THE ROLE OF SONIC SIGNALS IN THE SEXUAL COMMUNICATION OF PEACH TWIG BORERS, *ANARSIA LINEATELLA*, ZELLER (LEPIDOPTERA: GELECHIIDAE)

by

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ABSTRACT

I tested the hypothesis that female peach twig borers, *Anarsia lineatella*, Zeller (Lepidotpera: Gelechiidae), use acoustic signals in addition to known pheromone signals during sexual communication. I employed a digital system to record and playback sound. Males, flying on a tether, emitted signals of 12 dB above ambient sound intensity with wingbeats of 60 Hz and upper signal components at 2.5 and 10 kHz. Females produced sonic reply signals [50 Hz (wingbeat), 2 and 14 kHz) when exposed either to playback recordings of the males' signals or to signals from live males. The males' sonic signals provoked females to reduce pheromone emission. In field experiments, traps baited with sex pheromone and playback recordings of female sonic signals captured significantly more males than traps baited with sex pheromone alone. My data support the hypothesis that female and male *A. lineatella* use bioacoustic and pheromonal signals during sexual communication.

QUOTATION

You cannot acquire experience by making experiments. You cannot create experience.
You must undergo it.

Albert Camus

DEDICATION

In loving memory of Auntie Cath and Uncle Wes Thompson who helped me along the way, but departed before I completed this part of my life's journey.

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GLOSSARY

Acoustic: of or relating to the science of acoustics

Acoustics: a science that deals with the production, control, transmission, reception and effects of sound

Bioacoustic: of or relating to the science of acoustics in living organisms

Pheromone: an intraspecific chemical signal that benefits both the emitter and receiver

Scotophase: the dark phase in a 24-hour photoperiod

Sonic: frequencies between 0-20 kHz.

Sound: displacement of particles (typically in air) by pressure waves.

Ultrasonic: frequencies > 20 kHz.

INTRODUCTION

1.1 Sexual communication modalities in insects

To attract or locate mates, insects use sex pheromones (Gillott, 1980; Mankin et al., 2000; Triplehorn & Johnson, 2005), sound (Spangler, 1988; Stephen & Hartley, 1991), visual displays (Knuttel & Fiedler, 2001), and bioluminescence (Scoble, 1995; Gerhardt & Huber, 2002). Sex pheromones are typically emitted by females to attract males (Tamaki, 1985; Scoble, 1995; Phelan, 1997), and males usually orient anemotactically toward females (Kuenen & Baker, 1982; Willis & Baker, 1984; Justus & Cardé, 2002). Bioacoustic signals function similarly (Bailey, 2003), but males also utilize acoustic signal trains to broadcast their quality to potential mates (Hoikkala et al., 1998). Bioacoustic signals are commonly produced by one sex (Bennet-Clark et al., 1980; Crossley et al., 1995), but can be exchanged between prospective mates (Sanderford & Conner, 1990, 1995; Bailey, 2003).

Unlike pheromones or sonic signals, bioluminescence as a means of sexual communication is rare in insects. It is well studied, however, in nocturnal fireflies (Lloyd, 1971; Carlson & Copeland, 1985). For example, male and female American fireflies, *Photinus pyralis* (Coleoptera: Lampyridae), exchange single bioluminescent flashes that allow mates to find each other (Buck & Case, 2002).

Insects also use combinations of sensory modalities, most commonly pheromones and sound (Heller & Krahe, 1994; Čokl et al., 1999; Takács et al., 2002, 2003), during sexual communication. Male green stink bugs, *Nezara viridula* (Hemiptera:

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Pentatomidae), emit sex pheromones for long-range attraction of females, and both sexes use vibratory signals to locate each other on the plant (Miklas et al., 2003). Among moths, many species are already known to employ bioacoustics in addition to pheromones for mate-location (Table 1.1). For example, male webbing clothes moths, *Tineola bisselliella* (Lepidoptera: Pyralidae), emit pheromonal and acoustic signals that attract females (Takács, 2002). The lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae), has a system where males wingfan to attract females (Greenfield & Weber, 2000; Jia et al., 2001), while the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), has a more complex signaling system with wing-fanning males eliciting acoustic cues from females that, in turn, stimulate the males to emit pheromone that attracts females (Spangler, 1988).

1.2 Peach twig borer distribution

The first account of *A. lineatella* was published by Zeller in Germany in 1839 (Duruz, 1923). A second account of the insect led to its synonym, *Anarsia pruniella* (Clemens) (Ulenberg, 1989). *Anarsia lineatella* is thought to have originated in Asia or Western Europe (Marlatt, 1898, Duruz, 1923). It is an introduced pest to North America, first found in the United States in 1860 (Marlatt, 1895; Duruz, 1923), and in Canada in 1902 (Belton, 1988). It quickly became the primary lepidopteran pest of nectarines and peaches, and remained as such until the oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), was accidentally introduced (Daane et al., 1993). Along with the navel orangeworm, *Ameylois transitella* (Lepidoptera: Pyralidae), *A. lineatella* remains one of the main pests of almonds in California (Legner & Gordh, 1992). Today, *A. lineatella* is present in all the major growing areas of host trees in Asia, Europe and

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North America (Figure 1.1) (Marlatt, 1898; Jones, 1935; Bailey, 1948; Ahmad, 1988; Ponomarenko, 1990). When it was first discovered in North America, *A. lineatella* was erroneously believed to overwinter in the crowns of strawberry plants (Marlatt, 1898; Duruz, 1923). Although rectified later, this misunderstanding perpetuated by its appearance in Webster's dictionary under the definition of strawberry borer: "The root borer (*Anarsia lineatella*), a very small dark gray moth whose larv[ae] burrow both in the larger roots and crown, often doing great damage" (Webster's Revised Unabridged Dictionary, 1913).

1.2 Biology and crop damage

The entire life cycle of *A. lineatella* lasts from 31 to 44 days, except for the overwintering generation (Bailey, 1948). Adults live approximately two weeks after eclosion, feeding on juice exuding from damaged fruit and available water sources, and mating multiple times (Bailey, 1948). Adults are approximately 1 cm in length and silver grey in colour (Bailey, 1948). The forewings have alternating white and gray scales with two near-black spots at the costal margin of the wing: one in the middle and one close to the base (Ponomarenko, 1990). A gray stripe connects three short transverse streaks that extend from the costal margin toward the alar apex, while longitudinal, near-black streaks appear on the background of the wing (Ponomarenko, 1990). The wings are narrow and fringed with long hairs and have a wingspread of 13 - 14 mm (Bailey, 1948; Ponomarenko, 1990). The eyes and antennae are black. When resting, the moths position their wings and antennae along their back (Bailey, 1948). The palps differ morphologically between the sexes; females have a horn-like structure on their palps that is absent in males (Bailey, 1948; Park & Ponomarenko, 1996).

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Males and females are phototactic, anti-geotactic, and "hop" a lot, in addition to flying. How far adult moths can fly has not been recorded; however, from field work, it is believed that *A. lineatella* cannot, or does not, fly any long distances in the absence of foliage (Sziraki, 1984; Ahmad, 1989). The preoviposition period ranges from 1 - 4 days (Bailey, 1948). Once mated, females oviposit their eggs, normally the following night, but have been observed ovipositing during the day (Bailey, 1948); oviposition lasts up to 2 weeks (Ponomarenko, 1990). A female can lay up to 100 eggs (Bailey, 1948). Eggs are laid in clusters of 2-5 eggs on terminal shoots, bark, the base of buds, fruits and in leaf axils (Bailey, 1948; Sarai, 1966; Ponomarenko, 1990).

Freshly oviposited eggs are small, white, iridescent, ovoid structures (Marlatt, 1898; Bailey, 1948). The eggs are approximately 0.4 mm long and 0.2 mm wide, with reticulations covering their surface (Bailey, 1948; Marlatt, 1898). They are attached to the substrate by a glue-like substance, and become deep orange before the larva hatches (Marlatt, 1898; Bailey, 1948).

Newly hatched larvae are 0.5-1 mm long (Marlatt, 1898; Bailey, 1948), and have a light yellow-to-brown body colour and black head (Marlatt, 1898; Sarai, 1966). Larvae develop through 4 - 5 instars (Bailey, 1948). Fully grown instars are about 13 mm long and reddish-brown in colour, with cream-coloured intersegmental membranes (Bailey, 1948; Sarai, 1966).

Overwintering 1st and 2nd instars larvae feed to some extent and moult at least once before they emerge in the spring, typically at the balloon stage (first flowers with petals forming) of peach flower development (Treherne, 1923; Proverbs, 1954). When overwintering larvae emerge from their hibernacula, they feed on buds and developing

twigs, causing wilt or, in extreme cases, death of young trees (Duruz, 1923; Treherne, 1923; Bailey, 1948; Summers & Price, 1959). First and second instars spin a small white web to cover themselves (Marlatt, 1898; Ponomarenko, 1990). While many larvae attempt to enter the green fruit, few are able to penetrate the fruit successfully at this stage (Duruz, 1923; Treherne, 1923; Bailey, 1948; Summers, 1955). Summer generation larvae also feed on shoots, but more so on developing fruit and their kernels (Treherne, 1923; Ponomarenko, 1990; McElfresh & Millar, 1993). Most damage and highest trap captures of adult moths have been found in the upper levels of trees (Rice & Jones, 1975; Ahmad & Khadhum, 1986; Weakley et al., 1990). Burrowing by *A. lineatella* larvae allows weaker borers, such as *A. transitella*, to penetrate developing fruit (Curtis, 1983). The presence of feeding *A. lineatella* larvae within fruit can be detected by frass and sting marks on the fruit surface (Duruz, 1923; Treherne, 1923; Ponomarenko, 1990). *Anarsia lineatella* consume stony fruits of the *Prunus* genus (Family Rosaceae), including: peach, prune, almond, nectarine, plum, sweet cherry, and sour cherry (Bailey, 1948; Summers, 1955; Ponomarenko, 1990). Larvae of the summer generations may construct a hibernaculum from which they later leave to forage before constructing and entering another hibernaculum to pupate (Marlatt, 1898; Price & Summers, 1961; Ponomarenko, 1990). Larvae that hatch later in the summer and feed on fruit develop into adults, whereas those that feed on bark begin building hibernacula (Sarai, 1966).

Fully grown larvae pupate in small cracks in the tree bark, folded leaves, and sometimes within fruit (Marlatt, 1898; Treherne, 1923; Ponomarenko, 1990). When they pupate in wood, they create "chimneys" which are small reddish-brown tubes, consisting

of bark pieces fastened together with silk that protrudes from the bark surface (Duruz, 1923; Treherne, 1923). The purpose of this structure is unknown (Duruz, 1923).

The pupa is approximately 5 mm long and has a light tan colour (Duruz, 1923; Sarai, 1966; Ponomarenko, 1990) which turns from dark reddish brown to dark black-brown as it ages. The pupal stage lasts approximately 14 days for first generation insects and 2 - 4 days for summer generations, depending on temperature (Duruz, 1923).

In the course of the year, 3 - 4 generations have been observed in California (Price & Summers, 1961), and 2 - 3 in British Columbia (Sarai, 1966). Generations are not always distinct, because oviposition times often overlap, giving the impression that *A. lineatella* flight is continuous throughout the summer (Bailey, 1948; Brunner & Rice, 1984).

1.3 Control measures for peach twig borers

Organophosphate insecticides and dormant season oils for overwintering larvae are the recommended controls for *A. lineatella* (Zalom et al., 1992), and further applications of insecticides are used against subsequent generations (Summers, 1955; Rice et al., 1972). For almonds, Dipel™ at bloom and Lorsban™ at hull split provide good control (Cline, 2004). Due to the residual nature of insecticides, sprays cannot be applied safely at the ripening fruit stage, when much damage takes place (Summers, 1955). Moreover, insecticide resistance has occurred (Summers et al., 1959), and targeting the exact time for applications of chemical sprays can be very difficult (Rice & Jones, 1975). Because neonate larvae of summer generations immediately bore into shoots or fruit, they rarely ingest a lethal dose of insecticide, but overwintered larvae can be targeted as they will feed on several shoots before maturation (Barnett et al., 1993).

Unfortunately, organophosphate pesticides kill natural enemies of other pest insects (Pickel et al., 2002), such as green peach aphids (Tamaki, 1973), which can lead to other pest problems.

Bacillus thuringiensis Berliner is used against larvae emerging from overwintering hibernacula (Barnett et al., 1993), with 2 - 3 sprays at bloom appearing effective (Diver & Mumma, 2003). Dormant season applications of nematodes decrease population levels of *A. lineatella*, but not to a commercially acceptable level (Agudelo-Silva et al., 1995). *Anarsia lineatella* has numerous natural enemies (Bailey, 1948; Thompson, 1966; Diver & Mumma, 2003; Trandafirescu et al., 2004). The encyrtid wasp *Copidosoma pyralidis,* also known as *Paralitomastix pyralidis*, is a substantial parasitoid, infecting from 60 - 90% of developing larvae (Velimirovic, 1974; Trandafirescu et al., 2004). Both *C. pyralidis*[1] and *Gonizus pakmanus*[2], were introduced into the United States as control measures against *A. lineatella*. However, despite the presence of these and multiple other predators, *A. lineatella* populations still cause extensive damage.

Synthetic sex pheromone (Roelofs et al., 1975), has been tested for monitoring population densities, and for controlling populations by means of pheromone-based mating disruption (see Chapter 2); however mating disruption proved commercially ineffective against *A. lineatella* (Ahmad & Khadhum, 1986; Nicolli et al., 1990; Mayer & Lunden, 1996; Cravedi, 2000; Pickel et al., 2002).

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[1] Released into California, from France, in 1931 (Bailey, 1948).
[2] Released into the United States in 1985 (USDA, 1985).

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Figure 1.1. Worldwide distribution of *Anarsia lineatella*. Shading indicates countries in which host trees are attacked by *A. lineatella*.

Table 1.1 Moth species utilizing sound as part of their sexual communication

system.

FAMILY	GENUS AND SPECIES	REFERENCE
Arctiidae	*Cycnia tenera*	Conner, 1999
Arctiidae	*Euchaetes bolter*	Simmons & Conner, 1996
Arctiidae	*Euchaetes egle*	Simmons & Conner, 1996
Arctiidae	*Lycomorpha pholus*	Mumma & Fullard, 2004
Arctiidae	*Syntomeida epilais*	Sanderford & Conner, 1990
Noctuidae	*Amphipyra perflua*	Laphsin & Vorontsov, 2000
Noctuidae	*Autographa gamma*	Skals et al., 2003
Noctuidae	*Hecatesia exultans*	Alcock & Bailey, 1995
Noctuidae	*Heliothis armigera*	Xue et al., 1996
Noctuidae	*Mythimna separata*	Xue et al., 1996
Noctuidae	*Rileyana fovea*	Surlykke & Gogola, 1986
Nolidae	*Bena bicolorana*	Skals & Surlykke, 1999
Nolidae	*Psudoips prasinana*	Skals & Surlykke, 1999
Pyralidae	*Achroia grisella*	Spangler et al., 1984
Pyralidae	*Corcyra cephalonica*	Spangler, 1987
Pyralidae	*Ephestia cautella*	Trematerra & Pavan, 1993
Pyralidae	*Ephestia kuehniella*	Trematerra & Pavan, 1993
Pyralidae	*Galleria mellonella*	Spangler, 1988
Pyralidae	*Symmoracma minoralis*	Heller & Krahe, 1994
Pyralidae	*Tineola bissiella*	Takács et al., 2003

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MALE AND FEMALE SONIC SIGNALS: EVIDENCE, CHARACTERIZATION AND BEHAVIOURAL ACTIVITY

2.1 Introduction

The sex pheromone of female *A. lineatella* is a 2-component blend, consisting of

(*E*)-5-decenyl acetate (*E*5-10:OAc) and (*E*)-5-decenol (*E*5-10:OH) in a 7:1 ratio (Roelofs

et al., 1975). No other sex pheromone component has been elucidated (Millar & Rice,

1992; 1996). Pheromone derived from scales of females elicit contact by males (Schlamp

et al, 2005), but does not enhance long-range attractiveness of the sex pheromone. Traps

baited with live female *A. lineatella* captured significantly more males than traps baited

with synthetic sex pheromone (Schlamp, 2005), suggesting that females use additional

means of communication. Moreover, reduced pheromone emission by females in the

presence of males (Schlamp, 2005) suggests that males communicate their presence to

females. If true, the males' communication signals are unlikely to be pheromones. When

males fly upwind toward calling females, any potential male pheromone, or body odour,

would not be perceived readily by females. Moreover, large quantities of pheromone

may increase the distance over which potential mates are recruited (Schlyter, 1992), but

at short range may hinder rather than help mate-foraging insects to pinpoint the micro-

location of signallers. Instead, visual displays or bioacoustic signals could allow males to

announce their arrival prompting females to lower pheromone emission, or respond in

another way. Considering that *A. lineatella* is a darkly coloured, nocturnal moth,

signalling through visual displays would be rather difficult. However, bioacoustic signals

would be useful because their perception by females would not be as dependent on wind

direction as pheromones, and they might readily reveal the micro-location of the signaller. In this chapter, I will test the hypothesis that male and female *A. lineatella* use bioacoustic signals, in addition to pheromone, during sexual communication.

2.2 Materials and Methods

2.2.1 Experimental insects

Adult *A. lineatella* were obtained from a colony started in 2000 with moths from the southern interior of BC, Canada. The moths were kept in mesh-lidded 1-L glass jars in environmental chambers at 18-22 °C, 70-80% RH, and a 16L:8D photoperiod. Larvae were reared on diet (Sidney, 2005) adapted from McElfresh (McElfresh & Millar, 1993). Pupae were transferred to 9-cm Petri dishes. Adults were collected daily, separated by sex, and kept in separate Petri dishes until they were bioassayed (3 - 4 days old), after which time they were transferred to the environmental chambers described above.

2.2.2 Equipment for sound recording and playback

Sound recordings employed: a) a high-performance ultrasonic microphone (BT 1759; sensitivity 40 mV/Pa; frequency response: 100 Hz to 10 kHz; Knowles Acoustics, Itasca, Illinois, USA); b) AKG CK 61-ULS condenser microphones [sensitivity: 20.0 mV/Pa; flat frequency response: 20 Hz to 20 kHz +/- 1 dB (flat frequency response: 200 to 16,000 Hz +/- 0 dB)]; AKG Acoustics, Nashville, Tennessee, USA); c) signal amplification of 800 times with a National Instruments (NI) SC 2040 differential amplifier (National Instruments Corporation, Austin, TX, USA 78759-3504); and d) a sampling frequency of 43.2 kHz. Recordings were saved to the hard disk of a Panasonic CF-47 Toughbook equipped with an NI data acquisition board (DAQcard-6062E; 12 bit,

500 kHz maximum sampling rate). Intensities of sound signals were measured with a

1551-C sound-level decibel meter (General Radio Company, Massachusetts, USA) that

was placed ~1 cm from the recorded insect. Sound intensity measurements were based

on the wingbeat frequency as the loudest signal component and found to be 12 dB above

ambient noise of 50 dB. The same sound intensity was used in playback recordings in all

laboratory and field experiments. Signals were played back through Sennheiser 70

headphone speakers (Sennheiser Electronic Corporation, Old Lyme, CT, USA; matched

+/- 1 dB flat frequency response: 10 to 39,500 Hz, 0.05% THD) connected to laptop

computers with software programs developed in LabVIEW (NI).

2.2.3 Acquisition of acoustic signals

Sounds were recorded under red light 2 h before and until the end of the

scotophase, the period of sexual communication (Rice & Jones, 1975; Schlamp, 2005).

A sitting, pheromone-emitting female (Figure 2.1), or a flying male, were tethered near

(1-2 cm) a microphone using a strand of human hair tied directly behind the head so as

not to impede movements of antennae or wings. The female's behaviour was videotaped

with a Sony digital handycam (Model DCR-VX1000) acquiring 22 pictures per second to

an Intel Pentium 2.54 GHz computer equipped with an IMAQ PCI-1411 programmed

with Labview (NI). Concurrent acquisition of audio and picture files allowed me to

correlate sound and behaviour. Females were first exposed to silence, then either to

sound from live males or to 8-s recordings of male-produced sound, replayed repeatedly

after 1 s of silence. To investigate whether males and females might exchange signals,

and whether any exchange is triggered by specific characteristics of a signal, I analyzed

paired audio files for a) time of signal occurrence, and b) frequency components of the signal.

2.2.4 Capture of airborne pheromone

One day prior to conducting experimental replicates, 8-14 virgin females were placed in each of two Pyrex glass chambers (15.5 ID x 20 cm). An hour before the end of the 8-h scotophase, the headphone speaker and airflow in each chamber were turned on, exposing the females to playback of male-produced sound signals or silence (Experiment 1), or to white noise (sound uniformly distributed over all sonic frequencies) or silence (Experiment 2) (Figure 2.2).

Exposure to white noise in Experiment 2 determined whether potential changes in pheromone emission by females could be in response to random noise. To simulate dawn, when sexual communication takes place, a custom-designed computer program (Ray Holland, Electronic Supervisor, Science Technical Centre, Simon Fraser University, unpublished) increased the intensity of a 60-watt Philips light bulb from 0 to 600 lux over 30 min. in the last half of the experimental time. During the 60-min experimental period, a water aspirator drew air at 2 L/min through the chamber, and then through a glass column (140 x 10.1 mm ID) filled with Porapak Q (0.5 g, 50-80 mesh, Waters, Milford, Mass., USA). Volatiles were eluted with 1 mL of redistilled pentane to which an internal standard, (E)-8-undecenyl acetate (100 ng), was added. The eluent was concentrated to 100 μL under a stream of nitrogen, and aliquots were analyzed by gas chromatography-mass spectrometry (GC-MS) employing a Varian Saturn 2000 Ion Trap equipped with a GC column (30 m x 0.25 mm ID) coated with DB-5 (J&W Scientific, Folsom, California, USA). Results were analyzed using a Wilcoxon paired t-test (JMP 5.1).

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2.2.5 Field experiments

Field experiments were conducted in a commercial almond orchard near Willows, California (N 39° 52', W 122° 20') and a commercial apricot orchard near Cawston, British Columbia (N 49° 18', W 119° 5'). Experiments employed paired traps in each of three trees with 1 m between paired traps, and 20 m between trap pairs. Traps were made of 2-L milk cartons (Gray et al., 1984) coated on the inside with adhesive (The Tanglefoot Co., Michigan, USA). Traps were suspended 1-1.5 m above ground in a straight line, with a single "guard" trap at the beginning and end of the line, separated by 20 m from trap pairs. By random assignment, one of the two paired traps was baited with a rubber septum (The West Company, Pennsylvania, USA) impregnated with E5-10:OAc (1,000 μg) and E5-10:OH (100 μg) (stimulus 1), and the other with stimulus 1 plus sound previously recorded from female *A. lineatella* and played back at biologically relevant levels (see 2.2.2). The speakers for playback sound were connected to one of three laptop computers and amplifier systems, supplied with electricity from line-powered AC current (120 volts) (Cawston) or battery (MotoMaster Eliminator, Canadian Tire Corporation Ltd., Toronto, Canada) (Willows). Experiments were run from dusk until dawn (Cawston) and from midnight until dawn (Willows). Traps and functioning of sound equipment were checked at dusk (Cawston), and at 24:00, 03:00 and dawn (Cawston and Willows). Six and 12 trap-pair replicates were run in Willows and Cawston, respectively. Trap captures of moths were analyzed using ANOVA ($\alpha = 0.05$) (JMP 5.1).

2.3 Results

Tethered flying males produced sound signals at 60 +/- 10 Hz (wingbeat), 114 +/-
10 Hz, 2.5 +/- 3 kHz, and 10 +/- 5 kHz (Figures 2.3, 2.5). Tethered females exposed to
playback of male signals, or live males, produced sound signals at 50 +/-10 Hz
(wingbeat), 2 +/- 5 kHz, and 14 +/- 5 kHz (Figures 2.4, 2.5). These reply signals were
interspersed with signals from males, up to 15 times per second (Figure 2.6). There was
neither a pattern of signal exchange, nor evidence for specific signal components
triggering such exchange in plots depicting the time of signal occurrence and the
frequency components of a signal (Figure 2.7). Pheromone emission by females
significantly decreased in the presence of played-back male signal recordings (Figure
2.8), but was not affected by white noise or silence (Figure 2.9).

In the presence of silence, females remained motionless. However, after exposure
to the male sound (live or played-back), females fanned their wings, jumped, and
engaged in short, looped flights, as depicted in Figure 2.10.

In Willows (Figure 2.11, Experiment 3) and Cawston (Figure 2.11, Experiment
4), traps baited with synthetic sex pheromone and played-back recordings of female-
produced sonic signals captured two to three times as many males as traps baited with
pheromone alone.

2.4 Discussion

My data show that *A. lineatella* use bioacoustic signals as part of their sexual
communication system. This conclusion is based on the findings: 1) that male sonic
signals provoke sonic reply signals from females (Figure 2.4) and lower the females'

pheromone emission (Figure 2.8), and 2) that playback recordings of the females' reply

signals plus pheromone are more effective in attracting males than pheromone alone

(Figure 2.11). These results might explain the stronger attraction of males to live females

than to synthetic sex pheromone (Schlamp, 2005).

When both female and male insects produce sound, males commonly initiate the

exchange of signals (Robinson, 1990). This applies to *A. lineatella*. Females remain

silent until they receive a male's signal. This tactic may help them minimize attraction of

predators that eavesdrop on bioacoustic communication of potential prey (Cade, 1976;

Burk, 1982; Acharya, 1995; Skals et al., 2003). Females also gain the opportunity to

judge a male's fitness based on his acoustic signals (Wagner et al., 1995; Jang &

Greenfield, 1996; Tuckerman et al., 1993; Jia et al., 2001).

The signal reply latency [sensu Bailey & Hammond (2003)] in *A. lineatella*

appears exceptionally quick, with up to 15 "signal exchanges" per second between a male

and a female. This would surpass the fastest known reply latency (15 ms) between

"conversing" bush crickets, *Ancistrura nigrovittata* (Orthoptera: Phaeneropteridae)

(Dobler et al., 1994). However, with many of the male- and female-produced signals

overlapping in *A. lineatella* (Figures 2.6, 2.7), the entire signal train may be a means of

exchange, rather than individual signals. Insects that exchange signals typically use

established signal trains (Surlykke & Gogala, 1986; Trematerra & Pavan, 1995; Jang &

Greenfield, 1996; Jones et al., 2002). The signal repertoire may vary but the set nature of

the signals and replies remains the same (Sanderford & Conner, 1995; McBrien et al.,

2002). None of the recordings from female and male *A. lineatella* revealed characteristic

sets of signals, or patterns of signal exchange in terms of time of, or between signals

(Figures 2.6, 2.7). Similarly, there was no evidence for specific signal components triggering reply signals (Figure 2.7). The signals in both analyses (Figures 2.6, 2.7) were so short that they are unlikely to contain detailed information (Bailey, 2003; Bailey & Hammond, 2003; Dobler et al., 1994). Rather, they may be used by the signaller to brief the receiver about its respective location, much like remote-control car keys that allow owners to locate their car in a parking lot through brief bursts from the car horn. As such, female-produced signals would facilitate the male's orientation toward her, whereas male-produced signals would keep the female informed about his progress. This rapid exchange of signals may fall under the category of dialogue (Greenfield, 1994; Buck & Case, 2002), with loosely structured signal exchanges instead of the category of duet (Bailey, 2003), with predictable temporal associations between caller and replier. Analyses of paired audio files from males and females (Figures 2.6, 2.7) did not reveal a duet type temporal pattern. However, there are several factors, including the intensity of signal components, the males' flight progress (or lack thereof), and the females' mate choice, that could mask a potential temporal pattern and may have complicated the interpretation of results.

Unlike the loud signals emitted by relatively large insects such as crickets and cicadas, sounds produced by small insects are relatively quiet (Bennet-Clark, 1971). *Anarsia lineatella* falls into this latter category. Because the wingbeat is louder than the upper signals, it can be difficult to reveal all upper signal components. Even after filtering out the wingbeat signals, there is the possibility that a signal will not be visible. The detection of these upper signals may also be influenced by the male's orientation in flight. Although the male was tethered to stay within 1-2 cm of the microphone, he was

still able to fly in any direction. Thus, it is likely that some directional orientations were more conducive than others to signal recording. This may be comparable to a conversation between humans where the speaker is difficult to hear when not facing the listener. Another possible problem with the flight of a tethered male is that he does not progress toward the female. Thus, the female may choose not to reply to otherwise good signals.

The female's mate choice may also affect her signalling. Assuming that a female may assess signal characteristics of an approaching male, she may choose not to respond to a signal that appears to be of low quality, as reported in the katydid *Conocephalus nigropleurum* (Morris and Fullard, 1983). Conversely, a female that is anxious to mate may respond to even low-quality male signals. All these aspects may explain the lack of a pattern in the audio recordings of male and female pairs.

That female *A. lineatella* lowered pheromone emission in response to sonic signals from conspecific males (Figure 2.8) appears counterintuitive. However, high concentrations of pheromone hinder rather than help anemotactic orientation of foraging insects (Willis & Baker, 1984). With large amounts (100 ng) of pheromone in pheromone glands (Schlamp, 2005), it is likely that female *A. lineatella* emit significant quantities of pheromone during sexual communication. Finally, because female pheromone and sonic signals are synergistic, females can probably afford to reduce pheromone emission (Schlamp, 2005) while engaging in bioacoustic communication with approaching males.

Production of bioacoustic communication signals in *A. lineatella* appears associated with wing movement. Low frequency (< 60 Hz) components of the males'

signals were present continuously and can be attributed to the movements of wings. Upper frequency (> 2 kHz) components, in contrast, occurred intermittently (Figures 2.3, 2.7), and thus are likely decoupled from the mechanics of moving wings. Nonetheless, these signal components were recorded only from females and males that moved their wings, suggesting that the sound-producing organs are associated with or near the wings. The type and location of sound-producing and receiving organs in moths is diverse (Scoble, 1995; Conner, 1999). As examples, bollworms, *Heliothis zea* (Lepidoptera: Noctuidae), produce sound whereby their forewings strike each other during flight (Agee, 1971), whereas males of the noctuid *Thecophora fovea* rub a tarsal segment against a stridulatory swelling on the hindwing (Surlykke & Gogala, 1986). Sound is received by air-filled tympanal organs at the base of wings (Minet, 1988), on the thorax (Spangler, 1988), or abdomen (Scoble & Edwards, 1987; Cook & Scoble, 1992). How male or female *A. lineatella* perceive sound, and where the receptors are located, remains unknown.

Mate attraction and location in *A. lineatella* is unusually complex and appears to proceed in a sequence, as follows: 1) females emit long-range sex pheromone that attracts males (Roelofs et al., 1975); 2) approaching males emit sound "announcing" their arrival; 3) females lower pheromone emission, and emit sonic reply signals that help males orient toward them; 4) males alight near females and emit pheromone from hairpencils during courtship behaviour (unpublished); and 6) close-range scale-derived pheromone components from females induce contacts by males (Schlamp et al., 2005). Similarly complex sexual communication and elaborate exchange of pheromonal and sonic signals are known only from the greater wax moth, *Galleria mellonella* (Lepidoptera, Pyralidae)

(Spangler, 1987). Male *G. mellonella* produce sonic signals that cause conspecific females to emit a wingfanning signal which, in turn, provokes males to release pheromone that attracts females (Flint & Merkle, 1983; Spangler, 1987).

Control of *A. lineatella* populations in commercial orchards by means of pheromone-based mating disruption has not been very effective (McElfresh & Millar, 1993, Millar & Rice, 1992). I propose that a combination of pheromonal and bioacoustic signals could be developed for more effective control.

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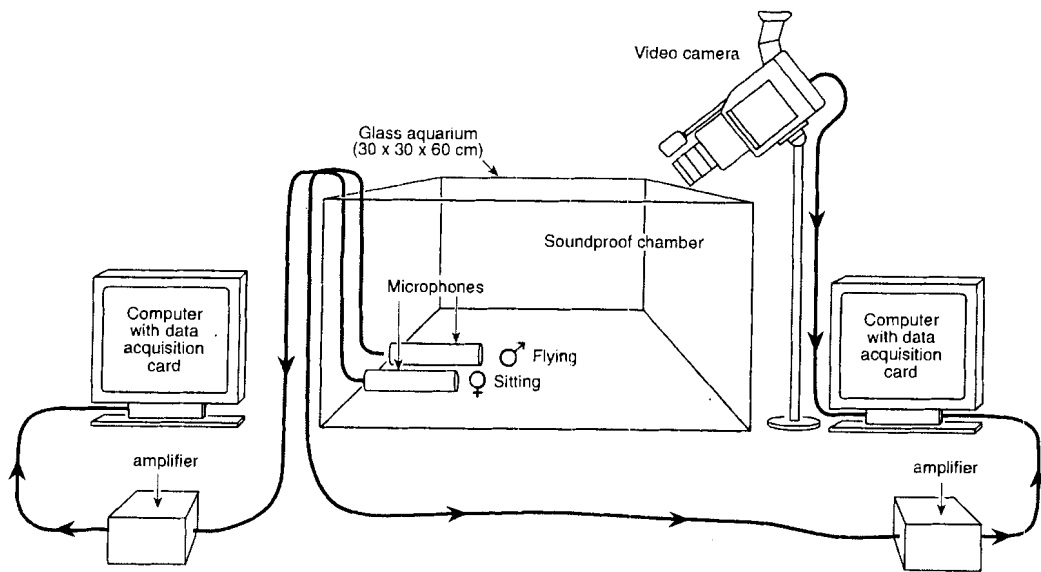
Figure 2.1. Graphical illustration of the experimental set-up for recording acoustic signals from female *Anarsia lineatella*.
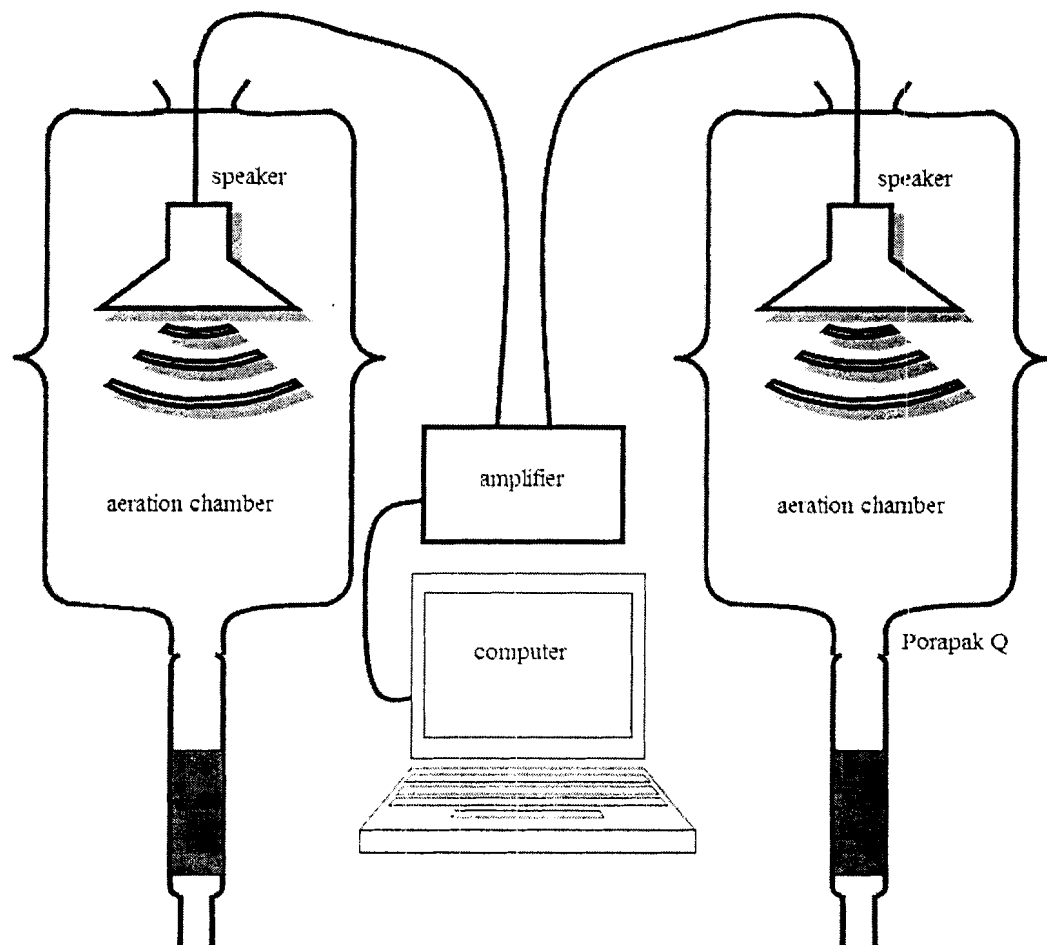
Figure 2.2. Graphical illustration of the experimental set-up for the capture of pheromone from female *Anarsia lineatella* exposed to male-produced sound or silence (Experiment 1), or to white noise or silence (Experiment 2).
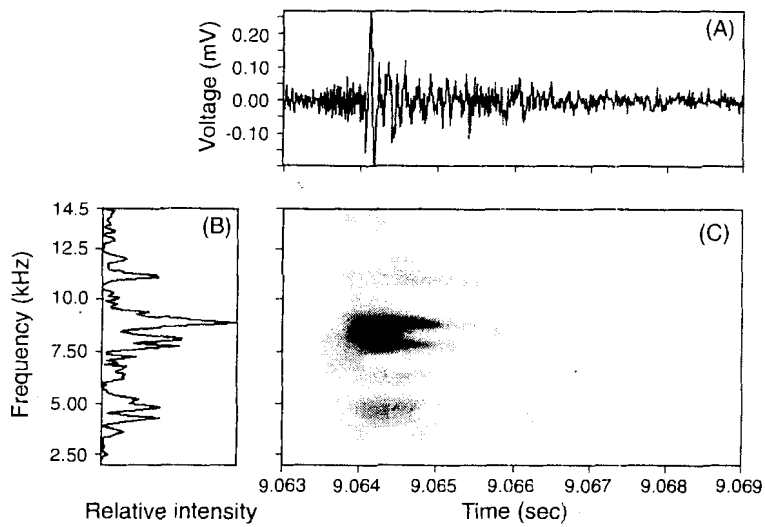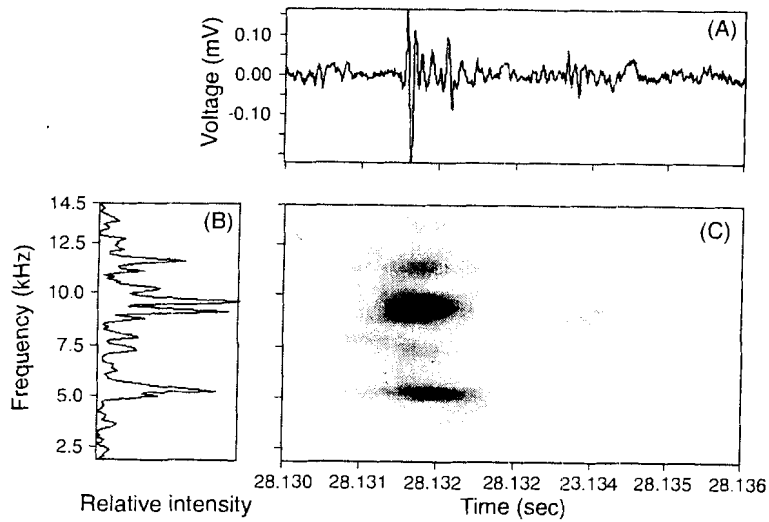
Figure 2.3. Analyses of waveform (A), frequency (B), and time-frequency sound intensity (sonogram) (C) of bioacoustic signals produced by one representative male *Anarsia lineatella* **during flight.**

Figure 2.4. Analyses of waveform (A), frequency (B), and time-frequency sound
intensity (sonogram) (C) of bioacoustic signals produced by one representative
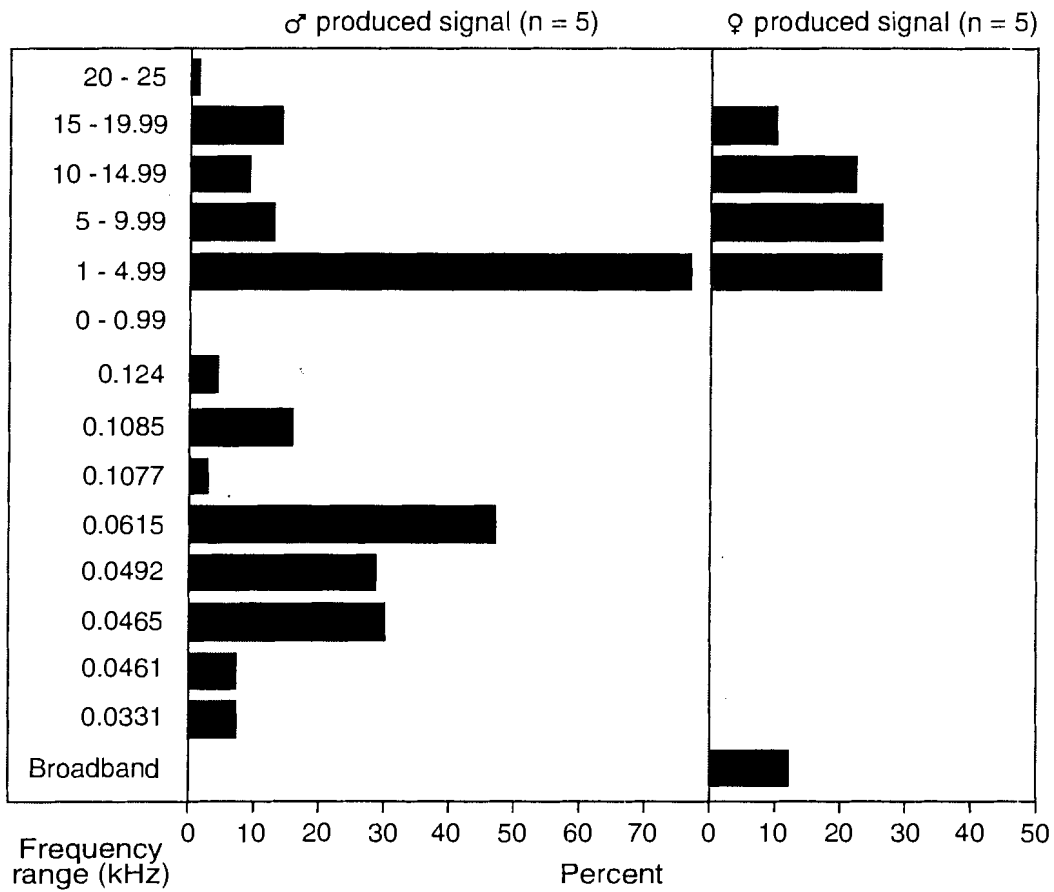female *Anarsia lineatella* in response to male-produced sound.

Figure 2.5. Frequency distributions of sonic signal components produced by male (left) and female (right) *Anarsia lineatella*. For male- but not for female-produced signals, two separate frequency distributions cover frequencies > 1 kHz (top) and < 1kHz (bottom).
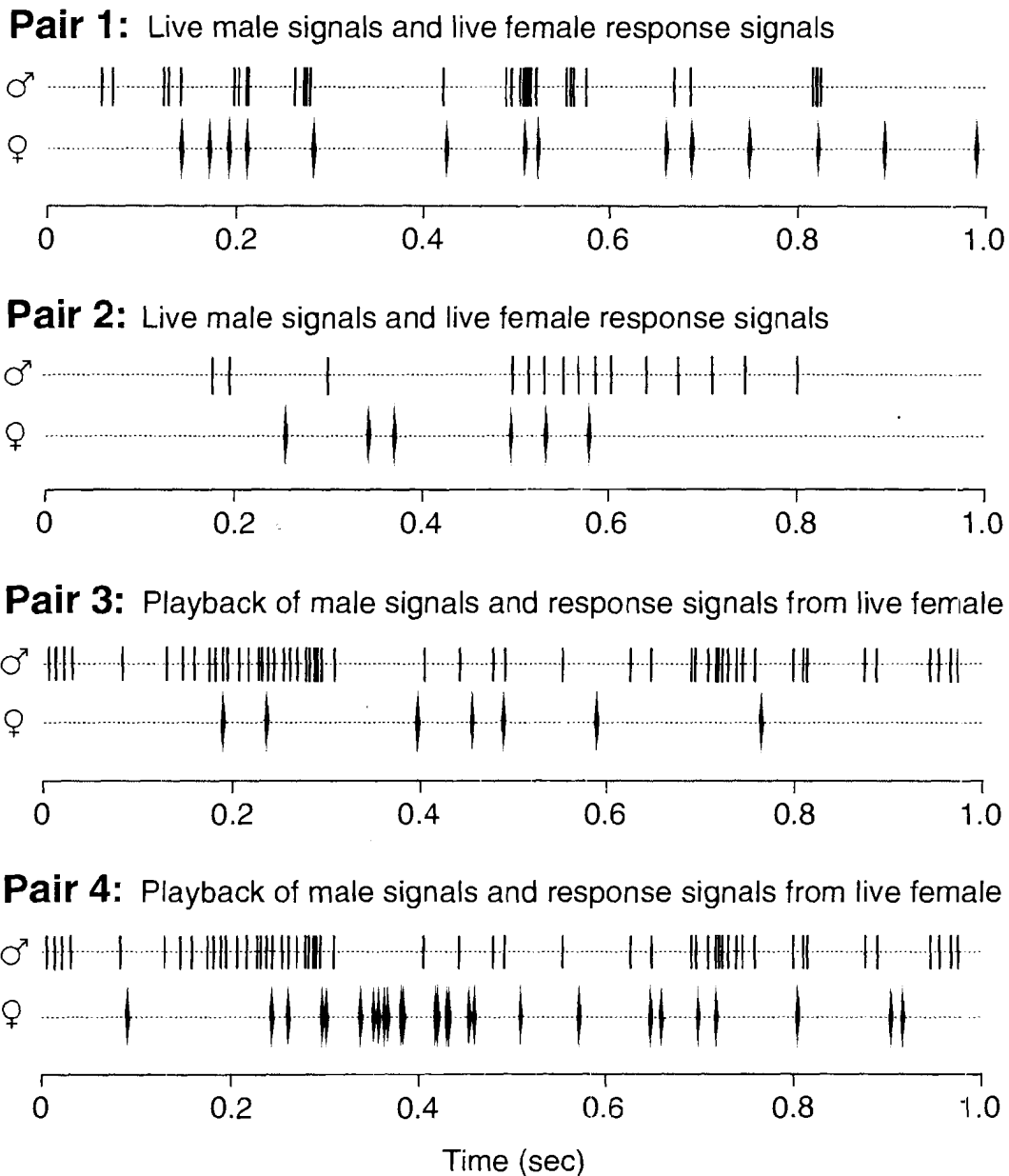
Pair 1: Live male signals and live female response signals

Pair 2: Live male signals and live female response signals

Pair 3: Playback of male signals and response signals from live female

Pair 4: Playback of male signals and response signals from live female

Time (sec)

Figure 2.6. Synchronized recordings of sonic signals produced by a live tethered female *Anarsia lineatella* in response to a tethered flying conspecific male (Pairs 1, 2) or in response to playback of male sonic signal recordings (Pairs 3, 4). Vertical lines represent a sonic signal comprising frequency components > 2.5 kHz.
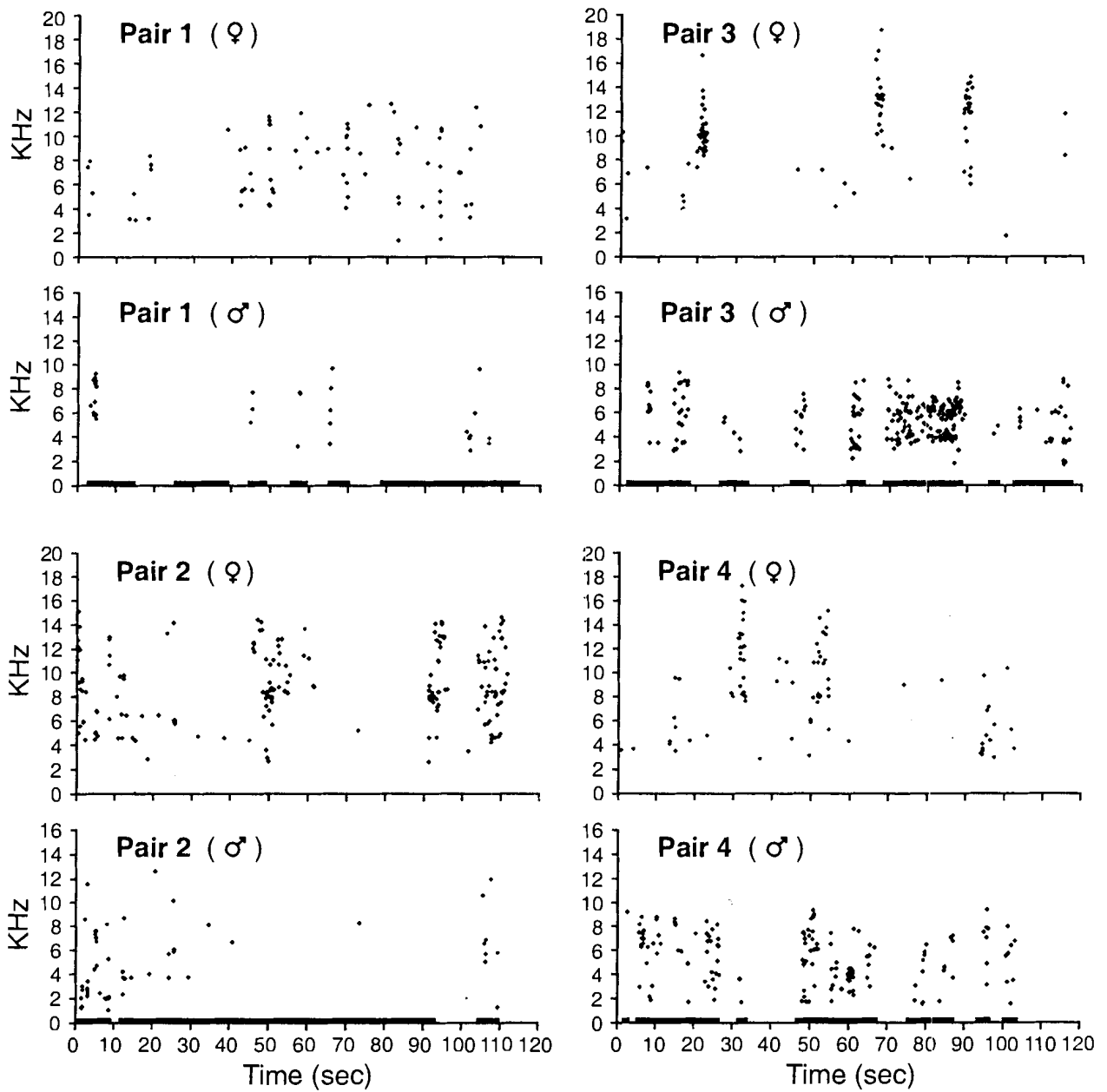
Figure 2.7. Synchronized recordings of sonic signals produced by a live tethered female *Anarsia lineatella* in response to a tethered flying conspecific male. Dots represent the dominant frequency component of the signal at that time. Horizontal black bars above the x-axis in the males' recordings depict time during which the male was flying. Note: all frequency components, except the wingbeat, are depicted.
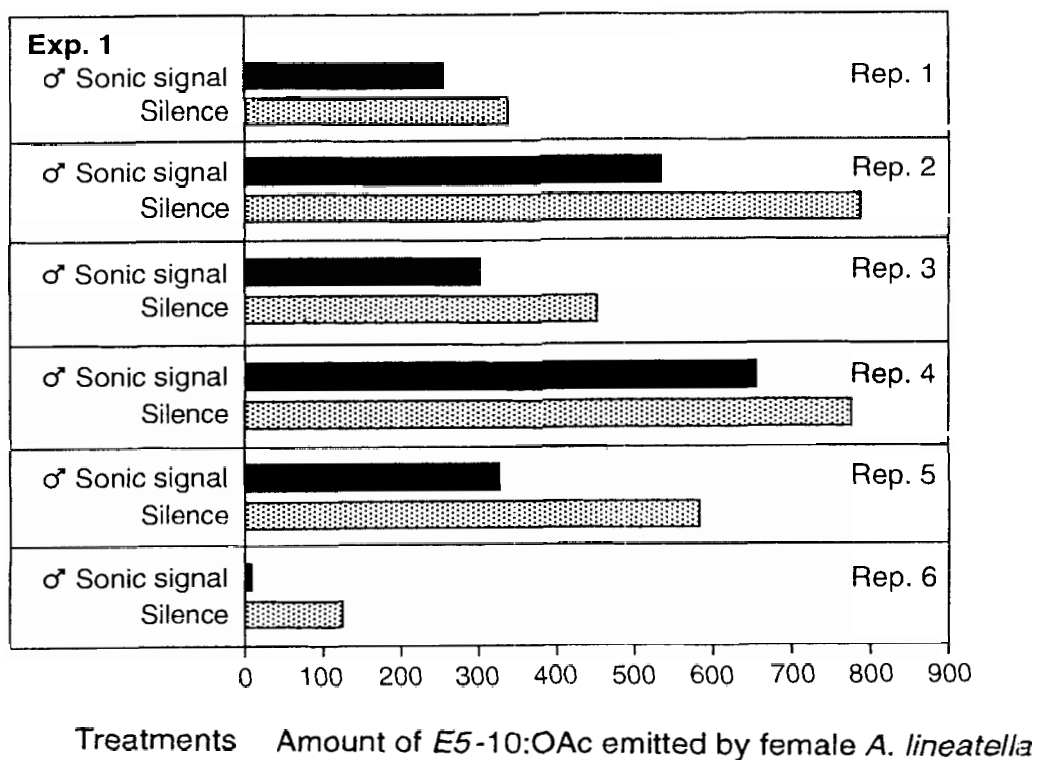
Treatments   Amount of *E5*-10:OAc emitted by female *A. lineatella*

Figure 2.8. Amounts (pg) of (*E*)-5-decen-1-yl acetate (*E*5-10:OAc) emitted by female *Anarsia lineatella* in response to playback of male sonic signals recordings or silence. In each of six replicates, less *E*5-10:OAc was emitted by females exposed to playback of male sonic signals than by females exposed to silence; Wilcoxsin paired t-test, *P* < 0.05
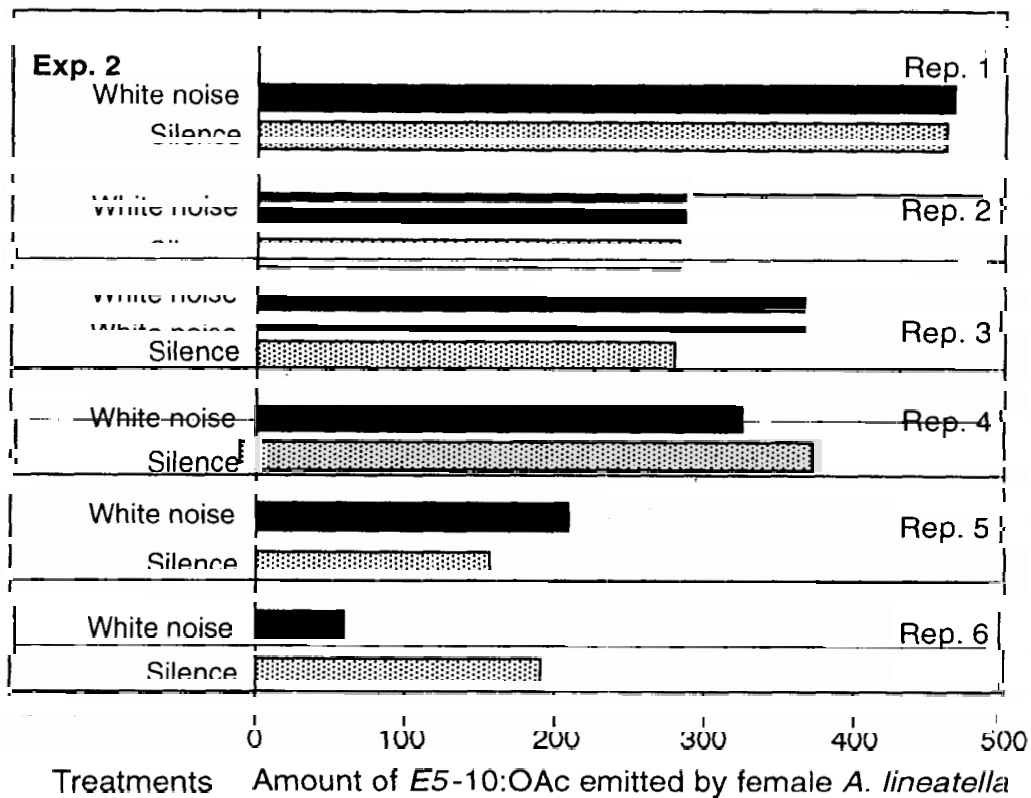
Figure 2.9. Amounts (pg) of (*E*)-5-decen-1-yl acetate (*E*5-10:OAc) emitted by female *Anarsia lineatella* in response to playback of white noise or silence. Emission of *E*5-10:OAc in response to white noise was not significantly reduced; Wilcoxsin paired t-test, *P* > 0.05
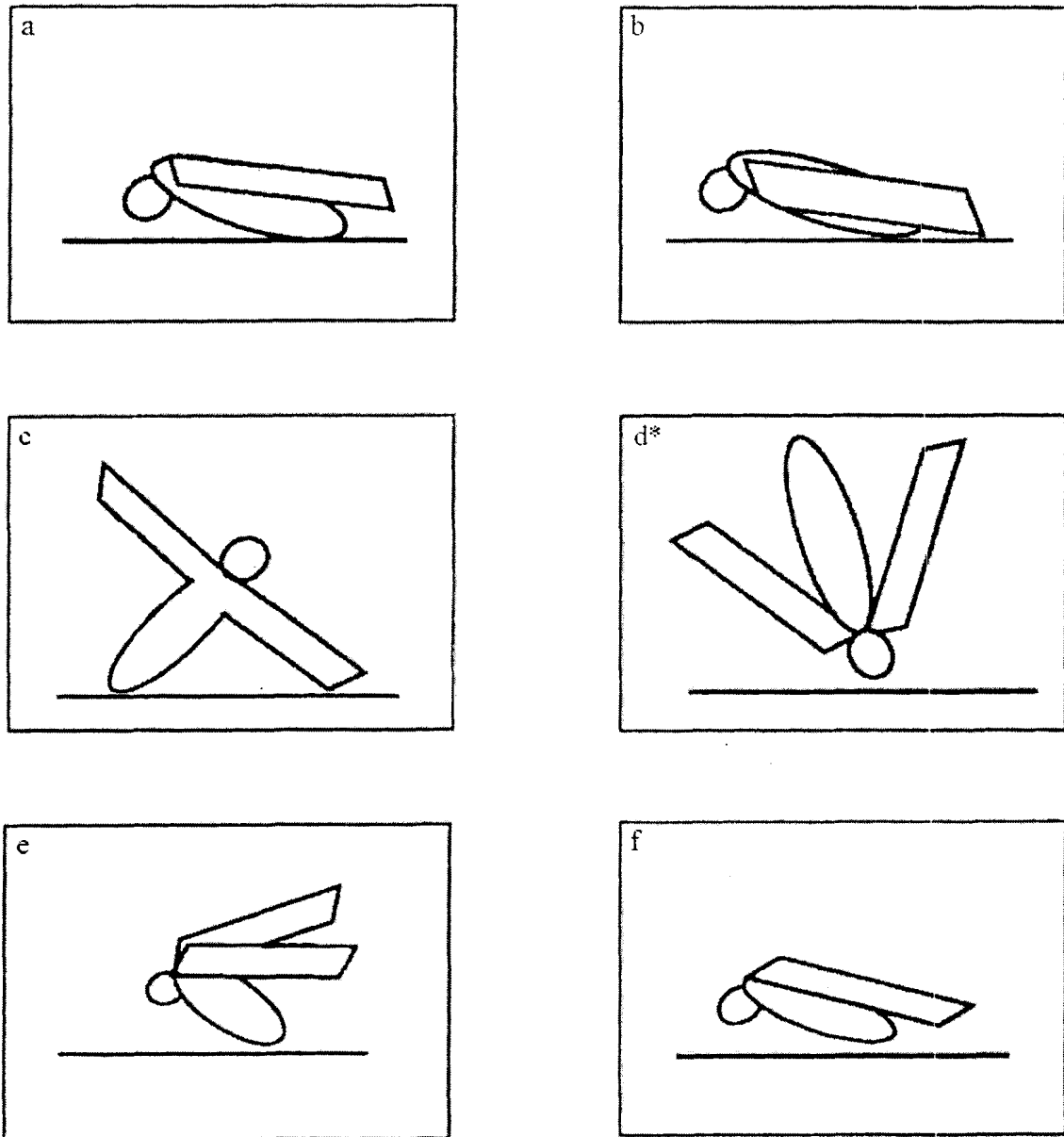
Figure 2.10. Graphical illustration of a behavioural sequence exhibited by a female *Anarsia lineatella* in response to a male-produced sonic signal. The asterik (*) demarcates the behaviour that is associated with the female's emission of a sonic reply signal. Drawings produced from synchronized video and audio recordings.
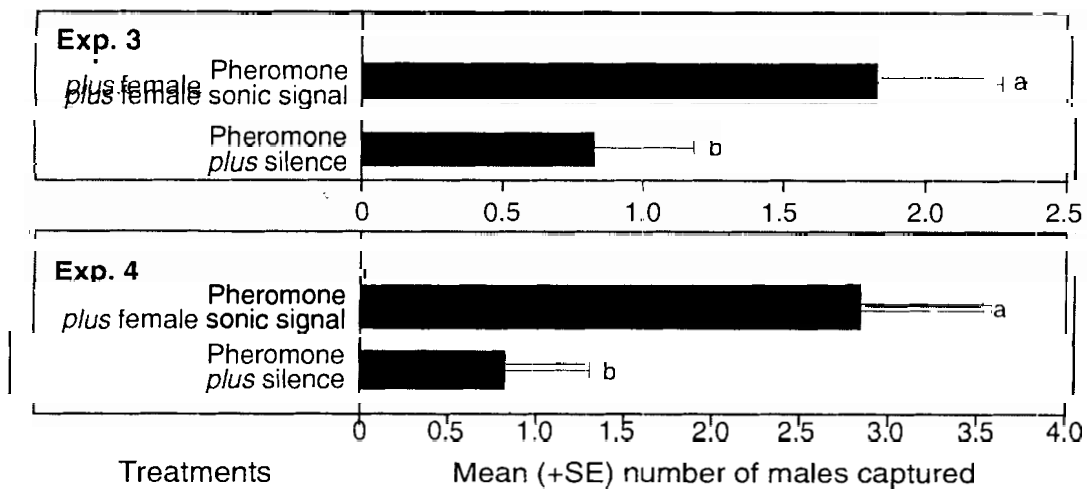
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Figure 2.11. Mean number (+ SE) of male *Anarsia lineatella* captured in Exp. 3 (Willows, California; June 21-30, 2004; 6 replicates) and in Exp. 4 (Cawston, British Columbia; July 15-22, 2004; 12 replicates) in traps baited with synthetic pheromone [(E)-5-decen-1-yl acetate (1000 µg) and (E)-5-decen-1-ol (100 µg)] alone or in combination with playback of female sonic signal recordings. In each experiment, bars with different letter superscripts are significantly different; ANOVA, $P < 0.05$.