al.*, 1977; Nordlund & Brady, 1974; see Chapter 2.2). *M. convecta* females started exhibiting extrusion only after they had been calling for at least two days. Moreover, the two behaviours were qualitatively different. When genuinely calling, *M. convecta* females did not move

their fully protruded ovipositors from side to side. It appeared that during extrusion females were preparing to lay eggs by searching for suitable oviposition sites. This was indicated by the sideward movement of their ovipositors which were usually curved forward, on the surface of the containers. Guppy (1961) described similar pre-oviposition behaviour in *P. unipuncta* on oat stubble. Palaniswamy & Seabrook (1978) observed the sideways movement of the abdominal tips in ovipositing but not in calling *C. fumiferana* females.

Another type of behaviour exhibited by *M. convecta* may have represented "weak calling", as described for other species. At the start of the first calling bout, females partially protruded and retracted their ovipositors for a few seconds before fully extruding them. This behaviour was clearly distinguishable from both extrusion and genuine calling, but it was not recorded as a separate behaviour type because it only lasted for a very short period.

#### Oviposition

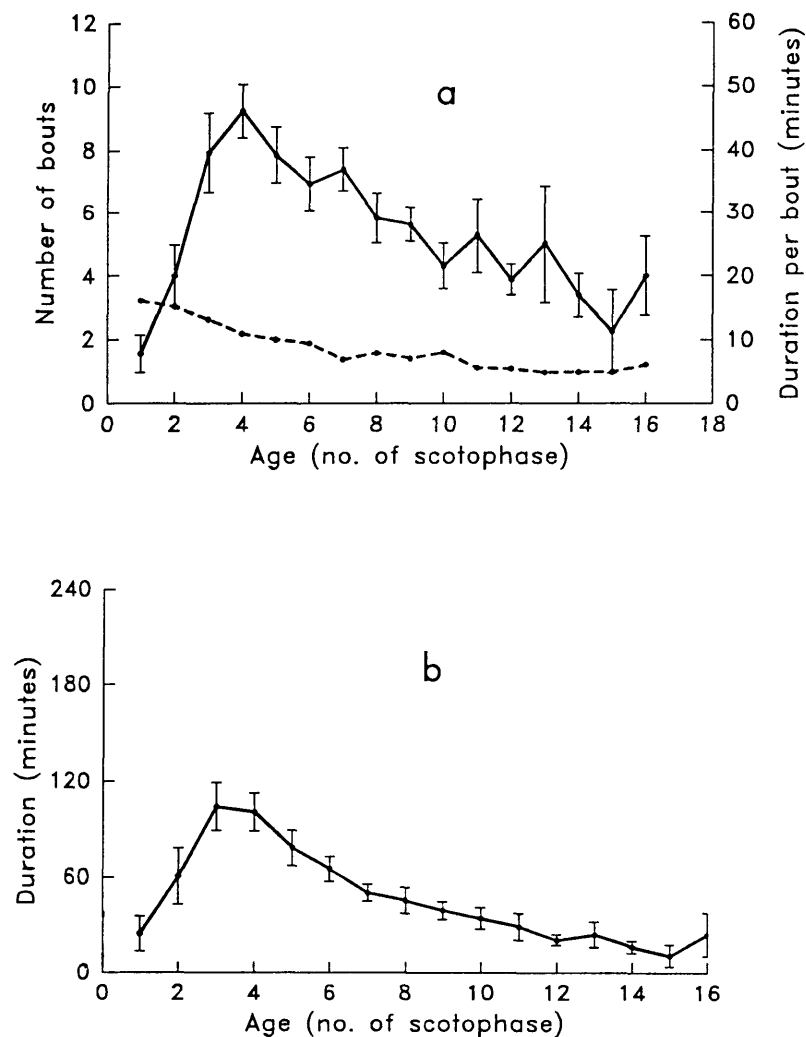
Moths began ovipositing from the 6th scotophase onwards. The numbers of eggs laid were much lower than those which were laid by mated moths in the stock cultures, and none of them ever hatched. As with extrusion behaviour, the mean number of oviposition bouts and the mean duration per bout increased with age but were variable during the 13th-16th scotophases (Fig. 4.9a). The mean duration generally increased as the moths got older until the 12th scotophase (Fig. 4.9b). Like extrusion, oviposition occurred throughout the scotophase with a single broad peak in the mean duration during the 3rd to 7th hours of the scotophase (Fig. 4.10).

#### **4.3.2.2. Calling behaviour**

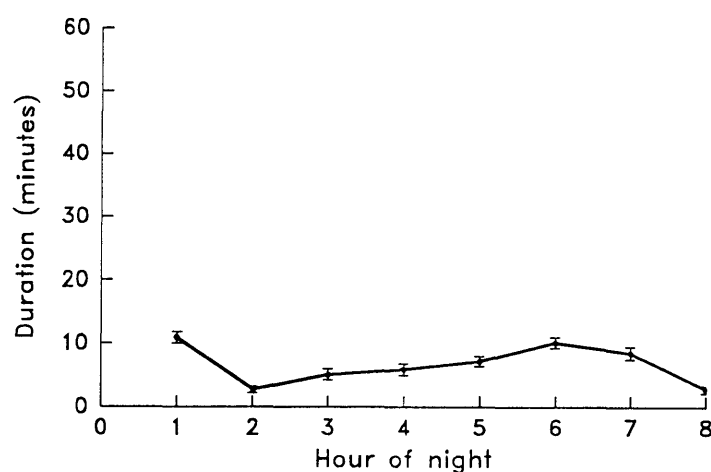
*M. convecta* exhibited a calling posture similar to that in other noctuids, characterised by the full protrusion of the ovipositor with the wings slightly raised above the abdomen (Plate 4.1).

#### *Age at first calling*

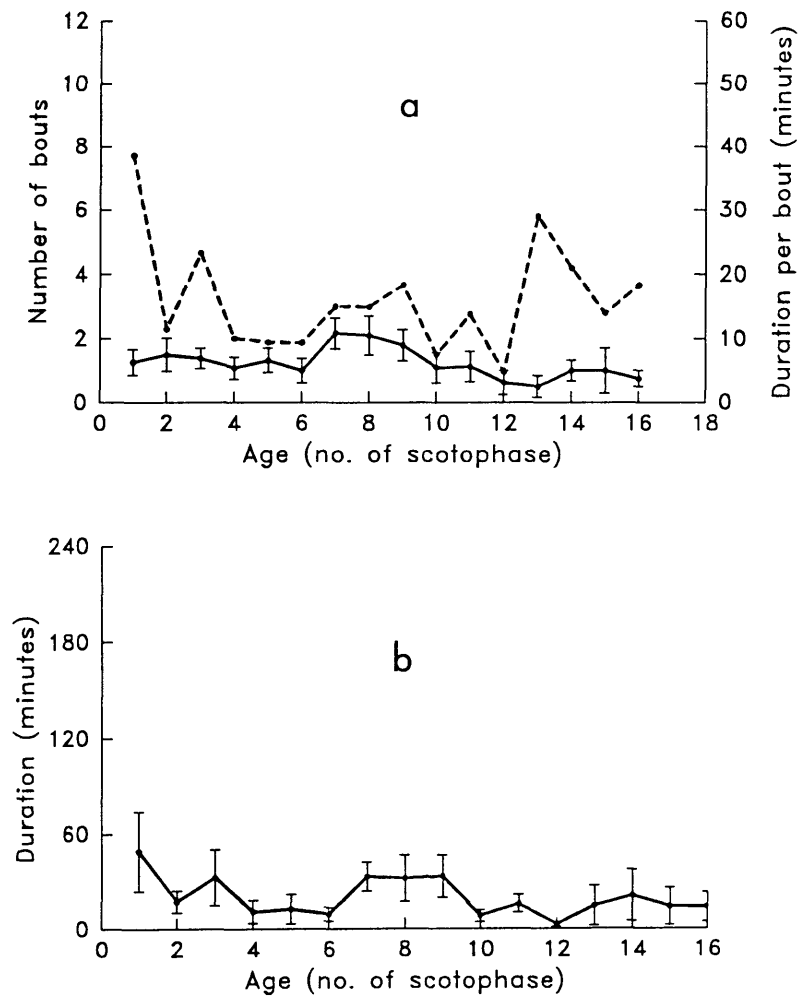
The number of moths calling at different ages and the number calling for the first time at each age during the first 10 scotophases of the experiment are shown in Table 4.2.



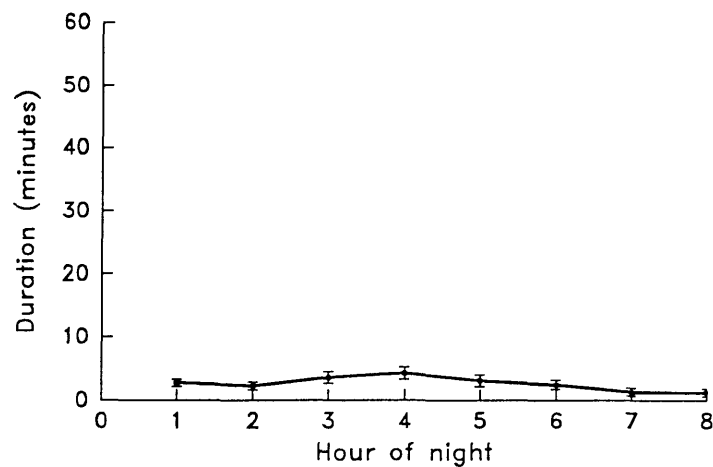
**Fig. 4.1.** Experiment 1. Mean number of activity bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females at different ages (a) and mean duration of activity per scotophase at different ages (b). Values in Fig. 4.1b are the product of mean number of bouts and mean duration per bout shown in Fig. 4.1a. Temperature - 20°C, photoperiod - 16:8 light:dark (LD), N=13. Bars are s.e.'s.



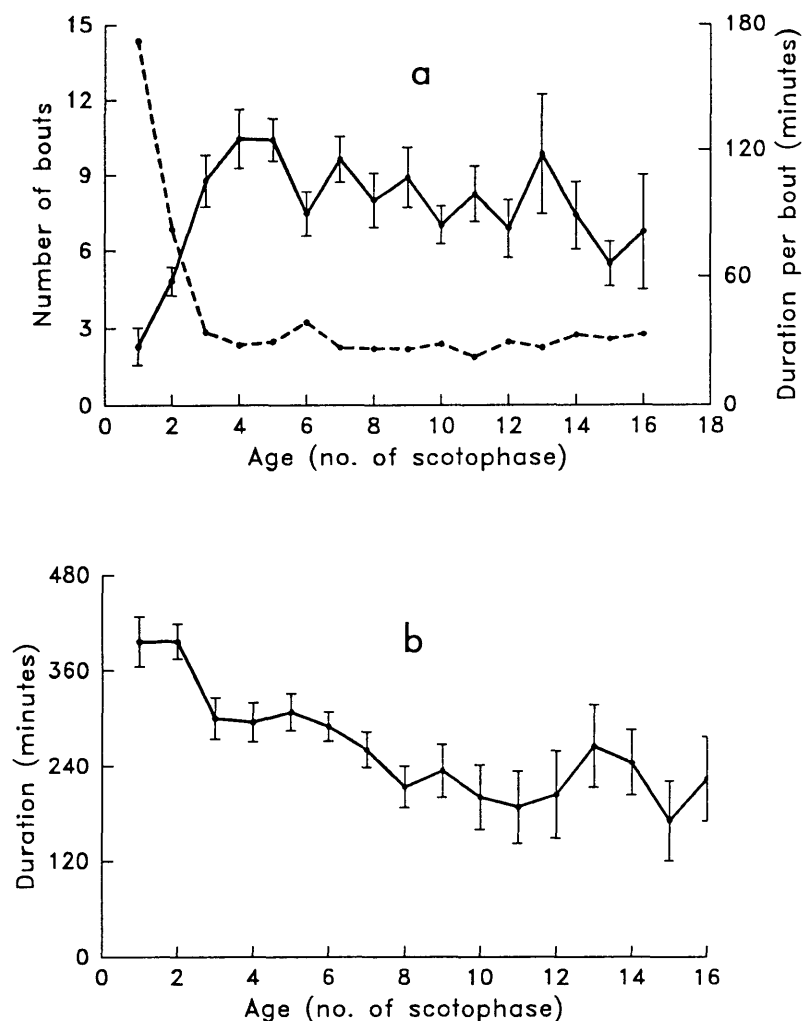
**Fig. 4.2.** Experiment 1. Mean duration of activity in *M. convecta* females during each hour of the scotophase. Data from all females of all ages combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



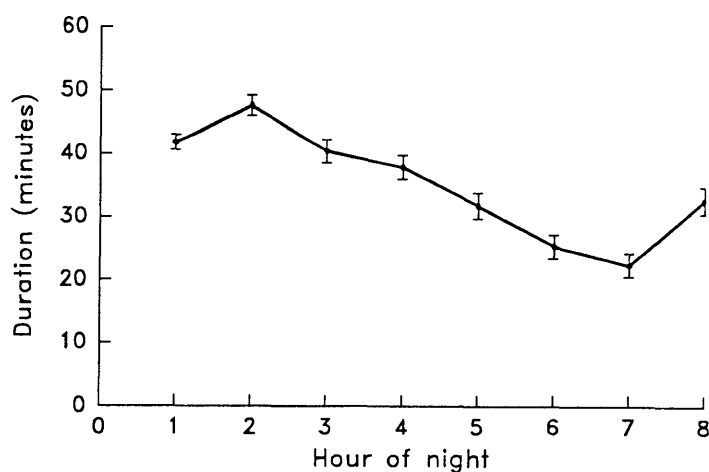
**Fig. 4.3.** Experiment 1. Mean number of feeding bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females at different ages (a) and mean duration of feeding per scotophase at different ages (b). Values in Fig. 4.3b are the product of mean number of bouts and mean duration per bout shown in Fig. 4.3a. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



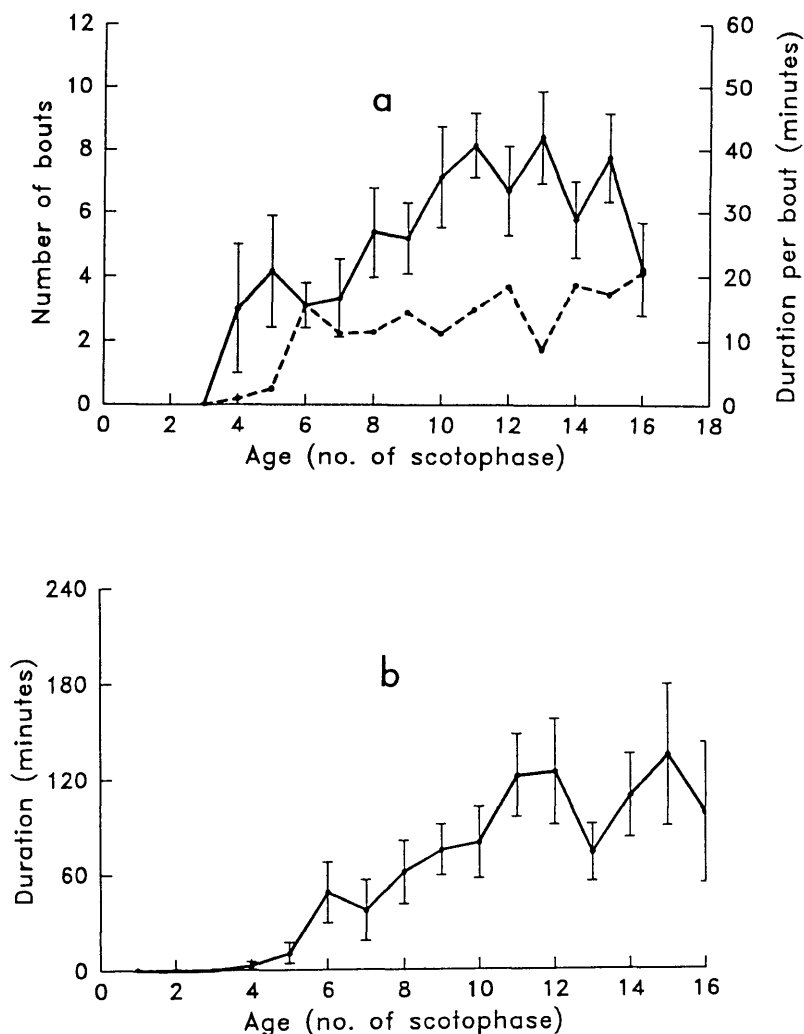
**Fig. 4.4.** Experiment 1. Mean duration of feeding in *M. convecta* females during each hour of the scotophase. Data from all females of all ages combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



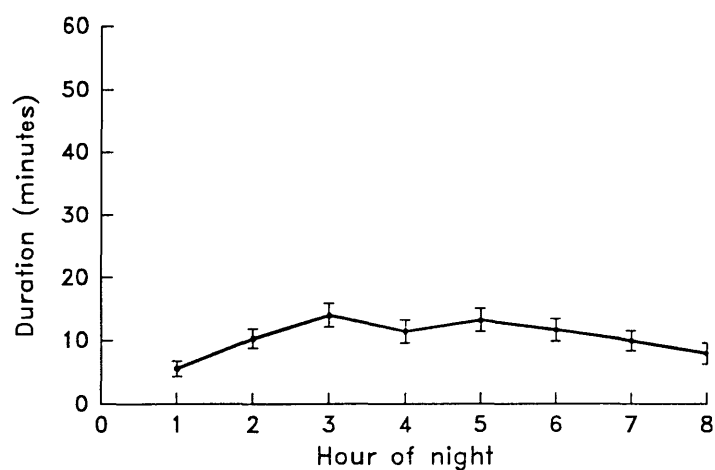
**Fig. 4.5.** Experiment 1. Mean number of resting bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females at different ages (a) and mean duration of resting per scotophase at different ages (b). Values in Fig. 4.5b are the product of mean number of bouts and mean duration per bout shown in Fig. 4.5a. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



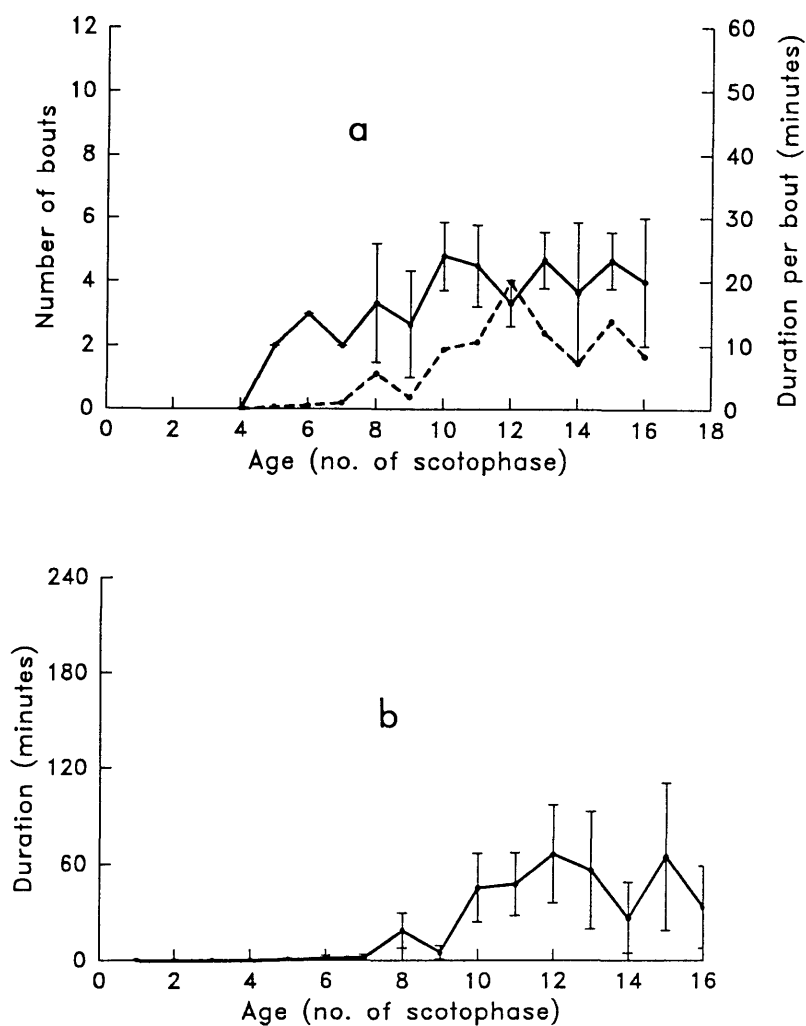
**Fig. 4.6.** Experiment 1. Mean duration of resting in *M. convecta* females during each hour of the scotophase. Data from all females of all ages combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



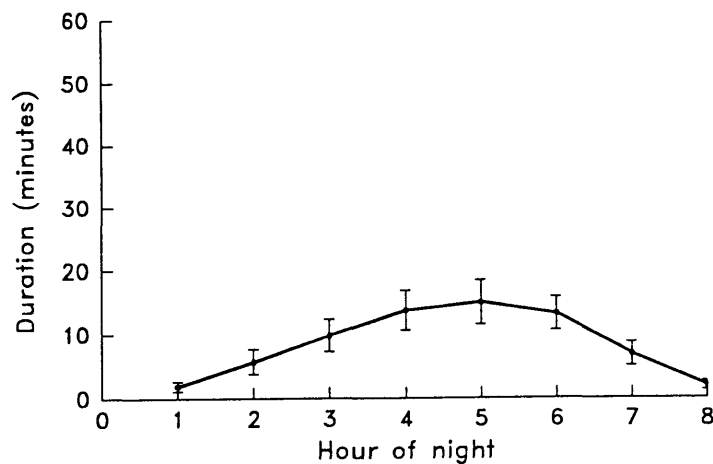
**Fig. 4.7.** Experiment 1. Mean number of extrusion bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females at different ages (a) and mean duration of extrusion per scotophase at different ages (b). Values in Fig. 4.7b are the product of mean number of bouts and mean duration per bout shown in Fig. 4.7a. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



**Fig. 4.8.** Experiment 1. Mean duration of extrusion in *M. convecta* females during each hour of the scotophase. Data from all females of all ages combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



**Fig. 4.9.** Experiment 1. Mean number of oviposition bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females at different ages (a) and mean duration of oviposition per scotophase at different ages (b). Values in Fig. 4.9b are the product of mean number of bouts and mean duration per bout shown in Fig. 4.9a. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



**Fig. 4.10.** Experiment 1. Mean duration of oviposition in *M. convecta* females during each hour of the scotophase. Data from all females of all ages combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.

Age	Number of moths		
	a	b	c
2	12	2	2
3	13	6	4
4	13	9	3
5	12	9	1
6	11	8	0
7	11	9	1
8	10	9	1
9	10	8	0
10	10	9	1

**Table 4.2.** Experiment 1. Number of *M. convecta* females observed (a), number calling (b), and number calling for the first time (c) at different ages.

Although most moths called for the first time at 3 or 4 days of age, there was considerable variation. One moth took 10 days to begin calling. Although the sample size was small, this result suggested that the distribution of age at first calling might vary significantly from the standard normal distribution (Devore & Peck, 1993). To statistically test this hypothesis, a larger sample size was required. Accordingly, data from this experiment and the one described in Chapter 4.5 were combined. The analysis is presented in Chapter 4.6, along with a discussion of the possible sources of variation in age at first calling, and its implications for the ways in which data from experiments such as these are analysed.

*Analysis by calling age or by chronological age?*

The wide variation in age at which *M. convecta* females called for the first time is likely to cause problems in statistical analysis, because any sample of moths of a given age is likely to contain some which have begun calling and others which have not. To overcome this problem, Turgeon & McNeil (1982), working with *P. unipuncta*, which also shows considerable variation in age at first calling, proposed using "calling age" (the number of scotophases after the time of first calling), rather than chronological age.

While using calling age might help overcome some of the problems caused by such heterogeneity, it might not always be appropriate. Exocrine compounds, including pheromones, may be biosynthesised at specific times after eclosion, and their qualitative and quantitative compositions may be critically correlated with chronological age (Blum, 1985). The chronological age of moths having the same calling age may vary widely. For example, in *M. convecta*, the chronological age in females of calling age 1 varied from 2 to 11 scotophases. It is very likely that the general physiological state of a 2-day-old moth with calling age 1 would be different from that of an 11-day-old moth of the same calling age, in ways which might affect calling patterns. Factors such as the level of energy reserves and endocrine balances might vary with chronological age (Sohal, 1985).



Plate 4.1. Calling posture of *M. convecta* female.

Thus, using calling age might provide a homogenous basis for comparison of calling behaviour but the general physiological condition of individual moths at different chronological ages might be a confounding factor. In this thesis, both chronological and calling ages of *M. convecta* females were used in analysing calling parameters, and the resulting patterns were compared. In addition, the relationships between age at first calling and other calling parameters such as duration and onset calling time on subsequent scotophases were specifically tested.

#### *Duration of calling at different ages*

The duration of calling appeared to increase with moth age from the 2nd to the 10th scotophases after which it declined until the 13th scotophase following emergence (Fig. 4.11). Before the statistical significance of these trends could be assessed, however, it was necessary to take into account some potential confounding factors.

Regression analyses showed that the average duration of calling per night was significantly correlated with the age at which females called for the first time. Two data sets were used. The first one contained calling durations for all moths on all nights, including those prior to the night of first calling, which were naturally zero. This yielded the regression equation:

$$D = 139 - 15.7c \quad (F_{1,149} = 39.57, p < 0.001, R^2 = 0.21)$$

where D = calling duration

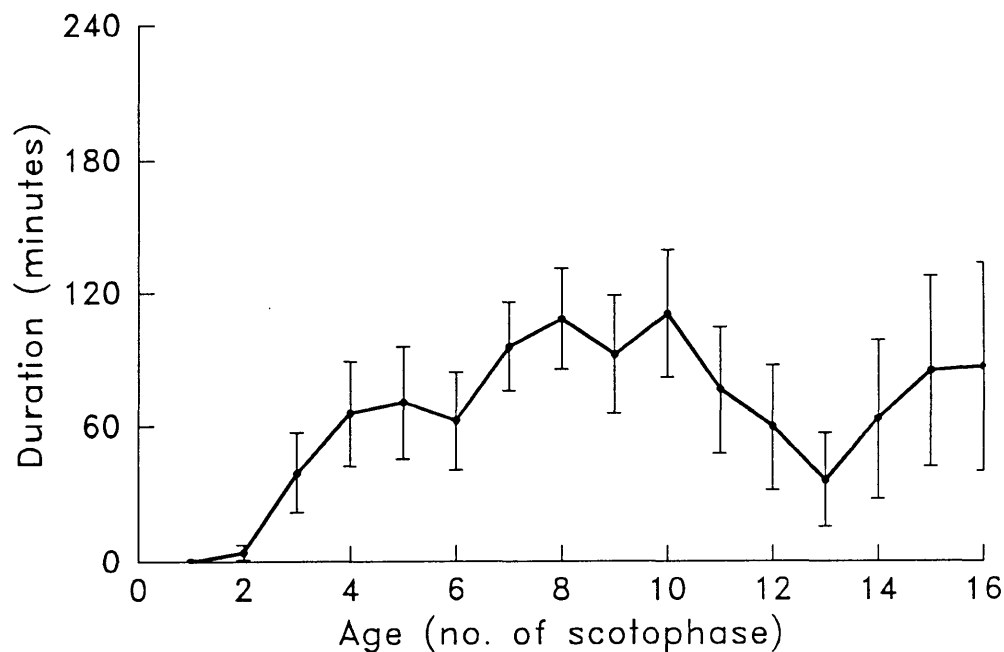
c = age at first calling

This regression was highly significant, indicating that moths which began to call later in their lives spent less time calling than those which began calling earlier. This result might be expected since such moths had fewer scotophases available in which to express their calling behaviour.

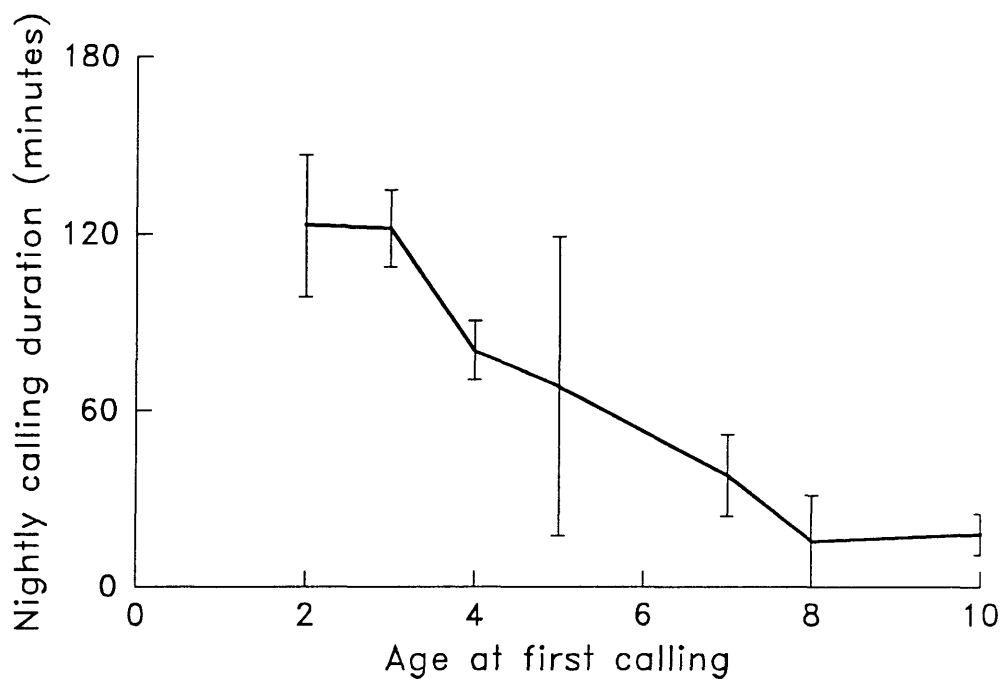
It was also of interest to determine whether moths which began calling later in their lives showed less calling per night after they began calling than did those which began calling earlier. For this analysis, all zero values prior to the night of first calling were omitted. This second regression analysis also gave highly significant result. The regression equation was:

$$D = 160 - 17.2c \quad (F_{1,108} = 18.23, p < 0.001, R^2 = 0.14).$$

This analysis showed that the age at which females started to call was correlated with the amount of calling they did on subsequent nights (Fig. 4.12).



**Fig. 4.11.** Experiment 1. Mean duration of calling per scotophase in *M. convecta* females at different chronological ages. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



**Fig. 4.12.** Experiment 1. Mean duration of calling per scotophase in *M. convecta* females of different ages when first calling occurred. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s. Nights prior to first calling have been excluded.

*Relationships between age at first calling, chronological and calling ages, and calling duration*

Further analysis of the same two data sets for which the above regressions are given was done to explore the relationships between the duration of calling by any one moth on any one night, the age of the moth on that night (expressed as either chronological age or calling age) and the age at which the moth called for the first time. The generalised linear model (GLM) routine in the MINITAB package (Ryan *et al.*, 1992) described in Chapter 3.2.1 was used. For these analyses, the age at first calling was treated as a factor and the chronological or calling age of the moth was treated as a covariate. The GLM procedure in MINITAB was used to fit models of the form:

$$\text{GLM } D = C \ A \ C*A;$$

$$\text{COVARIATE } A.$$

where D = calling duration

C = age at first calling

A = age (either chronological or calling).

In the first data set, with chronological age as the covariate and the zero values for nights before calling began included, the effects of both the factor C (age at first calling) and the covariate A (chronological age) were highly significant ( $p < 0.001$ ). It made no difference whether the factor or the covariate was fitted first (that is, the two pathways illustrated in Fig. 3.1 (Chapter 3) yielded identical conclusions). The interaction term was not significant, indicating that, in the terms of Fig. 3.1, Model 5 did not describe the data significantly better than did Model 4.

In this analysis, it is likely that the significance of chronological age is to some extent an artifice resulting from the inclusion of data prior to calling, because at low values of A, the dependent variable (calling duration) will often be zero. The analysis was therefore repeated, excluding these zero values. The factor term (age at first calling) remained significant ( $p < 0.01$ ), but the significance of chronological age disappeared. Again, the interaction was not significant and the order of fitting the terms did not matter. This result indicated that, once calling had begun, the duration of calling was not affected by chronological age. It confirmed the hypothesis that the significance of chronological age in the first analysis was substantially an artifice of the experimental design.

With calling age as the covariate, the zero values prior to calling were automatically excluded. In this analysis, only age at first calling was significant ( $p < 0.01$ ). The

interaction was not significant, and the order of fitting the terms did not matter. This result confirms the relative unimportance of age (whether expressed as chronological or calling age), in determining the duration of calling on any one night, by comparison with the age at which calling began.

The pattern revealed by the above analyses suggests that the moths used in this experiment came from a continuum between two extreme types. One type might be called "dedicated callers". They begin calling early in life, and (if they are not mated, as in the present experiment) they call for a similar long period each night throughout their lives. The other type might be called "reluctant callers". They do not begin calling until late in life, and even then do not call as much as the "dedicated callers". They clearly do not attempt (at least under the conditions of the present experiment) to compensate for their late start by extensive calling late in life.

*Number of calling bouts and duration per bout.*

The number and duration of calling bouts at different ages are shown in Fig. 4.13. The number of bouts was highest in 4-day-old moths. After the 4th scotophase, there was a significant decrease with age. Regression analysis for the subset of the data between 4 and 16 days yielded the equation:

$$B = 10.7 - 0.788a$$

where B = number of calling bouts

a = chronological age

The regression was highly significant ( $F_{1,76} = 40.59$ ,  $p < 0.001$ ,  $R^2 = 0.35$ ), indicating that moths had fewer calling bouts as they became older. The same trends were observed when the number of bouts was regressed against calling age. The regression equation was:

$$B = 6.12 - 0.339a \quad (F_{1,76} = 6.21, p = 0.015, R^2 = 0.08)$$

The total duration of calling was not significantly affected by moth age (see the previous section). In conjunction with the trends for the number of bouts, this suggests that frequent bouts of short duration occurred in young moths while older moths had fewer but longer calling bouts. This interpretation was confirmed by analysis of the mean duration per bout per night. There was a highly significant increase in calling duration per bout until the 10th scotophase.



**Fig. 4.13.** Experiment 1. Mean number of calling bouts (—) and mean duration per bout (---) for the entire scotophase in *M. convecta* females of different ages. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.

The regression equation obtained was:

$$D = 3.5 + 4.32a \quad (F_{1,70} = 8.49, p = 0.005, R^2 = 0.11)$$

where  $D$  = mean duration/calling bout

$a$  = age

A similar result was obtained when calling age was used. The regression equation was:

$$D = 2.05 + 5.65a \quad (F_{1,70} = 20.82, p < 0.001, R^2 = 0.23)$$

Between the 10th and 12th scotophases, bout duration seemed to decrease before rising again in very old moths, but these trends were not statistically significant. The sample size at this time was reduced because of moth mortality, and the standard errors were quite large.

Further analyses of the number of bouts and average duration per bout were conducted using the GLM procedure in MINITAB with age at first calling as the factor and age as the covariate. The models were:

$$\begin{aligned} \text{GLM } B &= C \ A \ C*A; \\ &\text{COVARIATE } A. \end{aligned}$$

where  $B$  = number of calling bouts

$C$  = age at first calling

$A$  = age (either chronological or calling)

and

$$\begin{aligned} \text{GLM } D &= C \ A \ C*A; \\ &\text{COVARIATE } A. \end{aligned}$$

where  $D$  = mean duration/calling bout

$C$  = age at first calling

$A$  = age (either chronological or calling)

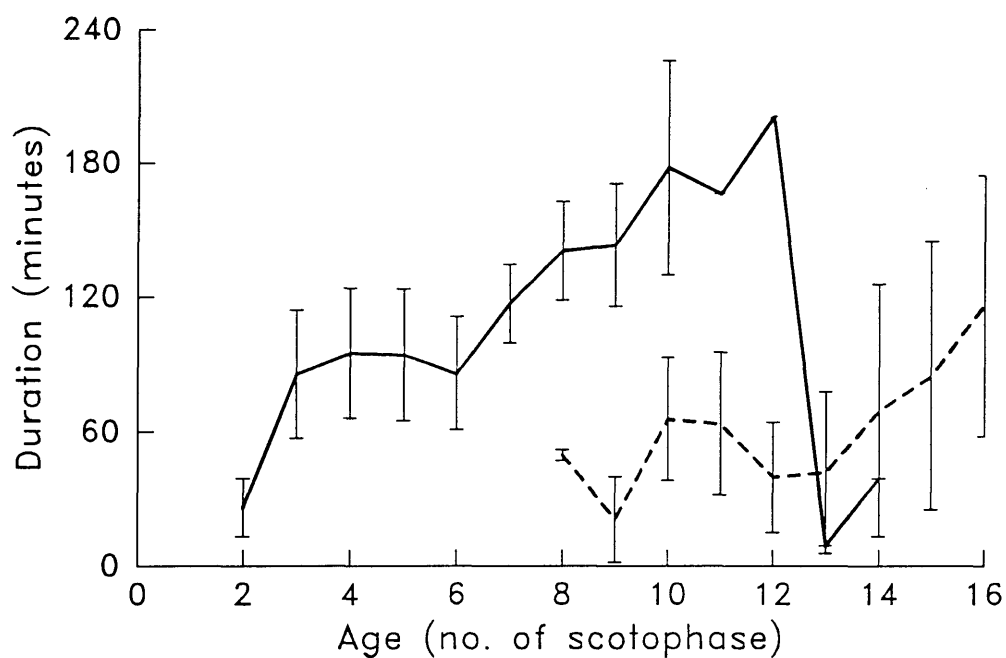
For the number of calling bouts, both the factor (age at first calling) and the covariates (chronological and calling ages) were highly significant ( $p < 0.001$ ). For average duration per calling bout, age at first calling was not significant but both chronological and calling ages were highly significant ( $p < 0.001$ ). This indicated that the number of calling bouts on each scotophase was influenced by both the age at which individual moths initiated calling as well as their current ages, whereas the average duration per bout was not affected by their age at first calling but by their current ages only. Thus, early callers appeared to have more bouts than the late callers and younger moths had more frequent bouts but bout duration was longer in older moths.

#### *Calling in ovipositing versus non-ovipositing moths*

Lawrence & Bartell (1972) suggested that reduced calling in older *E. postvittana* moths may be related to the presence of large numbers of mature eggs in the ovarioles, or to the act of oviposition itself. Their moths were unmated, as were those in this experiment. In some species, mating reduces calling (e.g., Howlader & Gerber, 1986b; Raina *et al.*, 1986; Sasaki & Riddiford, 1984; Webster & Cardé, 1984).

The duration of calling at different ages was compared in ovipositing and non-ovipositing *M. convecta* females (Fig. 4.14). This comparison suggested that non-ovipositing females generally called for significantly longer periods than ovipositing ones of the same age. The statistical significance of this effect was tested by a one-way analysis of variance using MINITAB. Data from moths of various chronological and calling ages, and various ages at first calling, were pooled for this analysis. The analysis showed that there were significant differences between the ovipositing and non-ovipositing groups ( $F_{1,108} = 10.59$ ,  $p = 0.002$ ). Non-ovipositing females had an average calling duration of  $109.1 \pm 9.4$  minutes while ovipositing ones had a mean of  $63.7 \pm 10.0$  minutes per night.

The number of calling bouts and average duration per bout in ovipositing and non-ovipositing females was compared by one-way analysis of variance. Non-ovipositing females had significantly more bouts than ovipositing females ( $F_{1,108} = 36.75$ ,  $p < 0.001$ ). The mean number of calling bouts in non-ovipositing females was  $5.4 \pm 0.4$  while ovipositing ones had a mean of  $2.0 \pm 0.3$  bouts. The average duration per calling bout was not significantly different between these two groups. This suggested that the significant difference in their calling duration was mainly due to variation in the number of their calling bouts rather than the length of these bouts.



**Fig. 4.14.** Experiment 1. Mean duration of calling in non-ovipositing (—) and ovipositing (---) *M. convecta* females at different chronological ages. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s. Points for the non-ovipositing group after scotophase 11 are based on only one moth.

It is possible that there were interactions or confounding effects between the ovipositing status of the female and her age, or her age at first calling. These could have been investigated using GLM models, but the data available for analyses involving age and oviposition status were limited because oviposition did not begin until late in the life of the females (age 6 onwards, and ages 9-11 for most moths), by which time mortality had reduced the sample sizes (Table 4.1). A regression was carried out using MINITAB to determine whether there was any relationship between the age of the female at first calling, and the age at first oviposition. Only 10 moths provided data for this analysis, because three died before any oviposition was recorded. The regression was not significant ( $F_{1,8} = 1.86$ ,  $p = 0.24$ ,  $R^2 = 0.19$ ), indicating that moths which began calling early did not necessarily begin ovipositing early. In such moths, there was a period of between 6 and 13 days between the initiation of calling and oviposition, whereas in moths which began calling late, this period was only 0-2 days. Moths used in this experiment were not mated. In mated moths, as indicated by the timing of oviposition in the stock cultures, the deposition of fertile eggs usually follows mating within a day or two. Since calling is usually indicative of the presence of mature eggs in noctuid moths (e.g., Swier *et al.*, 1976; Howlader & Gerber, 1986b) it seems likely that the unmated moths in this experiment had mature eggs from about the time they first called, but often did not lay them for several days. During this period, their calling duration was not noticeably lower than at the time of first calling. This suggests that it is not the presence of mature eggs, but the act of oviposition which reduced the amount of calling in older *M. convecta* females. It may simply be that oviposition occupies time which would otherwise be spent calling. Of course, in the field, moths are likely to mate and lay fertile eggs well before the age at which they did so in this experiment. The effect of this on calling has not been determined.

#### *Onset time of calling*

The mean onset times of calling (minutes after lights-off) for each night are shown in Figure 4.15. Between the 2nd and 12th scotophases there was a linear regression with a negative slope. The regression equation was:

$$OT = 357 - 11.9a$$

where OT = mean onset time of calling

a = age

This regression was highly significant ( $F_{1,79} = 9.75$ ,  $p = 0.003$ ,  $R^2 = 0.11$ ) indicating that the mean onset time of calling advanced with age.

A similar trend was obtained when calling age rather than chronological age was used. The regression equation was:

$$OT = 317 - 10.0a \quad (F_{1,79} = 6.89, p = 0.01, R^2 = 0.08).$$

Although these regressions were statistically significant, the  $R^2$  values for both of them were quite low, indicating that much of the variance could not be explained by age.

A regression analysis showed that the onset time of calling was not significantly correlated with the age at first calling ( $F_{1,79} = 1.01, p = 0.319, R^2 = 0.013$ ). This indicated that, across all ages, the onset time of calling in females that started to call for the first time when young did not significantly vary from that in females that called for the first time when they were older.

As with the analysis of total duration, relationships between age at first calling and chronological or calling ages were explored for the onset time data using GLM models. Analyses were confined to moths with chronological ages between 2 and 12 scotophases, because of the limited data for older moths. The GLM procedure in MINITAB was used to fit models of the form:

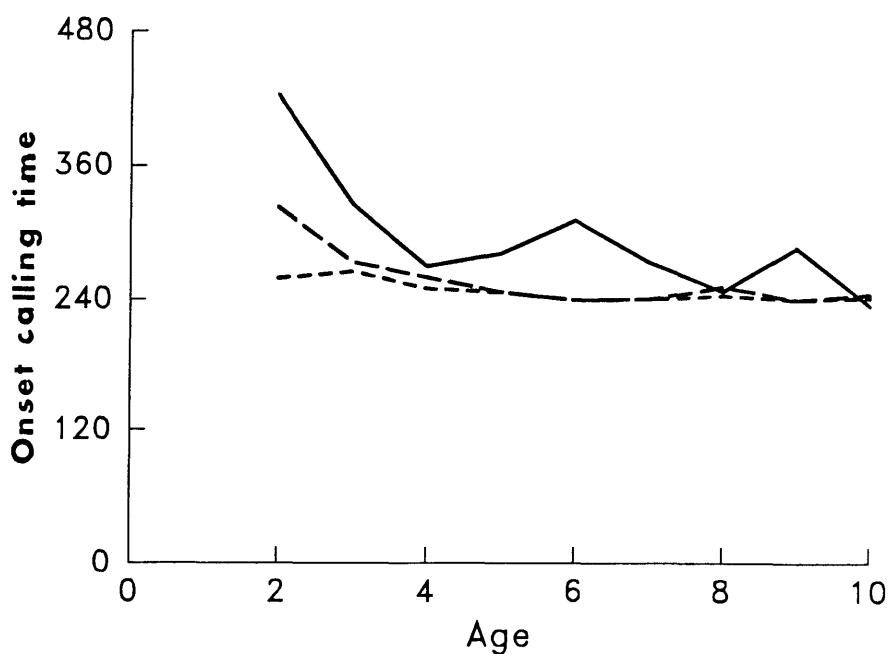
$$\begin{aligned} \text{GLM } OT &= C \ A \ C*A; \\ &\text{COVARIATE } A. \end{aligned}$$

where OT = onset time of calling  
C = age at first calling  
A = age (either chronological or calling).

When chronological age was used as the covariate, it was significant ( $p < 0.01$ ), but the age at first calling was not. Similar results were obtained when calling age was used as the covariate ( $p < 0.001$ ). There were no significant interactions and the order of fitting the terms was not important. These results indicate that for onset time, in contrast to total duration, the important factor is the current age of the moth, not the age at which it first began calling. This suggests that, at any given age, the greater calling duration of "dedicated callers" is not achieved by an earlier start to calling during the night, but by more persistent calling after calling is initiated.



**Fig. 4.15.** Experiment 1. Mean onset time of calling in *M. convecta* females at different chronological ages. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.



**Fig. 4.16.** Experiment 1. Comparison of simulated onset calling time in *M. convecta* females assumed to acquire the capacity to call within the photophase and scotophase (---) and within the scotophase only (-.-) with actual onset calling time of females (—) observed at ages 1-10 (Data from Fig. 4.15).

It might be argued that the advance in onset calling time with age is an artifice, resulting from the combination of a "calling gate" and gradual acquisition of the capacity to call (i.e., reproductive maturation). To investigate this, a small QuickBASIC program was used to generate 13 random numbers representing the time at which each of the moths in Table 4.2 acquired the capacity to call. Two sets of calculations were conducted. In the first one, it was assumed that the capacity to call was acquired at random throughout the photophase and scotophase, i.e., random numbers between 1-1440 (minutes within a 24-hour period). In the second one, it was assumed that the capacity to call was acquired only during the scotophase, i.e., random numbers between 1-480 (minutes within 8-hour period). In both, "calling gate" was assumed to open at 4 h (240 min after lights off) into the scotophase. A moth will call at 240 min if it acquires the capacity before the gate opens, and later if it acquires the capacity after the gate has opened. The following equation was used to calculate the onset calling time when the moths should have called for the first time:

$$OT_i = (\sum OT_{\text{new } i} / N_{\text{new } i} + 240 * N_{\text{cont } i}) / (N_{\text{new } i} + N_{\text{cont } i})$$

where  $OT_i$  = average onset time at age  $i$

$OT_{\text{new } i}$  = onset calling time of moths calling for the first time at age  $i$

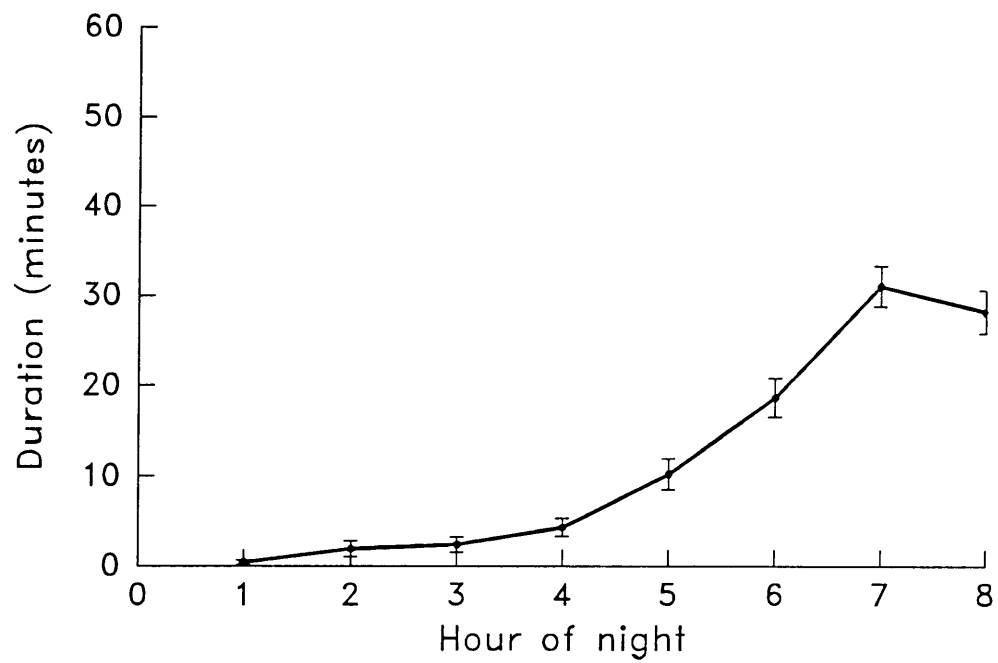
$N_{\text{new } i}$  = number of moths calling for the first time at age  $i$

$N_{\text{cont } i}$  = number of moths calling at age  $i$  but not for the first time

This equation was applied to the moths which generated the data shown in Fig. 4.15. The simulation was run 10 times, and comparison of the means of these 10 runs with the actual onset calling time at ages 1-10 is shown in Fig. 4.16. It is clear that the trend of advanced onset calling time with age is too strong to be accounted for by such an artifice.

#### *Duration of calling at different hours of the scotophase.*

Calling duration varied with the time of the scotophase (Fig. 4.17). Calling was minimal during the 1st half and increased towards the 2nd half of the scotophase. Peak calling was recorded during the 7th hour then declined on the last hour of the scotophase. Once the lights came on, females immediately ceased calling and retracted their ovipositors.



**Fig. 4.17.** Experiment 1. Mean duration of calling in *M. convecta* females during each hour of the scotophase. Data from all females of all ages are combined. Temperature - 20°C, photoperiod - 16:8 LD, N=13. Bars are s.e.'s.

A GLM analysis in MINITAB was used to investigate relationships between the timing of peak calling and the age at which females called for the first time. The model used was:

$$\text{GLM PH} = \text{C} \text{ A} \text{ C}^* \text{A};$$

$$\text{COVARIATE A.}$$

where PH = peak hour of calling

C = age at first calling

A = age (either chronological or calling)

Only the age at first calling was significant when either chronological or calling age was used as the covariate ( $p < 0.05$ ). There were no significant interactions between the age at first calling and chronological or calling age. This indicates that the time of peak calling during the scotophase was correlated with the age at which females initiated calling rather than with their current chronological or calling ages. A regression analyses indicated that early callers had significantly later peak calling time than late callers ( $F_{1,82} = 11.44$ ,  $p = 0.001$ ,  $R^2 = 0.12$ ). The regression equation was:

$$\text{PH} = 8.05 - 0.246a$$

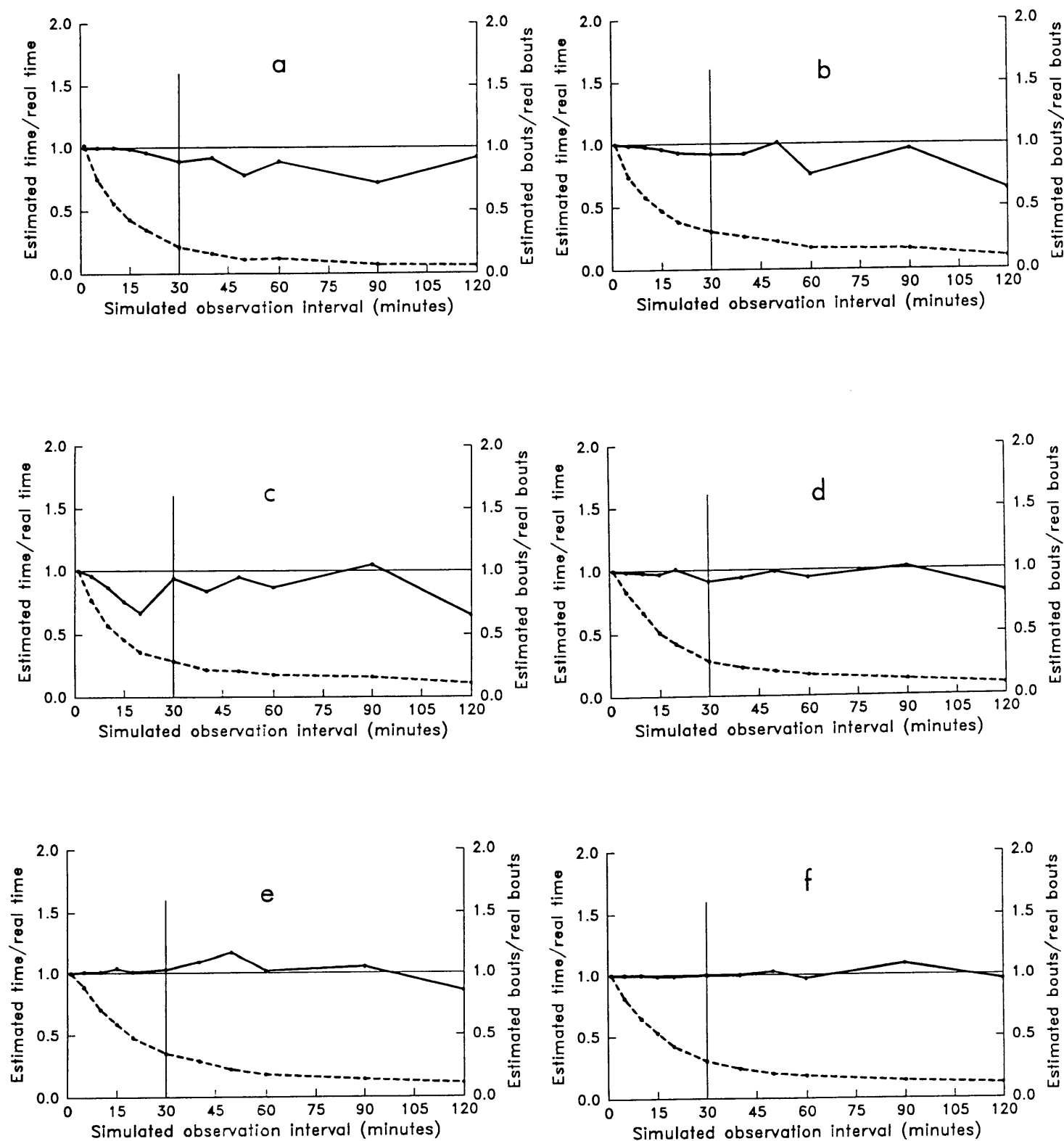
where PH = peak hour of calling time

a = age (either chronological or calling)

Moths that started to call for the first time between the 2nd and 5th days following emergence usually had peak calling time between the 6th and 8th hours of the scotophase while those with age at first calling between the 7th and 10th days, had peak calling time between the 5th and 6th hours of the scotophase.

#### **4.4. Simulated effects of non-continuous observation of moth behaviour**

Turgeon & McNeil (1982) pointed out that observations in behavioural experiments should be made as frequently as possible to avoid either overestimating or underestimating parameters. An important consideration, however, is that frequent observations are labour intensive. Some compromise between these considerations may be necessary. To determine whether non-continuous observations in future experiments could be done instead of continuous ones, without substantially underestimating or overestimating parameters, the STRAT3 program (Chapter 3.2.1), was used to simulate observation intervals ranging from 1 to 120 minutes on the estimated duration and number of bouts of each behaviour. The results are shown in Figure 4.18.



**Fig. 4.18.** Experiment 1. Simulated mean total duration (—) and number of bouts (---) of various behaviours in *M. convecta* females at different observation intervals compared with continuous observation: a - activity; b - feeding; c - calling; d - extrusion; e - oviposition; f - resting. A computer program, STRAT3, was used to simulate observation intervals ranging from 1 to 120 minutes on the estimated duration and number of bouts of each behaviour.

There was an exponential decline in the number of bouts "detected" by the model as the observation interval increased. The estimates of the total time spent in each behaviour did not differ greatly from the real time. There was some indication that activity, feeding and calling might have been slightly underestimated by non-continuous observation, but the stochastic variation was considerable. At 30-min intervals, the simulation model significantly (but predictably) underestimated the number of bouts but the estimates of the duration of behaviour were relatively unaffected. These results suggested that observation at 30-min intervals was a reasonable compromise between labour requirements and accuracy and this procedure was adopted for the experiments described in Chapter 5.

## **4.5. Experiment 2 - Behaviour of younger moths in the presence of older moths**

### **4.5.1 Introduction**

The presence of conspecific sex pheromone influences the calling behaviour in some female moths. Noguchi & Tamaki (1985) reported a delay in the onset of calling in the tortricids *Adoxphytes* sp. and *H. magnanima* when exposed to their sex pheromone. In *C. fumiferana*, the presence of conspecific synthetic sex pheromone resulted in earlier calling by about 2 to 3 hours (Palaniswamy & Seabrook, 1985). These examples suggest that in experiments to study the effects of environmental factors on calling it might be desirable to confine moths in close proximity only with other moths of the same calling status. Failure to do this might result in effects due to the factor under study being confounded with those due to variation in the amount of conspecific pheromone being released by adjacent moths. On the other hand, it is extremely difficult to establish experiments which meet this design. Due to variation in larval and pupal development, it may be difficult to obtain large numbers of moths at the same time. Even if this can be done, the large variation in age at first calling (Table 4.2) will ensure that on some nights moths which have not yet called might be exposed to pheromone.

It is generally not practical to isolate moths from pheromones produced by their neighbours, while still maintaining consistent environmental conditions. In the first experiment described in this chapter, *M. convecta* females which varied in both chronological and calling ages were observed in separate containers but in the same experimental chamber. Since a similar situation was likely to arise in future experiments, it was considered useful to investigate the possibility that females in the dark might influence the behaviour of their neighbours in separate containers. Although pheromones seem the most likely medium for such communication (e.g., Noguchi & Tamaki, 1985; Palaniswamy & Seabrook, 1985), the further possibility of auditory/acoustic communication cannot be ruled out (Ewing, 1984).

Accordingly, a second experiment was conducted to study the behaviour of younger *M. convecta* females in the presence of older females in the same experimental chamber. The comparison of these two groups of females provided a means of assessing the extent of communication between them. If there were no differences between the duration of calling (or other behaviours) between the groups, or in the ages at first calling or the mean onset times, it could be concluded that effects due to communication were unlikely to be important. If, on the other hand, females were shown to influence each other's behaviour, caution would have to be used in analysing results from subsequent experiments, because each behavioural event could not be considered independent.

## **4.5.2. Materials and methods**

### **4.5.2.1. Experimental design**

Two groups of unmated females were used in Experiment 2. Older moths (Group 1) were observed for the first 6-8 scotophases and were thereafter in the presence of younger moths (Group 2) in separate but adjacent containers. Group 2 moths were in the presence of older moths throughout the experiment. Group 1 moths had a total of 21 scotophases while Group 2 moths had a total of 15 scotophases in the experimental chamber. Observations were done at 30-min intervals.

### **4.5.2.2. Statistical analyses**

The age at first calling of females in the two groups of moths were compared using a 7 x 2 contingency table (Devore & Peck, 1993). Differences in the behavioural patterns (hourly durations per night) of the two groups of moths were tested by an F test of the reduction in deviance obtained by comparing models consisting of separate cubic regression equations for each group with models using a single cubic regression. The Generalised Linear Interactive Model (GLIM) package (Payne, 1985) was used to fit the models. Differences between the two groups were further compared using models describing different intercepts but similar curve shapes, and models describing different intercepts and curve shapes. Differences in the intercepts alone indicate parallelism while differences in the curve shapes indicate different time-related patterns, and therefore interaction between the two groups. Further GLM analyses using MINITAB were conducted to investigate relationships between calling duration, age at first calling, and chronological or calling age, as described for Experiment 1 (Chapter 4.3.2.2).

Raw data for each type of behaviour for Group 1 and Group 2 moths are provided in the sub-directory A:\CHAP4 of the floppy disk.

### 4.5.3. Results and discussion

The number of moths observed and the number calling at different ages in Experiment 2 is shown in Table 4.3.

Age (no. of scotophase)	Group 1	Group 2
1	21 (0)	13 (0)
2	21 (0)	13 (0)
3	21 (0)	13 (2)
4	21 (9)	13 (8)
5	21 (16)	13 (9)
6	21 (16)	13 (5)
7	20 (7)	13 (6)
8	20 (11)	13 (6)
9	20 (14)	13 (10)
10	20 (11)	13 (12)
11	20 (15)	13 (9)
12	20 (13)	12 (12)
13	17 (13)	12 (8)
14	17 (16)	12 (10)
15	17 (17)	6 (3)
16	17 (15)	-
17	17 (11)	-
18	16 (15)	-
19	16 (14)	-
20	12 (12)	-
21	7 (3)	-

**Table 4.3.** Experiment 2. Number of moths observed at different ages in old (Group 1) and young (Group 2) *M. convecta* females. Numbers in parentheses are number of moths calling for each age.

#### *Age at first calling*

The ages at which females called for the first time in Groups 1 and 2 are shown in Table 4.4. The pattern of ages at first calling was similar to that found in Experiment 1. Most moths began calling at between 3 and 5 days, but there were a few which did not begin calling until they were much older. A chi-square analysis of the data in Table 4.4, as a 7 x 2 contingency table, showed that the differences between the two groups were not statistically significant ( $\chi^2_6 = 12.10$ ,  $p > 0.05$ ). A Mann-Whitney test of the same data (applied because of suggestions that the distribution of age at first calling was not normal, see Chapter 4.6) also yielded a non-significant result ( $p = 0.536$ ).

Age at first calling	Number of moths	
	Group 1	Group 2
3	0	2
4	9	6
5	8	1
6	1	0
7	0	0
8	1	1
9	0	3
10	0	0
11	1	0
Total	20	13

**Table 4.4.** Experiment 2. Number of moths calling for the first time in old moths (Group 1) and young (Group 2) *M. convecta* females at different ages. One female in Group 1 did not call.

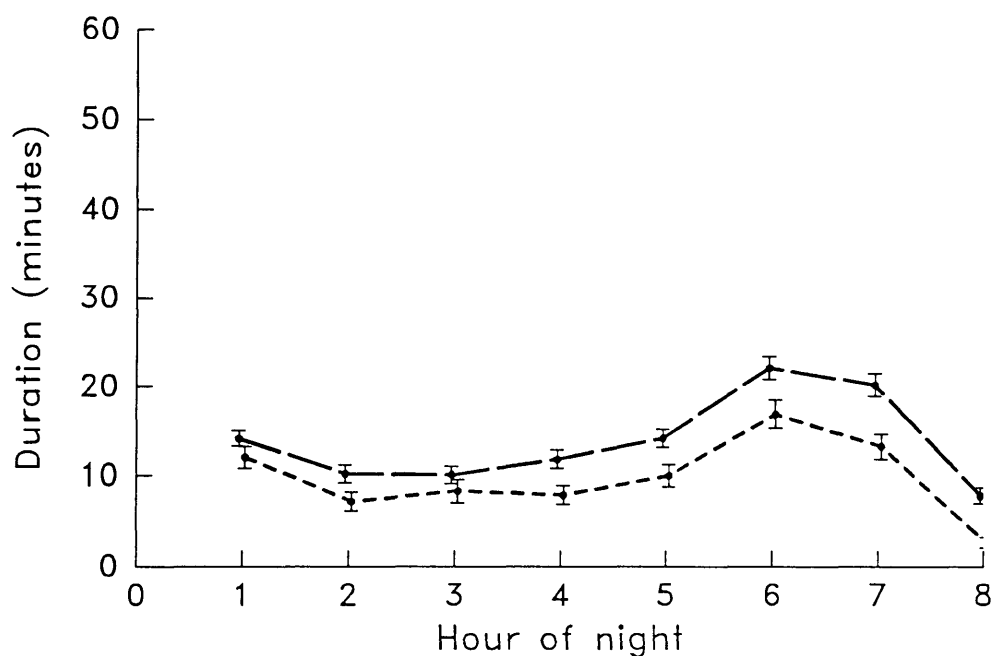
*Duration of behaviour at different hours of the scotophase*

The results from the GLIM analyses on the calling duration at different hours of the scotophase in Groups 1 and 2 are shown in Table 4.5.

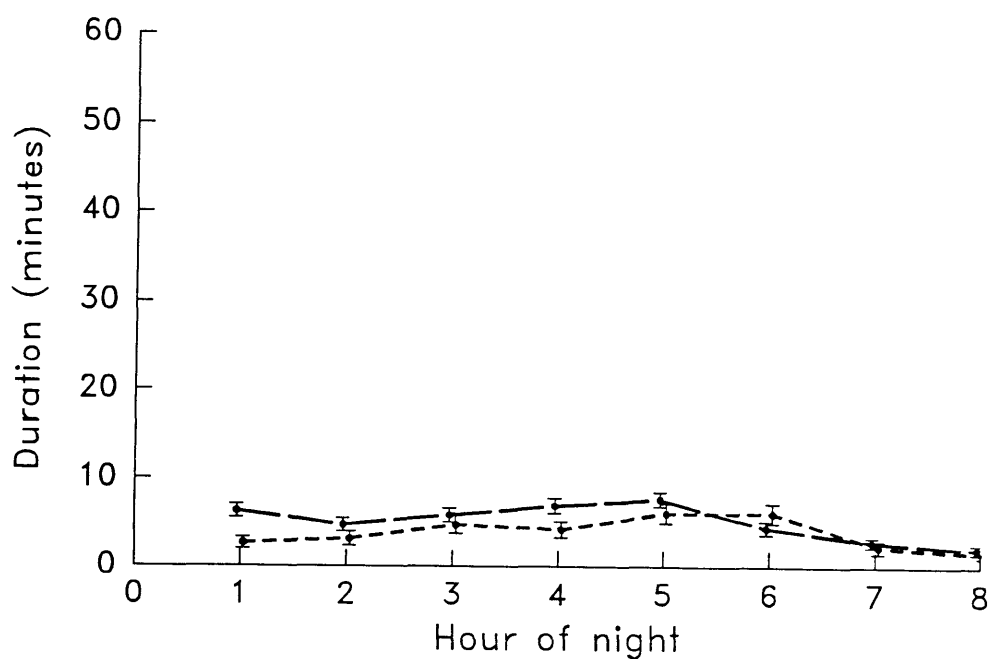
Behaviour	F ratios and levels of significance	
	Intercept ( $F_{1,11}$ )	Curve shape ( $F_{3,8}$ )
Activity	12.57, $p < 0.001$	0.5, n.s.
Feeding	7.13, $p < 0.05$	0.65, n.s.
Calling	0.0004, n.s.	0.05, n.s.
Extrusion	5.57, $p < 0.05$	1.41, n.s.
Oviposition	0.32, n.s.	0.65, n.s.
Resting	2.73, n.s.	0.04, n.s.

**Table 4.5.** F ratios and levels of significance for the different behaviours obtained by using the Generalised Linear Interactive Model (GLIM) analysis to test differences between older (Group 1) and younger (Group 2) *M. convecta* females.  $N = 21$  and  $13$ , for Groups 1 and 2, respectively.

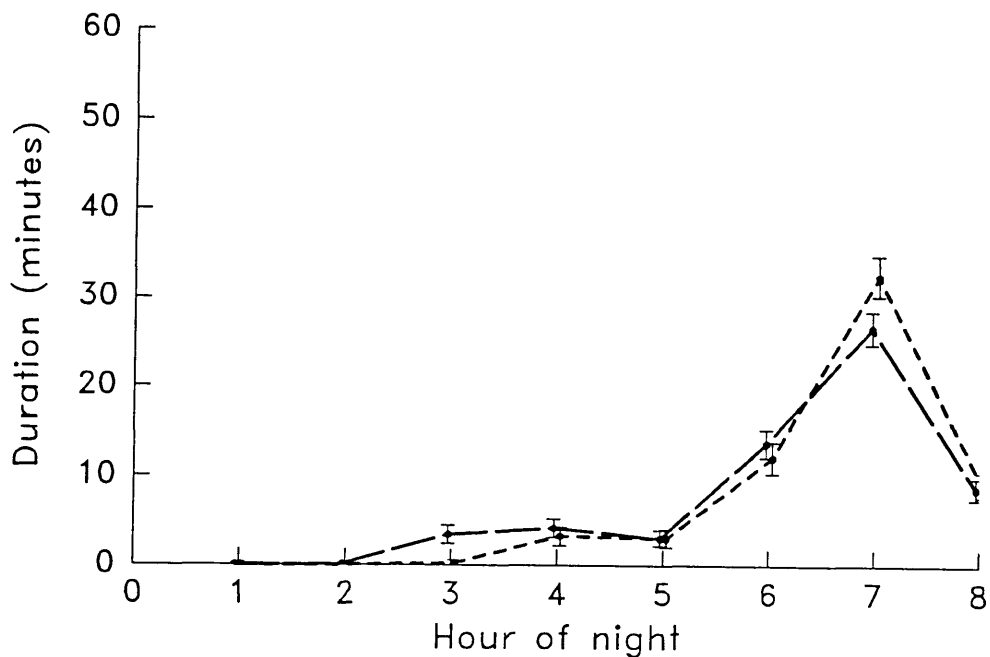
Significant differences in intercepts between the two groups were obtained for activity, feeding and extrusion behaviours. There were no significant differences in their curve shapes for all behaviours. Group 1 females showed significantly more activity (Fig. 4.19) and feeding (Fig. 4.20) than Group 2 females. Extrusion was significantly longer in Group 2 females than that in Group 1 females (Fig. 4.22). No significant differences were detected for calling (Fig. 4.21), oviposition (Fig. 4.23) and resting (Fig. 4.24).



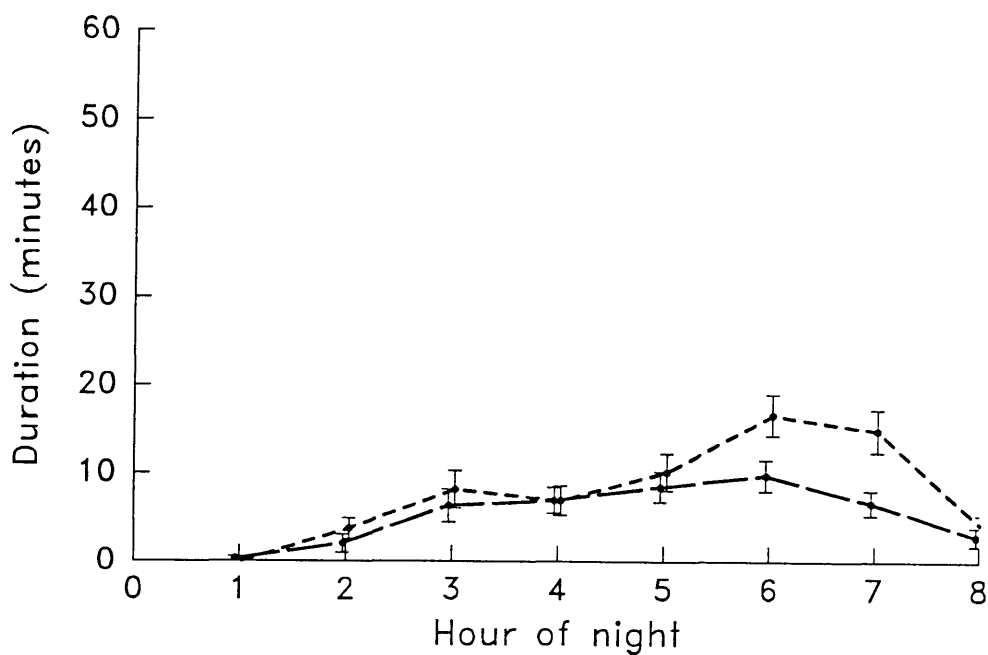
**Fig. 4.19.** Experiment 2. Mean duration of activity during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



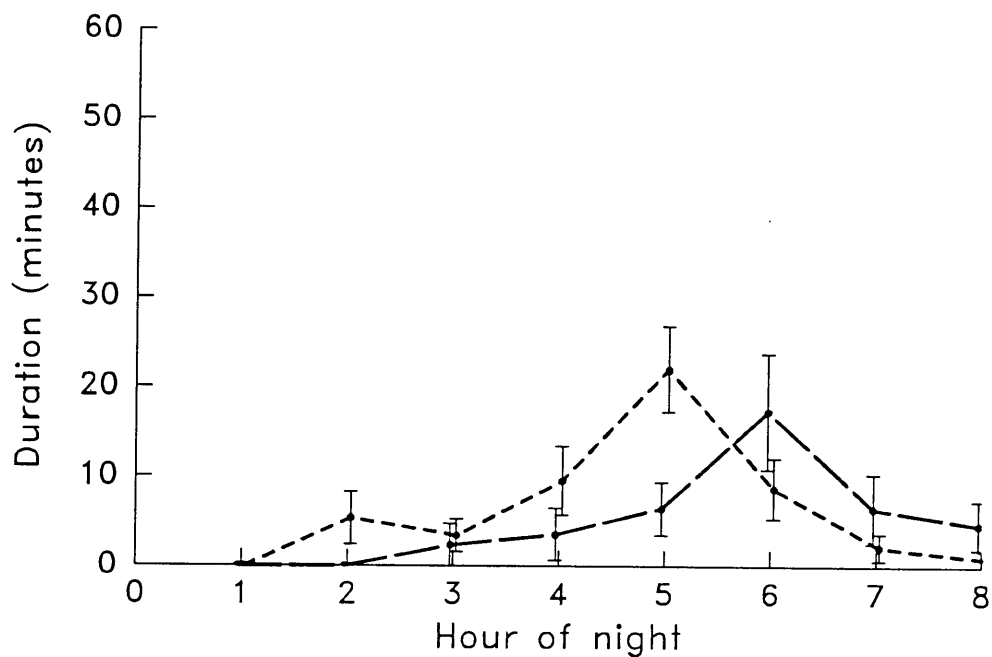
**Fig. 4.20.** Experiment 2. Mean duration of activity during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



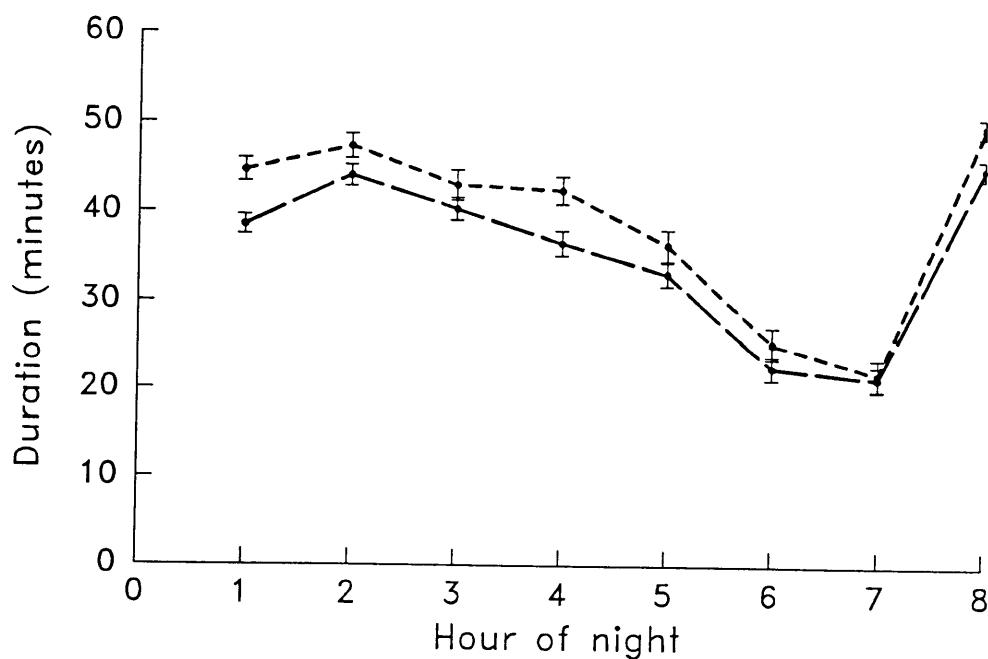
**Fig. 4.21.** Experiment 2. Mean duration of calling during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



**Fig. 4.22.** Experiment 2. Mean duration of extrusion during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



**Fig. 4.23.** Experiment 2. Mean duration of oviposition during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



**Fig. 4.24.** Experiment 2. Mean duration of resting during each hour of the scotophase in Group 1 (—) and Group 2 (---) *M. convecta* females. Data from all females of all ages are combined for each group. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.

### *Onset time of calling*

The mean onset time of calling in Group 1 and Group 2 females at different ages is shown in Fig. 4.25. In both groups, moths generally started calling earlier in the scotophase when they were older. This trend however, occurred between the 3rd and 12th scotophases only. As moths became older, onset calling time was very variable. This pattern was similar to that found in Experiment 1 (Fig. 4.15). A one-way analysis of variance of onset calling time against group was used for the 3rd to 12th scotophases. The mean onset time of calling was not significantly different between the two groups and a regression analysis for the same data set showed that onset calling time was not significantly correlated with the age at first calling. This result is similar to that obtained in Experiment 1.

Regressions of onset calling time on either chronological age or calling age using the same data set above, were highly significant with a negative slope, indicating that the mean onset calling time during the scotophase advanced with age. The  $R^2$  values were however, very low. The regression equations were:

$$OT = 378 - 7.23a \quad (F_{1,188} = 12.89, p < 0.001, R^2 = 0.064)$$

where OT = onset calling time

a = chronological age

and

$$OT = 349 - 6.94a \quad (F_{1,188} = 11.46, p < 0.001, R^2 = 0.057)$$

where OT = onset calling time

a = calling age

As in Experiment 1, a GLM analysis of the same data set was done using age at first calling as the factor and either chronological or calling age as the covariate. The model was:

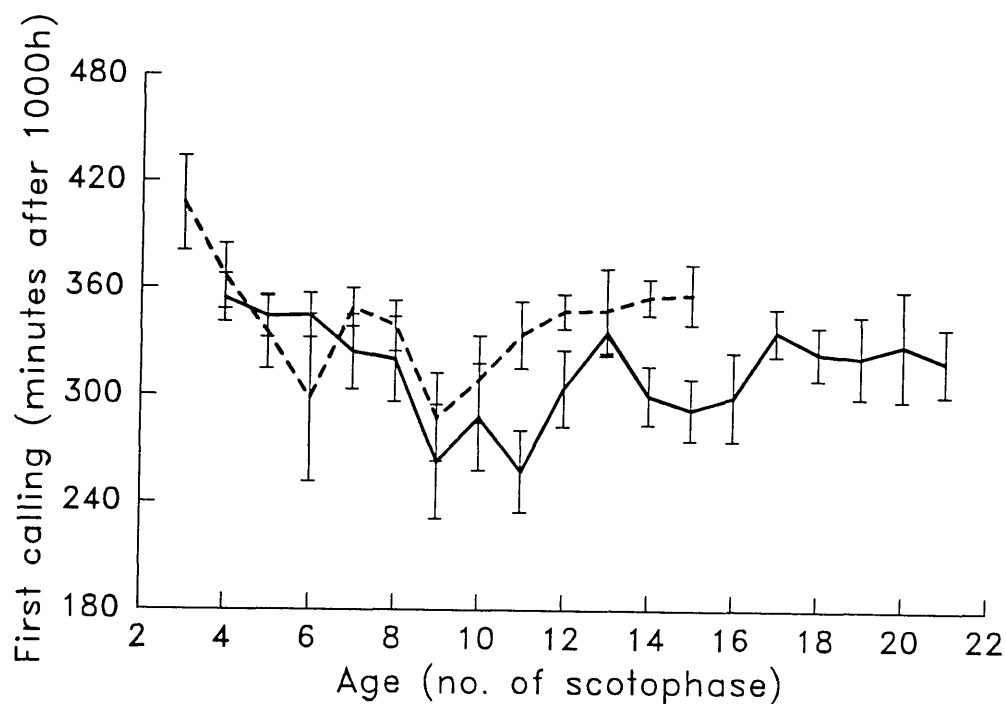
$$\text{GLM } OT = C \ A \ C*A;$$

$$\text{COVARIATE } A.$$

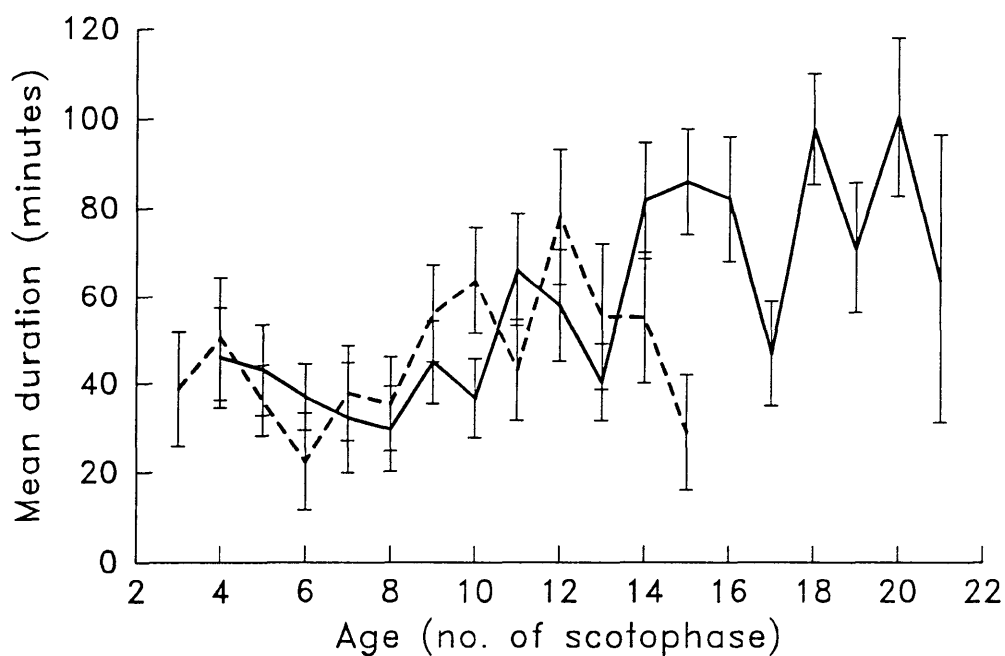
where OT = onset calling time

C = age at first calling

A = age (either chronological or calling)



**Fig. 4.25.** Experiment 2. Mean onset time of calling at different chronological ages in Group 1 (—) and Group 2 (---) *M. convecta* females. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.



**Fig. 4.26.** Experiment 2. Mean duration of calling at different chronological ages in Group 1 (—) and Group 2 (---) *M. convecta* females. Temperature - 20°C, photoperiod - 16:8 LD, N=21 (Group 1) and 13 (Group 2). Bars are s.e.'s.

When either chronological or calling age was used as the covariate, age at first calling was not significant while age was highly significant ( $p < 0.001$ ). This result indicates that the time at which moths initiated calling during the scotophase was not dependent on what age they called for the first time, but more so on their current ages at the time of calling. There were no significant interactions between age at first calling and age (chronological or calling), and the order of fitting the factor or the covariate was not important. These observations are again consistent with those found in Experiment 1 (Chapter 4.3.2.2).

*Duration of calling at different ages*

The mean duration of calling in Groups 1 and 2 moths at different ages is shown in Fig. 4.26. A one-way analysis of variance showed that calling duration was not significantly different between the two groups. A regression analysis of the same data set showed that calling duration was significantly correlated with age at first calling, though the  $R^2$  value was very low. The regression equation was:

$$D = 79.5 - 4.85c \quad (F_{1,412} = 11.36, p < 0.001, R^2 = 0.027)$$

where D = calling duration

c = age at first calling

The regression had a negative slope indicating that early callers had significantly longer calling duration than the late callers. This observation agrees with that found in Experiment 1 (Chapter 4.3.2.2).

Further analyses of the same data set using the GLM method in MINITAB, was done with group and age at first calling as the factors and age as the covariate. In the first analysis using group as the factor, the model was:

$$\begin{aligned} \text{GLM } D &= G \ A \ G*A; \\ &\text{COVARIATE } A. \end{aligned}$$

where D = calling duration

G = group

A = age (either chronological or calling)

With chronological age as the covariate, group was non-significant while chronological age was highly significant ( $p < 0.001$ ). Similar results were obtained when calling age was used as the covariate ( $p < 0.001$ ). In both models, interactions between group and age

were non-significant and the order of fitting the terms was not important. This indicated that calling duration in Group 1 females did not significantly differ from that in Group 2 females and that in both groups, the amount of calling was dependent on their current ages.

In the second analysis with age at first calling as the factor, the model was:

$$\text{GLM } D = C + A + C \cdot A;$$

$$\text{COVARIATE } A.$$

where D = calling duration

C = age at first calling

A = age (either chronological or calling)

When either chronological or calling age was used as the covariate, the age at first calling and age were both highly significant ( $p < 0.01$  and  $p < 0.001$ , respectively). In both models, there were no significant interactions and the order of fitting the factor or the covariate did not matter. The results indicate that mean duration of calling at different ages in both Group 1 and Group 2 females was significantly correlated with the age at which they called for the first time as well as with their current ages. This result is contradictory to what was observed in Experiment 1, where the same models showed that only the age at first calling was significant, and not either chronological or calling age. In Experiment 2, the number of moths observed at older ages (i.e., from scotophase 13 onwards) was higher than that in Experiment 1. The lack of a significant effect of age on calling duration observed in Experiment 1 may be because few very old moths were observed due to mortality. The significance obtained in Experiment 2, which had a larger sample size, might represent the real calling pattern in very old moths.

#### *Influence of moths of different ages*

The lack of significant differences in the calling pattern between Group 1 and Group 2 females (Fig. 4.21), despite the fact that each was in the presence of moths of different ages and therefore different age-related calling patterns, indicates that calling behaviour of individual females was not influenced by pheromonal or auditory/acoustic communication with other females. Similar observations were reported for *C. fumiferana* by Sanders & Lucuik (1972). The time and duration of calling and flight in younger females of this species were not significantly different from those in older females that had already called, suggesting the lack of chemical communication at close range between them.

There were significant differences in extrusion between Group 1 and Group 2 females during the 2nd half of the scotophase (Fig. 4.22) but none in the time spent in oviposition (Fig. 4.23). Since extrusion is a pre-oviposition behaviour this suggests that there may have been some communication between females related to oviposition. Such communication might serve to synchronise oviposition, which may be important for an insect which is at least partially gregarious. However, if this is so, it is likely that the younger Group 2 moths were prevented from responding (other than through extrusion) by lack of reproductive maturity.

#### 4.6. Age at first calling - analysis of data combined from the experiments 1 and 2

Data on the age at first calling for Experiments 1 and 2 were combined to obtain a larger sample size to test this parameter. The number of moths observed in the two experiments totalled 47. Females called for the first time between the 2nd and 11th scotophases, with the highest number of females calling for the first time in the 4th scotophase (Fig. 4.27). MLP analysis (Ross, 1987) was used to fit models to the data.

There was a bimodal distribution of age at first calling, with one group centred around the 4th scotophase and another during the 8th to 9th scotophases. The distribution shown in Fig. 4.27 differed significantly from the normal ( $\chi^2_9 = 33.7$ ,  $p < 0.01$ ) and from two skewed distributions, the lognormal ( $\chi^2_8 = 17.27$ ,  $p < 0.05$ ) and the gamma ( $\chi^2_9 = 21.95$ ,  $p < 0.01$ ). However, a double normal (bimodal) model fitted well ( $\chi^2_7 = 3.9$ ,  $p > 0.75$ ).

The result suggests that *M. convecta* females can either be "early" or "late" callers. In other species, the initiation of calling is associated with the degree of ovarian development and pheromone production in female moths (e.g., Cusson & McNeil, 1989b; Howlader & Gerber, 1986b; Raina *et al.*, 1986a). The levels of ovarian development and pheromone production in *M. convecta* females used in these experiments were unknown. Peak first calling in early callers, i.e., during the 4th-5th scotophases, may have occurred in females that had fully mature ovaries and high pheromone contents while the small proportion of females which called for the first time when they were older, i.e., during the 8th-9th scotophases, may be more adapted to dispersal or migration instead of reproduction early in their lives.