Disruption of Gypsy Moth\textsuperscript{1} Male Sex Pheromone Behavior
by High Frequency Sound\textsuperscript{2}

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ENVIRONMENTAL ENTOMOLOGY
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ABSTRACT

Males of the day-flying moth *Lymantria dispar* L. (gypsy moth), while flying upwind toward a pheromone source, respond to high frequency (>15 KHz) sounds by deviating sharply from the established flight course and flying away rapidly. Pheromone-stimulated males fanning their wings while walking on the substrate "freeze" momentarily in response to high-frequency sound. The puncturing of both tympana eliminates auditory behavior, whereas perforation of only one tympanum causes highly directional flight course deviations toward the perforated (silent) side. The retention of ultrasonic sensitivity in this essentially day-flying species may be explained by the selective advantage from bat predation gained by individuals active near dusk.

In July 1976, while observing male *Lymantria dispar* L. (gypsy moth) behavior in response to its sex pheromone, it appeared that observer movement could terminate pheromone searching behavior prematurely. Preliminary investigation revealed that males were responding to the sound of snapping twigs and leaves underfoot and not to the visual cues presented by the observer. Males in free flight along the roadside (presumably non-pheromone activated) also appeared to respond by abrupt changes in their flight pattern to the breaking of hand-held twigs. Direct observation of response to auditory stimuli has not been reported in the Lymantriidae previously. Treat (1962) observed a significant reduction in light trap catch of unspecified species of lymantriids when ultrasound was broadcast from the trap surface. Secondly, it seemed curious that a species of mostly diurnal flight habits (Cardé et al. 1973) should possess a behavior known to increase survival among its noctuid relatives, who avoid bat predation by evasive maneuvers after the early detection of the bat's ultrasonic emissions (Roeder 1967). Other than the work of Turner and Schwarz (1914) on *Catocala* (Noctuidae) and observations of grass-dwelling pyralids (Treat 1955) there appeared to be no other reports of acoustic sensitivity to high frequency sound by moths that exhibit some day-flying behavior, even though their period of sexual activity is nocturnal. Finally, the observation that sound could terminate a pheromone-stimulated male’s searching behavior seemed important from 2 aspects. One, it implied that defensive (evasive) behavior can take precedence over sexual behavior. Secondly, from a practical viewpoint, to minimize anomalous results observers of *L. dispar* male sexual behavior should be aware of the auditory sensitivity of their subjects.

Materials and Methods

Analysis of Auditory Stimuli

For field observations, the sound source was a spring-steel clicker consisting of a plastic cone 10.5 cm long, 4.5 cm diam at the mouth containing a piece of spring steel (4.5 x 1.3 cm) which was distorted to make a single audible click by depressing a lever on the outside of the cone. Although effective in the field, in preliminary laboratory experiments the clicker appeared to evoke evasive behavior in only ca. 50% of the males, possibly due to a background of ultrasound generated by the wind tunnel apparatus. Therefore, metal keys on a key-ring shaken for 0.8±0.1 sec SD were used as a laboratory stimulus. Both stimuli were analyzed for their ultrasonic components using a Holgate ultrasonic detector, which transforms ultrasound into clicks audible through a set of headphones. A variable band pass filter on the detector was set at 5 KHz increments from 10–120 KHz and the clicks from the ultrasonic detector recorded onto a Sony TC66 portable cassette recorder. The recorded audible clicks then were displayed on a Tektronix model 5103N storage oscilloscope for analysis of their frequency and amplitude.

Male Response to the Sonic and Ultrasonic Components

A recording of the key jingling sound played back on a Sony TC66 portable tape recorder was analyzed for its ultrasonic content as in the previous section. Males then were tested for their response to a playback of the recorded keys (no ultrasonic component) and the real keys (strong ultrasonic component). The stimuli were of equal intensity at a mutual frequency of 15 KHz when the recorded keys were at 6 cm and the real keys at 40 cm from the males as measured by the ultrasonic frequency detector, and so these distances were used to test flying males as described in the following section.

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\(^1\) Lepidoptera: Lymantriidae.
\(^2\) Published as Journal Article No. 8183 of the Michigan State Univ. Agric. Exp. Stn. Received for publication June 21, 1977.
Males in Flight

In the laboratory, observations of male response to auditory stimuli were made using laboratory-reared individuals from the Gypsy Moth Development Laboratory, USDA. Moths were maintained in plastic cups (4 cm high, 3 cm base) with dampened filter paper for 2 days after emergence on a 16:8 L:D photoperiod regime at 25°C. A wind tunnel, 2.4×1.2×0.8 m constructed of plexiglass and aluminum was used to study auditory responses of flying males orienting to a pheromone source placed at the upwind end of the tunnel. A 0.7 m diam fan drove air through the tunnel at a rate of 0.7 m/sec and an exhaust fan at the downwind end removed pheromone-laden air from the room and building. The pheromone source consisted of a filter paper disc (2.4 cm diam) impregnated with 100 ng of (+)-cis-7,8-epoxy-2-methyloctadecane (Mori et al. 1976), inserted into a clip on the end of a straight copper wire with a cork stopper as a base. This dispenser (9.5 cm high) then was placed on a plexiglass stand (7 cm high) at the extreme upwind end of the tunnel.

To initiate flight, a male was removed from its cup during photophase and placed in a cylindrical screen cage (20 cm long, 10 cm diam) open at the upwind end, stationed on a plexiglass stand (9 cm high) in the tunnel 1.7 m downwind of the pheromone source. Once a pheromone-responding moth was airborne, a motor-driven floor of alternating white and green stripes was used to control a male's progress toward the source, due to their tendency to use the apparent movement of the ground as a guide in upwind orientation (Kennedy and Marsh 1974). This procedure was necessary to ensure that enough of a time interval had elapsed before males reorienting to the source were retested with the auditory stimulus.

An airborne male was allowed to orient to 10 cm downwind of the pheromone-impregnated filter paper. Orientation at this distance from the source was very precise, usually with lateral oscillations of less than 5 cm magnitude and forward progress of only a few cm per second. Either the recorded or real key jingling stimulus was then given to the male from 6 cm and 40 cm, respectively, from the immediate left of the male. If a male's position changed more than 20 cm immediately after the stimulus had been given then the initial direction of the deviation (combinations of up, down, left, right, forward, or backward flight) was noted. Whenever possible the same male was optically guided downwind, at least 10 sec allowed to elapse, and then presented with a stimulus from a different direction. Many times males would not re-enter the pheromone plume, and so unequal numbers of test directions and males appear in the results. To provide an experimental control, the keys were wrapped with a rubber band to prevent sonic and ultrasonic jingling and shaken 40 cm from the left of the males in flight.

Males With Punctured Tympanic Membranes

Each of the 2 tympanic membranes of L. dispar males are located in a smooth sclerotized channel on the postero-lateral margin of the metathorax (Fig. 1). They are directed obliquely posteriorly toward hair-covered counter-tympanic hoods on the 1st abdominal segment which cover all but the extreme lateral edges of the scaleless channels. A sensillum similar to that described by Roeder (1967) for Agrotis ypsilon (L.) (Noctuidae) appears to be attached to each membrane at a light-colored region in the center of the membrane, and to extend internally into the tympanic air sac (Fig. 1). Unanaesthetized males were hand-held under a binocular microscope, the tympanic hoods retracted with an insect pin, and one or both tympanic membranes and associated sensilla destroyed with the pin. Sham operated males were treated identically to operated males except that the membrane and sensillum were not touched by the pin. The operated males were tested for response to the real key jingling stimulus as described above for intact males.

Females in Calling Position

Female L. dispar were examined and found to possess tympanic membranes along with apparently intact sensilla in the same location as the male. However, the tympanic hoods appeared to be much more membranous and lacked the cup-like form found in the male. Consequently, they appeared to close nearly completely the channel to the tympanic membrane with their flaccid form.

Females extruding their ovipositor (calling) were tested for response to the key jingling stimulus. Twenty-five females maintained in 31×22×11-cm covered plastic boxes were tested at various times during photophase by giving the stimulus at a distance of 20 cm. Evidence of ovipositor retraction or other responses was noted.

Males Wing Fanning on Substrate

A typical response to pheromones in many species of male Lepidoptera is wing fanning in which a male maintains tarsal contact with the substrate and either is stationary or walks toward the source while rapidly vibrating its wings as if in flight. In our wind tunnel this behavior occurred both before flight and
after arrival at the pheromone source. Response to the auditory stimulus while wing fanning in both situations was investigated in the following manner. A single male in the pre-flight cylindrical cage was observed until wing fanning behavior had commenced and continued for 20 sec. The auditory stimulus then was presented at a distance of 40 cm and the response observed. A cessation of wing fanning within 1 sec was considered a positive response.

Usually the male became quiescent or "froze" with its wings in the resting position (held flat over the abdomen parallel to the substrate) for a period of seconds. This interval was measured using a stopwatch. When fanning had resumed for a period of not less than 10 sec, the male was tested again.

Males not tested in pre-flight fanning were allowed to orient to and reach the pheromone source, upon which they often landed and fanned their wings while walking. When a male made tarsal contact with the filter paper the auditory stimulus was presented from 40 cm away and the response recorded. No less than 10 sec were allowed to elapse between the resumption of wing fanning and presentation of subsequent stimuli. Many males leaving the pheromone stimulus were induced to fly downwind using the moving floor and then allowed to orient upwind to the pheromone source to be retested.

Field Observations

Observations of feral male auditory behavior both during flight in response to sex pheromone and in free flight (apparently not responding to pheromone) were made at Pomfret, Conn. on July 22 and 23, 1976. The sound source was the spring steel clicker described earlier. Males flying toward a synthetic pheromone source (Cardé et al. 1977) were tested with a single click when they were within ca. 1 m of the source. Male flight before testing was usually very steady, consisting only of small "casting" or oscillating movements, and so the response criterion, greater than 1 m deviation from the flight path, was easily observed. Videotape recordings of the responses were made using a Sony AVC-3450 camera and videorecorder deck and later replayed on a Con-

Fig. 1.—Postero-lateral view of the tympanic membrane (TM) of L. dispar male with sensillum (S) visible as a thin white line projecting inward toward the tympanic air space. The scaleless cuticular channel (Ch) is visible in the foreground. The location of the counter-tympanic hood is marked (TH).
Table 1.—The number of responses to real and recorded key jingling stimulus presented at intensities equivalent at 15 KHz to male L. dispar flying in the laboratory wind tunnel at 10 cm from the pheromone source (N = 10 males for both stimuli).

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>No. of tests</th>
<th>No. of responses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real keys</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Recorded keys</td>
<td>35</td>
<td>0</td>
</tr>
</tbody>
</table>

- Response criterion was a greater than 20 cm deviation from the established flight course immediately (< 1 sec) after presentation of the auditory stimulus.

Table 2.—The initial direction of response by L. dispar males with both tympanic organs intact to the auditory stimulus presented from 3 directions at 40 cm while the males were flying in the wind tunnel at 10 cm from the pheromone source.

<table>
<thead>
<tr>
<th>No. of stimuli and direction</th>
<th>To left</th>
<th>To right</th>
<th>Other</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 (45 males)</td>
<td>8**</td>
<td>57**</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>82 (30 males)</td>
<td>61**</td>
<td>2**</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>51 (26 males)</td>
<td>18 NS</td>
<td>16 NS</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>50 (25 males)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

- Response criterion was greater than 20 cm displacement from the established flight course immediately (< 1 sec) after presentation of the auditory stimulus.
- Significant left or right deviations mean that along with possible significant deviations in other directions, there occurred a significant leftward or rightward deviation. Responses marked "other" are those significant deviations from the flight course not containing a significant left or right component (e.g., straight upward or downward flight).
- For a given stimulus direction, the number of left-right deviations is significantly different from that proposed by the null hypothesis at the 0.05 level by \( x^2 \) (\( P < 0.01 \)). NS: Does not differ from the null hypothesis of equal numbers of left and right deviations by \( x^2 \) (\( P > 0.05 \)).
but for a significantly shorter period of time than males in the pre-flight cage. Some males also flew from the filter paper in response to the stimulus and it appeared (though not investigated directly) that whether a male flew or “froze” depended upon the degree of contact that was maintained by the tarsi with the substrate. Males “brushing” the dispenser with their legs appeared more likely to fly in response to the auditory stimulus than males walking while wing fanning.

Responses by Calling Females

No observable response was given by any of the calling females to the auditory stimulus. No evidence of retraction of the ovipositor was seen in response to the stimulus, but this behavior occurred frequently when the box was accidently jostled before or after testing.

**Table 3.** The initial direction of response by *L. dispar* males with one or both tympanic organs destroyed to the auditory stimulus presented from 3 directions at 40 cm while the males were flying in the wind tunnel at 10 cm from the pheromone source.

<table>
<thead>
<tr>
<th>Initial response direction*</th>
<th>To left</th>
<th>To right</th>
<th>Other</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of stimuli and direction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both tympana destroyed (N = 11 males)</td>
<td>20 From left</td>
<td>14 From right</td>
<td>22 From above</td>
<td>0 0 0 20</td>
</tr>
<tr>
<td></td>
<td>0 0 0 14</td>
<td>0 0 0 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right tympanum destroyed (N = 9 males)</td>
<td>10 From left</td>
<td>8 From right</td>
<td>42 From above</td>
<td>0 10 0 0</td>
</tr>
<tr>
<td></td>
<td>0 8 0 0</td>
<td>0 38 2 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left tympanum destroyed (N = 11 males)</td>
<td>21 From left</td>
<td>10 From right</td>
<td>44 From above</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>21 0 0 0</td>
<td>10 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operated (N = 10 males)</td>
<td>16 From left</td>
<td>12 From right</td>
<td>11 From above</td>
<td>0 11 4 1</td>
</tr>
<tr>
<td></td>
<td>0 9 2 1</td>
<td>5 4 2 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Response criterion was a greater than 20 cm displacement from the established flight course immediately (< 1 sec) after presentation of the auditory stimulus. Significant left or right deviations mean that along with possible significant deviations in other directions there occurred a significant leftward or rightward deviation. Responses marked “other” are those significant deviations from the flight course not containing a significant left or right component (e.g., straight upward or downward flight).
plume, to which they returned within a few seconds. For such males, repeated presentation of the stimulus seemed to cause a diminution or elimination of response to the sound stimulus.

**Discussion**

The fact that an ultrasonic stimulus can interrupt response to sex pheromone by causing evasive flight or cessation of wing fanning in males is to our knowledge the 1st report of its kind. Treat (1962) demonstrated the ability of ultrasound and recorded bat cries to interrupt phototactic flight to an ultraviolet light. In his study, among the species inhibited by the sound were unspecified members of the Lymantriidae. Eggars (1920) described the tympanic organs of individuals of the Lymantriidae including *Lymantria monacha* (L.), but did not test their responsiveness to sound.

The initial direction of the auditory response of flying males in the laboratory immediately following the sound stimulus was fairly predictable when a left or right side stimulus was presented to males with both tympana intact. The response was usually away from the stimulus, often with an upward component as well. That responding males use the relative intensities of the sound striking the left and right tympana to obtain directional information was indicated by the responses of males possessing only one intact tympanic membrane; their initial flight direction was always toward the atympanic (quiet) side, even when the sound originated from that side. Not surprisingly, bilaterally atympanic males were completely unresponsive to sound. Occasionally we tested what we thought was an intact male and found that it always responded in the same direction, regardless of the stimulus location. Examination of such individuals revealed a damaged tympanum on the side they favored. Previous reports (Treat 1955) for other species have indicated that response direction of acoustically stimulated moths is highly unpredictable. However, Roeder (1967) showed that the form of a response is fairly predictable depending upon the intensity of the stimulus; a weak (distant) stimulus caused feral flying moths to turn and fly away from the source, whereas a strong stimulus caused the moths to power-dive and take cover in the foliage. Observations in the controlled situations in our laboratory wind tunnel seem to agree with those of Roeder (1967) for low sound intensities; initial left-right directional responses depended upon the relative sound intensity striking the left and right tympana.

Males that were fanning their wings while in tarsal contact with the substrate also exhibited fairly predictable behavior to our sound stimulus; they immediately became quiescent with their wings in the resting position. This occurred whether or not they already had flown, either in the pre-flight release cage or on the pheromone source. However, the duration of quiescence was significantly shorter for males at the source. Some males that flew evasively rather than becoming quiescent appeared not to be in firm contact with the substrate with their tarsi, and essentially may have been more in the flying than the fanning mode prior to the stimulus.

The extensive work of Roeder and Treat (1961) and Roeder (1967) leaves little doubt that the ultrasonic emissions of insectivorous bats have been a strong selective force governing the evolution of ultrasonic-sensitive tympanic organs in the Lepidoptera. However, *L. dispar* is a largely diurnal species with its main sexual activity occurring during late morning and throughout the afternoon (Cardé et al. 1973), although males are sometimes captured in light traps. Since bats are crepuscular or nocturnal, it may appear that the sensitivity to high frequency

<table>
<thead>
<tr>
<th>Type of male</th>
<th>No. of stimuli</th>
<th>Quiescent (mean duration ± SD)</th>
<th>Flight</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both tympani intact; fanning on pre-flight cage</td>
<td>21 (9 males)</td>
<td>18 (21.9 sec ± 37.9 SD)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Both tympani intact; fanning on pheromone dispenser</td>
<td>26 (10 males)</td>
<td>15 (1.4 sec ± 0.6 SD)*</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Both tympani destroyed; fanning on pre-flight cage</td>
<td>25 (5 males)</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Both tympani destroyed; fanning on pheromone dispenser</td>
<td>29 (7 males)</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
</tbody>
</table>

*Mean duration of quiescence is significantly different from the other entry in the table according to the t-test (**P < 0.05**).
sound in *L. dispar* should not have been retained. However, some pheromone attraction occurs in the 1st h after sunset (Cardé et al. 1973) and this activity may be sufficient to maintain the auditory response. Thus nocturnal flight with attendant bat predation would appear to account for the maintenance of evasive response to ultrasound, as suggested for some pyralid species by Treat (1955) which are apparently quite active at night as well as during the day. Lack of behavioral reaction to sound by *L. dispar* females is not surprising since females do not fly. Because nominate *L. dispar* females in the Soviet Union are reported to fly (Leonard 1974), it would be of comparative interest to ascertain their reaction to ultrasound. Notwithstanding, it is apparent that the possible defensive survival advantages offered by the ability to hear and respond to ultrasound are strong enough to take precedence over male sexual behavior, thereby interrupting sexual communication. In the daytime such interruption from natural ultrasonic sources should be minimal and ultrasonic-sensitive males should be at no competitive disadvantage in the prompt and accurate location of calling females.

Acknowledgment

We thank Dr. J. King for the use of the ultrasonic detector, Dr. C. C. Doane for valuable discussions on the field aspects of this study, Dr. C. Schwalbe for supplying the laboratory-reared moths, and P. Castrovillo for drawing Fig. 3. This research was supported in part by a USDA sponsored pro-
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ENVIRONMENTAL ENTOMOLOGY