

# Role of ultrasound production and chemical signals in the courtship behaviour of *Ephestia cautella* (Walker), *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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## Abstract

Further details are given on the role of ultrasound production in the courtship behaviour of *Ephestia cautella* (Walker), *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) during wing-fanning and on the role of other signals in pair forming.

Sounds produced by these moths consist of regular sequences of ultrasonic pulses with a frequency range up to 80 kHz, emitted by wing-fanning males during courtship behaviour. The pulses are emitted in regular sequences at 14–24 ms, corresponding to pulse-repetition rates ranging from 41–72 pulses per second.

Tests to evaluate the reproductive success in muted males and deaf females revealed a lower success in muted males and deaf females than in intact individuals. This is probably related to the suppression of the acoustic signals.

When antennae were surgically removed from males and females a lower reproductive success (from 40–78%) was observed in males. Female *E. cautella* and *E. kuehniella* without antennae showed no fall in their reproductive success, whilst a drop of 22% was observed with *P. interpunctella*.

Ultrasonic communication in these moths plays a significant role in mating behaviour. Thus courtship behaviour must be reconsidered with greater emphasis on the interaction between multiple modalities. Further work is needed to understand the role played by ultrasounds in phycitidae mating behaviour and the relative importance of chemical, visual and acoustic stimuli.

## Introduction

Many insects have airborne sound receptors, which function primarily in intraspecific communication, whilst in others hearing serves to warn of the potential threat of a predator (Roeder and Treat 1961; Spangler 1988; Spangler and Hippenmeyer 1988).

Certain pyralid moths, such as the Galleriinae wax moths *Achroia grisella* F. and *Galleria mellonella* L., and the rice moth *Corcyra cephalonica* (Staint.), have acquired the additional ability to generate sounds by wing-fanning for intraspecific communication and pair forming (Trematerra 1992).

With other pyralid moths infesting stored products, the hearing ability of *Ephestia cautella* (Walker), *Ephestia elutella* (Hübner), *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) was reported by Mullen and Tsao (1971) and by Pérez and Zhantiev (1976).

The courtship of these Phycitinae has been examined and several studies have been made on the male orientation to the pheromone produced by the calling female and also on the behaviour of the male after the female has been located (Traynier 1968; Kennedy and Marsh 1974; Barrer and Hill 1977, 1980).

These pyralids, Galleriinae and Phycitinae, have tympanic hearing organs located on the pleural-ventral surface of the first abdominal segment. The morphological similarity of these insects led us to suspect that the hearing capabilities of the moths are similar, and that they are capable of developing acoustical communication systems (Trematerra and Pavan 1994).

This paper gives further details on the role of ultrasound production in the courtship behaviour of *E. cautella*, *E. kuehniella* and *P. interpunctella* during wing-fanning and discusses the role of other signals in pair forming.

## Materials and Methods

### Insect rearing

*E. cautella*, *E. kuehniella* and *P. interpunctella* adults were obtained from laboratory cultures reared using the method of Hoppe (1981) on a *pabulum* of maize flour (28.6%), whole meal flour (14.3%), wheat flour (14.3%), oat meal (9.5%), brewer's yeast (4.8%), wheat germ (4.8%), honey (7.9%), and glycerol (7.1%) in standard conditions of  $25 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  r.h.; with a natural photoperiod. Virgin males and females were obtained sexing the IV-V instar larvae by observing the dark male gonads through the cuticle.

### Sound recording and analysis

Ultrasonic signals were acquired by means of a Bruel & Kjaer (B&K) 2231 phonometer with a B&K 4135 transducer (flat-frequency response up to 100 kHz) and a B&K 1627 filter unit (set as a high-pass at 15 kHz). Signals were fed into a NF P61 amplifier and an NF P86 anti-aliasing low-pass filter (125 dB/oct) to be digitally recorded on a PC-based Digital Signal Processing Workstation. Sixteen seconds of continuous signal at 192000 s/s or 24 seconds at 128000 s/s were stored, thus allowing a bandwidth of 90 kHz and 56 kHz respectively, for each experiment. Recorded signals were played back at a reduced sample rate (32000 s/s) to reduce their bandwidth by

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6 and 4 times respectively to be audible and recordable on a DAT (Digital Audio Tape) recorder. Spectral and temporal analyses were performed to reveal the structure and the temporal patterning of the signals.

Ultrasonic emissions were tested on virgin males stimulated by means of synthetic pheromones or by putting virgin females in a Petri dish (20 cm in diameter and 2 cm in height) covered with an acoustically transparent thin grid.

Wing-fanning males were followed (tracked) manually with the microphone at a distance of about 1 cm; sounds were typically picked up from the dorsal side. Some recordings were made from the ventral side of the moths when they were walking on the covering grid.

Recordings were performed at a temperature of about 22°C, 65% r.h. and in a low light intensity.

The same procedures were used on males from which the tegulae were surgically removed to verify our hypothesis on the structures involved in sound production.

### Biological tests

The mating capacity in the adults of *E. cautella*, *E. kuehniella* and *P. interpunctella* was tested by isolating, for each species, a one day old virgin male and a virgin female, in a cage (9 × 17 × 8.5 cm) containing a Petri dish (5.5 cm in diameter) with some *pabulum*. The evaluation of fertility by examination for the presence of larvae was carried out after 7–10 days. The test was repeated at least 13 times.

The same procedure was used in tests with adult males with their tegulae removed (muted males), with adult females with their timpanus pierced (deaf females) and with males and/or females with their antennae surgically removed.

The environmental conditions in the room housing the cages were kept at 25 ± 1°C, 65 ± 5% r.h. and with a natural photoperiod.

**Table 1.** Ultrasonic emissions in males of *E. cautella*, *E. kuehniella* and *P. interpunctella*.

Species	Maximum frequency	Pulse interval
<i>E. cautella</i>	50–80 kHz	15–20 ms
<i>E. kuehniella</i>	50–80 kHz	20–24 ms
<i>P. interpunctella</i>	50–70 kHz	14–18 ms

**Table 2.** *E. cautella*, *E. kuehniella* and *P. interpunctella* biological tests with intact (M) or muted males (Mm) and with intact (F) or deaf females (Fd).

Species	Group	Tests (n)	Reproduction success (%)	Drop in reproduction (%)
<i>E. cautella</i>	M × F	22	93.34	
	Mm × F	21	50.20	46.22
<i>E. kuehniella</i>	M × F	24	83.33	
	Mm × F	21	44.14	47.03
<i>P. interpunctella</i>	M × F	34	94.13	
	Mm × F	21	60.54	35.68
<i>E. cautella</i>	M × F	15	86.66	
	M × Fd	20	66.66	23.08
<i>E. kuehniella</i>	M × F	27	81.48	
	M × Fd	13	61.53	24.48
<i>P. interpunctella</i>	M × F	32	93.75	
	M × Fd	21	61.54	34.36

## Results and Discussion

### Sound recording and analysis

The courtship sequence for *E. cautella*, *E. kuehniella* and *P. interpunctella* proved to be similar as reported by Barrer and Hill (1977) for *E. cautella*.

Males started wing-fanning and females searched on perceiving chemical stimuli, i.e. the artificial pheromones or the female sexual pheromones. The male wing-fanning slowed progressively when the genitalia of the two sexes come into direct contact. The male then came to rest facing the female with their heads facing and then the male proceeded with the initial stages of copulation.

During the entire phase of wing-fanning, ultrasonic pulses were detected in the three species (Trematerra and Pavan 1994). The wing-fanning males emitted trains of ultrasonic pulses extending in frequency range up to 80 kHz. The pulses were emitted in regular sequences, spaced and with the pulse-repetition as reported in Table 1.

The recordings of *E. cautella*, *E. kuehniella* and *P. interpunctella* appeared very similar to the pulses clearly recognisable (Fig. 1).

Males with the tegulae removed showed no modification in their courtship behaviour but no ultrasonic emissions were detected.

### Biological tests

The test to evaluate reproductive success in muted males and in deaf females revealed a lower reproductive success than in intact individuals as shown in Table 2. This is probably related to the suppression of the acoustic signals.

Evaluation of the reproductive success in males or females without antennae (Table 3) showed a lower reproductive success in males with their antennae removed. With *E. cautella* and *E. kuehniella* females without antennae showed no drop in reproductive success. This compares with a drop of 22% with *P. interpunctella*. In the experimental cages, *P. interpunctella* showed heightened activity in the search for a partner by increased walking and flying.

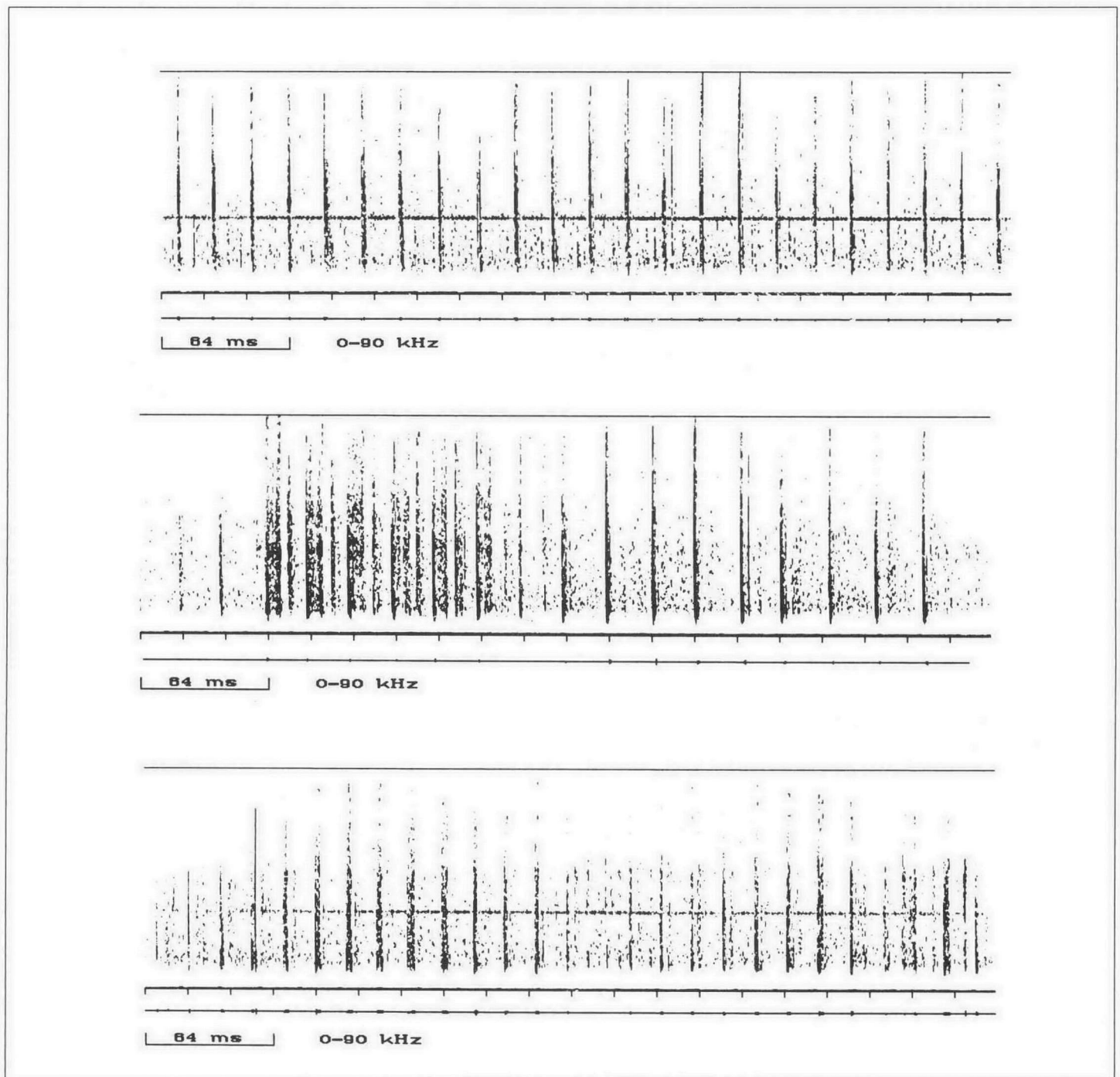


Fig. 1. Spectrograms showing the ultrasonic pulses produced by wing-fanning *Epehstia cautella*, *Epehstia kuehniella* and *Plodia interpunctella* males. Frequency range 0–90 kHz, time span 427 ms, tic marks 21.33 ms, bandwidth 1 kHz.

### Conclusion

Research on moth courtship behaviour has focused primarily on female pheromones and male behavioural responses to them. Although several studies on moth sex pheromones have demonstrated that the complete sequence of courtship behaviour also involves male pheromones and other types of communication, relatively little work has been done on the connection between pheromones and extrusible organs or with sound production (Birch and Hefetz 1987; Spangler 1988; Trematerra 1992; Trematerra and Pavan 1994).

Many insects have airborne sound receptors; in Diptera, Hemiptera, Lepidoptera and Orthoptera these receivers function primarily for intraspecific communication (Spangler and Hippenmeyer 1988) whereas in Coleoptera, Dictyoptera,

Lepidoptera and Neuroptera hearing serves to warn the bearer of the potential threat of a predator (Roeder and Treat 1961; Spangler 1988).

In view of the preliminary results presented here, moth courtship behaviour in *E. cautella*, *E. kuehniella* and *P. interpunctella* must be reconsidered with greater emphasis on the interaction between multiple modalities. The experiments reported here show that the ultrasonic and chemical communications in these species play a significant role in mating behaviour.

Further experiments are needed to deepen our knowledge of the role played by ultrasound in phycitid mating behaviour and the relative importance of chemical, visual and acoustic stimuli.

**Table 3.** *E. cautella*, *E. kuehniella* and *P. interpunctella* biological tests with intact males (M) and females (F) or with males without antennae (Ma) and females without antennae (Fa).

Species	Group	Tests (n)	Reproduction success (%)	Drop in reproduction (%)
<i>E. cautella</i>	M × F	19	89.47	
	Ma × F	20	20.00	77.65
	M × Fa	20	100.00	0
<i>E. kuehniella</i>	M × F	27	81.48	
	Ma × F	17	35.29	56.69
	M × Fa	18	83.33	0
<i>P. interpunctella</i>	M × F	32	93.75	
	Ma × F	16	56.25	40.00
	M × Fa	13	72.72	22.43

In a preliminary examination of the hearing capabilities of these moths a quick response to bat-like ultrasonic signals was revealed. Males halted wing-fanning or terminated their flight when given short ultrasonic signals at frequencies of 40–50 kHz similar to those produced by some echolocating bats. A further investigation is thus required to better understand the role played by ultrasounds in anti-predation behaviour of pyralid moths.

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