Competition Among Courting Male Moths: Male-to-Male Inhibitory Pheromone

Abstract. *The behavioral function of a pheromone released by males of the armyworm moth* Pseudaletia unipuncta *was investigated both in laboratory wind-tunnel experiments and in experiments with moth-baited traps in the field. Such male moth scents have been thought to act at close range as sexual stimulants for females of the same species. However, the only obvious effect of the* P. unipuncta *male pheromone was upon other males, decreasing their tendency to approach sexually receptive, pheromone-releasing females and to exhibit copulatory behavior when near those females. The adaptive significance of the male pheromone may be related to the increased reproductive efficiency that results if multiple males are prevented from competing for a single female.*

Male noctuid moths possess a variety of brushlike structures associated with glandular organs that secrete volatile chemicals. These "scent brushes" are often conspicuous and have attracted considerable attention from morphologists and taxonomists (*1*, *2*). The chemicals that evaporate from the brushes have generally been speculated to play an aphrodisiac role, stimulating a female so that she more readily accepts an odor-releasing male in copulation. Although several experiments have suggested that the scents of male noctuid moths may indeed influence female behavior, the data supporting this proposed role are scant and sometimes conflicting (*1*, *3*).

In studies with the armyworm moth, *Pseudaletia unipuncta* (Haworth) (Noctuidae), we have found a marked influence of a male-produced odor on the behavior of other males. This male pheromone inhibits the approach and copulatory behaviors that are normally exhibited by the males when they are stimulated by the sex pheromone released from sexually receptive females. By contrast, we have found no inhibitory influence of the male pheromone on the premating behavior of nearby females, as measured by their tendency to release pheromone.

Males of the armyworm moth have a distinct almondlike odor, which is due to benzaldehyde. The benzaldehyde, plus benzyl alcohol and benzoic acid, have been identified from the pair of scent brushes found on the anterior portion of the moth abdomen (*4*). The males also have smaller scent brushes at the posterior of the abdomen. In our experiments we used living, intact moths and we have not intended to elucidate which organs release the pheromone that causes the male-inhibition effects (*5*).

An armyworm colony was maintained in the laboratory (*6*). Males and females were separated from each other when in the pupal stage and were thereafter maintained in separate chambers having independent air supplies. All laboratory experiments were performed at the normal time of mating, between hours 7 and 9 of the dark period, under a light intensity of 0.2 lux (*7*). Sexually mature male and female moths were placed in various combinations in small wire-screen cages situated in series in a glass wind tunnel (*8*). Air was continuously pulled through the tunnel so that moths in the downwind cages were exposed to any odors released from moths in the upwind cages.

In one experiment, seven cages were placed in the tunnel. The three upwind cages were loaded with ten males each, and the four downwind cages were loaded with five males each. For comparison, three empty cages were placed in the upwind part of the tunnel, followed by four five-male cages. At 30-minute intervals for each of these treatments, a cage having one armyworm female with her sex pheromone gland obviously exposed was selected from a group of such cages containing three females each and was placed in the tunnel upwind of all the male cages. Observations of responses induced by sex pheromone were then made for all of the males in the tunnel (*9*). The female cage was removed, and the positions of the male cages in the tunnel were rotated, preserving the 10,10,10,5,5,5,5-, or 0,0,0,5,5,5,5-male sequence, in preparation for the next stimulation by female sex pheromone 30 minutes later (*10*). Males downwind from other males exhibited a decrease in wing-fanning response when all the males were exposed to female sex pheromone, with the degree of inhibition of this response being proportional to the number of upwind males (Fig. 1). Similarly, the upwind males directed more copulatory attempts toward each other than did the downwind males.

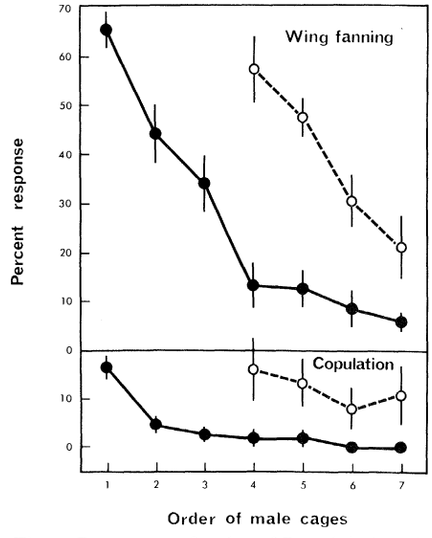

Fig. 1. Responses of males of *Pseudaletia unipuncta* to female sex pheromone. Closed circles represent responses of males confined in seven cages arrayed in series in a wind tunnel, with numbers of males per cage, starting at the unwind end of the tunnel, being 10,10,10,5,5,5,5. Open circles represent responses of males in a series consisting of 0,0,0,5,5,5,5 males per cage. Vertical lines indicate the magnitudes of standard errors.

In another experiment, some of the cages contained three virgin males plus three virgin females, and others contained ten males only. The sequence of cages in the tunnel, starting at the upwind end was three cages with both sexes, three cages with males only, and three cages with both sexes. The cages containing both sexes were observed for 2 minutes at 30-minute intervals throughout the period of mating on eight different nights to determine the number of copulatory attempts or completed copulations between males and females. There were 26 attempts among the moths upwind of the ten-male cages and eight attempts among those downwind (*11*). This decrease in copulatory behavior must have been caused by an inhibitory effect exerted from upwind to downwind males and not from upwind males to downwind females, because no inhibition of female pheromone-releasing behavior in the downwind cages was noted (*12*).

Two traps, each baited with four virgin female moths confined in a wire-screen enclosure, were placed in a cornfield on nine separate nights. The female enclosure was surrounded by a larger enclosure which, in one of the traps, contained ten males that were prevented by the screen barrier from being able to copulate with the females (*13*). Means of 4.3 ± 0.9 (standard error) and 1.3 ± 0.3 male moths were captured each night in the traps baited with females alone and females plus males, respectively. On the basis of the laboratory data presented above, we propose that this 70 percent

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reduction in the male capture in the male-plus-female-baited traps was caused by a male-to-male inhibitory pheromone that interfered in some way with the normal approach response of males to females that were releasing sex pheromone (*14*).

Interactions among male moths probably occur only at those times when two or more males approach and attempt to copulate with the same female simultaneously. Thus, the biological significance of this male-produced pheromone might be associated with the increased reproductive efficiency that results when multiple males are prevented from competing for a single female.

Electrophysiological evidence indicates that males of a variety of noctuid species may be able to sense their own scent-brush odors (*1*, *4*, *15*). Whether such scents could be dispensed into the air of fields containing males and females of certain pest lepidopterous species, so as to prevent mating and thus provide a selective means of pest control, remains to be determined.

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References and Notes

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5. In other experiments (unpublished), we have found that either an extract of the anterior abdominal scent brushes of male *P. unipuncta* or synthetic benzaldehyde also cause an inhibition of the precopulatory behavior exhibited by males in response to pheromone-releasing females.
6. The colony was maintained by a method similar to that described by H. H. Shorey and R. L. Hale [*J. Econ. Entomol.* **58**, 522 (1965)]. All stages were held at 25° ± 2°C, under a photoperiod of 12 hours of light and 12 hours of darkness.
7. A red light provided illumination in the experimental room. The light intensity was measured with a Photovolt photometer, model 200M. Observation was also aided by occasional use of a dim red flashlight.
8. The cylindrical tunnel measured 7 cm (inside diameter) by 100 cm, and each cage, which tightly fit in the tunnel, measured 6.5 cm (diameter) by 8.5 cm. Air velocity in the tunnel was maintained at 25 cm/sec.
9. Two types of response, characteristic for sex pheromone-stimulated male moths, were measured, namely, wing fanning and attempted copulations. Because no females were present in the male cages, copulatory attempts were directed from one male toward another. These responses were measured at 15 and 60 seconds after the female cage was placed in the tunnel, and the percentage of response for any one cage was calculated as follows:

$$\frac{N_1 + N_2 - 2B}{2(N_0 - B)} \times 100$$

where N_0 is the number of males in the cage, N_1 and N_2 are numbers responding after 15 and 60 seconds, respectively, and B is background number responding immediately before stimulation.
10. For the 10,10,10,5,5,5,5-male sequence, 26 replicates (separate stimulations) were conducted over 5 days, and for the 0,0,0,5,5,5,5 sequence, 20 replicates were conducted over 4 days. All moths used in this and other experiments were 3 to 6 days old.
11. Numbers of observed behaviors in the first three consecutive cages were 10,10,6, and in the last three were 5,2,1.
12. During the experiment, the numbers of females with pheromone glands extruded and maintaining the typical receptive posture were 26 and 41 in the upwind and downwind cages, respectively. This lack of inhibition of female pheromone-releasing behavior by male scent was seen in an experiment, in which seven cages containing four males or four females each were placed in the tunnel, starting with a female cage and with sexes then alternating downwind. During 21 different 2-minute observation periods conducted over three nights, the percentages of females observed in the typical receptive posture were 39 ± 6 (standard error); 57 ± 6; 45 ± 7; and 52 ± 8 from the most upwind to the most downwind cage. In addition, we have observed that antennectomized females accept males in copulation as readily as do females having intact antennae.
13. Double cone traps, identical to those described by R. K. Sharma, H. H. Shorey, and L. K. Gaston [*J. Econ. Entomol.* **64**, 361 (1971)], were supported at 1-m elevation in the field. During three of the nine experimental nights, two traps of each type were placed in the field. The minimum distance between the traps was 20 m. Positions of the traps were changed at random nightly, and the females were replaced once during the experiment. The female enclosure was a 5 cm (diameter) by 12 cm wire-screen cage, and the male enclosure was a 8.5 cm (diameter) by 12 cm cage. Female and male enclosures were provided with 10 percent sucrose solutions.
14. C. J. Sanders [*Can. Entomol.* **110**, 43 (1978)] has reported that sex attractant traps for the male spruce budworms, *Choristoneura fumiferana* (Clem.) have a reduction in attractancy over time, apparently because the odor of males initially trapped repels other males.
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Echo Detection and Target-Ranging Neurons in the Auditory System of the Bat *Eptesicus fuscus*

Abstract. *Some of the neurons in the nucleus intercollicularis and auditory cortex of the echolocating bat* Eptesicus fuscus *respond selectively to sonar echoes occurring with specific echo delays or pulse-echo intervals. They do not respond for a wide range of other types of sounds or for sonar echoes at longer or shorter pulse-echo intervals; they may, therefore, be specialized for detection and ranging of sonar targets.*

We have made single-unit recordings from the auditory cortex, inferior colliculus, and nucleus intercollicularis in lightly anesthetized bats of the species *Eptesicus fuscus* (big brown bat, Vespertilionidae). These bats orient themselves and seek prey by echolocation. The responses of neurons were studied with pairs of acoustic stimuli simulating sonar sounds and echoes, and several types of response patterns were observed. Some of these neurons responded exclusively to echoes following more intense pulses and then only when the echo time delay or pulse-echo interval was within a restricted range. These units exhibited properties that were expected from knowledge of echolocation behavior and the acuity of target range (echo time delay) perception by bats (*1*).

Each bat was initially anesthetized with sodium pentobarbital (25 mg per kilogram of body weight). The top of the skull was exposed and dried, the head of a small nail was glued to the skull with acrylic adhesive (Eastman 910) and dental cement, and a small hole was made through the skull to insert a recording electrode. The bat was placed in a Plexiglas holder, which was suspended from an aluminum rod with a rubber band to absorb mechanical stress from the bat's movements, and the bat's head was rigidly held by clamping the attached nail to another aluminum rod. Both rods were mounted on a small steel platform used for supporting the preparation. The bat and the platform were placed in a soundproof room at 34°C for 3 to 4 hours before physiological recordings were begun. Generally, the bat's eyes were open, and pinching a leg elicited a withdrawal reflex, indicating that the bat was lightly anesthetized or awake. Either a tungsten microelectrode (tip diameter 2 to 5 μm) insulated with Insl-X, or a 3*M* KCl-filled glass micropipette (tip broken to about 1 μm diameter) was then lowered onto the surface of the brain overlying the desired recording site. Subsequent advance of the electrode into brain tissue was controlled from outside the soundproof room with a calibrated hydraulic microdrive. The stability of

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