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PHEROMONE-MEDIATED FLYING AND WALKING ORIENTATION AND FACTORS PROMOTING MATE RECOGNITION IN THE GYPSY MOTH, LYMANTRIA DISPAR (L.)

A Dissertation Presented

by

RALPH ERNST CHARLTON

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Entomology

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DEDICATION

To my mother and father,
Else and Floyd, with love

ACKNOWLEDGMENTS

There are many people to thank for their help in bringing this protracted affair to an end. First and foremost, I owe a special debt of gratitude to my mentor and major professor, Dr. Ring Cardé. Throughout our long association, Dr. Cardé has provided me with constant financial and material support, a stimulating intellectual climate, and the freedom to pursue some of my admittedly unorthodox ideas as well as a multitude of 'side projects'. He, in large measure, helped transform a green work study student with nebulous career plans into someone able to pursue scientific investigation with at least a modest degree of expertise - not an easy task!

I am also grateful to the other members of my committee, Drs. Vincent Dethier, Ron Prokopy, John Clark, and Dave Leonard for their critiques of the design and presentation of this research. Their suggestions and incisive comments substantially improved the quality of the finished product.

Most of this work could not have been accomplished without the steady stream of experimental insects provided by the USDA-APHIS Methods Development Center at Otis ANG Base, Massachusetts. I thank all of the personnel of that facility and, in particular, Dr. Charles Schwalbe, Vic Mastro, and Jessie Baker for their assistance and generosity.

Originally I had intended to include in this dissertation work done in collaboration with Dr. William Bell at the University of Kansas. Unfortunately, a combination of bad luck and timing, a.k.a. the

'servosphere blues', prevented the completion of this research before going to press. Nevertheless, the experiments conducted there provided important insights into the mechanisms used by walking male gypsy moths to locate pheromone sources. Thus, I wish to express my appreciation to Dr. Bell for giving me the run of his research facility and to Greg Vogel, Tom Tobin, and Ken Sorenson for keeping a complicated and oft-times obstinate piece of machinery up and running.

Many colleagues, friends, and relatives have provided moral support and given freely of their time and expertise during the gestation period of this work. It is with especial pleasure and appreciation that I thank the following individuals: Juliet Tang, Coby Schal, Mark Willis, Reggie Webster, and Bob Collins, to name a few, for many enlightening discussions as well as for making the lab a much more pleasant place; Kim Spencer, Sheri Rosenblum, and Nancy Haver for coordinating the efforts of the 'cast of thousands' involved in the insect rearing effort; Bob Collins for writing the data analysis programs, and Hiroo Kanno for assistance in experiments; Beth Owens and Andrea Hurley for showing a neophyte how to make spectral radiometric measurements and for recovering the film seized by overzealous security guards; Nancy Haver for expertly drawing many of the figures; Lucy Yin for taking a number of SEM photomicrographs; and finally, Steve and Beth Mallone-Woods for graciously making available a bed in their congenial home, no matter what outrageous hour I stumbled in.

My sincere gratitude to all these people and to my many other friends in Amherst.

ABSTRACT

PHEROMONE-MEDIATED FLYING AND WALKING ORIENTATION AND FACTORS PROMOTING MATE RECOGNITION IN THE GYPSY MOTH, LYMANTRIA DISPAR (L.)

SEPTEMBER 1988

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The behavioral mechanisms inherent to male flying and walking orientation, courtship interactions, and recognition of female gypsy moths by the males were investigated. Airspeeds, groundspeeds, and wingbeat frequencies of flying males increased as pheromone concentration was lowered, as did the magnitudes of course and interleg angles. The tracks of males exposed to higher pheromone concentrations also were narrower. An inverse relationship existed between the width of track reversals elicited by different pheromone concentrations and the corresponding dimensions of the time-averaged active space as estimated by a male wing-fanning assay. When males were flown at different ambient temperatures but equivalent pheromone release rates, airspeeds and groundspeeds were elevated at the higher temperature but the track widths were similar. The mean turning frequency was identical at the two test temperatures despite the 5 °C difference in thoracic temperature.

Visual cues presented by the female at close range were not critical to either the males' choice of landing site or the efficiency with which the walking male located the female. Both the presence and absence of pheromone influenced male walking orientation. When stimulated by pheromone, males oriented anemotactically towards the source; loss of the odor cue prompted an area-restricted local search characterized by primarily vertical and oblique movements with frequent reversals in direction. These maneuvers enhance the likelihood of recontacting the plume or serendipitously encountering a female.

Analyses of transition probabilities indicated that courtship interactions were highly stereotyped for both sexes. Males executed the majority of courtship behaviors whereas the behavioral repertory of the female was limited. The fact that all courtships were successful coupled with the rarity of discernible rejection behavior suggested that females were not choosy about prospective mating partners, although potential cryptic mate choice is discussed.

Models invested with virgin female wings or body scales elicited male copulatory responses comparable to those evoked by virgin females. Solvent, acid, or base extraction of female abdominal scales did not diminish the copulatory response whereas pulverization of the scales eliminated it, indicating that tactile cues serve as primary releasers of copulatory behavior.

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CHAPTER I

INTRODUCTION AND OVERVIEW

Ever since the gypsy moth, *Lymantria dispar*, debuted in North America in 1869 through the inattention of the aspiring sericulturist, Leopold Trouvellot, this insect has been the focus of deserved attention. Its rapid and extensive spread from the introduction site in Medford, Massachusetts coupled with its well deserved notoriety as a major defoliater of forests both in Eurasia and North America have created public concern and spawned an immense body of research as evidenced by the over 4100 entries in the most recent bibliography of gypsy moth literature (Griffiths, 1980). Although these reports consider numerous aspects of this insect's physiology, life history, ecology, and behavior, the majority are concerned, not surprisingly, either directly or indirectly with methods of control.

A particularly promising means of achieving species-specific management, and seemingly without the undesirable side effects of pesticides, is through the use of sex attractant pheromones. With the advent of increasingly sensitive analytical tools, and enhanced knowledge of the types of compounds commonly produced, isolation and identification of insect pheromones is proceeding at an increasingly rapid pace (Inscoe, 1982; Tamaki, 1985). Unfortunately, our understanding of the roles of these chemicals in sexual communication continues to lag far behind. Consequently, the considerable potential of these compounds in pest-management schemes has not been realized.

The gypsy moth was one of the first insect species documented to use a chemical attraction system for mate location (Hasse, 1888; Forbush and Fernald, 1896). As early as the late nineteenth century, Forbush and Fernald (1896) noted that "as the female does not fly, the male is guided to her by the odor which she gives off, and which is disseminated by currents of air."[1]

Furthermore, the potential for exploiting this phenomenon in control strategies was also recognized early. Thus, Forbush and Fernald performed experiments "for the purpose of determining whether it is possible to trap the males of the gypsy moth, in any infested region, to such an extent that there would not be enough left to mate with any considerable number of females, so that a large proportion of the females would be compelled to lay infertile eggs, and therefore greatly reduce the number of gypsy moths in that locality the following season."[2]

Substantial progress in exploiting the chemical communication system for monitoring and control purposes had to await the isolation and correct identification of the pheromone. The female-emitted pheromone was characterized by Bierl et al. (1970) as consisting of a single component, *cis*-7,8-epoxy-2-methyloctadecane which was given

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[1] E.H. Forbush and C.H. Fernald, The Gypsy Moth. (Boston, Wright and Potter, 1896), p. 345.

[2] Forbush and Fernald, pp. 357-358.

the trivial name disparlure. Because this molecule possesses a chiral center, it is optically active and therefore can exist in two enantiomeric forms. Following synthesis of the enantiomers in relatively pure form (Iwaki et al., 1974; Mori et al., 1976; Farnum et al., 1977), a number of field tests were conducted in Europe and North America to assess the relative attractiveness to males of different enantiomeric mixtures (Iwaki et al., 1974; Vité et al., 1976; Cardé et al., 1977; Miller et al., 1977; Plimmer et al., 1977). These tests demonstrated that the (+)-enantiomer of disparlure induces 5 to 10-fold higher trap catch than the racemic mixture. Admixture of even low percentages of the (-)-enantiomer suppressed trap catch and traps baited with pure (-)-disparlure failed to capture males. In addition, Hansen (1984) through single cell antennal recordings of the male showed that females produce almost exclusively the (+)-enantiomer. Therefore, the combined evidence indicates that (+)-disparlure in the naturally-emitted pheromone.

Following identification of disparlure, monitoring traps baited with synthetic pheromone came into widespread use and offered a sensitive, selective and inexpensive means of monitoring the dispersion and spread of gypsy moth populations. Moreover, the potential utility of disparlure and other moth sex attractants as direct population control agents had also been acknowledged. Some time before, it was proposed (Beroza, 1960; Babson, 1963) and later demonstrated experimentally with *Trichoplusia ni* (Gaston et al., 1967), that broadcast of relatively large quantities of pheromone over infested areas could disrupt the natural mating process. The successful commercial use of gossyplure to disrupt the mating and achieve effective population control of the pink bollworm, *Pectinophora gossypiella* (Brooks et al., 1979) spurred similar investigations on a wide variety of lepidopteran pests (reviewed in Kydonieus and Beroza, 1982).

Not surprisingly, the air permeation technique has also been explored for gypsy moth control. A number of field trials with racemic disparlure have demonstrated that effective suppression of mating can be achieved at low to moderate population densities but at higher densities control is generally not effective (Cameron, 1981; Plimmer, 1982; and references therein). Many factors have been implicated as contributing to the inconsistent results obtained to date. These include the impracticality of using (+)-disparlure due to its prohibitive expense, release characteristics of dispensers, and difficulties inherent to monitoring and evaluation of tests. Certainly, another important contributing factor is our inadequate basic comprehension of certain pheromone-mediated behaviors. Understanding these behaviors is central to explaining why disruption of communication with pheromone is not totally effective in this and many other species and perhaps to devising more effective disruption systems.

It is with the objective of gaining a clearer understanding of gypsy moth mate-finding and mate recognition behaviors that the research presented here is concerned. This dissertation examines in four chapters several facets of sexual communication in *L. dispar.*

What follows is a brief summary describing, in general terms, the behaviors inherent to the signaling and mate-seeking process in *L. dispar* and other moths and the specific facets of precopulatory behavior addressed in each of the chapters.

The adult male's search for the virgin female encompasses a number of different behaviors mediated to an appreciable extent by the female-emitted pheromone. The flightless and sedentary female commences pheromone emission 1 to 2 hours following eclosion (Doane, 1968; Charlton and Cardé, 1982). Pheromone release and the attendant 'calling' behaviors involve the rhythmic protrusion and retraction of the terminal abdominal segments containing the gland (Doane, 1968; Hollander et al., 1982; Tang et al., 1987). The liberated pheromone is disseminated by air currents, and transported downwind to potentially encounter a male.

In the gypsy moth, as with other moths, it is not known how often resting males are enveloped by a pheromone plume and then become 'activated' or if males initiate flight without pre-exposure to pheromone and then 'search' for pheromone plumes in so-called 'appetitive' or 'ranging' flight. Possibly both strategies come into play under natural conditions. Activation of quiescent males has been studied extensively in the laboratory (Cardé and Hagaman, 1979, 1983; Hagaman and Cardé, 1984). A male stimulated by pheromone can react by antennal movements, stepping, and most noticeably and diagnostically, initiation of wing fanning; wing fanning can occur while the male is stationary or walking. The latency to wing fanning varies inversely with pheromone concentration (Cardé and Hagaman, 1979; Hagaman and Cardé, 1984) or temperature (Cardé and Hagaman, 1983).

Following male exposure and reaction to the pheromone plume four basic behavioral patterns that culminate in mating can be discerned (Fig. 1): (1) pheromone-modulated anemotactic flight toward the odor source; (2) vertical flight in the immediate vicinity of the tree bole harboring the calling female, whereby the male intermittently contacts the bark with his tarsi; (3) landing on the trunk after which the male walks while wing fanning until he arrives at the female and; (4) contact with and recognition of the female with attendant expression of courtship behaviors that climax in copulation. Even though it is convenient to subdivide *L dispar* precopulatory behaviors into such broad categories, it must be emphasized that these behaviors can cycle and some behaviors of the sequence may be omitted. For example, walking males will occasionally take flight and reorient in the pheromone plume before landing anew and resuming walking, or males will sometimes land directly on the female, thus obviating walking maneuvers.

Certainly the most extensively studied components of premating behavior in moths are the orientation maneuvers exhibited by males as they fly toward a pheromone source. Field and wind tunnel observations on several species have clarified the complex mechanisms involved in pheromone source location. The zigzag path that is characteristic of upwind flight was earlier thought to be due to the male exiting the pheromone plume and then turning back toward the direction in which the pheromone was last sensed (Kennedy, 1977). Current evidence indicates that these tacking maneuvers are manifestations of an internally generated program of self-steered counterturns elicited and modulated by contact with the pheromone plume. These counterturns are expressed in concert with the optomotor response to wind-induced drift that provides polarity to the counterturns and results in generally upwind displacement (Kennedy, 1983; Baker et al., 1984). Further research has also demonstrated that fluctuations in stimulus intensity stemming from the filamentous nature of the plume and/or movements in and out of the plume are necessary for a moth to maintain upwind flight (Kennedy et al., 1980, 1981; Willis and Baker, 1984; Baker et al., 1985).

Pheromone concentration has been shown to modulate a number of characteristics of flight tracks, notably flight speed and the expression of counterturns (Cardé and Hagaman, 1979; Kuenen and Baker, 1982). The effects of temperature independent of pheromone on moth flight to pheromone have hitherto not been investigated. Chapter II provides a detailed quantitative analysis of the effects of pheromone concentration and ambient temperature on various flight parameters of male gypsy moths assayed in a sustained-flight tunnel.

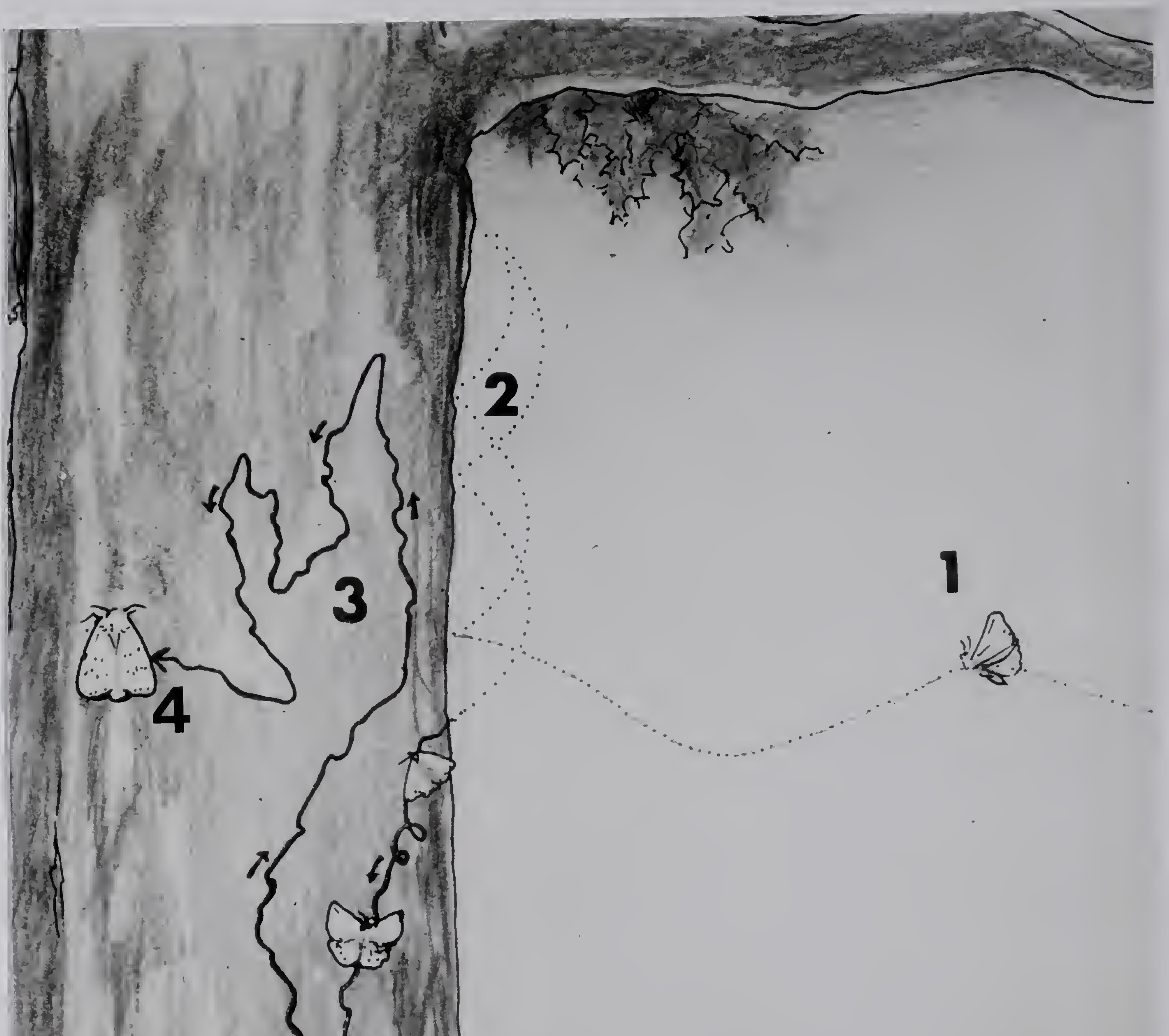
Although flight reactions of pheromone-stimulated male gypsy moths have been reasonably well studied, other aspects of orientation and mating behavior remain poorly understood. Walking behavior constitutes another important element of the mate-seeking process.

Several studies have documented that once males land on trees harboring females, they navigate the intervening distance to the female or synthetic pheromone source by walking while wing fanning (Doane, 1968; Richerson, 1977; Cardé and Hagaman, 1984). The resultant walking-search paths are characteristically convoluted, predominantly vertically directed, and may require several minutes to complete (Fig. 1). Among the potential mechanisms which could allow the male to locate the source are pheromone-modulated anemotaxis, pre-programmed search, visual orientation or a combination of these tactics. However, the cues and mechanisms inherent to this process have not been systematically evaluated. To this end, Chapter III examines the orientation mechanisms used by walking males to locate pheromone sources, with a particular emphasis on the relative roles of olfactory and visual inputs as they pertain to the orientation process.

After the walking male negotiates his path to and approaches within several cm of the female, a series of behaviors, collectively termed precopulatory or courtship behaviors, ensue. Courtship involves a rapid and sometimes subtle exchange of information between male and female which culminate in copulation. Here again, the major steps involved in courtship have been outlined in general terms by several authors (Doane, 1968; Schröter, 1976; Richerson, 1977; Cardé, 1981) but the individual behaviors that comprise the sequence and their interactions have not been quantitatively described. Chapter IV provides a detailed conditional probability analysis of gypsy moth courtship interactions, emphasizing both male and female behaviors. The possible role of mate choice as it relates to successful execution of courtship behaviors is also discussed.

Having described mating behaviors, other important questions naturally arise. How do males actually recognize a conspecific female? More specifically, what factors associated with the female release male copulatory behaviors and through which sensory modalities are these reactions mediated? Chapter V addresses these questions by describing experiments that use models incorporating various female attributes to identify the specific factors that evoke male copulatory behavior. In addition, the sensory basis of the male response is investigated through bioassays that elucidate the relative contributions of chemical, tactile, and visual components to the recognition process.

Figure 1. Generalized sequence of male gypsy moth mate-seeking behaviors: (1) pheromone-modulated anemotactic flight toward the pheromone source; (2) vertical flight in the immediate vicinity of the tree bole harboring the calling female, whereby the male intermittently makes tarsal contact with the bark; (3) landing on the trunk after which the male walks while wing fanning until he arrives at the female and; (4) contact with and recognition of the female followed by mating.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CHAPTER II

INFLUENCE OF PHEROMONE CONCENTRATION AND AMBIENT TEMPERATURE ON FLIGHT OF THE GYPSY MOTH IN A SUSTAINED-FLIGHT WIND TUNNEL

Introduction

The maneuvers that flying moths and other insects employ to locate a 'calling' female or a synthetic pheromone source have been the subject of considerable study and continuing debate. Current concepts of how moths navigate a course to a point source of pheromone involve the following integrated elements: (1) a self-steered counterturning producing a zigzag flight that is elicited and modulated by contact with the pheromone plume and; (2) an optomotor response to wind-induced drift that provides polarity to the counterturns and results in generally upwind displacement (Kennedy, 1983; Kuenen and Baker, 1983; Baker, 1985; Kennedy, 1986).

A major factor influencing numerous characteristics of these flying maneuvers is pheromone concentration. For example, in studies of several lepidopteran species, increases in stimulus intensity elicited decreases in upwind groundspeed (Farkas et al., 1974; Kuenen and Baker, 1982; Murlis et al., 1982; Sanders, 1986), and modified the propensity to initiate and sustain upwind flight (Baker and Roelofs, 1981; Baker et al., 1981; Sanders et al., 1981; Linn and Roelofs, 1983). Moreover, pheromone concentration has been shown to modify the expression of the counterturning program, affecting both the width and frequency of counterturns (Cardé and Hagaman, 1979; Kennedy et al., 1980, 1981; Kuenen and Baker, 1982; Willis and Baker, 1984). Another factor that potentially could influence the expression of flight behaviors is ambient temperature. Increases in ambient temperature have been shown to elicit higher wingbeat frequencies in several lepidopterans including *Lymantria dispar* (Casey, 1980), and *Danaus plexippus* (Kammer, 1970). However, to date no studies have assessed the effects of temperature on the flight reactions of pheromone-stimulated moths.

In the gypsy moth, pheromone concentration affects both preflight behaviors (Cardé and Hagaman, 1979; Hagaman and Cardé, 1984) and aspects of flying orientation (Cardé and Hagaman, 1979). Cardé and Hagaman (1979) reported that increases in pheromone concentration modulated groundspeed of flight and duration of sustained flight as well as the magnitude of lateral displacement of counterturns. In addition, the latencies of male wing fanning in response to pheromone decreased at higher ambient temperatures independent of pheromone emission rate (Cardé and Hagaman, 1983).

In this chapter I extend these findings to provide a detailed analysis of the effect of pheromone concentration and ambient temperature on various flight parameters of male gypsy moths as recorded in a sustained-flight wind tunnel. Male flying maneuvers were examined via two approaches: (1) a causal analysis designed to identify the strictly behavioral mechanisms in operation, utilizing the calculable relations between the moth's sensory inputs and its motor outputs; and (2) a functional analysis which quantifies the indirect outcome of these actions and reactions by the moth in a given configuration of wind and pheromone, i.e. the resultant aerial maneuvers which the moth achieves along its ground track and which eventually allow it to attain the odor source.

Materials and Methods

Moths

Lymantria dispar were received as egg masses from the USDA-APHIS Methods Development Center, Otis Air National Guard Base, Massachusetts. Larvae were reared on wheat germ diet (Bell et al., 1981) at 24 °C and 60-70 % relative humidity on a 16L:8D photoperiod. Males were segregated from females in the pupal stage, transferred for emergence to screen cages (30 x 30 x 36 cm), and held in an environmental chamber regulated at conditions identical to those used in rearing. All moths used for experiments were 36-48 hr old and tested between the 6th and 10th hours of photophase.

Pheromone

The female-emitted pheromone inducing attraction in *L. dispar* was identified as *cis*-7,8-epoxy-2-methyloctadecane and named disparlure (Bierl et al., 1970). The (+)-enantiomer of disparlure has proved to be many fold more effective in eliciting trap catch than the racemate (Miller et al., 1977; Cardé et al., 1978; Plimmer et al., 1977) and, on the basis of antennal receptor studies (Hansen, 1984), this enantiomer appears to be the natural pheromone.

The synthetic (+)-disparlure (Farnum et al., 1977) used in these experiments had an enantiomeric purity of > 96 % as determined by analysis of intermediates. GLC analysis using a 30 m SP-2340 fused silica capillary column indicated the presence of < 1 % of the *trans* isomer. A 10 ug/ul solution of (+)-disparlure in hexane was formulated gravimetrically and serially diluted to obtain the desired concentrations.

Wind Tunnel and Data Recording

Behavioral assays were conducted in a Plexiglass™ sustained-flight tunnel, 240 cm long, 100 cm wide and 72 cm high as described by Cardé and Hagaman (1979). A fan pushed air through a set of screen baffles to provide a relatively laminar air flow with a velocity of 70 cm/sec as measured with a hot-wire anemometer (W-141 A 1/5; Weather Measure Corp.) located at the position of the pheromone source. Pheromone was vented from the downwind end of the tunnel to the outside by an exhaust fan. Illumination was provided by eight 40 watt DC-mains fluorescent bulbs diffused by reflection from a matte-white ceiling. The resultant light intensity, measured in the center of the tunnel, was 450 lux. A canvas floor with alternating 10-cm width yellow and white stripes located below the clear floor provided optomotor feedback for the moths. This color scheme was chosen because the moth was clearly discernible against the background of the yellow stripes during the black-and-white video playback.

A SONY RSC 1050 rotary-shutter video camera with a 8.5 mm wide-angle lens linked to a SONY SLO 340 videorecorder recorded the horizontal flight tracks of moths. The camera was attached to a platform which was moved by rollers along a track mounted on the ceiling. The track was located above the center of and aligned with the long axis of the wind tunnel. To eliminate potential visual cues associated with the setup, the track was painted matte white and the camera and platform were masked with white cardboard. During runs the camera was manually moved in concert with, and aligned as nearly as possible with the vertical plane of the moth to minimize optical distortion of the moth's flight path. The flight path, superimposed on a 100 cm wide x 160 cm long section of the tunnel floor extending from 20 cm to 180 cm downwind of the pheromone source (the 'observation area'), was videorecorded and subsequently analyzed.

General Testing Procedure

Moths were randomly selected from emergence cages, transferred individually into 30 ml clear plastic cups resting on screen squares, and held in the wind tunnel room for at least 1 hr pretest acclimation period. The different pheromone doses were dispensed onto 0.7 cm diam filter paper (Whatman No. 1) discs and aerated in an exhaust hood for 1 hr prior to use in bioassays. Pheromone was released from 15 cm high disposable stands constructed from 10 ul pipets anchored in a cork and into the top of which an insect pin was inserted to hold the filter paper; the negligible cross-sectional area of this design minimized air turbulence. At the onset of each trial, the appropriate pheromone source was introduced into the tunnel. The screen holding a moth was placed on a 15 cm high metal stand 195 cm downwind of the pheromone source such that the moth was facing directly upwind, and the restraining cup was removed. Following activation and initiation of upwind flight, the moth was allowed to fly up the tunnel over a stationary floor pattern until it either attained the predetermined endpoint of the observation area or exited the pheromone plume.

Data Analysis

Individual flight tracks were re-recorded onto and displayed frame-by-frame on a SONY SVM-1010 motion analyser. Consecutive positions of the moth at 0.05 or 0.10 sec time intervals were marked on transparent acetate sheets placed on the analyzer screen. Reference marks corresponding to the distance markers on the wind tunnel floor were added to each sheet to facilitate proper alignment and comparison between tracks. Successive points comprising the flight track were converted to X,Y coordinates using an X-Y digitizer (Apple Graphics Tablet) interfaced with an Apple IIe microcomputer. Flight tracks were then analyzed using computer programs (in BASIC) written specifically for this purpose.

Turns in the flight track were defined as occurring when the accumulated angular deviation from a straight-line course reached or exceeded 70 °. This filtering procedure served to eliminate small transcription errors while preserving the salient features of the flight path (Fig. 2A). Consideration of additional criteria for designating turns, such as changes in the direction of turning (clockwise versus counterclockwise) as suggested by Kuenen and Baker (1982), did not affect the outcome of the analyses and were not used.

The following flight track parameters were calculated for each flight track based on the filtered data: (1) total flight duration (sec), (2) total number of turns, (3) turning frequency (number of turns/sec), (4) groundspeed (velocity along the actual track), (5) windline groundspeed (the windline component of the groundspeed, regardless of direction), (6) upwind groundspeed (the upwind component of the groundspeed), (7) airspeed, (8) length of track leg (=inter-reversal leg length; the distance traveled (cm) between turns along the actual track), (9) track width (the cross-wind component of the inter-reversal track legs), (10) angular velocity (degrees/sec), (11) track angle (deviation of track from the windline), (12) interleg angle (the angle defined by two consecutive track legs), (13) course angle and, (14) drift angle. Definitions of terms used here to describe a flight track are presented graphically in Fig 2B. To promote interspecific comparisons they are generally consistent with those used by Kuenen and Baker (1982) and Marsh et al. (1978). Course parameters (i.e. (7), (13), and (14) above) were calculated using the triangles of velocities method (Kennedy, 1940; Marsh et al., 1978); the males' course and drift angles and airspeeds were derived geometrically based on the measured upwind groundspeed and track angles combined with the known wind direction and velocity.

Frequency distributions were determined for the following flight track parameters: (1) windline groundspeed, (2) groundspeed, (3) airspeed, (4) track width, (5) length of track leg, (6) track reversal distance (the distance from the plume centerline of initiated turns) (7) track angle, (8) interleg angle, and (9) course angle. Values were assigned to equal size classes and the values for individual classes were converted to percentages. This allowed equal weighting of individual flight tracks in the derivation of combined distributions for each treatment.

Dimensions of the Pheromone Plume

Two approaches were used to assess horizontal plume dimensions. Plume size was estimated in the conventional manner by emitting 'smoke' from titanium tetrachloride dispensers configured identically to those used in behavioral assays and in the same wind speed. In addition, pheromone active spaces were mapped using a bioassay technique similar in concept to that used by Aylor et al. (1976) and Elkinton et al. (1984) to delimit pheromone plumes in a forest setting. This test utilized latency of wing fanning from quiescence to estimate the dimensions of the pheromone active space. Previous studies (Cardé and Hagaman, 1979; Hagaman and Cardé,

1984) have established the wing-fanning assay as a sensitive indicator of the presence of pheromone. Furthermore, the latency of wing fanning is inversely correlated with the atmospheric pheromone concentration. Male response was assayed 20, 100, and 180 cm downwind of 10, 100 and 1000 ng pheromone sources at 24 ± 1 °C. Males, resting on screen squares, were placed on top of a 15 cm high metal platform so that the male was facing directly upwind. Each moth was observed for 2 min, and then the stimulus was inserted into the windstream through a hole in the bottom of the tunnel. Moths wing fanning during the pre-stimulus observation period (ca. 3 %) were discarded. Observation continued until 3 min after stimulus introduction or initiation of wing fanning. Males were tested individually to preclude either response facilitation or turbulence and attendant pheromone spread induced by wing fanning. Moths were assayed at the plume centerline and at predetermined increments transverse to the wind flow: 15 cm downwind of the dispenser the increment was 1 cm; at 80 and 160 cm downwind, the spacing was 3 cm except at the edge of the plume (as indicated by at least a 50 % decrement in proportion responding) where the moths were tested at 1 cm intervals to more explicitly define the boundary. Because of the symmetrical nature of the pheromone plume, responses were measured only on one side of the centerline. Ten males were scored for occurrence of and latency to wing fanning at each position for the three concentrations.

Effects of Concentration

To compare the flight maneuvers of individual moths to differing pheromone concentrations, males were assayed at 24 ± 1 °C to filter paper dispensers impregnated with 10, 100, or 1000 ng of the pheromone. This range of concentrations was chosen because males rarely exhibit sustained flight to 1 ng sources (Cardé and Hagaman, 1979). Moreover, in preliminary tests, no complete flights were recorded from moths exposed to 10 ug sources; in-flight arrestment always occurred before the moths traversed the entire length of the observation area. For comparative purposes, a female gypsy moth emits ca. 12-16 ng pheromone per hour at ca. 24 °C during periods of maximal release (Charlton and Cardé, 1982; Tang et al., 1987); these values are intermediate between those produced by the 100 ng and 1000 ng filter paper dispensers used in this test. Fifteen moths were tested to each treatment in a randomized-complete block design. Only the tracks of those moths that traversed the entire length of the observation area were included in the comparative analysis.

In addition to the track parameters described previously, wing-beat frequency was determined for the 15 moths tested at each concentration. Two segments were selected from each track analysis; zones extending 60-100 and 100-140 cm downwind of the pheromone source. The pertinent flight sections were re-recorded on the motion analyzer and the flight track monitored at 1/60 sec intervals to ascertain wing-beat frequency.

Effects of Temperature

To assess the influence of ambient temperature on flight, *L. dispar* males were flown at 20 and 26 °C. Because disparlure emission rates vary with temperature (Cardé and Hagaman, 1983), dispenser loadings were calibrated to provide identical release rates at the two test temperatures. To establish release rates, 0.7 cm filter paper dispensers, pre-ventilated in an exhaust hood for 1 hr, were aerated in the wind tunnel for 24 hr at a wind velocity of 0.7 cm/sec. GLC analysis of residual pheromone indicated that dispenser loadings of 100 ng at 26 °C and 210 ng at 20 °C provided equivalent emission rates (1.2 ng/hr).

Prior to assays, moths were acclimated for 1 hr to the test temperature. Five moths were tested in succession to one temperature and then the temperature was changed and another 5 moths tested. Ten moths were tested per day and the presentation order of temperature regimes was reversed on alternate days. Immediately following completion of flights and within 3 sec of capture, the thoracic temperature of males was measured using a Bailey BAT-4 amplifying thermometer with a microprobe (MT-3) accurate to ± 0.5 °C. The probe was inserted into the ventral side of the mesothorax.

Results

Dimensions of the Pheromone Plume

As visualized by titanium tetrachloride 'smoke', the crosswind plume dimensions at 20, 100, and 180 cm downwind of the pheromone source were 4, 9, and 15 cm, respectively. Estimation of plume size using the male wing-fanning assay yielded substantially different results (Fig. 3). The crosswind dimensions of the active space emanating from the 10 ng source were essentially identical to those obtained using the 'smoke' tracer. However, as pheromone dosage increased the resultant active spaces became progressively wider, particularly at greater downwind distances, as indicated both by initiation of and latency to wing fanning. These findings are consistent with theoretical calculations (Bossert and Wilson, 1963) for a time-average active space which predict that elevating the emission rate of pheromone widens the crosswind expanse of above-threshold concentration of pheromone.

The proportion initiating wing fanning is useful for delimiting the boundaries of the active space. However, substantial differences in latency to wing fanning evident within the active space (Fig. 3) indicate that the pheromone becomes increasingly dilute at greater downwind distances and especially as the crosswind distance from the plume centerline increases. Because this attenuation in concentration occurs gradually, it is not possible to precisely specify the 'edge' of the plume. Moreover, this assay considers male reactions over time intervals of several to tens of seconds whereas a flying moth encounters the plume 'edge' over fractions of a second (Cardé and Charlton, 1985). Notwithstanding, the disparity between plume dimensions estimated from visible markers and male activation indicate that caution must be exercised when attempting to correlate behavioral reactions with plume features (such as apparent boundaries) visualized by 'smoke' techniques.

Effects of Concentration

Substantial differences were evident in flight tracks of moths exposed to different pheromone concentrations (Fig. 4). Increases in pheromone concentration elicited significant decreases in velocity, regardless of whether the parameter considered was groundspeed, windline groundspeed, upwind groundspeed, or airspeed. Mean frequency distributions of groundspeed, windline groundspeed, and airspeed are shown in Figs. 5A, 5B, and 5C, respectively. Nevertheless, angular velocities did not differ significantly among the three concentrations (Table 1), possibly because the decreased turning frequency at higher doses was offset by a concomitant decrease in distance between reversals.

The tracks of males exposed to higher pheromone concentrations also narrowed significantly (Table 1). Examination of the mean frequency distributions of track width (Fig. 5G) reveals that for 1000 ng, the track width rarely exceeded 15 cm with the great majority of reversals less than 5 cm wide. At lower concentrations most reversals were still less than 5 cm wide but this peak was successively less pronounced and the reversal distances encompassed a wider range. The narrowing of tracks was attributable primarily to reductions in the length of track legs (distance between reversals) at higher concentrations (Fig. 5H) because the mean track angle of moths flying toward 1000 ng sources was marginally but significantly larger than the track angles measured from moths exposed to either 10 or 100 ng sources (Table 1). In addition, a higher proportion of turns were initiated closer to the plume centerline as pheromone concentration increased (Fig. 6). For example, 90 % of the turns were initiated within 12.5, 10, and 7.5 cm of the plume axis for 10, 100, and 1000 ng sources, respectively.

Kuenen and Baker (1982), citing the theoretical calculations of Bossert and Wilson (1963) for a time-average active space and their own measurements on the effect of concentration on reversal distance from the plume axis, suggested that an inverse relationship exists between the dimensions of active spaces of more concentrated plumes and the width of reversals. Our results are consistent with this contention. Figure 7 shows the frequency distribution of inter-reversal leg lengths measured at 16 cm increments along the windline for the three pheromone concentrations. It is evident that in plumes of greater strength, inter-reversal leg lengths are reduced along the entire longitudinal plume axis. In addition, as the moths progress nearer to the source there is a trend toward narrowing of the reversals which is particularly evident in 100 and 1000 ng plumes. Comparison of Fig. 3 and Fig. 7 suggests that, irrespective of the concentration, a high percentage of turns occur within the borders of the time-averaged plume. Moreover, as pheromone concentration increases, proportionally more turns are initiated within the active space.

Paralleling the inverse relationship between velocity parameters and stimulus concentration was a slight but significant reduction in wingbeat frequency with increased pheromone concentration (Table 1). These results are in agreement with those recorded for *Pectinophora gossypiella* which exhibited a ca. 11 % reduction in wingbeat frequency in response to a 100-fold increase in pheromone dosage (Farkas et al., 1974). The more modest 5 % reduction in wingbeat frequency shown here for *L dispar*, and elicited by a similar range of odor dosages could account, at least in part, for the concomitant 20 % decrease in airspeed. Comparison of wingbeat frequencies measured from track segments nearer to (60-100 cm downwind) and further removed (100-140 cm downwind) from the pheromone source revealed no significant differences regardless of the concentration ($P < 0.05$; Wilcoxon Matched-Pairs Test); wingbeat frequencies for track segments nearer vs. further from the pheromone source (± SD) were 27.2 (0.6) vs. 26.8 (0.9) at 10 ng, 26.2 (1.3) vs. 26.4 (1.0) at 100 ng, and 25.6 (1.0) vs. 25.5 (1.2) at 1000 ng.

Differences in pheromone concentration affected the propensity of males to lock-on to plumes and display in-flight arrestment (Table 2). Arrestment within the plume was characterized by a decrease in upwind groundspeed, often to values near zero, so that the moth appeared to be hovering, with narrow lateral movements directed essentially perpendicular to the windline. This behavior generally persisted for several seconds after which the moth typically flew abruptly upward out of the plume. All moths took flight, and locked-on to the plume in response to 100 and 1000 ng loadings. However, as concentration increased, a greater proportion of moths exhibited in-flight arrestment and the mean up-tunnel distance traversed before arrestment was reduced. Fewer moths locked-on to plumes from 10 ng sources, but all those moths that did so successfully flew the entire distance to the source.

Effects of Temperature

Ambient temperature modulated several features of the flight tracks of males flying to sources emitting pheromone at equivalent rates. Examination of representative flight tracks (Fig. 8), suggests that at 20 °C more turns were executed compared to 26 °C tracks, but the overall width of the tracks was similar at the two temperatures. The most marked differences evident among the measured parameters were the differences in groundspeed, windline groundspeed, upwind groundspeed, and airspeed, all of which increased at the higher temperature (Table 3). As a result, the mean total flight duration was correspondingly reduced.

At 20 °C the moths executed nearly twice the number of turns recorded from 26 °C tracks. Yet the turning frequency was identical at the two temperatures because the moths flying at the lower temperature required nearly twice as much time to traverse the observation area. Consequently, the angular velocities also remained constant at the two temperatures (Table 3).

Neither the mean track nor interleg angles recorded from 20 and 26 °C flights were significantly different from each other (Table 3). Only the course angle was significantly reduced at 20 °C (Table 3). The more upwind course heading in combination with the decreased airspeed contributed to the significantly larger drift angle at the lower temperature. In addition, track width increased marginally, albeit not significantly, at the higher temperature (Table 3). Curiously, this was the case even though the track legs recorded from 26 °C tracks were significantly longer than their counterparts at the lower temperature while the corresponding mean track angles were statistically indistinguishable. Still, the modest decrease in the magnitude of the track angle probably counterbalanced the increased distance between reversals, thus maintaining essentially the same track width.

When the values of parameters recorded from moths flying to 100 ng sources at 24 °C (Table 1) are compared with those obtained in the temperature experiment (Table 3), it is apparent that the 24 °C results are almost invariable consistent with the trends evident when temperature was varied. For example, turning frequencies and angular velocities are similar at the three temperatures whereas all of the velocity measurements recorded at 24 °C are intermediate in value to those obtained from 20 ° and 26 °C tracks. Interestingly, the emission rate from 100 ng dispensers at 24 °C was 1.25 ng/hr (Cardé and

Hagaman, 1983) and therefore virtually identical to the release rate used in the temperature trials.

Thoracic temperatures of individual males caught in flight were consistently elevated over the ambient temperature, ranging from 22.8 to 24.1 °C with a mean of 23.4 °C (SD 0.4) when the ambient temperature was 20 °C. At 26 °C the mean thoracic temperature was 28.2 °C (SD 0.3) with a range of 27.8 to 28.8 °C. These values are in accordance with previous measurements (Casey, 1980; Cardé and Hagaman, 1983) on *L. dispar* relating ambient temperatures to thoracic temperatures.

Discussion

The alterations seen in the slowing and narrowing of the zigzag flight path as pheromone concentration was increased are similar to those shown in *Grapholita molesta* (Kuenen and Baker, 1982). In agreement with the inverse orthokinetic trends demonstrated with *G. molesta*, gypsy moth males flew at lower groundspeeds and lower upwind groundspeeds toward pheromone sources emitting higher quantities of pheromone. Both species displayed reduced track widths and track leg lengths in response to increased concentrations. Airspeeds and course angles decreased, while elevated drift angles accompanied these changes. In the gypsy moth, however, counterturning frequency decreased as the plume concentration was increased. The increased drift angle evident in flight at higher concentrations for both species would provide more lateral drift and hence more visual information on the direction of wind flow (Cardé, 1984). These paths are reminiscent of the tracks of *Plodia interpunctella* as it closed in on a pheromone source with concomitant elevation of pheromone concentration (Marsh et al., 1978).

Elevated air temperatures evoked substantial increases in the velocity parameters measured; a 6 °C increase in ambient temperature elicited an 8 % increase in mean airspeed and a 66 % enhancement of mean upwind groundspeed. The augmentation of flight occurred even though the moths flying to the two test temperatures were exposed to identical pheromone concentrations and plume configurations. Thus, either the gypsy moth does not adjust its airspeed to maintain a 'preferred' flux of pheromone as may be the case in *Choristoneura fumiferana* (Sanders, 1985; Sanders, 1986) or the 'preferred' flux is temperature-dependent.

To determine the way in which the concentration of a pheromone plume modifies the zigzag maneuver, it would be ideal to vary the stimulus concentration within a 'discrete' plume specifically a plume of uniform size and homogeneity and possessing a sharp demarcation between the above and below threshold regions. In this fashion any differences in flight paths could be ascribed solely to the intensity of pheromone within a given plume size. But models of the active spaces generated by point sources of pheromone emitting at differing rates predict (and our direct measurements corroborate) that plume isopleths (lines of equal concentration) expand laterally to the plume axis and the plume becomes more dilute over the 160 cm of downwind mixing. The gradients present within the active space thus may be substantial. Furthermore, because a flying male could encounter several plume edges per sec as he zigzags upwind and because the wing-fanning assay relies on a behavioral reaction that occurs over several to tens of sec, it is not possible to precisely define the active space and the position of the plume's edge as perceived by a flying male. Indeed, the pheromone threshold of quiescent gypsy moths for activation is substantially higher than the concentration of pheromone necessary to sustain upwind flight (Cardé and Charlton, 1985).

Counterturning in the gypsy moth tends to be initiated closer to the plume's centerline in more concentrated plumes, as Kuenen and Baker (1982) have emphasized in analysis of *G. molesta*. Thus as pheromone concentration is increased there is an inverse relationship between the tendency to initiate a counterturn and the probability of encountering a sharp decrease in pheromone concentration as the moth reaches (or flies beyond) the pheromone plume's edge. Further, many of these turns (Figs. 3 and 7) seem to be executed within the *time-averaged* plume. These correlations would seem to argue against the plume's edge serving as a cue that increases the probability of counterturning (cf., Cardé, 1986). Instead, the narrower and 'more accurate' track may depend solely on the self-steering program instigated by higher pheromone concentrations (Kuenen and Baker, 1982). Additional trials that employ plumes of differing dimensions and concentrations will be needed to validate this contention.

The counterturning maneuver itself is of particular interest. The zigzag path initially was viewed by Kennedy (1977) as a 'reversing anemomenotaxis' in which the program of left and right reversals was initiated when the flyer reached "the margins of the plume". Later experiments by Kennedy et al. (1980, 1981) using wide homogeneous clouds in a wind tunnel showed that the zigzags of male *Adoxophyes orana* could occur either along the border between the cloud and the clean air or within the cloud itself. Thus, the zigzag is not a reaction to loss of the scent and a turn toward the direction in which it was last detected; rather, the turns appear to be self-steered and generated by an 'internal program' (Baker, 1986; David, 1986; Kennedy, 1986). Moreover, Kennedy et al. (1980, 1981) showed that upwind flight entirely within the cloud never persisted for more than two sec., suggesting that an intermittent signal, such as would be encountered when zigzagging along a narrow plume, was necessary to sustain upwind flight. The importance of a 'flickering' signal to upwind flight has been confirmed in *G. molesta* by the ingenious strategy of presenting pheromone in a train of pulsed clouds (Baker et al., 1985). Males fly a zigzag path upwind in such a fluctuating signal, whereas a uniform cloud of pheromone does not evoke upwind flight.

Kennedy (1986) characterized the counterturning mechanism as "driven by a central nervous turn generator, which alternates between left and right at a frequency governed by the concentration and pulsing of the pheromone input". The existence of central pattern generators for walking and flight in insects has been questioned, in part because sensory input seems to be integral to the pattern generating system (Pearson, 1985). For the motor pattern output to be appropriate it must be coordinated with the position and movements of the associated structures. This view of rhythmic motor activity, however, still provides for an internal generation of counterturning. Given a certain level of pheromone input, turns could be initiated after moths had flown either a characteristic distance or time interval along a leg. Such a feedforward system could thus rely on optomotor or mechanoreceptor input to initiate the counterturn for flying and walking insects, respectively. The striking regularity and low variance associated with the frequency of counterturns (Tables 1 and 3) seems inconsistent with the interpretation that counterturns observed in *tethered* gypsy moths in simulated free-flight are explicable solely by "internal noise functions" (Preiss and Kramer, 1986a).

In *L. dispar* the turning frequency was elevated at the lowest pheromone concentration tested (10 ng source, Table 1), but constant at matched stimulus intensities when the body temperatures differed by 5 °C. That the rhythms of counterturning were indistinguishable in the two test temperatures suggests that if a central pattern generator controls counterturning, its output is not modulated by a 5 °C lowering of thoracic temperature.

Although these experiments provide insight into the steering maneuvers that a male gypsy moth uses to navigate a flight along a narrow pheromone plume aligned with the wind, other conditions remain to be investigated if we are to understand how a gypsy moth locates a point source of pheromone at a distance of tens of meters in a natural forest habitat. There the upwind direction and the long axis of the plume are infrequently aligned, so that a moth flying upwind in an above threshold concentration of pheromone usually is not aimed directly toward the pheromone source and may often head out of the plume (Elkinton et al., 1987). Strategies for recontacting the plume, either by casting flight lateral to the windline (David et al., 1983) or hovering in a restricted area until pheromone again envelops the male, may be crucial to successful plume following. Secondly, the structure of a plume generated in a forest may be quite different from the discrete plumes used in my experiments. Tens of meters downwind of the pheromone source the plume is fenestrated, dilute, and expanded compared to its initial state. Even close to a calling female the plume may be quite broad. A gypsy moth female typically calls while perched on a tree trunk (Doane, 1968). When a female is on either the wind or lee side of a trunk, the plume becomes roughly equal to the tree's diameter. Such plumes may produce a quite different series of behavioral reactions than observed in the present tests. But when a female calls either from a crosswind side of the trunk or from an isolated twig, a narrow plume will issue, evoking the very upwind maneuvers seen in response to a narrow plume in the sustained-flight wind tunnel.

Table 1. Mean orientation and track parameters (± SD) of *L. dispar* males flying toward pheromone sources of three concentrations.

Flight Parameter	Pheromone Dosage (ng)		
	10	100	1000
Total Flight Duration (sec)	10.9^{a} (2.3)	18.2^{b} (6.8)	34.4^{c} (13.7)
Total Number of Turns	51.6^{a} (13.2)	73.5^{a} (33.3)	145.4^{b} (69.9)
Turning Frequency (turns/sec)	4.7^{a} (0.7)	3.9^{b} (0.6)	4.1^{b} (0.6)
Overall Velocity (cm/sec)	50.6^{a} (11.7)	30.8^{b} (5.1)	22.7^{c} (3.9)
Absolute Ground Velocity (cm/sec)	23.4^{a} (4.8)	15.0^{b} (2.7)	11.2^{c} (2.1)
Net Ground Velocity (cm/sec)	15.3^{a} (3.4)	10.0^{b} (3.5)	5.4^{c} (2.2)
Air Velocity (cm/sec)	96.0^{a} (8.6)	83.0^{b} (3.6)	76.9^{c} (2.6)
Length of Track Leg (cm)	11.3^{a} (3.4)	7.8^{b} (1.8)	5.5^{c} (1.4)
Track Width (cm)	9.2^{a} (3.0)	6.0^{b} (1.5)	4.2^{c} (1.3)
Angular Velocity (degrees/sec)	498.4^{a} (88.9)	449.3^{a} (92.3)	487.6^{a} (73.5)
Track Angle	68.7^{a} (4.6)	69.0^{a} (5.6)	75.7^{b} (5.0)
Interleg Angle	72.3^{a} (6.9)	64.9^{b} (9.5)	61.6^{b} (5.4)
Course Angle	26.9^{a} (5.2)	17.6^{b} (3.1)	13.5^{c} (2.4)
Drift Angle	41.8^{a} (5.5)	51.2^{b} (5.9)	61.8^{c} (5.5)
Wingbeat Frequency (beats/sec)	27.0^{a} (0.5)	26.3^{b} (1.1)	25.6^{c} (1.1)

Fifteen moths were tested at each concentration. Means in the same row having no letters in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test. Wingbeat frequencies represent means measured from a zone extending 60-140 cm downwind of the pheromone source.

Table 2. Numbers and percentages of male *L. dispar* locking-on to plume and displaying in-flight arrestment at different pheromone concentrations.

Concentration (ng)	No. Moths Observed	No. Moths Locking-on	No. Moths Arrested	Distance Flown (cm) Before Arrestment (± SD)
10	28	18 (64.3 %)^{a}	0 (0 %)^{a}	----
100	20	20 (100 %)^{b}	4 (20 %)^{b}	82.5 ± 53.2
1000	20	20 (100 %)^{b}	7 (35 %)^{b}	68.6 ± 34.9

Numbers in parentheses indicate proportion of moths exhibiting response. Percentages in same column having no letters in common are significantly different ($P < 0.05$) according to a method of adjusted significance levels for proportions (Ryan, 1960).

Table 3. Mean orientation and track parameters (± SD) of *L. dispar* males flying toward pheromone sources providing identical release rates at two ambient temperatures.

Flight Parameter	Ambient Temperature (°C) 20°	Ambient Temperature (°C) 26°
Total Flight Duration (sec)	24.8^a (8.1)	14.4^b (4.3)
Total Number of Turns	103.1^a (44.6)	57.6^b (20.4)
Turning Frequency (turns/sec)	4.0^a (0.6)	4.0^a (0.5)
Overall Velocity (cm/sec)	28.7^a (4.5)	36.4^b (8.1)
Absolute Ground Velocity (cm/sec)	14.4^a (1.7)	18.0^b (4.3)
Net Ground Velocity (cm/sec)	7.3^a (3.1)	12.1^b (3.6)
Air Velocity (cm/sec)	79.8^a (3.5)	85.9^b (4.8)
Length of Track Leg (cm)	7.2^a (1.6)	9.0^b (2.4)
Track Width (cm)	5.4^a (1.4)	6.7^a (2.0)
Angular Velocity (degrees/sec)	476.4^a (78.7)	453.2^a (68.1)
Track Angle	74.1^a (4.9)	69.7^a (5.9)
Interleg Angle	61.0^a (5.4)	64.0^a (8.1)
Course Angle	16.7^a (2.8)	20.6^b (4.7)
Drift Angle	57.4^a (6.0)	49.1^b (6.2)

Fifteen moths were tested at each temperature. Means in the same row having no letter in common are significantly different ($P < 0.05$) according to Mann-Whitney *U* test, two-sided.

Figure 2. Terminology and conventions used for analysis of *L. dispar* flight tracks. (A) Representative flight track: (1) original - solid line, and (2) filtered - dashed line (70 degree minimum cumulative turn); (B) Flight track parameters indicating the notation used throughout.

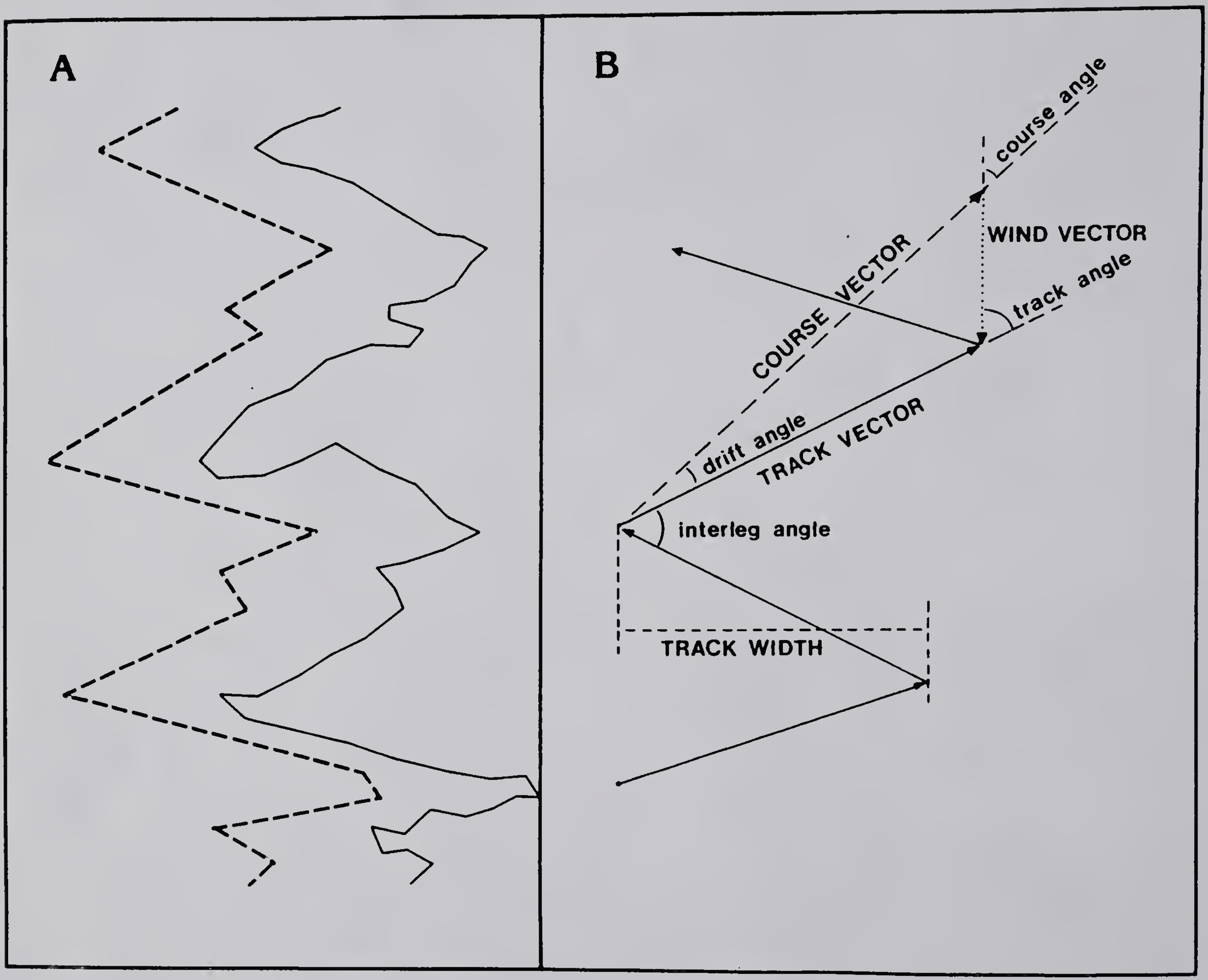

A
B
course angle
WIND VECTOR
COURSE VECTOR
track angle
drift angle
TRACK VECTOR
interleg angle
TRACK WIDTH

Figure 3. Dimensions of pheromone plumes of three concentrations as estimated by male wing fanning response. Vertical bars represent proportion of moths responding (N=10 at each position); associated numbers denote wing fanning latency (± SD). Dashed lines demarcate the time-average pheromone active space as indicated by at least a 50% decrement in numbers responding. For each concentration, the latency to wing fanning means along the same crosswind line having no letters in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test

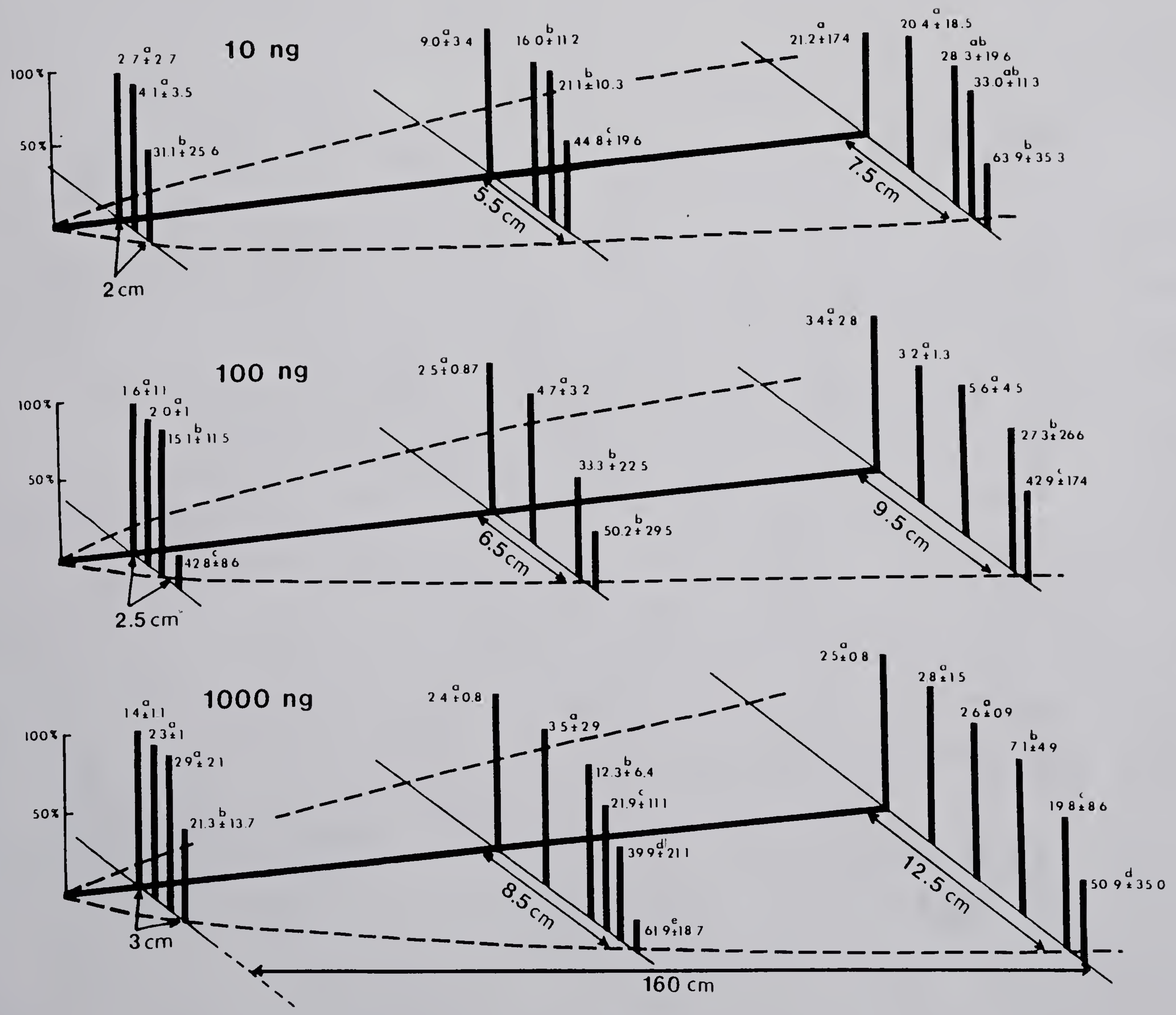

10 ng
100%
50%
2.7±2.7 a
4.1±3.5 a
31.1±25.6 b
2 cm
9.0±3.4 a
16.0±11.2 b
21.1±10.3 b
44.8±19.6 c
5.5 cm
21.2±17.4 a
20.4±18.5 a
28.3±19.6 ab
33.0±11.3 ab
63.9±35.3 b
7.5 cm
100 ng
100%
50%
1.6±1.1 a
2.0±1 a
15.1±11.5 b
42.8±8.6 c
2.5 cm
2.5±0.87 a
4.7±3.2 a
33.3±22.5 b
50.2±29.5 b
6.5 cm
3.4±2.8 a
3.2±1.3 a
5.6±4.5 a
27.3±26.6 b
42.9±17.4 c
9.5 cm
1000 ng
100%
50%
1.4±1.1 a
2.3±1 a
2.9±2.1 a
21.3±13.7 b
3 cm
2.4±0.8 a
3.5±2.9 a
12.3±6.4 b
21.9±11.1 c
39.9±21.1 d
61.9±18.7 e
8.5 cm
2.5±0.8 a
2.8±1.5 a
2.6±0.9 a
7.1±4.9 b
19.8±8.6 c
50.9±35.0 d
12.5 cm
160 cm

Figure 4. Representative flight tracks of *L. dispar* males flying to three different pheromone concentrations. Flight tracks were recorded from overhead with the moth progressing from bottom to top. Open asterisks denote the moths' positions at 1 sec intervals.

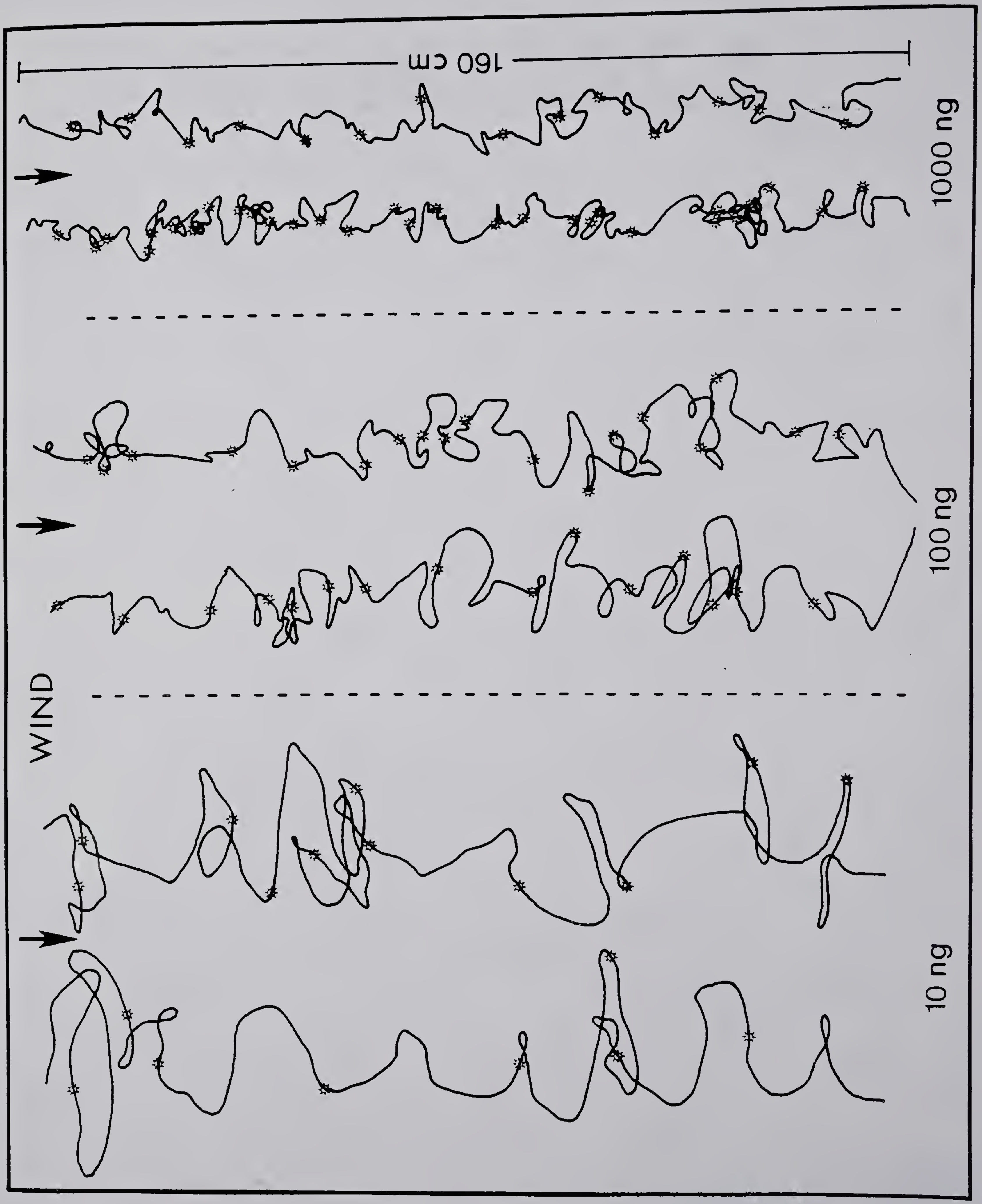

160 cm
1000 ng
100 ng
10 ng
WIND

Figure 5. Mean frequency distributions of; A, groundspeeds; B, windline groundspeeds; C, airspeeds; D, track angles to wind; E, interleg angles; F, course angles; G, track widths; H, length of track legs; at three concentrations.

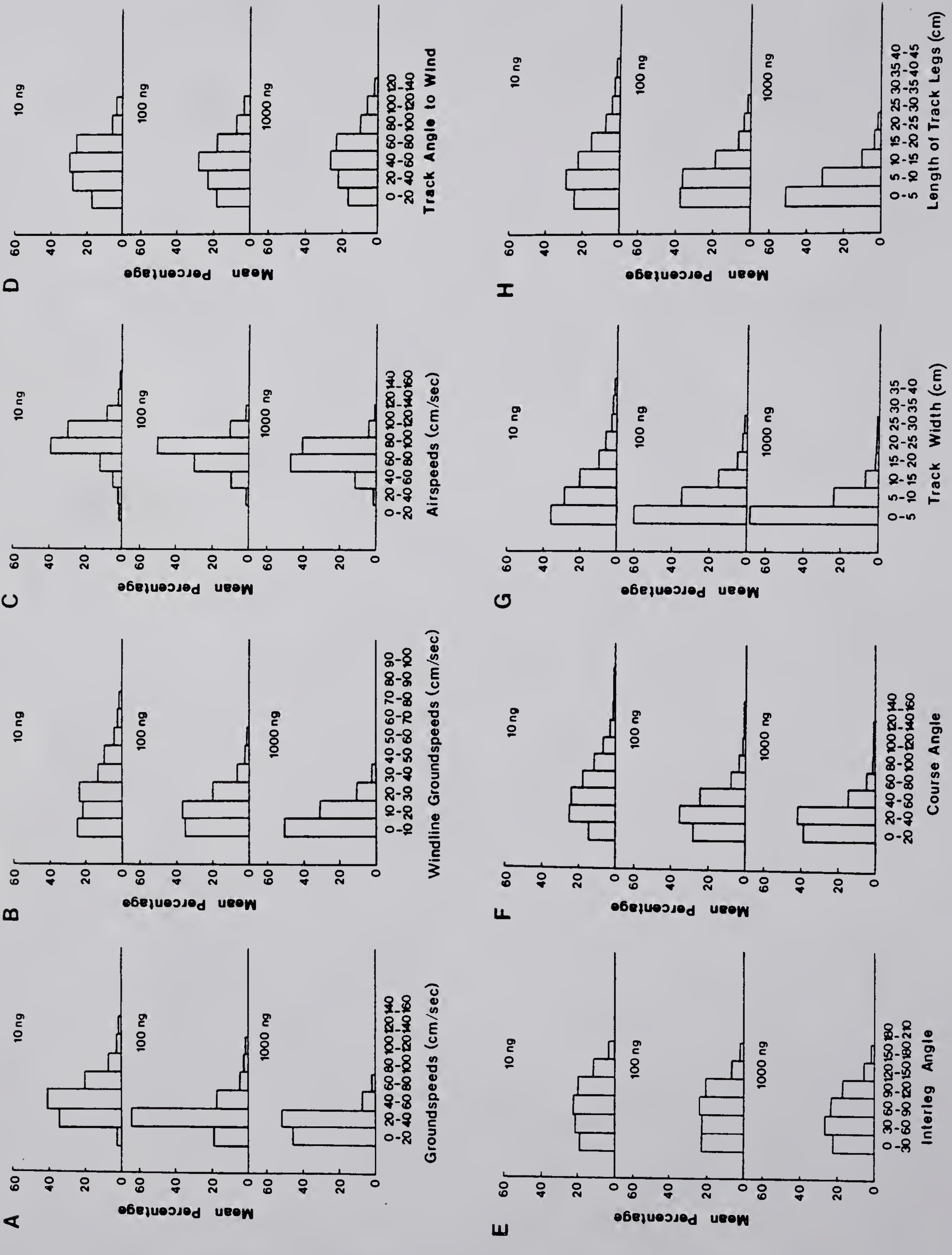

A
B
C
D
E
F
G
H
Mean Percentage
10 ng
100 ng
1000 ng
0
20
40
60
Groundspeeds (cm/sec)
Windline Groundspeeds (cm/sec)
Airspeeds (cm/sec)
Track Angle to Wind
Interleg Angle
Course Angle
Track Width (cm)
Length of Track Legs (cm)

Figure 6. Mean frequency distribution of inter-reversal leg lengths measured from moths flying to different pheromone concentrations. Solid lines represent the cumulative percentage of turns initiated at increasing distances from the plume centerline.

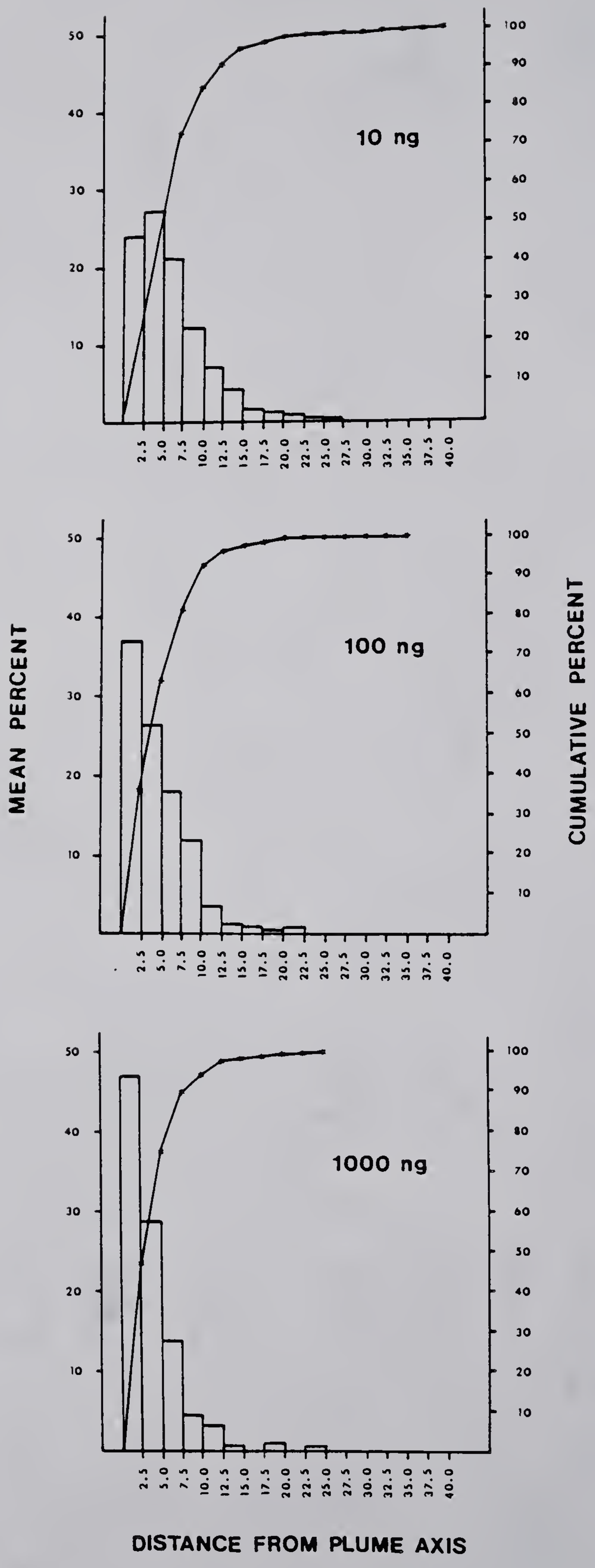

10 ng
100 ng
1000 ng
MEAN PERCENT
CUMULATIVE PERCENT
10
20
30
40
50
10
20
30
40
50
60
70
80
90
100
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5
40.0
DISTANCE FROM PLUME AXIS

Figure 7. Mean frequency distributions of inter-reversal leg lengths measured at regular increments along the windline. Distributions represent composites of 15 complete tracks for each concentration with each track given equal weighting. The moths' tracks proceed from left to right.

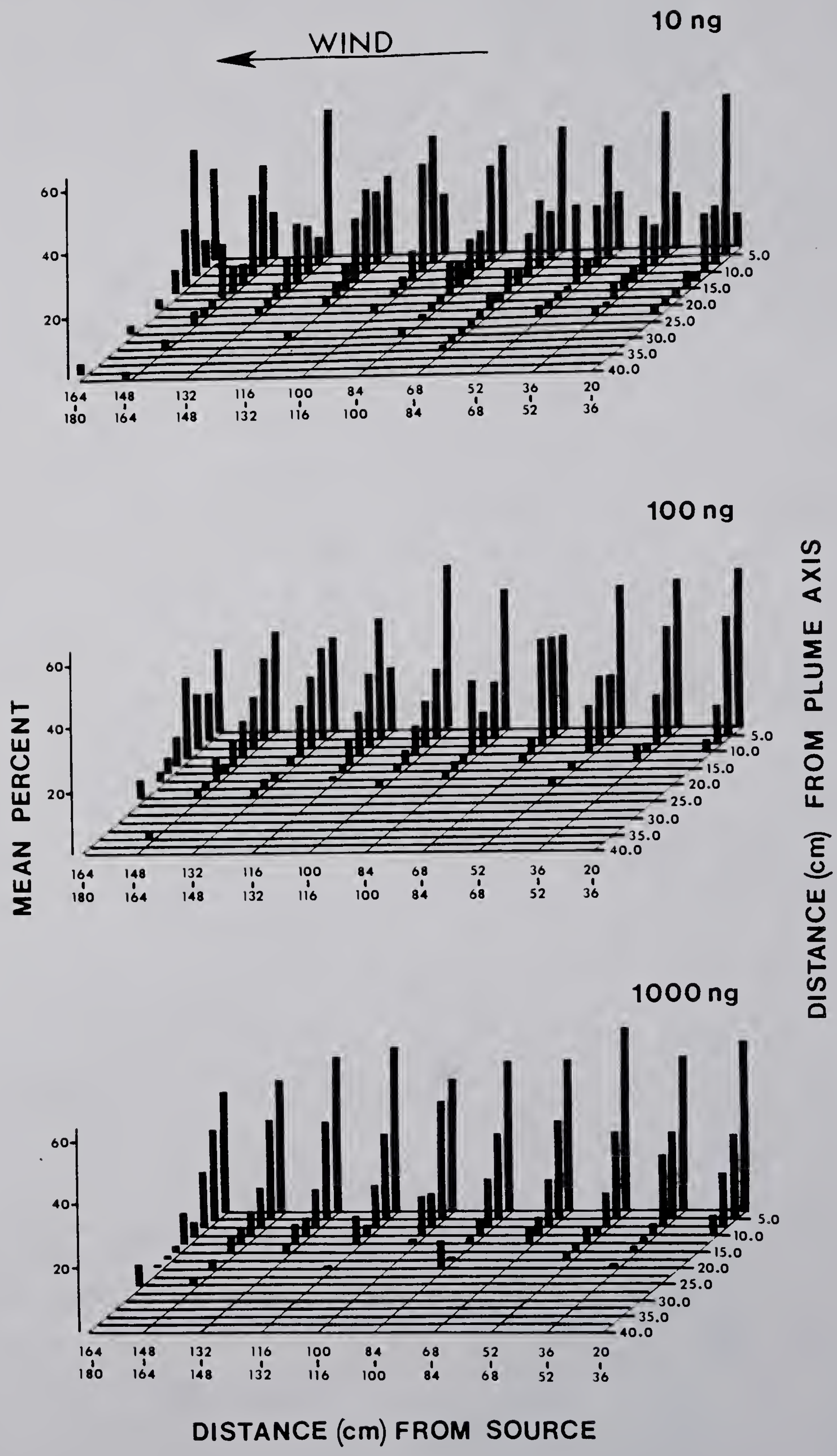
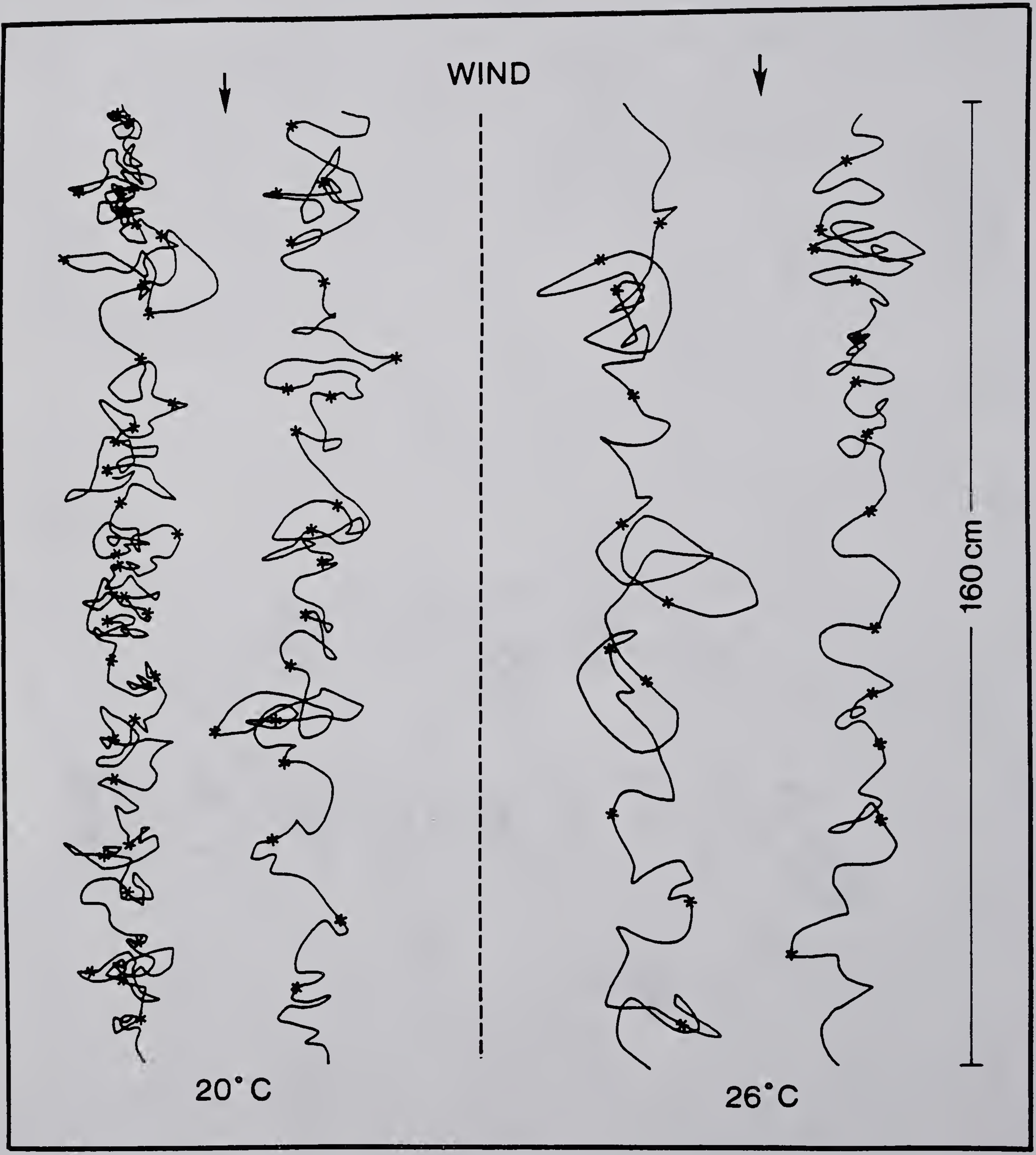

10 ng
WIND
100 ng
1000 ng
MEAN PERCENT
60
40
20
DISTANCE (cm) FROM PLUME AXIS
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
164 180
148 164
132 148
116 132
100 116
84 100
68 84
52 68
36 52
20 36
DISTANCE (cm) FROM SOURCE

Figure 8. Typical flight tracks of *L. dispar* males flying at two temperatures and identical pheromone emission rates. Flight tracks were recorded in the plan view with the moth progressing from bottom to top. Solid asterisks indicate the moths' positions at 1 sec intervals.


WIND
160 cm
20°C
26°C

CHAPTER III

ORIENTATION OF MALE GYPSY MOTHS TO PHEROMONE SOURCES: THE ROLE OF OLFACTORY AND VISUAL CUES

Introduction

The adult male gypsy moth's quest for a virgin female encompasses several different behaviors mediated in large part by a female-emitted pheromone, identified as (7R,8S)-*cis*-epoxy-2-methyloctadecane (Bierl et al., 1970; Iwaki et al., 1974; Hansen, 1984) and named (+)-disparlure. Following locking on to the pheromone plume by the male, four basic behavioral patterns that culminate in mating can be discerned: (1) upwind flight toward the odor source; (2) vertical flight in the immediate proximity of the tree bole harboring the female, the male intermittently contacting the bark with its tarsi; (3) landing on the trunk whereupon the male walks while wing fanning and orients to the female, and; (4) contact with and recognition of the female followed by mating (Doane, 1968; Richerson et al., 1976a; Richerson, 1977; Cardé, 1981).

Considerable effort has been invested in the study of flying orientation in this species (Cardé and Hagaman, 1979; David et al., 1983; Elkinton and Cardé, 1984; Charlton et al., 1989a), but other elements crucial to location of the female including the factors involved in the choice of the landing site and behaviors subsequent to landing, such as walking orientation have received comparatively little attention. In particular, the relative contribution of visual and olfactory inputs to the male orientation process remains poorly understood. Doane (1968) reported that males oriented to and attempted to copulate with non-calling females which were located downwind of calling, virgin females, suggesting that the males were responding to visual cues from the female. However, it is not clear whether these pheromone-stimulated males were responding visually to the female or to the vertical stakes on which they perched. Conversely, Cardé (1981) found that feral males located (+)-disparlure dispensers as quickly as pheromone dispensers presented in combination with dead females, implying that visual cues associated with the female were not crucial to the mate-seeking process.

In this study, we describe field and laboratory analyses of male close-range flying and walking orientation designed to decipher the behavioral reactions and sensory inputs that promote successful location of female gypsy moths by the males. Ideally, behavioral studies should be conducted under natural conditions where full expression of the behavioral repertory is not hindered. The often highly variable wind field and resultant pheromone dispersion patterns prevalent under these conditions, however, render the factors mediating certain behaviors difficult, if not impossible to decipher. Therefore, close-range flying orientation and walking behaviors were investigated further in a sustained-flight wind tunnel where the appropriate variables could be more precisely controlled.

Materials and Methods

Moths

Lymantria dispar were received as egg masses from the USDA-APHIS Methods Development Center, Otis Air National Guard Base, Massachusetts. Larvae were reared on wheat germ diet (Bell et al., 1981) at 24°C and 60-70% relative humidity on a 16L:8D photoperiod. Males were segregated from females in the pupal stage and transferred for emergence to screen cages (30 x 30 x 36-cm) which were held in an environmental chamber regulated at conditions identical to those used in rearing.

Pheromone

The synthetic (7R,8S)-*cis*-epoxy-2-methyloctadecane (Farnum et al., 1977) used in these experiments had an enantiomeric purity >99% as determined by analysis of precursors. GLC analysis using a 30m SP-2340 fused silica capillary column indicated the presence of <1% of the *trans* isomer. A 1 ug/ul solution of (+)-disparlure in hexane was formulated gravimetrically and serially diluted to obtain the desired concentrations.

Wind Tunnel

Laboratory behavioral assays were conducted in a Plexiglas™ sustained-flight tunnel, 240 cm long, 100 cm wide, and 72 cm high, as described in Cardé and Hagaman (1979) and Charlton et al. (1989a). A voltage-regulated 1/2 hp fan pushed air through a set of screen baffles to provide a relatively laminar air flow with a velocity of 50 cm/sec as measured with a hot-wire anemometer (Weather Measure Corp; W-141 A 1/5) at the position of the pheromone source. Pheromone was vented from the downwind end of the tunnel to the outside by an exhaust fan. Illumination was provided by eight 40 watt DC-mains fluorescent bulbs diffused by reflection from a matte-white ceiling. The resultant light intensity, measured in the center of the tunnel was 450 lux. A canvas belt with alternating 10 cm wide yellow and white stripes located immediately beneath the clear tunnel floor provided optomotor feedback for the moths.

Data Recording and Analysis

Behaviors were recorded either from above or from the side view, according to the experimental design, with a SONY RSC 1050 video camera and a SONY SLO-340 video recorder. For analysis, tracks of walking and flying moths were re-recorded onto a SONY SVM-1010 motion analyzer and displayed back frame-by-frame. The moths' successive positions every 0.1 sec were marked on a clear acetate sheet affixed to the analyzer screen. The X,Y coordinates comprising the tracks were transferred to an Apple IIe microcomputer via an Apple Graphics Tablet and selected parameters were calculated using computer programs (BASIC) written for this purpose (Charlton et al., 1989a).

General Testing Procedure

Moths were randomly selected from emergence cages, transferred individually into 30-ml clear plastic cups resting on screen squares, and held in the wind tunnel room for at least a 1 hr pretest acclimation period. At the onset of each trial, the appropriate pheromone source was introduced into the tunnel. Then the screen holding a moth was placed on a 25 cm high metal stand 160 cm downwind of the pheromone source and within the pheromone plume. Following activation and intiation of upwind flight, the moths flew up the tunnel over a stationary floor pattern. All moths used for experiments were 36-48 hr old and tested between the 6th and 10th hours of photophase.

Effect of Female Visual Stimuli on Efficiency of Male Location of Pheromone Sources in the Forest

Initial experiments evaluated the role of visual cues presented by the female in promoting efficiency of location of (+)-disparlure sources by the males. Experiments were conducted on July 27-28, 1984 in a mature oak-maple forest with sparse understory vegetation in Pelham, Massachusetts. This site supported a low density, non-defoliating population of wild gypsy moths. Experiments were conducted from 1200 to 1600 hours e.d.s.t. to coincide with the interval of peak male flight (Cardé et al., 1974).

Male response to three different treatments was compared: (1) 1000 ng (+)-disparlure on a 0.7 cm diam filter paper disc; (2) 1000 ng (+)-disparlure in conjunction with an acetone-rinsed dead female, and; (3) a dead, acetone-rinsed female alone. The filter paper dispensers were dyed to blend in with the bark background. Treatments were placed at a height of 1.5 m on a ca. 0.7 m diameter white oak (*Quercus alba*) trunk. The tests involving the female alone were conducted first to avoid the possibility of residual pheromone influencing the results. The remaining two treatments were alternated following each successful location by a male. Males not locating treatments were not included in the analysis.

To facilitate transcription of walking paths, the observation tree was invested with a 10 x 10 cm grid fashioned with white cotton thread. The grid encompassed the entire circumference of the tree and extended from 0.5 m to 3 m above the ground. A SONY RSC 1050 rotary-shutter video camera linked to a SONY SLO-340 videorecorder was used to record the walking pathways. The following indices of pheromone source location were derived from the transcribed paths: (1) initial distance between landing position and the stimulus; (2) time required from landing until direct contact with treatment, and; (3) the linearity of the walking path, defined as the straight-line distance between the landing point and the stimulus divided by the total distance traversed along the actual walking track (Bell et al., 1983).

Spectral Properties of Females

To human eyes, female gypsy moths appear white, so consequently the females contrast sharply with the dark background of the tree trunks on which they typically call. However, the color and level of contrast that the male gypsy moth perceives may differ substantially, depending on the sensitivity of its visual system relative to the spectrum of available reflectance, as well as differences perceived in the reflectance components of female wings vs. the background (Prokopy and Owens, 1983). Photographic techniques were used to provide relative estimates of the visual contrast of females in their natural environment. Females were placed on a white oak trunk (*Quercus alba*) and photographed under sunny conditions from a distance of ca. 1 m to emulate the scene confronting a male as he approaches a calling female on a tree bole. This scene was photographed with black-and-white film using either a UV filter (which transmits only UV light), no filter, or the following narrow band-pass filters (half-bandwidth = 50 nm): 450, 500, 550, and 600 nm. This range of wavelengths was chosen to encompass the electroretinogram response curve of the male compound eye, which is characterized by a peak between 480-590 nm, approximately half the maximum response in the UV range (350-390 nm) and greatly diminished response in the red region (600-700 nm) (Brown and Cameron, 1977).

Male Response to Pheromone and Visual Cues on Vertical Silhouettes

This series of experiments assessed whether, in a choice situation, a female visual model alone or in combination with a pheromone source on a vertical silhouette, would elicit an increase in the likelihood of male flying and walking orientation over that evoked by a pheromone source alone.

These tests employed two simulated tree trunks constructed from 70 cm high x 20 cm diam cylinders wrapped with dark brown construction paper. The cylinders were positioned transverse to the wind flow such that their centers were 40 cm apart, and 40 cm from the upwind end of the flight tunnel. The pheromone source comprised a 0.7 cm diam filter paper disc, dyed to match the appropriate background and impregnated with 100 ng of (+)-disparlure. A female gypsy moth, killed by freezing ca. 1 hour after eclosion and thus prior to commencement of pheromone emission (Charlton and Cardé, 1982) provided the visual cue. Males were released from a platform centered at the downwind end of the tunnel and allowed to fly upwind over a stationary floor. Trials were terminated either when the male contacted the screen at the upwind end of the tunnel or exited the observation area. Twenty-five males were flown to each treatment combination and the female was alternated between trunks following each successful male flight. Horizontal flight tracks were videorecorded in the plan view over a zone extending 90 cm downwind of the trunks. Supplemental verbal descriptions of behaviors were recorded onto the audio track of the videotape.

Male response to four different stimulus configurations was assayed: (1) The pheromone source was suspended 30 cm high and equidistant between the two trunks by a taut thread. Pheromone was not deployed on either trunk; the female was pinned to one of the trunks at the height of the pheromone while the other trunk was barren. The female was alternated between trunks following each successful male flight. (2) The pheromone source was pinned to one of the trunks and the other trunk held only the female. A filter paper hexane control was positioned between the trunks as descibed in (1). The position of the trunks was switched after each male flight. (3) Same configuration as (2), except that the female was attached directly above and on the same trunk as the pheromone source. The other trunk held neither pheromone nor female. (4) The trunks were positioned as described previously. However, now a pheromone source was affixed to both trunks and the female was pinned on one of the trunks directly above the pheromone dispenser. The female was switched between trunks following each male flight. Visible vapors from titanium tetrachloride-impregnated filter papers positioned identically as the pheromone sources indicated that the individual plumes coalesced ca. 80 cm downwind of the trunks. Thus, a male moth orienting upwind in the pheromone plume could respond to the pheromone plume emanating from the trunk either with or without the associated female visual stimulus.

The following behaviors were derived from the video and verbal record: (1) contact (initial and subsequent) with either the pheromone source, the female, the trunk harboring the female, or the empty trunk, (2) landing and walking on either trunk, and (3) time from landing until contact with the pheromone source or the female. In addition, the moths' net up-tunnel ground velocities of flight along the windline were measured with a stopwatch to the nearest 0.1 s as they traversed the section of the tunnel extending 10-90 cm downwind of the trunks.

Effect on Walking Orientation of Spatial Segregation of Visual and Olfactory Cues

This experiment assayed the consequences on male orientation of separating a female visual stimulus from the chemical source. A 30 cm diam x 70 cm high surrogate tree, constructed of brown paper supported by a metal cylinder, was positioned with its center 50 cm from the upwind end of the tunnel. A 2 x 2 cm grid was drawn in pencil over the entire paper surface to aid in transcription of walking tracks. Two concentric circles, 10 cm and 20 cm in diam were marked on the paper, centered at a height of 35 cm. These circles were divided into four sectors designated upwind, downwind, upper, and lower. Pheromone was dispensed at the center of the circles from a 0.7 cm diam filter paper disc loaded with 100 ng of (+)-disparlure and dyed to match the background. 'Smoke' trials using titanium tetrachloride on filter paper indicated that this arrangement provided a thin pheromone plume directed downwind and perpendicular to the vertical axis of the cylinder. An acetone-rinsed dead female gypsy moth was pinned in one quadrant at a time either 5 or 10 cm away from, or directly above the pheromone source; the control utilized the pheromone source alone. Females deployed at or downwind of the pheromone source were not reused in tests due to potential contamination with pheromone. Males were released from the downwind end of the tunnel and their behaviors subsequent to landing on the cylinder were videorecorded. For each male, (1) the time from landing until contact with the female and/or the pheromone dispenser, (2) the distance between landing position and pheromone source, and (3) the percentage of time spent in each quadrant both near (<5 cm) and removed from (5-10 cm) the pheromone source prior to contact with either the pheromone source or the female were derived from a frame-by-frame analysis of the tapes.

Relative Importance of Visual and Chemical Stimuli

Results of the previous experiment suggested that males could respond visually to females over short distances (<10 cm) only when the males were enveloped by the pheromone plume. To examine this response in greater detail and to elucidate the relative importance of the olfactory and visual cues in this situation, a test was devised whereby the visual and olfactory stimuli were uncoupled as the male approached within 10 cm of the female. This experiment utilized the same surrogate tree described in the previous experiment except that the cylinder sat atop a thin metal revolving platform to allow for rotation. An acetone-rinsed female was pinned in combination with 100 ng (+)-disparlure on a 0.7 cm diam filter paper disc at a height of 35 cm. Males were released individually at the downwind end of the tunnel, whereupon they flew upwind, landed on, and walked while wing fanning over the surface of the cylinder. The male was permitted to continue walking until he entered an area ca. 5-8 cm downwind of the female, where normally the probability of negotiating a direct approach to the female was very high (0.96 probability from previous tests). At this juncture, the wind was abruptly shifted by rotating the cylinder via an attached string through a ca. 60° arc (Fig. 9); the shift was accomplished in <1 sec. The consequence of this maneuver was that the male maintained the same spatial relationship with the female, but now he was no longer in the pheromone plume. To guard against the possibility that the actual movement of the cylinder induced particular male reactions, a control was devised whereby the female and pheromone dispenser were initially positioned such that the male was downwind of the pheromone source both prior and subsequent to rotation of the cylinder. The cylinder was rotated through a similar arc and at the same velocity as described previously. Video cameras positioned on both sides of the flight tunnel recorded the males' behaviors prior to and immediately following the loss of the plume. The following were recorded for each moth: (1) time elapsed between rotation of the cylinder and contact with the female; (2) length of the walking path, and; (3) the linearity of the walking path.

Results

Effect of Female Visual Stimuli on Efficiency of Male Location of Pheromone Sources in the Forest

Under forest conditions, visual stimuli alone, in the absence of pheromone, were not sufficient to elicit male orientation. Thus, when the female was deployed alone, only 3 of 34 males that passed through a 2 m zone around the observation tree engaged in vertical flight near the tree, and of these, only 1 male landed and eventually contacted the female. Conversely, when pheromone was dispensed, the majority of the males (40 of 46) that entered the 2 m zone landed and walked while wing fanning over the tree bole. The presence of female visual cues apparently did not influence the males' choice of landing spot since the mean initial distances between the landing position and the pheromone locus did not differ significantly whether or not the female was present (Table 4). In addition, the time required to negotiate the walking path to the stimulus was not affected by the presence of the female, nor did the mean magnitudes of the associated linearity indices differ (Table 4).

All of these indices of source location had high associated levels of variability (Table 4). This reflected the diversity of search patterns exhibited by the males (Fig. 10). In some cases, the approach to the female/pheromone was quite direct. More typically, walking males adopted a vertical heading on the tree bole with relatively few intervals of directed movement toward the female with pheromone (Fig. 10). The tortuous nature of these paths with their attendant low linearity values suggested that visually-mediated orientation was probably not involved. Instead, random search, preprogrammed search (i.e., based on internally-derived information), response to an intermittent odor signal, or elements of all three strategies appeared to be implicated. However, the precise contribution of these alternatives to the search process remained nebulous because it was difficult, under forest conditions, to instantaneously correlate pheromone dispersion patterns with male behavioral reactions. The problem was exacerbated because the windfield prevalent under forest conditions can be extremely variable. Figure 11 depicts pheromone dispersion patterns as visualized by titanium tetrachloride 'smoke' in the forest over a 1 min interval and indicates how dramatically the wind can shift even over relatively short time intervals.

Spectral Properties of Females

Females photographed using various narrow bandpass filters contrasted with and were visually discernible against the natural bark background over the entire spectral sensitivity range of the males (Fig. 12). The intensity of contrast appeared most pronounced when no filter or 550 and 600 nm filters were employed and diminished at shorter wavelengths (450 and 500 nm). Females were also distinctive when photographed through a UV filter, chiefly due to their increased intensity of reflectance relative to the bark background; the tree bole appeared relatively darker and thus reflected less radiation in the UV range compared to higher wavelengths.

Male Response to Pheromone and Visual Cues on Vertical Silhouettes

Experiment 1: When the pheromone source was deployed separate from the trunks, all the males initially oriented to the pheromone source. The majority of these males first contacted the pheromone dispenser, whereas only a small number exited the pheromone plume to contact either trunk; none of the latter males landed and walked on either trunk (Fig. 13A).

Experiment 2: When the pheromone dispenser was affixed to one of the trunks and a female alone to the other, virtually all the males initially oriented to and contacted the trunk with the pheromone (Fig. 13B); all the males that contacted this trunk landed and walked while wing fanning over its surface. Only one male oriented to and contacted the female trunk and this male did not alight on the trunk. No orientations or contacts were recorded for the isolated filter paper control.

Experiment 3: Males oriented exclusively to the trunk holding the female and pheromone together. All of these males subsequently landed and walked while wing fanning on this trunk (Fig. 13C).

Experiment 4: When pheromone was dispensed simultaneously from both trunks and the female alternated between the trunks, oriented flights, contacts and walking were evenly divided between the two trunks (Fig. 13D). This lack of bias was not attributable to a propensity for males to fly preferentially on either side of the wind tunnel since 56% of the flights were directed toward the trunk on one side vs. 44% to the trunk on the opposite side of the tunnel ($P>0.05$; Ryans Comparison Test for Proportions).

The results of these experiments indicate that regardless of the stimulus configuration, the presence of pheromone was the principal factor eliciting male orientation. Males did not orient to trunks harboring only the female, and pheromone was requisite to evoke landing and walking behavior on the trunks. The time required to negotiate the walking path to the pheromone source was not affected by the presence of associated female visual cues; the mean time ± SD) between landing and contact was 14.7 ± 8.6 sec (N=38) for the pheromone alone compared with 14.7 ± 9.9 sec (N=34) when pheromone and female were presented together.

In addition, appreciable differences were evident in mean net ground velocities recorded from moths flying toward the isolated pheromone source (7.8 ± 2.8 cm/sec) compared with flights directed toward either the trunk with pheromone alone (43.6 ± 14.5 cm/sec) or pheromone and female together (43.2 ± 23.5 cm/sec). Furthermore, there were substantial qualitative differences evident in flights directed toward pheromone on the trunk (Fig. 14A) versus those to the isolated pheromone (Fig. 14B). The flight paths to the detached pheromone source were characterized by a comparatively much higher number of turns and reduced track width and were similar qualitatively and in terms of ground velocity to tracks recorded by Charlton et al. (1989a) of gypsy moth males responding to a 100 ng pheromone source.

Effect on Walking Orientation of Spatial Segregation of Visual and Olfactory Cues

Irrespective of the position of the female, males landed approximately the same distance from the pheromone source. In addition, there were no significant differences in the amount of time required to contact the pheromone dispenser (Table 5). Conversely, there were substantial differences in the time needed to contact the female, depending on the distance between the female and pheromone and the position of the female relative to the pheromone source. When the female was deployed 5 and 10 cm from the source, males required 2 to 3-fold and 4 to 7-fold more time, respectively, to contact the female than to contact the pheromone. Males took longest to locate females upwind or below the odor source, whereas females deployed at, downwind, or 5 cm above the pheromone dispenser, were contacted comparatively rapidly (Table 5).

This trend was mirrored in the proportion of initial contacts by the males with either the female or pheromone (Fig. 15). When females were deployed upwind of or below the dispenser, virtually all of the initial contacts were with the pheromone, whether the female was 5 or 10 cm from the source. Similarly, if the female was 10 cm above the dispenser, all initial contacts were with the pheromone, whereas when the female was downwind, the intial contacts were evenly divided between the female and the pheromone. If the female was 5 cm from the source, the proportion of initial contacts with the pheromone was significantly reduced when the female was above or downwind of the pheromone source.

A complementary pattern was evident in the proportion of males that never contacted the pheromone or female (Fig. 15). The likelihood of not contacting the female was greatest when the female was positioned 10 cm from the pheromone source, particularly when she was directly upwind. The percentage of males that never contacted the pheromone was always identical to the percentage making initial contact with the female. This was the case because contact with the female terminated search behavior; males that encountered a female would remain in contact with and attempt to copulate with the female for prolonged periods, responding principally to tactile cues associated with her body and wing scales (Charlton and Cardé, 1989b).

The proportion of time that males spent in a given sector did not differ significantly, regardless of the juxtaposition of the female and the pheromone source (Fig. 16). For all stimulus configurations, males generally spent significantly more time in the downwind sector than in the sectors below, above, or upwind of the source. This was true for both the near and far zones. The tendency for males to spend more time in the downwind sector was a direct reflection of their typical upwind walking approach direction. Independent of the spatial arrangement of female and pheromone, the majority of male approaches were upwind and thus through the downwind sector (Fig. 16).

Males typically alighted at or below the level of and, on average, ca. 12-16 cm removed from the source in the downwind direction. At this distance, the males were not enveloped by pheromone even if they were directly downwind of the source. This was verified by 'smoke' trials, which indicated that the plume separated from the cylinder after travelling ca. 10 cm downwind, so consequently, the plume passed over the male. The initial walking movements frequently were directed vertically and once the male contacted the odor plume, he proceeded to walk horizontally upwind. This is illustrated in Fig. 17A, which shows a representative walking path when the pheromone was presented alone.

In most cases, such an approach pathway to the pheromone was also recorded even when the pheromone and female were deployed together. In fact, correlations between the time to contact and inital distance to the stimulus were weak whether the pheromone was deployed alone (R = 0.014; N.S.) or with the female (R = -0.320; N.S.) (Sokal and Rohlf, 1981). However, when the female was placed downwind of the pheromone, two alternate approach patterns were noted. Most males walked directly upwind and thereby encountered the female before the pheromone (Fig. 17B), but males that alighted below and between the female and the source generally bypassed the female and oriented preferentially toward the pheromone source (Fig. 17B). This suggests that encounters with the female were incidental as the males oriented to the pheromone source. Nonetheless, when the female was pinned 5 cm above the pheromone locus, males appeared to modify their approach path. In this situation, the males adopted a more vertical heading and frequently bypassed the pheromone as they headed directly toward the female, suggesting that there was some visual input or an interaction between olfactory and visual stimuli (Fig. 17C). However, if the female was placed 10 cm above the pheromone, males typically contacted or passed within 2 cm of the pheromone dispenser and then continued walking upward several more cm before looping in the reverse direction (Fig. 17D). Thus, the putative visual response to the female positioned 5 cm above the pheromone may simply reflect the movement tendencies of males in the vicinity of the source or alternately the visual response distance of pheromone stimulated males was only on the order of 5 cm.

Relative Importance of Visual and Chemical Stimuli

When pheromone stimulation was interrupted, the large majority of males did not proceed directly to the female, despite the fact that the female was only 5-8 cm away and presumably in the male's line of sight (Table 6). Rather, males typically reacted initially by walking upwards or backing down and then exhibited the convoluted, primarily vertically, directed movements (Fig. 18) reminiscent of responses seen in the forest. Following initiation of this response, males would either revert to the bouncing vertical flight and realight on the cylinder, encounter the female during the course of the vertical walking movements, or locate the female after walking to the downwind side, contacting the pheromone plume and proceeding upwind to the pheromone source (Fig. 18). During expression of the vertical maneuvers, males often passed within 5 cm upwind of the female, but would not orient to her, again corroborating that visual attributes of the female, at least in the absence of pheromone, did not provoke oriented responses.

By contrast, virtually all of the the control males who were enveloped by the pheromone plume both prior to and following rotation of the cylinder, walked directly to the female. This was reflected in the relatively short time required to contact the female and the high linearity of the paths (Table 6). By comparison, males that lost contact with pheromone took much longer to find the female and the values of the associated path linearities were consistently low (Table 6). Thus, it is evident that the response to loss of pheromone overrode any propensity to orient visually to the female.

Discussion

The flying and walking search behavior of male gypsy moths was influenced primarily by olfactory stimuli. The presence of pheromone on the trunk was necessary to elicit orientation to the trunk as well as subsequent landing and walking. Both wind tunnel and forest tests indicated that vertical silhouettes devoid of pheromone almost never evoked orientation or landing, even if a female was associated with the silhouette. Furthermore, males alighted at comparable distances from the pheromone source whether or not the dispenser was supplemented with a female. These results suggest that interactions between pheromone and optical stimuli associated with the vertical silhouettes mediate male close-range orientation and landing, whereas visual cues associated with the female do not play a significant role in this process. Doane (1968) reported that male gypsy moths would readily locate a female perched on a stake positioned 15 cm downwind of a calling female. However, non-calling females deployed a mere 15 cm upwind of calling females were never found by the males. Doane (1968) concluded from these results that visual perception was not important in the absence of the pheromone, but that vision did play some role since males responded to females enveloped by the odor plume. Because male response to the stakes alone was not assayed, it was not resolved whether the males were responding visually to the female or to the vertical stakes in the presence of pheromone. The fact that pheromone-stimulated males did not respond to females only 15 cm upwind of the source suggests that visual cues associated with the stakes actually induced the males to land.

Investigations on the orientation behavior of several other moth species indicate that a combination of figural and pheromonal stimuli influence premating orientation behavior. In a study comparing flight reactions of *Lymantria monacha* males to different olfactory and visual stimuli, Schneider (1981) found that vertical silhouettes stimulated male searching flight when the silhouettes were coupled with pheromone; silhouettes without pheromone received substantially fewer visitations. In experiments comparing the response of *Cadra cautella* to figures varying in shape (Levinson and Hoppe, 1983), males few preferentially toward vertical rectangles and exhibited no response to open conical or prismatic figures. When the vertical rectangle was supplemented with the pheromone blend, male orientations were nearly five-fold higher as compared to the rectangles alone. *Coleophora laricella* males presented with synthetic attractant and twigs of several conifer species or models of larch twigs displayed strongly enhanced landing levels compared to situations where the attractant or visual cues were offered alone (Witzgall and Priesner, 1984). Similarly, arrestment of flight and landing were induced in *Pectinophora gossypiella* males by a combination of visual cues emanating from vertical silhouettes and relatively high pheromone concentration (Farkas et al., 1974).

The interaction of pheromone and vertical objects also influenced other aspects of close-range flight. The marked enhancement of net ground velocity evident when the pheromone source was associated with a vertical silhouette may be attributable to the turbulence and attendant dilution of pheromone concentration engendered by the cylinder. Decreases in pheromone concentation have been shown to elicit increases in ground velocity for several moth species, including *L. dispar* (Cardé and Hagaman, 1979; Charlton et al., 1989a), *Choristoneura fumiferana* (Sanders et al., 1981), and *Grapholita molesta* (Kuenen and Baker, 1982). Alternatively, either the structure of the pheromone plume itself, the visual cues emanating from the vertical silhouette, or an interaction of these factors and reduced concentration may have provoked the increase in ground velocity. The ca. four-fold elevation of net ground velocity over that elicited by an isolated 10 ng (+)-disparlure source (Charlton et al., 1989a) suggests that the increase in flight velocity is not explicable solely by the reduction in pheromone concentration. In this study, male response was assayed to basically only one configuration of pheromone dispenser on a vertical cylinder. However, plume characteristics are affected markedly, depending on how the pheromone source is positioned on a cylinder relative to the direction and velocity of the wind. Thus, additional experiments involving manipulation of odor cues on vertical silhouettes are necessary to resolve the factors responsible for the altered flight characteristics and to identify the cues promoting landing by the males.

Visual cues presented by the female at close range did not affect the efficiency with which the walking male located the pheromone source. In both forest and wind tunnel trials, the distance between the point of alightment and the pheromone dispenser, and the time required to locate the pheromone source were not affected by the presence of the female. These results are in agreement with those obtained in a similar study (Cardé, 1981) which showed that the speed of male location of (+)-disparlure sources was not enhanced when an acetone-rinsed female was presented in conjunction with the pheromone. Furthermore, when a female visual model was placed in various positions around a pheromone source, males oriented primarily to the chemical stimulus and spent most of their time in the sector downwind of the source. In addition, males which had lost contact with the odor plume frequently passed within a few cm of females without responding. Moreover, as was noted particularly in both the forest and wind shift experiments, the characteristically low linearity values associated with the often convoluted male walking pathways further argues against any significant role of visual responses to the orientation process, since visual orientation over short distances generally is highly directional.

Notwithstanding, there were indications that males would alter their walking paths in response to visual cues from females at distances of several centimeters. When females were deployed 5 cm downwind or above a pheromone dispenser, males generally approached and contacted the female directly, although it was not possible to distinguish these apparent visual responses from reactions to the pheromone plume. However, when males receiving concomittant olfactory and visual input suddenly lost contact with the pheromone plume, most responded to the loss of scent and adopted an essentially vertical walking heading rather than continuing along their former course to contact the female. Thus, the evidence suggest that the evident visual response is intimately tied to the concurrent perception of pheromone. These findings are in accord with those reported for *P. gossypiella*, for which visual stimuli in the absence of pheromone were relatively ineffective in prompting orientation by the males; models placed 2 cm downwind of 0.01 or 10 ug pheromone sources elicited ca. five times as many orientations than a model placed 2 cm upwind of the source (Colwell et al., 1978). Similarly, male *G. molesta* directed significantly more hairpencil displays toward visual models positioned downwind of or at a pheromone source than at models upwind or at the side of the source (Baker and Cardé, 1979).

Field studies of other diurnally active moths indicate that males rely on a combination of olfactory and visual inputs to guide them to the female. In the six-spotted burnet moth, *Zygaena filipendulae*, long-range attraction is mediated by the female-emitted pheromone. Once males close to within 50 cm of a female, they switch to casting flight and utilize visual cues associated with the female to guide them the intervening distance to the female (Zagatti and Renou, 1984). In an analogous manner, dawn-active male *Hyphantria cunea* engage in rapid straight line flights until they encounter a pheromone active space which was estimated to extend ca. 3 m from calling females. Within this zone, the male changes to a slow casting flight and searches visually for the white female (Hidaka, 1972). Pheromone-releasing females covered by dark paper or with their wings stained blue elicited far fewer male contacts than did their normal, visible counterparts.

Comparatively, little is known of the relative roles that olfactory and visual stimuli play in mediating mate location in nocturnal Lepidoptera. When Carpenter and Sparks (1982), in wind tunnel tests, offered *Heliothis zea* males a choice between a female model and an acetate filter impregnated with pheromone, the males flew toward and contacted the female preferentially. The propensity to respond to the female was dependent on the distance between the stimuli, with the maximum visual response distance calculated to be 16-22 cm. In contrast, the presence of a female visual cue did not affect the amount of time male *Laspeyresia pomonella* spent in oriented flight near the pheromone source (Castrovillo and Cardé, 1980).

Given the visual apparency of the white gypsy moth females against the background of their preferred calling sites, it is curious that the males apparently do not utilize these cues to locate the female. Possibly, the lack of visual response may be a relic of an ancestral search strategy evolved when gypsy moths had not yet shifted from a nocturnal to the present diurnal flight habit. Several lines of evidence support this contention. Despite their day flying habit, male *L. dispar* retain morphological and behavioral characteristics generally affiliated with nocturnal insects. For example, males possess well developed tympana sensitive to ultrasound (Baker and Cardé, 1978; Cardone and Fullard, 1988), an adaptation usually associated wtih nocturnal insects that allows detection of sound frequencies produced by foraging bats. Moreover, their compound eyes are of the superposition type typical of dark-active insects (Brown and Cameron, 1977). Indeed, with few exceptions, members of the Lymantriidae are nocturnal as adults (Ferguson, 1978).

In addition, it has been suggested that since its introduction to North America, the gypsy moth may have extended its flight period further into the evening and night (Cardé et al., 1974). Such a shift in diel periodicity could be partially attributable to relaxation of selective pressures in the absence of its congener, the nun moth, *L. monacha* (Cardé et al., 1974). Both species occur synchronously and sympatrically in parts of Europe (Fuester et al., 1975; Schröter, 1981). Although the two species possess overlapping diel activity rhythms, *L. monacha* is primarily nocturnal with peak male flight extending from ca. 1900 to 2400 hr (Schröter and Lange, 1975; Skuhravy and Zumr, 1981), whereas *L. dispar* flight occurs principally during the daylight hours (Schröter, 1976).

Furthermore, due to similarities in their sex pheromones, there exists the potential for cross-attraction between these species. Results of antennal receptor studies indicate that *L. monacha* females produce about 10% (+) and 90% (-)-disparlure, whereas *L. dispar* produce almost 100% (+)-disparlure (Hansen, 1984). Numerous trapping experiments have shown that even though male *L. dispar* are optimally attracted to (+)-disparlure, they will also respond to racemic disparlure (cf. Miller et al., 1977; Cardé et al., 1977; Plimmer et al.,1977; Klimetzek and Schönherr, 1978). In addition, Schröter (1976) has determined that males of both species will readily attempt to copulate with females of the other species although mating is not feasible because of genitalic incompatibility. Given the potential for interference in pheromonal communication, differences in diel periodicities of male responsiveness may be a major factor thwarting inter-specific communication. Thus, selective pressure to partition the communication channels may have provoked a shift in *L. dispar* from an ancestral nocturnal state to the diurnal flight habits in evidence now, with retention of mate-seeking behaviors adapted for nocturnal conditions. In this regard, it would be worthwhile to compare the flight periodicities of *L. dispar* and *L. monacha* in zones of sympatry and allopatry. More pronounced shifts of *L. dispar* male activity periods into the daylight hours in areas where the two species have traditionally coexisted would lend support to the character displacement hypothesis.

Walking males observed in the forest generally did not approach the pheromone source directly. Instead, the walking paths over the tree bole were characterized by predominately vertical and oblique movements with frequent reversals in direction. During these maneuvers, the male's body axis was aligned essentially perpendicular to the ground with the head upward; downward movements were accomplished by the male backing down the tree. The vertical movements were also interspersed with episodes of essentially horizontal displacement, particularly as the males approached the pheromone source. Males that were walking while wing fanning generally remained in this mode as they navigated their course to the female although males would frequently revert to bouncing vertical flight up and down the tree trunk before realighting. Possibly, this vertical flight serves as a means of localizing the pheromone and thus enables the males to land closer to the pheromone source. All of these behaviors were expressed faithfully by males assayed in the wind tunnel using surrogate trees.

It is not clear how important walking maneuvers are in the context of natural mate seeking behaviors of other moths. A walking component has been documented in the courtship sequence of a number of lepidopteran species spanning several families (cf. Grant and Brady, 1975; Colwell et al., 1978; Baker and Cardé 1979; Zagatti, 1981; Haynes and Birch, 1984; Ono, 1985). This response, variously termed the ground phase or mating dance, ensues when typically the male, or in a few species, the female (Barrer and Hill, 1980; Spangler et al., 1984) lands on the substrate and walks while wing fanning in the vicinity of the prospective mate. The final ambulatory approach can be mediated by different cues, including olfactory, visual, and auditory stimuli acting alone or in concert. However the unrealistic and/or confined conditions prevalent in many laboratory investigations likely do not allow faithful expression of certain behaviors, such as the walking response, and thus their contribution to the natural mate-seeking process may be underestimated.

The results reported here imply that both the presence and absence of pheromone influence male walking orientation. Studies on several lepidopterans, including *L. dispar* (Preiss and Kramer, 1986b), *G. molesta* (Willis and Baker, 1987), *Bombyx mori* (Kramer, 1975), and *P. gossypiella* (Shorey and Farkas, 1973) have shown that males walking on horizontal surfaces orient anemotactically upwind when stimulated by pheromone. However, under natural conditions, gypsy moth males walk predominately on vertical objects, such as tree boles. Further studies utilizing a locomotion compensator (servosphere) have examined the response to various temporal patterns of pheromone stimulation of male *L. dispar* walking on a vertical surface (Charlton et al., 1989b). These results verified that males display positive anemotaxis when enveloped by a pheromone plume. Interruption of pheromone stimulation by either still air or a pheromone-free airstream elicited an area-restricted walking response involving primarily vertical and oblique movements with frequent reversals in direction and thus similar to the maneuvers seen in the experiments described here.

The response to loss of pheromone may serve at least two functions. First, these maneuvers could enhance the likelihood of recontacting an elusive odor cue. Although wind direction can vary widely in a forest, the predominant wind flow under the canopy is parallel to the ground. Therefore, males walking primarily vertically and thus crosswind should have an enhanced probability of recontacting the plume. Such a strategy would be particularly advantageous in the forest where walking males frequently lose contact with the pheromone plume. Secondly, the intensive local search could increase the probability of serendipitously encountering a female. Results reported here indicate that when females were disjunct from the pheromone, males frequently contacted the female during the course of movements not obviously oriented toward the female, such as when they were backing down the trunk or moving upwards. This could account for the finding that in high-density populations, females are frequently mated before they commence calling, and often before their wings are expanded (Cardé and Hagaman, 1984).

The walking response also has potential implications for gypsy moth control strategies using disparlure as a mating disruptant. Numerous field trials have demonstrated that effective suppression of mating can be achieved at low to moderate population densities, but at higher densities control breaks down (Cameron, 1981; Plimmer, 1982; and references therein). Our findings demonstrate that the presence of pheromone actually stimulates males to orient to, land, and walk on tree boles. Other field observations also imply that elevated ambient pheromone levels influence male searching behavior. Richerson et al. (1976b) found that more males were captured on unbaited sticky panels attached to trees in plots treated with racemic disparlure than in untreated plots. Similarly, observations by Cardé and Hagaman (1984) of adult gypsy moths in dense populations indicated that the majority of males were either engaged in vertical flights near tree trunks, or less frequently, were walking over the bark surface. Naturally, the likelihood of encountering a chemically camouflaged female during the course of intensive local search walking maneuvers would be enhanced as the number of searching males and the density of available females increases. Once a male contacts the female, mate recognition is mediated principally by tactile cues associated with female wing and body scales (Charlton and Cardé, 1989b) and mating quickly ensues. Therefore, under moderate to high-density conditions, search by pheromone-stimulated males of trees potentially harboring females could yield sufficient matings to maintain or augment population levels.

Clearly, walking behaviors constitute a critical element of the mate-seeking process and their inherent flexibility facilitates location of females in a variable environment. Used in concert, the anemotactic and local search maneuvers allow the male to locate an intermittent pheromone signal or even females rendered cryptic because they are either newly emerged non-calling individuals or must compete with elevated ambient pheromone levels, such as occurs at high population densities or when pheromone disruptant treatments are deployed.

Table 4. Role of visual cues presented by the female on parameters associated with male location of pheromone sources in the forest.

Treatment	Time (sec) from landing until source contact	Initial distance (cm) from source	Total path length (cm)	Path linearity index	N
Acetone-rinsed dead female + pheromone	$31.4^{a} \pm 15.8$	$48^{a} \pm 21$	$176^{a} \pm 74$	$0.29^{a} \pm 0.10$	20 (22)
Pheromone alone	$35.0^{a} \pm 22.4$	$52^{a} \pm 25$	$185^{a} \pm 73$	$0.31^{a} \pm 0.15$	20 (24)
Acetone-rinsed dead female alone[1]	1 contact				1 (34)

Tests were conducted July 27-28, 1984 in Pelham, Massachusetts. Pheromone source was 1000 ng (+)-disparlure on filter paper. [1]Due to the low number of males responding to the female alone, these results were not subjected to statistical analysis. N = number of males that contacted the treatment; numbers in parentheses indicate males that flew within a 2 m radius surrounding the observation tree. Means (± SD) in each column followed by the same letter are not significantly different according to Mann-Whitney *U* Test, two sided ($P > 0.05$).

Table 5. Effect upon male *L. dispar* orientation responses of different spatial relationships of females with the pheromone source.

Spatial Arrangement	Time from Landing to Contact (sec)				Initial Distance from Pheromone[2] (cm) (N = 20)	Close Encounters with Female[3] (N = 20)	Contacts with Pheromone[4] (N = 20)
	Pheromone	N	Female[1]	N			
Pheromone (P)	8.1^{a} (7.7)	20	------	--	11.6^{a} (5.2)	------	-------
Female at P	9.0^{a} (10.5)	20	9.0^{a} (10.5)	20	13.8^{a} (4.6)	------	------
Female 5 cm above P	3.3^{a} (2.2)	11	6.4^{a} (4.7)	20	13.3^{a} (5.4)	0.2^{a} (0.4)	0.7^{a} (0.7)
Female 10 cm above P	11.8^{a} (11.4)	20	50.8^{bcd} (60.3)	14	14.0^{a} (5.2)	2.5^{b} (3.1)	3.4^{bc} (3.3)
Female 5 cm upwind of P	7.0^{a} (4.9)	20	23.4^{bc} (18.3)	19	13.9^{a} (5.5)	0.4^{a} (0.5)	2.4^{b} (1.4)
Female 10 cm upwind of P	8.1^{a} (6.0)	20	59.7^{d} (42.1)	5	15.6^{a} (4.8)	2.3^{b} (2.1)	5.3^{c} (4.6)
Female 5 cm below P	12.5^{a} (16.1)	19	37.1^{bcd} (30.1)	15	12.3^{a} (4.7)	0.9^{a} (0.7)	2.9^{bc} (2.3)
Female 10 cm below P	8.7^{a} (6.6)	20	47.4^{cd} (37.4)	12	13.8^{a} (6.3)	0.8^{a} (1.2)	4.1^{bc} (3.2)

Continued next page

Table 5. continued

Female 5 cm downwind of P	4.9^{a} (4.6)	3	10.6^{a} (10.3)	20	13.8^{a} (4.9)	0.7^{a} (1.3)	0.7^{a} (1.9)
Female 10 cm downwind of P	4.7^{a} (3.7)	10	29.0^{b} (36.4)	16	15.6^{a} (5.7)	1.3^{ab} (1.8)	2.3^{ab} (3.5)

All tabular values are means (± SD). [1] Defined as time elapsed between landing and contact with the female, or for males that contacted the pheromone but never located the female, the time from landing until taking flight; [2] represents the straight-line distance between the point of alightment and the pheromone source; [3] close encounters with the female were defined as passage by a male through a sector and zone (near vs. far) where a female was deployed without contacting the female; [4] represents the number of contacts with the pheromone source before contacting the female or taking flight. Means in any column having no letters in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test with a log $(x + 0.5)$ transformation.

Table 6. Effects on male walking orientation responses of disassociating pheromone from female visual cues by inducing a 180° wind shift.

Stimulus condition	No. of males proceeding directly to female[1]	Time(sec) to contact female	Total path length(cm)	Path linearity index
Shift in pheromone plume	4 (20%)**	$37.2^{a} \pm 27.8$	$132.8^{a} \pm 101.4$	$0.20^{a} \pm 0.30$
Constant pheromone plume	18 (90%)	$3.5^{b} \pm 3.4$	$12.1^{b} \pm 10.0$	$0.75^{b} \pm 0.22$

Plume shift was accomplished by rotating cylinder on which the female and pheromone source was placed; see text for details. [1]A direct approach was defined as one with an associated linearity value >0.70. N=20 for each stimulus condition. **Entries in the same column significantly different according to X^2 2 x 2 test of independence with Yates' correction (P<0.01). Means (± SD) in each column followed by different letters are significantly different according to Mann-Whitney *U* test, two sided (P<0.05).

Figure 9. Schematic overhead view of surrogate tree in wind tunnel showing the relative positions of the walking male and the female/pheromone combination with respect to the wind, prior to and following rotation of the cylinder. Arrow represents direction of rotation.

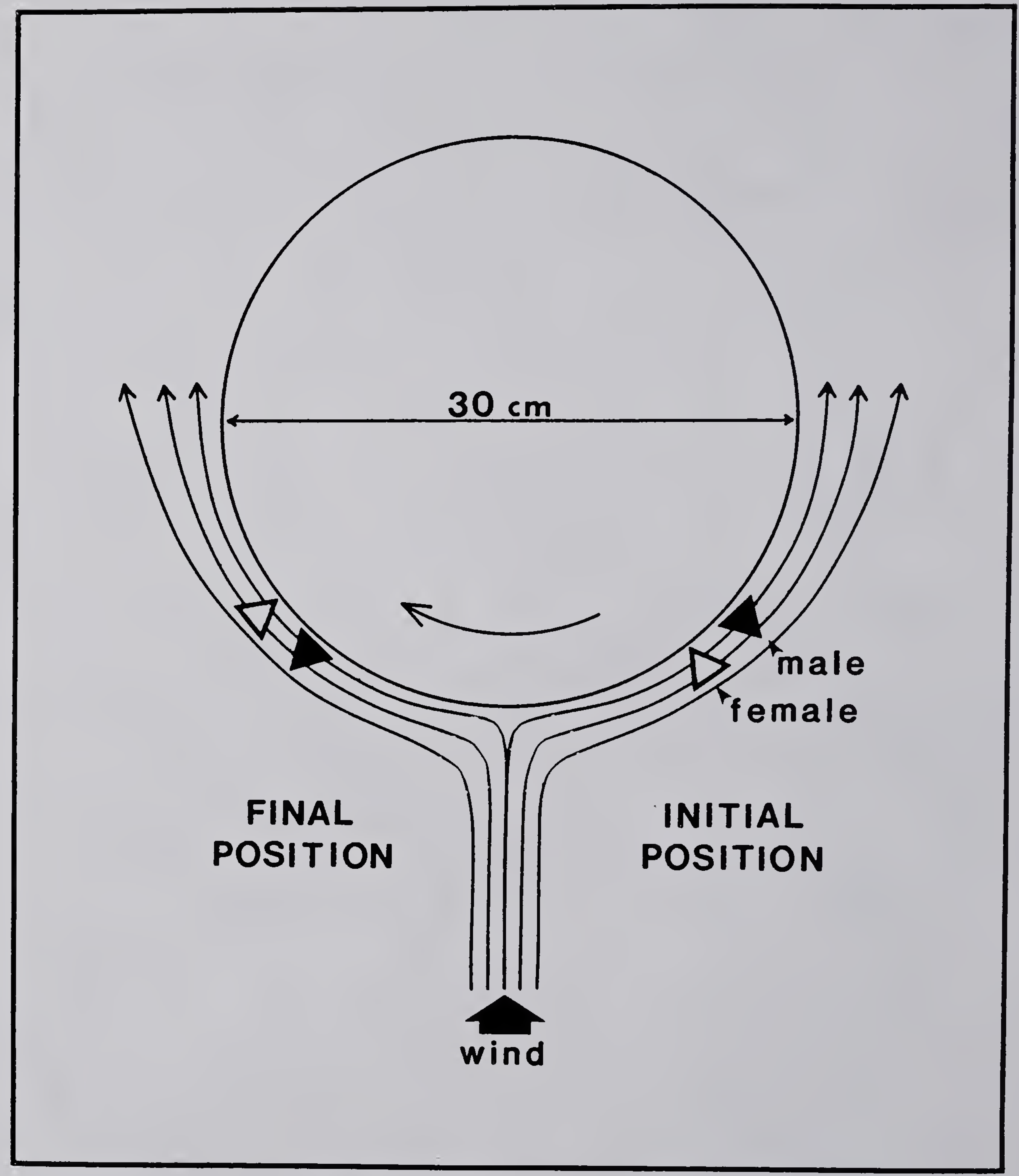

30 cm
male
female
FINAL POSITION
INITIAL POSITION
wind

Figure 10. Representative walking paths, on tree boles in the forest, of wild *L. dispar* males responding to a 100 ng (+)-disparlure source in combination with a female visual model (P). Solid triangle indicates landing point of male.

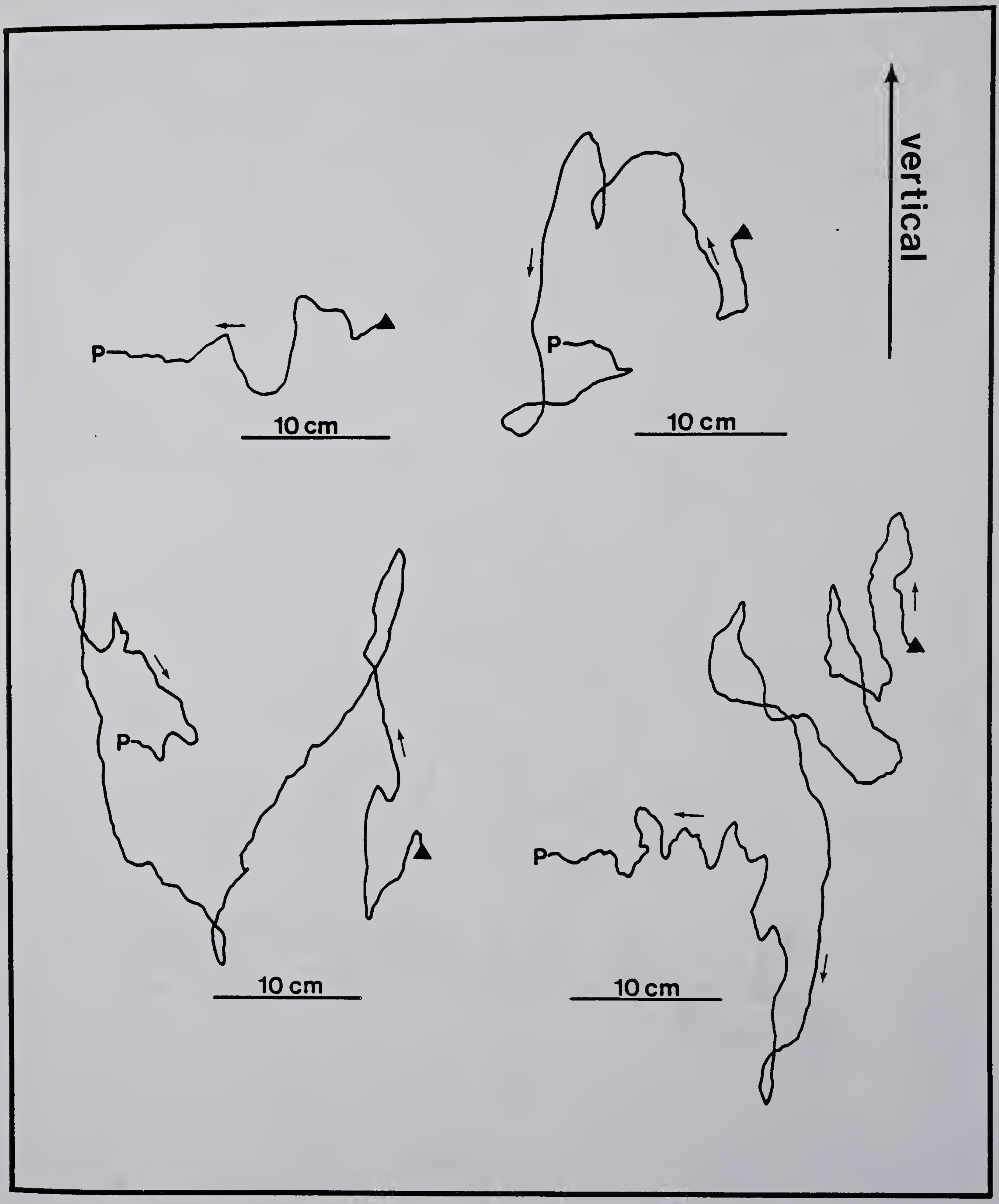

vertical
P
10 cm
P
10 cm
P
10 cm
P
10 cm

Figure 11. Successive photographs (taken during a 60 sec interval) of pheromone dispersion patterns visualized with titanium tetrachloride vapors illustrate the rapid shifts in wind direction that can occur in the forest.

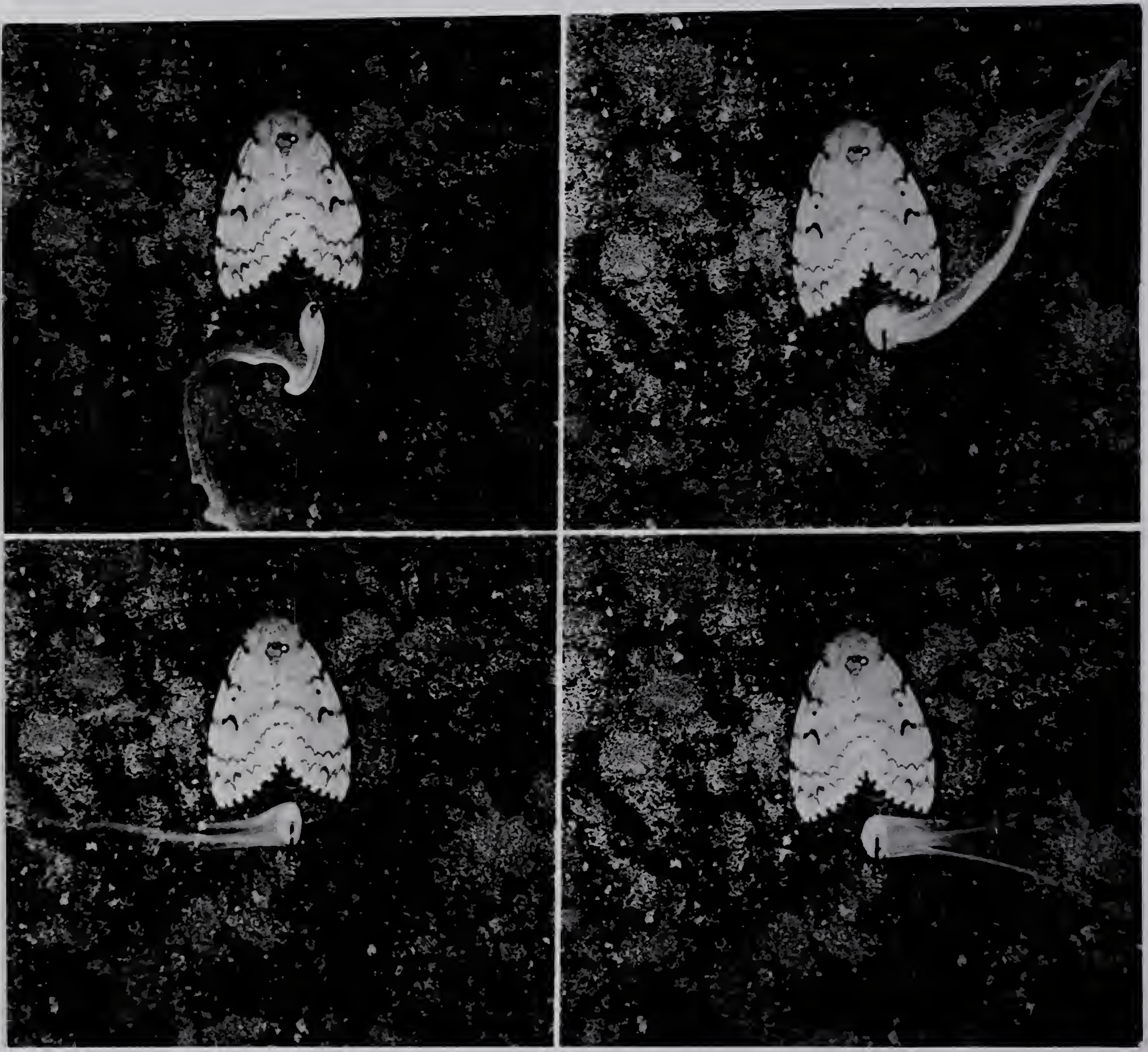
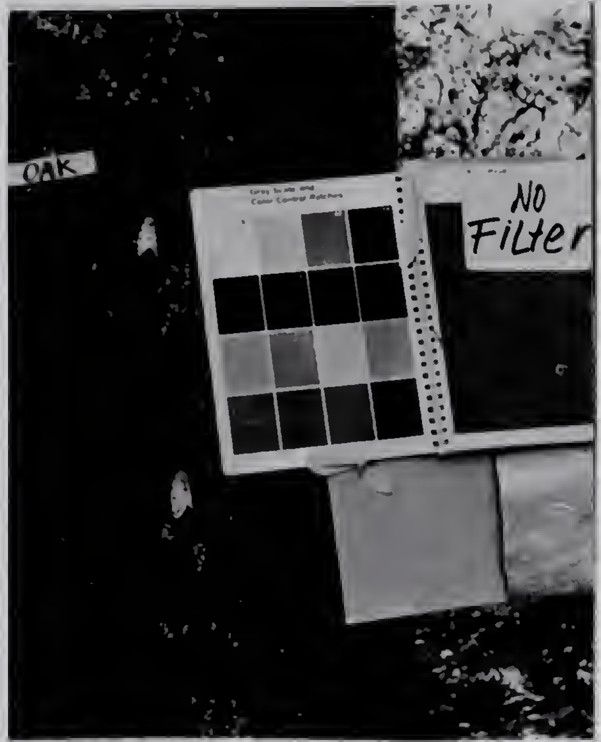


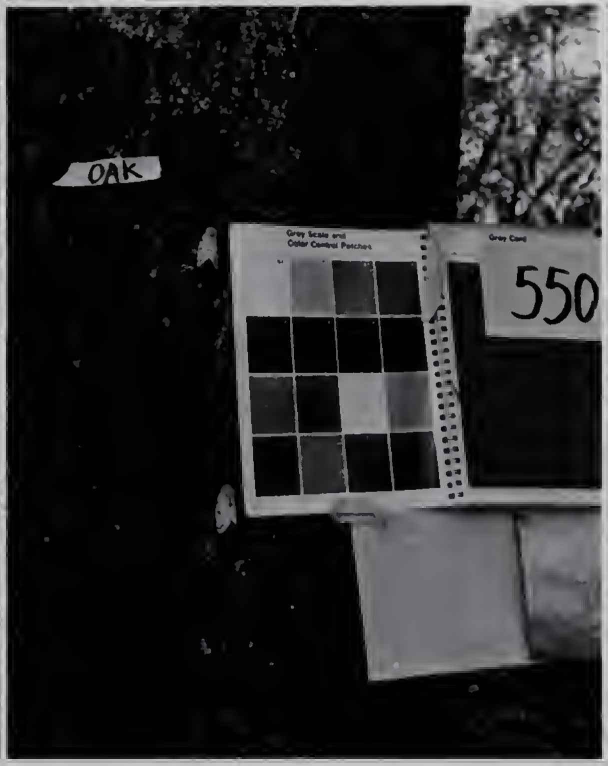
Figure 12. Visual contrast, at different spectral regions, of female gypsy moths photographed against the background of a white oak trunk. Photographs taken with SLR camera and 28 mm wide angle lens through various corion glass narrow bandwidth filters (HBW = 50 nm), a UV filter, or no filter. Print exposures were standardized using the Kodak gray card.


OAK
Gray Scale and Color Control Patches
NO Filter


OAK
UV


OAK
Gray Scale and Color Control Patches
450


OAK
Gray Scale and Color Control Patches
Gray Card
500


OAK
Gray Scale and Color Control Patches
Gray Card
550


OAK
Gray Scale and Color Control Patches
Gray Card
600

Figure 13. Behavioral responses of gypsy moth males to different combinations of pheromone sources, female visual cues, and vertical silhouettes. Shaded circles indicate cylinders with associated 100 ng (+)-disparlure source; P is 100 ng (+)-disparlure on filter paper dispenser suspended equidistant between the two cylinders; 0 denotes presence of a dead female on a cylinder; B indicates hexane blank on filter paper. Contact denotes touching the isolated pheromone source or either cylinder. Proportions associated with a particular behavioral category having no letters in common are significantly different ($P < 0.05$) according to Ryan's Multiple Comparison Test for Proportions (Ryan, 1960).

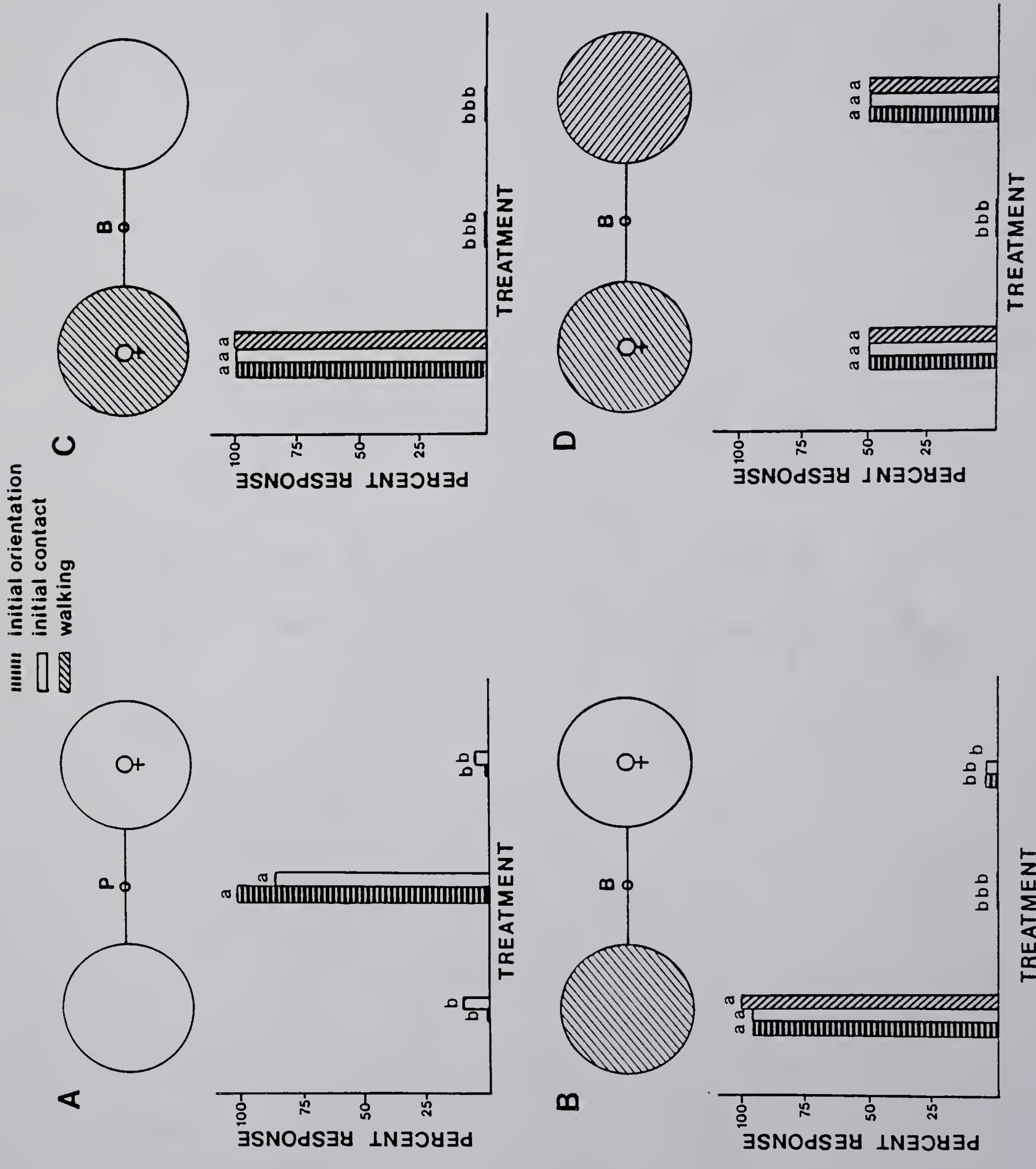

A
B
C
D
initial orientation
initial contact
walking
P
B
♀
PERCENT RESPONSE
100
75
50
25
TREATMENT
a
b
bbb
aaa

Figure 14. Typical flight tracks of *L. dispar* males flying to two configurations of pheromone (100 ng (+)-disparlure), female visual models, and vertical silhouettes: (A) Female model was pinned to the indicated trunk and the pheromone source was suspended 30 cm high between the silhouettes; (B) Female model was deployed on one trunk and pheromone source on the other, both 30 cm high. Flight tracks were recorded in a wind tunnel from above with the moth progressing from bottom to top. Solid asterisks denote the moths' positions at 0.5 sec intervals.

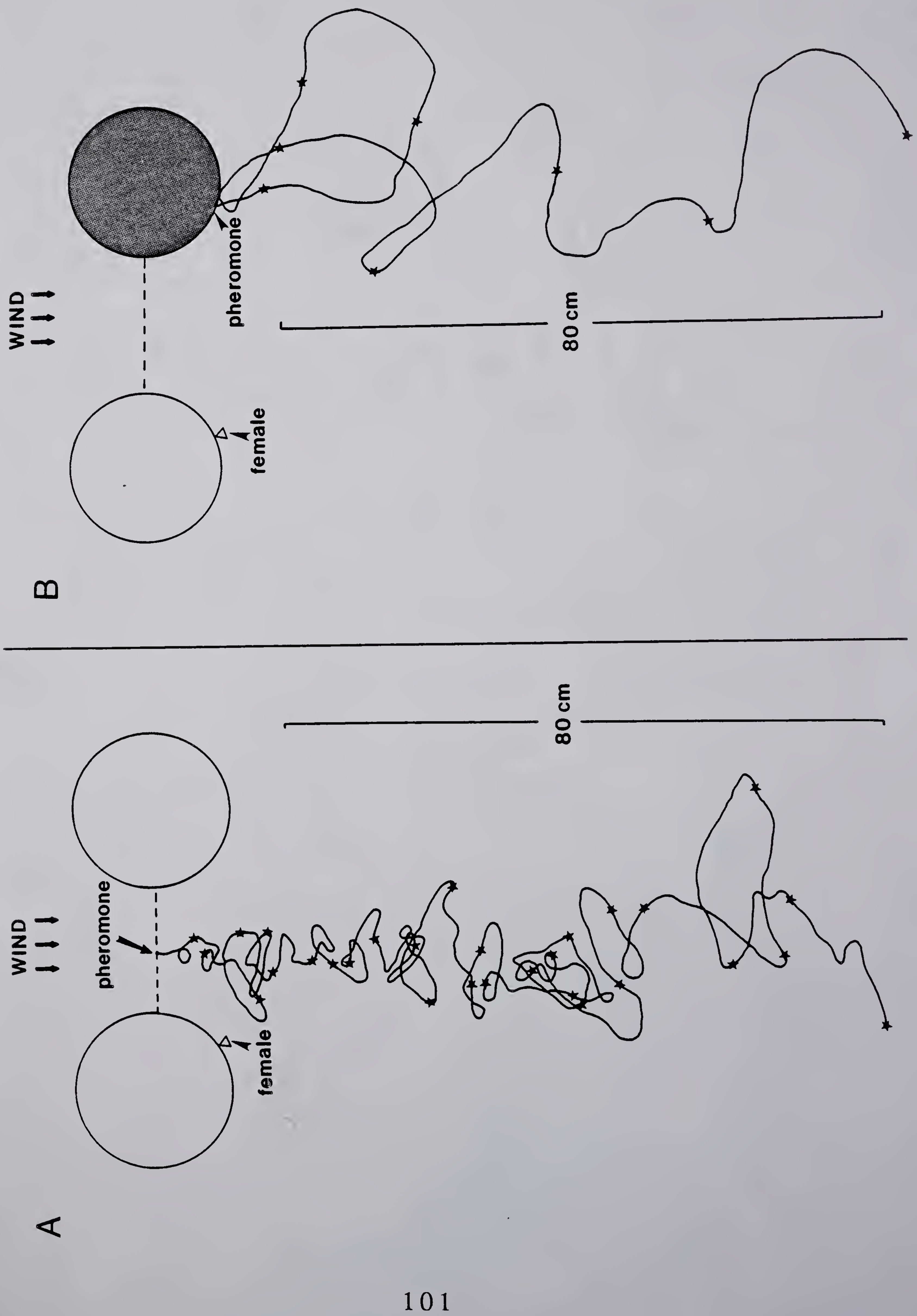

A
WIND
pheromone
female
80 cm
B
WIND
pheromone
female
80 cm

Figure 15. Effect of different spatial relationships of a dead female visual model and the pheromone source upon percentage of walking males making initial contact with the femal (ICF) or the pheromone source (ICP), and those not contacting either the female (NCF) or the pheromone source (NCP) during the observation period. Similar bars with no letters in common differ significantly ($P < 0.05$) according to Ryan's Multiple Comparison Test for Proportions (Ryan, 1960). Solid black triangular figure represents the model's position with respect to the pheromone source deployed at the center of the circles; wind direction was from left to right.

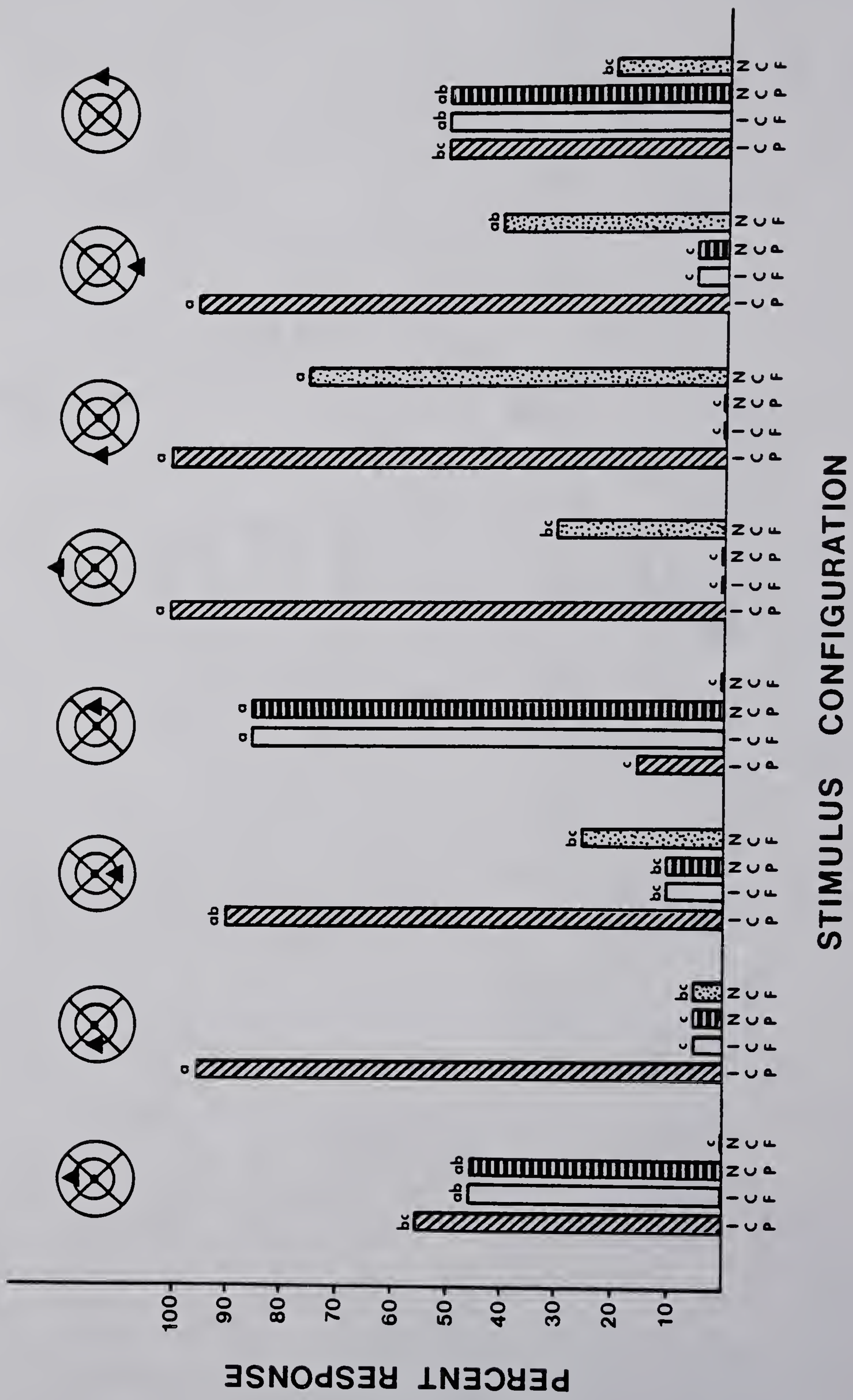

PERCENT RESPONSE
100
90
80
70
60
50
40
30
20
10
STIMULUS CONFIGURATION
ICP
ICF
NCP
NCF
bc
ab
ab
c
a
c
c
bc
ab
bc
bc
bc
c
a
a
c
a
c
c
bc
a
c
c
a
a
c
c
ab
bc
ab
ab
bc

Figure 16. Effect of different spatial arrangements of a dead, acetone-rinsed female and the pheromone source upon percentage of time spent by males in each of the sectors on the observation cylinder. The pheromone source (100 ng (+)-disparlure on 0.7 cm diam filter paper disc) was deployed at the intersection of the diagonal lines and the open quasi-triangular figure shows the position of the female. Widths of shaded areas and associated decimal numbers indicate the percentage time males walked in each sector both near (< 5 cm) and removed from (5 cm $< x <$ 10 cm) the pheromone source. Means followed by the same letter are not significantly different ($P > 0.05$) according to one-way ANOVA and Student-Newman-Keuls' test with an $\arcsin\sqrt{X}$ transformation: Lowercase letters compare sector means within a zone (near or far) for a particular configuration; uppercase letters compare means for a particular zone and sector across all treatments. $N = 20$ males for each configuration.

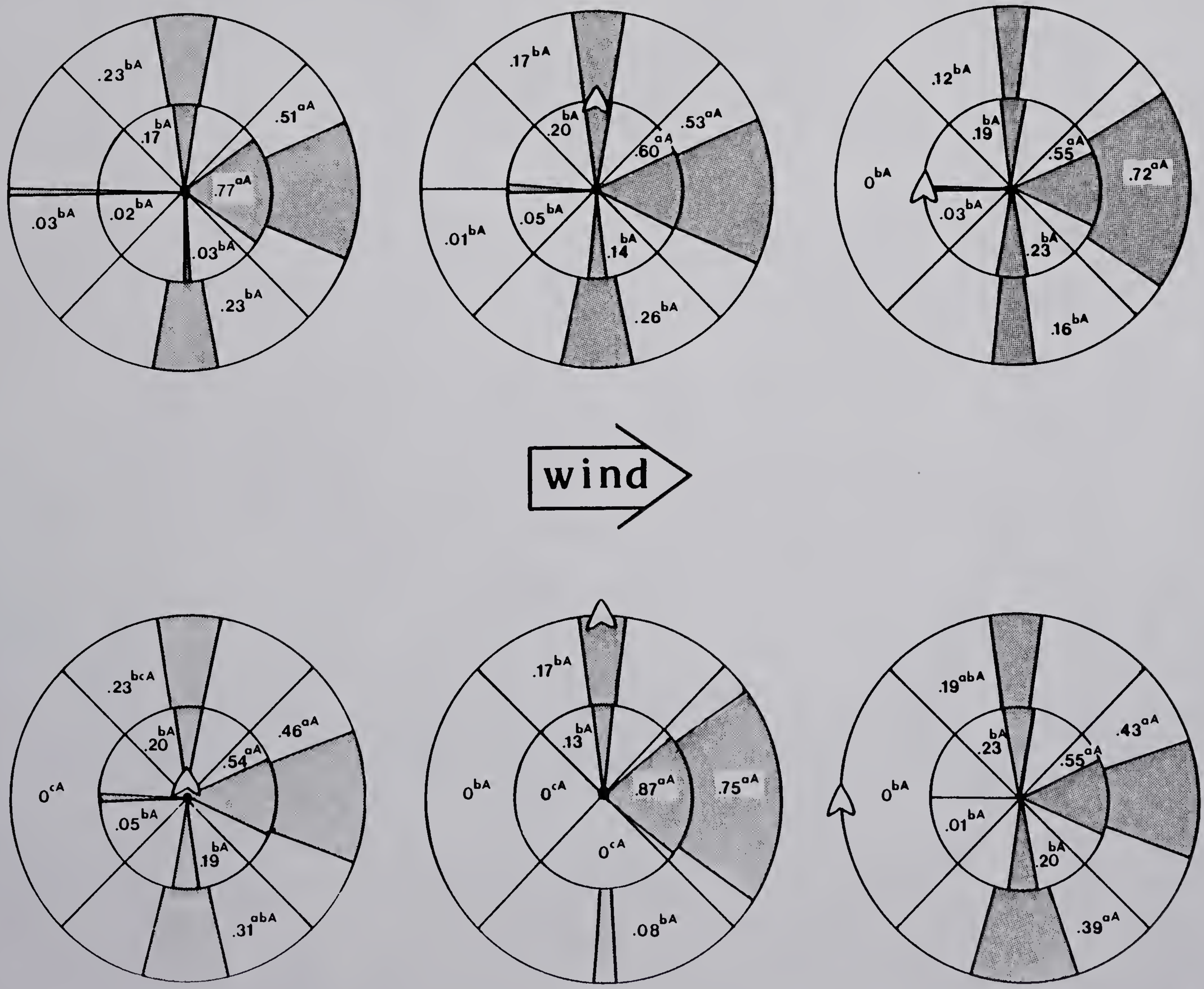


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Figure 16. continued

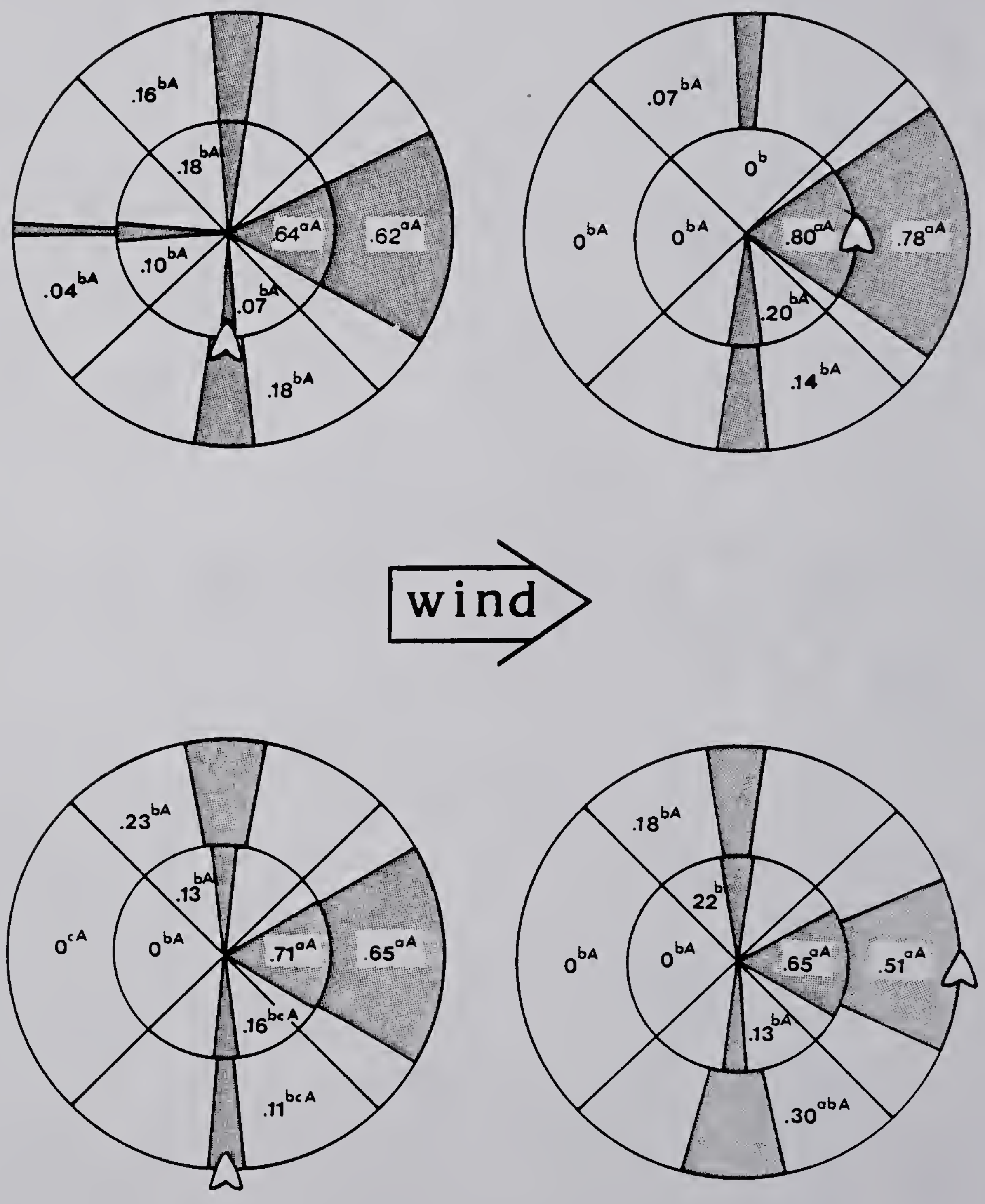


Figure 17. Representative walking paths on cylinder of male gypsy moths in response to different spatial arrangements of female models and pheromone sources. Pheromone (100 ng (+)-disparlure on filter paper) was dispensed at the position indicated by the solid triangle. Solid asterisks indicate position of the males at 1 sec intervals.

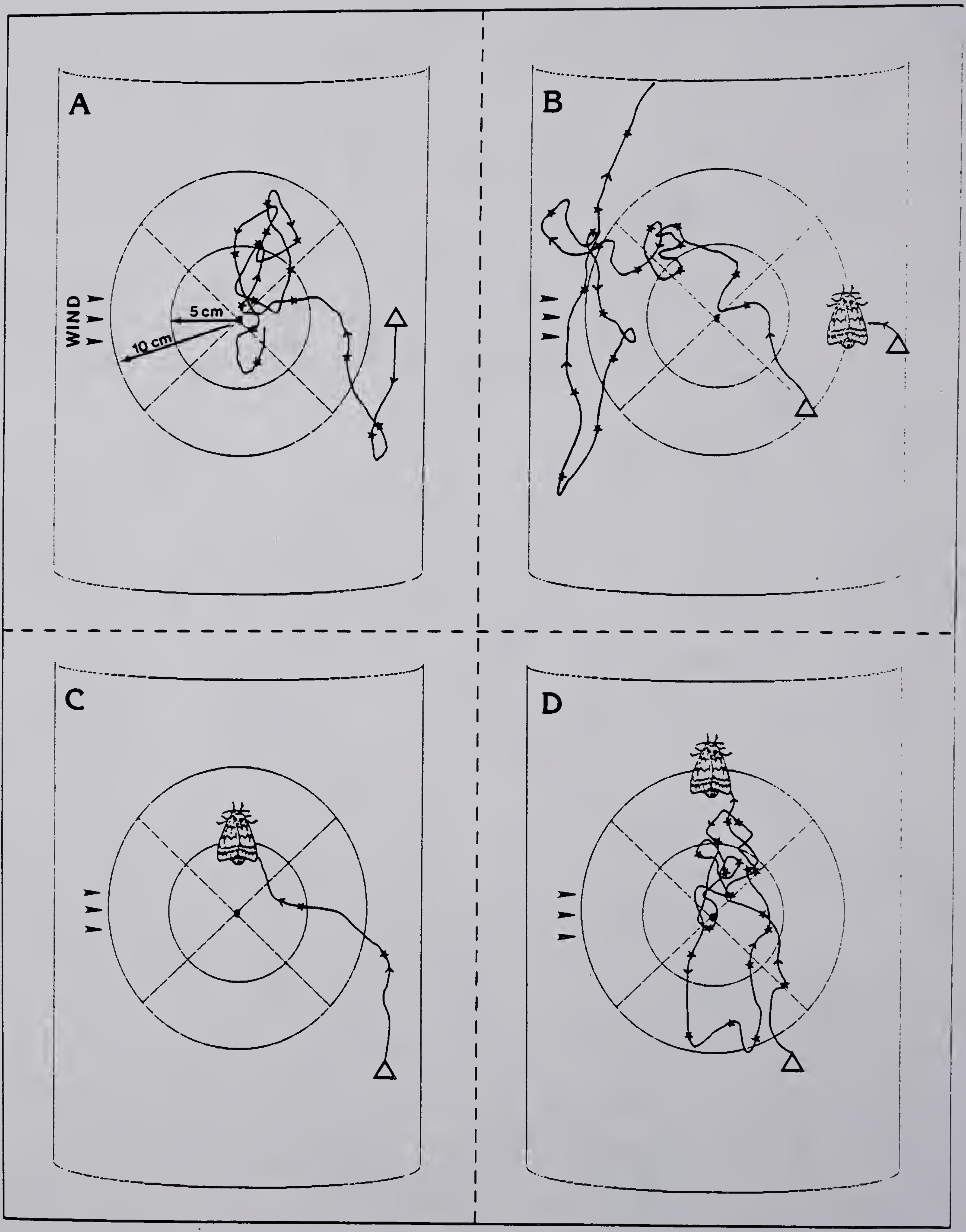
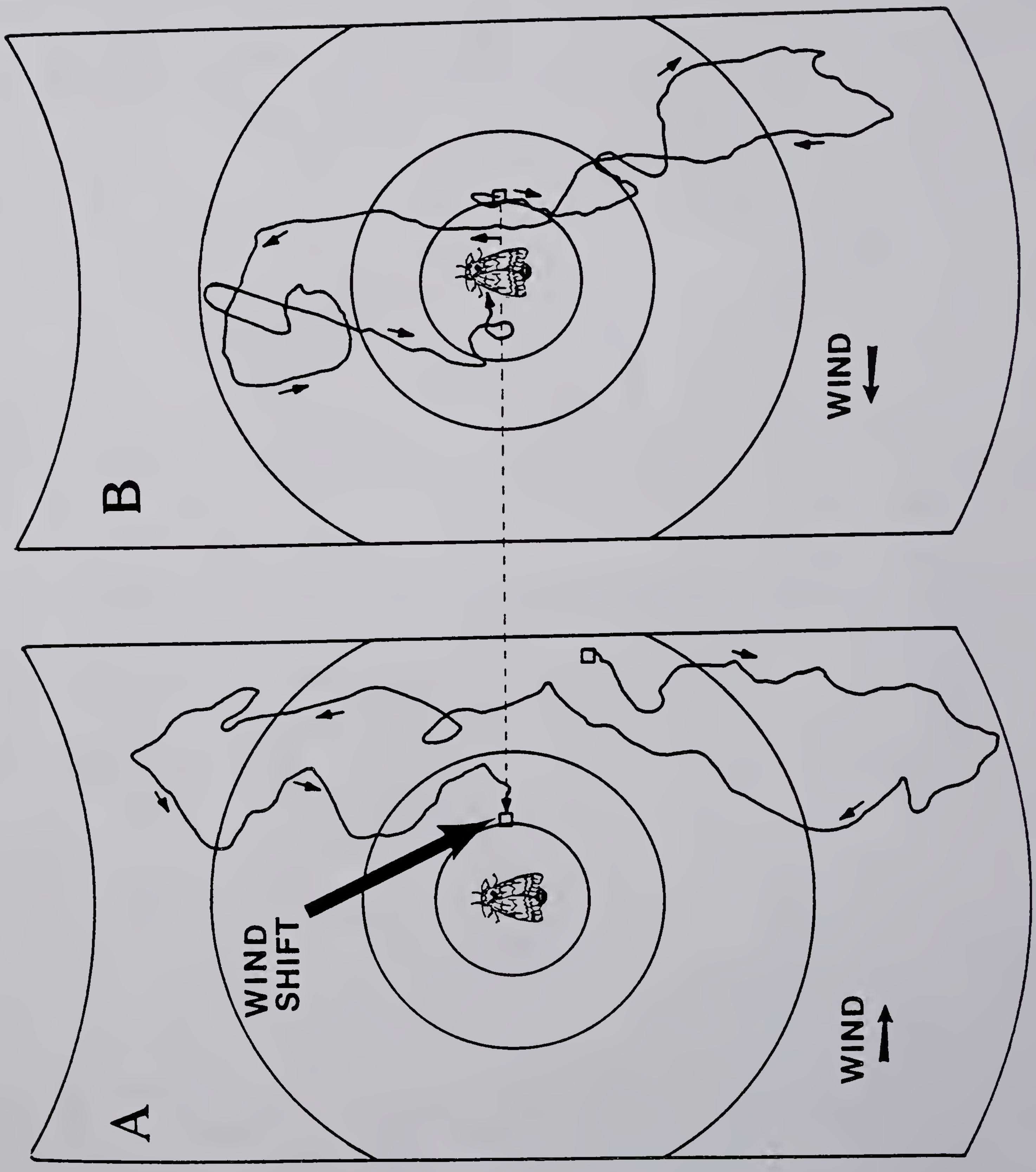

A
B
C
D
WIND
5 cm
10 cm

Figure 18. Typical walking path of male gypsy moth responding to female/100 ng pheromone combination; reactions (A) prior to and (B) following wind and pheromone plume shift induced by rotation of cylinder. Before wind shift, male was 5 cm directly downwind of the female and in the pheromone plume.


A
WIND SHIFT
WIND
B
WIND

CHAPTER IV

BEHAVIORAL INTERACTIONS IN THE COURTSHIP OF THE GYPSY MOTH

Introduction

The important role of pheromones in many aspects of moth mating behavior is well established. In the majority of species studied, the female emits and the male responds to a pheromone blend comprised of several compounds (Roelofs and Cardé, 1977; Tamaki, 1985). A notable exception is the gypsy moth, *Lymantria dispar*, for which only a single pheromone component, *cis*-7,8-epoxy-2-methyloctadecane has been identified (Bierl et al., 1970). Further field trapping experiments (Iwaki et al., 1974; Plimmer et al., 1977; Miller et al., 1977; Cardé et al., 1977) and antennal receptor studies (Hansen, 1984) have shown that males respond preferentially to, and females produce almost exclusively the (+)-enantiomer, 7R,8S-*cis*-epoxy-2-methyloctadecane (named (+)-disparlure). This compound mediates the major facets of mate-locating behavior including activation (Cardé and Hagaman, 1979; Hagaman and Cardé, 1984), upwind anemotactic flight (Cardé and Hagaman, 1979; David et al., 1983; Charlton et al., 1989a; Elkinton et al., 1987) and, to some extent, walking maneuvers (Preiss and Kramer, 1986b; Charlton and Cardé 1989c; Charlton et al., 1989b).

Comparatively, little is known of the potential role that the pheromone and other factors play once a male nears and then contacts the female. General descriptions of *L. dispar* precopulatory behavior have been provided by Doane (1968; 1976), Schröter (1976), Richerson et al. (1976), and Cardé (1981). However, the precise interplay of courtship behaviors and the stimuli that elicit these reactions have not been elucidated for this species. The present study provides a quantitative description of the behaviors comprising the courtship sequence of *L. dispar*. This information is prerequisite to identifying the factors associated with the female that provoke male copulatory attempts.

Materials and Methods

Insects

Lymantria dispar were received as egg masses from the USDA-APHIS Methods Development Center, Otis Air National Guard Base, Massachusetts. Larvae were reared on wheat germ diet (Bell et al., 1981) on a 16L:8D photoperiod at 24°C and 60-70% relative humidity. Individuals were sexed in the pupal stage and isolated by sex in different rooms. Adult males were held in screen cages (30x30x36 cm) and females in 470 ml paper cartons in environmental chambers regulated at conditions similar to those used in rearing.

Pheromone

The synthetic (+)-disparlure (Farnum et al., 1977) used in these experiments had an enantiomeric purity > 96% as determined by analysis of synthesis intermediates. GLC analysis using a 30 m SP-2340 fused silica capillary column indicated the presence of < 1% of the *trans* isomer. A 1 ug/ul solution of (+)-disparlure in hexane was formulated gravimetrically and serially diluted to obtain the desired concentrations.

Flight Tunnel and Testing Protocol

Behavioral assays were conducted in the sustained-flight tunnel described by Cardé and Hagaman (1979) and Charlton et al. (1989a). For all tests, the flight tunnel conditions were: 24±2°C, 60-70% RH, and 50 cm/sec air velocity. The flight tunnel was diffusely illuminated from above by DC-mains fluorescent lights, which produced a light intensity of ca. 450 lux.

Field observations of male gypsy moths have documented that walking search on tree boles and other objects that harbor females is an important element of the mate-seeking process (Richerson, 1977; Cardé and Hagaman, 1984; Charlton and Cardé, 1989c). Thus, to foster natural expression of behaviors, courtship sequences were recorded using a simulated tree trunk in the flight tunnel similar to the one described in Charlton and Cardé (1989c). The artificial tree comprised a 20 cm diam x 70 cm high glass cylinder over whose surface sheer, tan-colored nylon material (mesh size ca. 0.5x0.5 mm) was stretched. The cylinder was positioned in the center of and 40 cm from the upwind end of the tunnel. The nylon provided a surface for the moths to perch and walk on, and yet was sufficiently transparent to allow clear videotaping of behaviors from the ventral view through a window cut in the nylon on the opposite side of the cylinder. Another video camera simultaneously recorded behaviors from the dorsal side. Females were removed from the emergence cups and positioned ca. 30 cm high and perpendicular to the wind flow on the cylinder. The transfer appeared to cause minimal disturbance to the females since all resumed 'calling' within 2 min.

Males were randomly selected from emergence cages, transferred individually into 30-ml clear plastic cups resting on screen squares and held in the wind tunnel room for at least a 1 hr pre-test acclimation period. Fifteen minutes after the female was settled, the screen holding a male was placed on a 25 cm high metal release platform sitting 140 cm downwind of the pheromone source and the restraining cup was removed to expose the male to the female's pheromone plume. Following activation and initiation of upwind flight, the males flew up the tunnel over a stationary floor pattern. All moths used for experiments were 36-48 hr old and tested between the 6th and 10th hours of photophase to coincide with the peak intervals of female calling and pheromone release (Charlton and Cardé, 1982; Tang et al., 1987) and of male responsiveness (Cardé et al., 1974).

Data Recording and Analysis

Behaviors were recorded from the side of the flight tunnel with a SONY SLO-340 video recorder and a SONY RSC-1050 video camera outfitted with a SONY VCL-1206 zoom lens (f=12.5~75 mm). Tapes were played back on a black-and-white video monitor and behaviors were registered with a multi-channel event recorder (Observational Systems, Inc.; OS-3). For slow-motion analysis of rapidly executed behaviors, selected tape portions were re-recorded onto a SONY SVM-1010 motion analyzer and displayed back frame-by-frame (60 frames/sec).

Description and Analysis of Courtship Behaviors

Flow sequences of 33 individual courtships were derived from the video recordings. The frequencies associated with transitions from one behavior to another were tabulated and translated into a first-order transition probability matrix following established methods (e.g., Baerends et al., 1955; Halliday, 1975; Fagen and Young, 1978). These procedures were modified to provide equal weighting of individual courtships in the consolidated matrix. It is conventional in such analyses to combine the raw frequencies associated with behavioral transitions of all the individual sequences before calculating the overall probability that particular behavioral passages will occur. However, if only a small number of males repeatedly oscillate between two behaviors, (e.g., side stationary to abdominal probing in this analysis), then these transitions could be over-represented in the composite kinematic diagrams. To avoid this possibility, behavioral transition frequencies inherent to individual courtships were first converted to probabilities, and these probabilities were averaged across courtship sequences. Thus, the probabilities derived for the composite matrix represented the unweighted means of all courtship sequences. Self-transitions (i.e., when after a predetermined time interval, the animal remains in the same behavioral state) were not included in the analysis and impossible transitions were left as blanks in the table.

To assign an objective measure to the level of variability associated with particular behavioral transitions, stereotypic indices (SI) were calculated following the procedure described in Haynes and Birch (1984). The SI value can range between 0 and 1, with values near unity denoting "highly stereotyped" transitions. In addition, the SI for the overall courtship sequence was calculated by weighting the SI values inherent to the individual transitions by the associated frequencies of occurrence. To obtain a complete measure of variability, all observed transitions, including comparatively rare ones (i.e., those transitions deemed to be chance occurrences and thus non-significant in other studies; e.g., Hagaman and Cardé 1984), were used to calculate SI's associated with individual transitions and the overall sequence.

Results

Description and Analysis of Courtship Behaviors

As is typical for many moths, the mate-seeking behaviors of male gypsy moths are initiated in response to the pheromone emitted by the female. Calling females generally adopt a vertical posture with the head upward, elevate the wings slightly, and emit pheromone while rhythmically protracting and retracting the abdominal tip containing the gland (Doane, 1968; Hollander et al., 1982; Tang et al., 1987). In the wind tunnel, after a quiescent male is stimulated by the pheromone, he responds by wing fanning (Cardé and Hagaman, 1979; Hagaman and Cardé, 1984), taking flight, locking-on to the plume, and orienting anemotactically upwind in zigzagging flight (Cardé and Hagaman, 1979; Charlton et al., 1989a). When the male nears the vertical object on which the female is perched, he switches to vertical flight up and down the cylinder and the combination of pheromone stimulation and visual cues associated with the vertical silhouette induce landing (Charlton and Cardé, 1989c).

This study focused on courtship interactions, designated as the repertory of behaviors that occurred subsequent to landing. The basic sequence of courtship behavior, subdivisible into several discrete behaviors, is illustrated in Fig. 19. After alighting, the male wing fans while walking (WFW) over the substrate. Once the male encounters the plume emanating from the female, he orients anemotactically upwind, approaches (APP) (defined here as entering a 5 cm radius circle surrounding the female), and makes contact (CON) with the female. Initial male contact with the female was usually via the foretarsi (73% of 33 males), or less frequently, the antennae (24%) or the abdomen (3%). In response to this contact, the female retracts her pheromone gland, and draws her wings more tightly to the body. The male then moves forward parallel to the female, assumes the side position (SP) and begins to flex his abdomen (AF) almost 90° toward the female while exposing his claspers. At this point, the female usually begins tilting her body by extending the legs nearest to the male, thus partially exposing the ventral surface of her abdomen and providing the male access. This is an active response on the part of the female, and not simply the result of being pushed by the male, although force exerted by the male can accentuate the degree of tilt. After establishing genital contact with the female's abdomen, the male's full amplitude wing fanning changes abruptly to a low-amplitude wing quiver of roughly similar frequency as wing fanning. The male then probes the ventral surface of the female's abdominal tip with his genitalia (abdominal probing; PROB). After contacting the female's ostium bursa, the male stops quivering and then folds his wings, and directs both antennae posteriolaterally, thereby adopting the side stationary (SS) position. During copulation (COP), the male remains facing in the same direction as the female. The time that pairs spend *in copula* was not recorded in this study, but typically, the mean length of this phase varies between ca. 40-70 min (Doane, 1968; Waldvogel et al., 1981; Loerch and Cameron, 1984).

All of the courtships in which a male oriented to and made contact with the female were successful. The mean time from initial contact until genital engagement was 41 sec (range: 6-169 sec) with most sequences lasting less than 30 sec. Table 7 gives the frequency of occurrence of each transition summed across all courtships, and the proportion of courtships in which particular transitions occurred. The conditional probabilities and flow sequence of behavioral transitions are illustrated in Fig. 20. Both representations indicate that, for the most part, behaviors proceeded unidirectionally from male approach to copulation, although there was some recycling through the sequence. This is corroborated by the moderately high SI value of 0.76 associated with the overall courtship sequence.

Initial transitions in the sequence, including walking while wing fanning to approach, and approach to contact, were highly stereotyped with SI values exceeding 0.92. However, once contact was made, there was more variability in the male response as reflected in the lower SI values (Table 7). The nature of the subsequent transition hinged largely on the approach direction of the male. Males that approached from below and made initial contact with the female's abdomen generally flexed their abdomen before adopting the side position, whereas males that arrived from the side established the side position before flexing their abdomen. Regardless of the approach direction, the onset of male abdominal flexion occurred very rapidly (0.6±0.4 sec; x±SD) following initial contact. The transition following side stationary was also variable. Although some males (n=7) engaged their genitalia with the female's during the initial probing bout, more typically males shuttled between the side stationary position and abdominal probing. Observations from the ventral side indicated that the onset of this transition occurred when the male resumed probing with his abdomen. The abdominal movement was rapidly followed by a simultaneous forward lurch of the body and directing of the now quivering antennae anteriorly, followed almost immediately by resumption of wing quivering (behaviors collectively termed a paroxysm). The mean number of transitions between SS and PROB per courtships was 4.0±3.4 (SD) and the mean time between paroxysms was 10.0±9.4 (SD) sec. Because males in the pseudocopulatory SS pose were superficially indistinguishable from mating males, pairs were observed for 3 min after the last paroxysm and then the male was gently tugged to ensure that he was firmly attached and that mating was actually in progress. Thus, the time to copulation was designated as the elapsed time between initial contact and adoption of the final SS position.

Once the male assumed the SS position, the female generally remained quiescent except for an occasional antennal quiver. But when males required a relatively protracted period to achieve copulation, some females would resume calling; 5 of 33 females reinitiated calling after a mean of 72±8.5 (SD) sec had elapsed following initial male contact. In these courtships, the corresponding mean (±SD) time to mating was 109.8±47.9 sec compared to 28.2±18.6 sec for courtships wherein females never resumed calling.

During preliminary observations of courtship interactions, two females that called again while a male attempted to copulate were able to lure a second male. Interestingly, on both occasions, the last male to arrive displaced the original male and coupled with the female. In both cases, the first males remained in contact with the female and assumed a pseudocopulatory pose whereby they appeared to be attached to the female, but were, in fact, easily dislodged by a gentle tug.

Discussion

The courtship of the gypsy moth consists of a relatively uncomplicated series of behaviors which culminate in mating. The male orients to the calling female using flying and walking maneuvers and assumes the more active role in the courtship interactions that ensue following contact with the female. Conversely, the female has a relatively simple behavioral repertory, consisting basically of calling, retraction of the pheromone gland once males establish contact, and tilting the body after the male assumes the side position and initiates abdominal probing. During copulation, the male remains facing in the same direction as the female, a copulatory position also adopted by other lymantriids, including *Orgia leucostigma* (Grant, 19981), *Orgia pseudotsugata* (Swaby et al., 1987), and *Lymantria monacha* (Schröter, 1976), but atypical for most other male lepidopterans which assume the end-to-end or opposed position.

This simple type of mating pattern may be related to the fact that male gypsy moths do not possess scent-disseminating structures that have been shown or are presumed to play a role in the courtship of a number of moth species (Birch, 1970; Grant and Brady, 1975; Gothilf and Shorey, 1976; Grant, 1976; Barrer and Hill, 1977; Colwell et al., 1978; Baker and Cardé, 1979; Ellis and Brimacombe, 1980; Conner et al., 1981; Teal et al., 1981; Krasnoff, 1981; Zagatti, 1981; Farine, 1982; Hendrikse et al., 1984; Phelan, 1984; Hendrikse, 1986; Krasnoff, 1987).

There is a tendency in species in which male scent organs are evidently absent, for the females to remain stationary while the males execute most of the overt behaviors culminating in mating. This pattern has been documented for *O. leucostigma* (Grant, 1981), *O. pseudotsugata* (Swaby et al., 1987), *Platyptilia carduidactyla* (Haynes and Birch, 1984), *Laspeyresia pomonella* (Castrovillo and Cardé, 1980), *L. monacha* (Schröter, 1976), and in this study, on *L. dispar.* Moreover, in a detailed comparative study of the courtships of 10 phycitine species, Phelan (1984) found that the behavioral sequences exhibited by *Paramyelois transitella* and *Laetilia coccidivora* were comparatively simple; the females remained immobile and the males faced in the same direction as the female as they attempted to copulate, not adopting the head-to-head position displayed by the majority of the phycitines during courtship. Interestingly, males of neither species possess pheromone-emitting structure (Heinrich, 1956). This contrasts with the other 8 species Phelan (1984) described, all of which display complex courtships involving elaborate movements on the part of both males and females. Notwithstanding, there are exceptions to this generalization. *Pectinophora gossypiella* females remain stationary during courtship, even though the male everts hair pencils upon approach to the female (Colwell et al., 1978). However, the communicatory function of these structures has not been documented, nor has it been demonstrated that male scent dissemination affects the probability of successful mating.

Evidently, female gypsy moths exercise little mate choice, at least in the context of courtship behaviors. This lack of choosiness is perhaps surprising given that females typically mate only once and sperm from a single mating is sufficient to fertilize their entire egg complement (Doane, 1968). On the other hand, females generally live only a few days as adults, thus placing a premium on rapid mating and oviposition. Mate selection, if it is exercised, may simply involve mating with males that are most efficient at orienting to the pheromone while flying and walking and therefore, on average, arrive at the female first. It has been suggested (Greenfield, 1981; Cardé and Baker, 1984) that female lepidopterans could modulate their output of pheromone to emit reduced quantities, thereby selecting for the most sensitive males. Although intriguing, there exists no experimental evidence to support this hypothesis. Furthermore, the dilution of pheromone plumes engendered by varying wind direction and turbulence in the field probably is sufficient to challenge the sensory capabilities of males. Also to be considered is that males flying upwind in a pheromone plume frequently encounter shifts in wind direction such that the direction upwind is only infrequently aimed toward the pheromone source. Such discontinuities provide a major impediment to the ability of males to navigate an anemotactic course to the female, and a limitation to the effective distance of communication, irrespective of release rates and male response thresholds (Elkinton et al., 1987).

In this study, all the recorded courtships were successful and females exhibited few behaviors that could be construed as forms of rejection. Nevertheless, successful and rapid execution of courtship behaviors by males may be implicated in female choice. This was suggested by the finding that females would often resume calling if males required lengthy periods (>60 sec) to couple with the female. The renewed pheromone emission could simply serve to reinforce the response of the male already in contact. Alternatively, it may constitute a type of rejection behavior which functions to lure additional, possibly more adept males to the female.

Rejection behavior has been noted in mated female gypsy moths, taking the form of tucking their wings close to their abdomen, walking away, or dropping to the ground (Richerson et al., 1976). Females of other lepidopteran species commonly exhibit various forms of rejection behavior during courtship interactions. Rejection can be indicated by extensive wing fanning (Teal et al., 1981; Hendrikse, 1986), wing flicking (Colwell et al., 1978; Ellis et al., 1980), or most commonly, walking or flying away (Grant and Brady, 1975; Grant, 1976; Barrer and Hill, 1977; Colwell et al., 1978; Baker and Cardé, 1979; Ellis et al., 1980; Teal et al., 1981; Hendrikse, 1986). The flightless North American gypsy moth females do not possess the option of taking flight.

The rapid execution of courtship behaviors may have implications for male-male competition under field conditions, where several males can arrive at the female virtually simultaneously, particularly when population densities are high. Males that touch each other during vertical flight or walking movements frequently fly away and terminate search behavior (Doane and Cardé, 1973), or 'space themselves out' (Richerson et al., 1976). In contrast, males that encounter other males once in contact with the female, do not generally break off courtship. Instead, in this situation, males actively attempt to mate and compete for copulation (Charlton, personal observation). Thus, it appears that males most efficient at executing post-contact behaviors will likely garner the mating.

In fact, late-arriving or displaced males will, at times, stay attached to the female and assume a pseudocopulatory posture (Richerson et al., 1976; Cardé and Hagaman, 1984). The propensity for some males to remain in contact with a mating female could be construed as a type of satellite strategy whereby these males wait for the first male to disengage from the female and then attempt to copulate themselves. Although data supporting this contention are lacking, Richerson et al. (1976) described a situation wherein four "waiting" males, observed in a dense natural population, successively copulated with a single female following the first mating. This putative tactic could also help explain why males remain *in copula* for about an hour even though sperm sufficient to fertilize the female's entire egg complement is transferred during the first 5 to 7 minutes of copulation (Doane, 1968). Thus, extended copulation may constitute a form of mate guarding (see Drummond, 1984 for review) whereby the male ensures that the physiological transition of the female from a sexually receptive to a non-receptive state is initiated.

For male gypsy moths, the single pheromone component (+)-disparlure mediates, directly or indirectly, the flying and walking maneuvers used to locate the female. Other stimuli come into play after contact with the female is established; structural characteristics of the female wing and body scales elicit male copulatory behaviors (Charlton and Cardé, 1989b). The role of body scales as releasers of copulatory attempts has also been documented for several other moths (Ono, 1974, 1977, 1980, 1981; Shimizu and Tamaki, 1980; Grant, 1981, 1987). In addition, Grant et al. (1987) reported that the male copulatory response of *O. leucostigma* is reinforced by a series of straight-chain alkanes ranging from C-21 to C-29 extracted from the female cuticle. The relative roles of chemical and structural attributes of female gypsy moth wing and body scales in promoting male copulatory attempts, and the sensory modalities employed by the male to recognize these cues are considered elsewhere (Charlton and Cardé, 1989b).

Table 7. Summary of stereotypy indices associated with, and frequencies[a] (F) of occurrence of male behavioral transitions, and the proportion (P) of *L. dispar* courtships in which particular transitions occurred.

Preceding Behavior	Stereotypy Index[c]		Following Behaviors[b]							
			WWF	APP	CON	AF	SP	PROB	SS	COP
WWF	1.00	F	---	35	---	---	---	---	---	---
		P	---	1.00						
APP	0.89	F	1	---	32	2	---	---	---	---
		P	0.03	---	0.94	0.06				
CON	0.57	F	1	---	---	19	14	---	---	---
		P	0.03		---	0.58	0.42			
AF	0.56	F	---	---	2	---	18	13	---	---
		P			0.06	---	0.55	0.39		
SP	0.65	F	---	---	---	12	---	20	1	---
		P				0.36	---	0.61	0.03	
PROB	0.99	F	---	---	---	---	1	---	131	---
		P					0.01	---	1.00	
SS	0.64	F	---	---	---	---	---	97	---	33
		P						0.79	---	1.00
OVERALL	0.76									

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[a] Frequencies denote the total number of observations of a particular transition across all courtships.

[b] WWF, walking while wing fanning; APP, approach; CON, contact; AF, abdominal flexion; SP, side position; PROB, abdominal probing; SS, side stationary; COP, copulation.

[c] Stereotypy Index = $[(\Sigma(P_{ij})^2 - (\Sigma P_{ij})^2/r_i/(1-1/r_i)]^{1/2}$: P_{ij} is the probability of transitions from preceding behavior (i) to all subsequent behaviors (j) [ΣP_{ij} always equals either 0 or 1]; r_i represents the number of possible transitions from the preceding behavior (i) [Haynes and Birch, 1984].

Fig. 19. The sequence of events occurring during the courtship of *Lymantria dispar*. (A) Female in calling posture; inset: closeup view of exposed pheromone gland; (B) The male approaches from downwind by wing fanning while walking; (C) Male contacts and flexes abdomen toward female, female retracts gland; (D) Male assumes side position and probes with his abdomen, female has tilted to provide the male access; (E) Male probes the genital area of female, with wings quivering and antennae directed anteriorly; (F) Copulation, male's wings folded, antennae drawn back. Wind direction is from left to right.

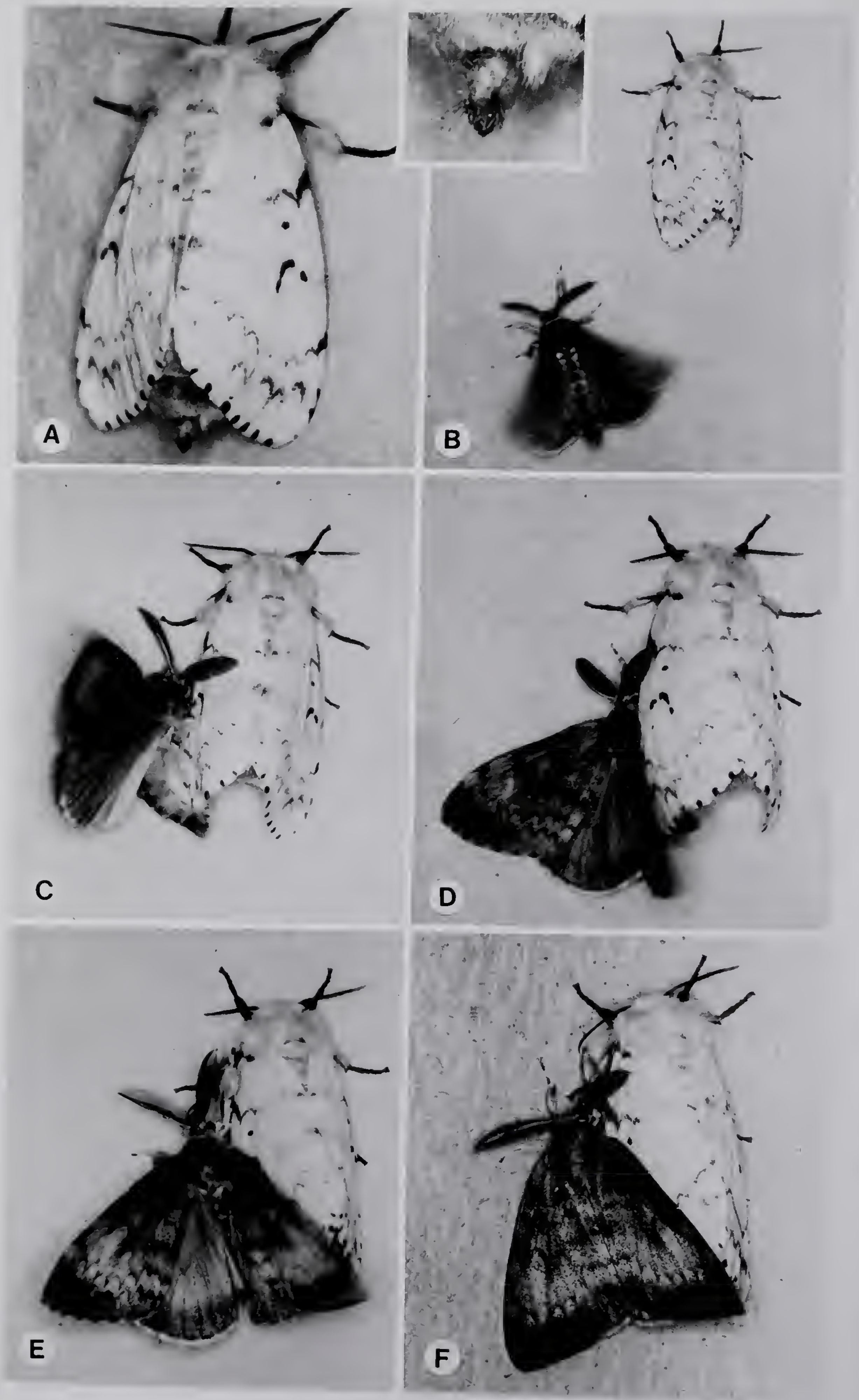
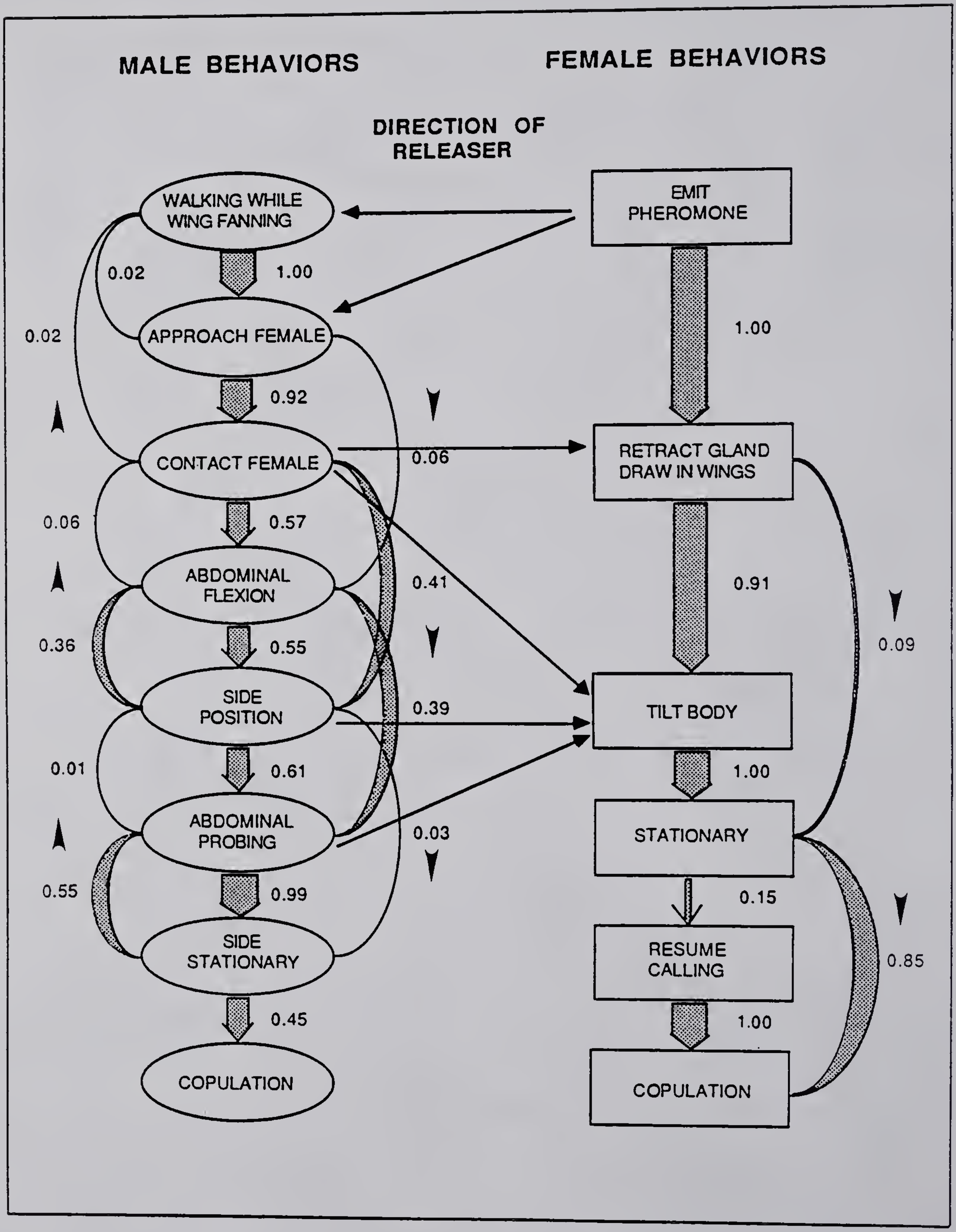

A
B
C
D
E
F

Fig. 20. Kinematic representation of *Lymantria dispar* courtship behaviors (based on 33 successful courtships). Widths of shaded bands or arrows and associated decimal numbers denote the conditional probability of a particular transition occurring between behaviors. Solid vertical arrows associated with bands indicate direction of behavioral flow


MALE BEHAVIORS
FEMALE BEHAVIORS
DIRECTION OF RELEASER
WALKING WHILE WING FANNING
APPROACH FEMALE
CONTACT FEMALE
ABDOMINAL FLEXION
SIDE POSITION
ABDOMINAL PROBING
SIDE STATIONARY
COPULATION
EMIT PHEROMONE
RETRACT GLAND DRAW IN WINGS
TILT BODY
STATIONARY
RESUME CALLING
COPULATION
0.02
1.00
0.02
0.92
0.06
0.57
0.06
0.41
0.36
0.55
0.39
0.01
0.61
0.03
0.55
0.99
0.45
1.00
0.91
0.09
1.00
0.15
0.85
1.00

CHAPTER V

FACTORS MEDIATING MATE RECOGNITION AND COPULATORY BEHAVIORS IN THE MALE GYPSY MOTH

Introduction

In the gypsy moth, as is typical for most moths, the female-produced pheromone mediates to a large extent the flying (David et al., 1983; Cardé and Hagaman, 1979; Charlton et al., 1989a) and walking (Preiss and Kramer, 1986b; Charlton and Cardé, 1989c; Charlton et al., 1989b) maneuvers used by the males to locate prospective mates. Once the male nears and contacts the female, courtship behaviors ensue (Schröter, 1976; Charlton and Cardé, 1989b); implicit to these courtship interactions is recognition of the female as conspecific and the subsequent release of male copulatory behaviors.

Relatively little is known of the potential role that the pheromone and other stimuli associated with the female play in the recognition process. As early as the late nineteenth century, Forbush and Fernald (1896) described some pioneering but rather modestly replicated experiments wherein females and males were adulterated in various ways and the effects on male mating behavior recorded. They found that coating the female's body with varnish did not diminish the ability of males to mate as long as the tip of the female's abdomen (containing the pheromone gland) remained uncovered. Similarly, Richerson (1977) assayed the mating response of feral males to females with altered physical and chemical characteristics and found that normal and apterous females elicited the best male mating response but even females painted various colors or rinsed with solvents prompted some mating attempts. These studies suggest that either the mating response is not highly specific or that males can rely on multiple sensory inputs to confer information about the species-identity of an encountered female. Still undetermined are the precise stimuli responsible for evoking these reactions.

For a number of other moth species, structural characteristics of the female scales have been shown to elicit the male copulatory attempts. These include *Phthorimaea operculella* (Ono, 1974, 1979), *Bombyx mori* (Ono, 1980), *Choristoneura fumiferana* (Sanders, 1979; Grant, 1987), *Adoxophyes* sp. (Shimizu and Tamaki, 1980), *Orgyia leucostigma* (Grant, 1981), and three phycitine species (Ono, 1981). In addition, Grant et al. (1987) recently reported that the male copulatory response of *O. leucostigma* is reinforced by a series of straight chain saturated hydrocarbons ranging from C-21 to C-29 extracted from female body scales.

The objective of this study was to describe in gypsy moths the specific factors associated with the female that provoke male copulatory behavior. Male behaviors were studied using models that incorporated different features of the female. The sensory basis of the male response was also investigated through behavioral assays designed to identify the relative contributions of chemical, tactile, and visual components to the recognition process.

Materials and Methods

Insects

Lymantria dispar were received as egg masses from the USDA-APHIS Methods Development Center, Otis Air National Guard Base, Massachusetts. Larvae were reared on wheat germ diet (Bell et al., 1981) on a 16L:8D photoperiod at 24 ± 2 °C and 60-70% relative humidity. Individuals were segregated by sex as pupae and held in separate rooms. Adult males were held in screen cages (30 x 30 x 36 cm) and females in 470 ml paper cartons in environmental chambers regulated at conditions similar to those used in rearing.

Pheromone

The synthetic (+)-disparlure (Farnum et al., 1977) used in these experiments had an enantiomeric purity > 96% as determined by analysis of intermediates. GLC analysis using a 30 m SP-2340 fused silica capillary column indicated the presence of < 1% of the *trans* isomer. A 1 µg/µl solution of (+)-disparlure in hexane was formulated gravimetrically and serially diluted to obtain the desired concentrations.

Flight Tunnel and Testing Protocol

Behavioral assays were conducted in the sustained-flight tunnel described by Cardé and Hagaman (1979) and Charlton et al. (1989a). For all tests the flight tunnel conditions were: 24 ± 2 °C, 450 lux, 70-80% RH, and 50 cm/sec air velocity. The flight tunnel was diffusely illuminated from above with DC-mains fluorescent lights reflected off a matte-white ceiling.

Field observations of male gypsy moths have shown that walking search on tree boles and other objects that harbor females is an important element of the mate-seeking process (Richerson, 1977; Cardé and Hagaman, 1984; Charlton and Cardé, 1989c). Thus, to promote natural expression of behaviors, male responses were assayed using a simulated tree trunk in the flight tunnel as described in Charlton and Cardé (1989c). The artificial tree consisted of a 30 cm diam x 70 cm high metal cylinder covered with dark brown poster board positioned in the center of the tunnel, 60 cm from the upwind end.

Males were randomly selected from emergence cages, transferred individually into either 6 x 6 cm cylindrical screen cages or 30-ml clear plastic cups resting on screen squares and held in the wind tunnel room for at least a 1 hr pretest acclimation period. At the onset of each trial the appropriate treatment was pinned 30 cm high on the cylinder. Then the screen square or cage holding a male was placed on a 25 cm high metal stand 160 cm downwind of the pheromone source and within the pheromone plume. Following activation and initiation of upwind flight, the moths flew up the tunnel over a stationary floor pattern, and landed on and walked over the surface of the cylinder until they contacted the test treatment.

Male behaviors were videorecorded for 3 min commencing with initiation of contact with a treatment. The following measures of male response were used to establish the suitability of treatments: contact duration, i.e. the total time spent in contact with the treatment; and copulatory attempt duration, defined as the amount of time the male flexed his abdomen toward and maintained genital contact with the stimulus (also termed 'probing'). In addition, a copulatory index defined as probing duration/contact duration was calculated for some experiments. Moths were tested in a randomized complete block design with three or four successful contacts per block per treatment; fifteen or twenty males were tested for each treatment. To prevent cross-contamination, each treatment was deployed on its own 30 x 30 cm paper which was affixed to the cylinder and removed after testing each treatment.

All moths used for experiments were 36-48 hr-old and tested between the 6th and 10th hours of photophase to coincide with the peak intervals of female calling and pheromone release (Charlton and Cardé, 1982; Tang et al., 1987), and of male responsiveness (Cardé et al., 1974).

Data Recording and Analysis

Behaviors were recorded from the side of the flight tunnel, with a SONY SLO-340 video recorder and a SONY RSC-1050 video camera outfitted with a SONY VCL-1206 zoom lens (f = 12.5 - 75 mm). Tapes were played back on a black-and-white video monitor and certain behaviors were registered with a multi-channel event recorder (Observational Systems Inc.; OS-3). For slow-motion analysis of some rapidly executed behaviors, selected tape portions were re-recorded onto a SONY-SVM-1010 motion analyser and displayed back frame-by-frame (60 frames/sec).

Factors Promoting Mate Recognition and Copulatory Behavior

To establish which stimuli associated with the female promote recognition and copulatory attempts by the male, various female features were incorporated onto models and their effectiveness as releasers of male copulatory behavior was assessed. Cylindrical grey plasticene (a soft clay-like material) models, simulating the female's body in shape and size (30 mm long x 10 mm diam) were used in assays. The following stimuli derived from virgin females were affixed individually to the models: (1) forewings alone; (2) forewing scales alone; (3) abdominal scales and forewings; and (4) abdominal scales alone. Wings were secured to the models with insect pins and scales were applied by lightly pressing them into the surface of the plasticene. Scales were replenished on models after each male tested, and forewings were replaced after every 3rd or 4th male. In addition, male response was assayed to a clay control and a normal, virgin female; prior to testing, the female's abdominal tip containing the pheromone gland (Hollander et al., 1982) and the genital area were sealed with molten dental wax to prevent mating and pheromone emission (as confirmed by the absence of activation responses of males exposed in an olfactometer to similarly operated females). All treatments were supplemented with 100 ng (+)-disparlure on a 0.7 cm diam filter paper disc pinned to the bottom of the model or beneath the female's abdominal tip. Behavioral assays were carried out using the same experimental design described previously and twenty males were scored for contact duration and probing duration in response to each treatment.

Effect of Model Size and Female Abdominal Scales on Male Response to Pheromone

In this experiment the effects on male copulatory responses of different configurations of pheromone, female abdominal scales, and variously sized models were evaluated. Males were tested to a pheromone source alone; abdominal scales applied flat over a 10 mm x 30 mm rectangular area directly above the pheromone source; and virgin female abdominal scales applied to small (5 mm diam x 30 mm long), normal (10 mm x 30 mm), and large (20 mm x 30 mm) clay models all supplemented with a 100 ng (+)-disparlure source. All treatments were pinned in the center of a 5 cm diam circle drawn on the paper; this area was designated as the 'arrestment zone' to facilitate computation of an arrestment index defined as the time a male spent inside the arrestment zone as a percentage of the total time the male walked on the cylinder following initial entry into the zone. To obtain an alternate measure of the 'arrestment' potential of a treatment, the number of excursions from the arrestment zone was also recorded for each male.

Effect of Chemical and Structural Alteration of Scales

Previous experiments established that factors associated with female abdominal scales evoked male copulatory behavior. To identify which attributes of the scales induce the copulatory response, scales were subjected to chemical and structural alterations. Abdominal scales from twenty virgin females were combined and treated as follows.

(1) Scales were sequentially extracted in a soxhlet apparatus with four solvents (12 hr per solvent) chosen to encompass the full range of polarities: nanograde quality (J.T. Baker Chemical Co.) hexane, methylene chloride, methanol, and distilled water. Following extraction, scales were oven-dried at 200 °C for 24 hr.

(2) Scales were stirred in either 200 ml hot acid (1N HCl in distilled water) or base (1N KOH in distilled water) solutions for 1 hr. The scales were then rinsed in a column with 500 ml distilled water to flush residual acid or base and dried at 200 °C for 2 hr.

(3) Scales were pulverized to destroy their structural integrity while preserving any chemical characteristics. The scales were crushed to a fine powder using a mortar and pestle.

Treated scales were applied to the surface of cylindrical clay models, 30 mm long and 10 mm in diameter. Male response was compared to bare control models and models covered with unadulterated virgin female abdominal scales. All treatments were supplemented with 100 ng (+)-disparlure.

Male Copulatory Response to Scale Extracts

To determine whether chemicals associated with female scales influence male copulatory reactions, solvent extracts of virgin female abdominal and forewing scales were tested. Extracts of each scale type were prepared by removing forewing and abdominal scales from ten females and extracting them sequentially in hexane, and 5% ethyl ether/hexane; 5 ml and 2 ml solvent were used for abdominal and wing scales, respectively. The extracts were filtered, combined and concentrated under a nitrogen stream to ca. 200 µl. To provide a suitable release substrate, it was necessary to use a different model for these experiments: the exterior sheath of a 10 mm diam x 30 mm long dental wick was removed with a razor blade, and impregnated with the total scale extract (10 FE). Following ventilation for 20 min in an exhaust hood, the sheath was inverted to expose its fuzzy interior (to better simulate the texture of female scales) and secured to the wick with insect pins inserted through the back of the model.

Controls consisted of wicks loaded with comparable quantities of clean solvent, and wicks coated with virgin female abdominal scales. A filter paper disc loaded with 100 ng (+)-disparlure was pinned to the bottom of each model.

Sensory Basis of Male Response

Male gypsy moths can potentially receive information about the identity of an encountered female through several communicatory channels involving a number of different sensory structures: these include olfaction (antennae), vision (compound eyes), contact chemoreception (tarsi, labial palps), mechanoreception (tarsi), and hearing (tympana). To determine which sensory structures and modalities are implicated in copulatory behavior, male sensory structures were selectively ablated and the effect on the male copulatory response was assayed. Regardless of the type of operation performed, male response was evaluated to a model coated with virgin female abdominal scales. All operations were performed ca. 2 hr before testing to allow recovery of the males. Unless otherwise noted, experiments followed the same protocol as described previously.

(1) <u>Antennectomy</u>: Males were chilled to immobility in a freezer and both antennae were excised at the base with iris scissors. Sham-operated males were handled identically except that the scissors were drawn several times across the base of the antennae. Because males lacking antennae are not able to orient to pheromone, a different experimental procedure was required. To facilitate intersexual

encounters, the pairs were assayed under confined conditions; the female model was pinned to the inside of a 470 ml paper carton and males were introduced individually into the carton. Following initial contact, behaviors were recorded for 3 min.

(2) Blinding: Males were chilled and red, water-soluble paint (Kodak opaque red) was applied over the entire surface of the compound eyes with a blunt insect pin. To ensure complete coverage this procedure was performed under a dissecting microscope. Sham-operated males received identical handling but the paint was applied to the frons area between the eyes. Since moths require optomotor feedback to orient to a pheromone source, the experimental procedure required modification. Cylindrical screen cages holding individual males were placed on the release platform and thus within the pheromone plume. After activation the males were allowed to wingfan for 2 min and then the cage was brought up to the cylinder and the male was released 10 cm directly downwind of the model. The time required to contact the model following release was recorded in addition to the usual copulatory behavior.

(3) Tarsal Ablation: Strong acids have been used to selectively destroy chemosensilla without impairing the response of mechanosensilla (Städler, 1977). An aqueous solution of 5N HCl was drawn by capillary action into the tip of a pipet and the tarsi of males were individually inserted into the solution for 10 sec per tarsus. Sham-operated males were handled similarly except that the tarsi were dipped into distilled water. We also attempted to ablate the tarsal mechanoreceptors by

either excising all tarsi or coating the tarsi with molten wax. However, the male's ability to walk was so severely impaired that reliable conclusions could not be drawn from the results of these experiments.

(4) Labial Palp Ablation: Unanesthetized males were held under a microscope and the labial palps were completely removed with scissors. Sham-operated males received similar handling but the scissors were merely drawn several times across the base of the palps.

(5) Genital Ablation: The claspers and genital area were exposed by exerting slight pressure on the male's abdomen. Molten dental wax was then applied to cover the entire genital area. Sham-operated males were handled similarly except that the wax was applied to the dorsal surface of the abdomen near the tip.

(6) Tympanal Ablation: Male *L. dispar* possess well-developed tympana on the metathorax sensitive to ultrasound (Baker and Cardé, 1978). Unanesthetized males were held under a microscope and the tympanic membranes and associated sensilla on each side were destroyed with an insect pin. Sham-operated males were subjected to the same treatment except that only the areas adjacent to the membranes were contacted by the pin.

Results

Factors Promoting Mate Recognition and Copulatory Behavior

Models incorporating either female abdominal scales alone, or a combination of forewings and abdominal scales elicited male copulatory behavior comparable to that evoked by a live female as measured by both total time spent in contact with and probing the models (Fig. 21). When forewings alone were tested, the durations of contact and probing were significantly reduced but still above control levels. However, when only forewing scales were tested, total contact durations were indistinguishable from the control but males displayed a significantly longer duration of probing. Although the lowest response levels were seen with bare models, even this treatment elicited some copulatory attempts (Fig. 21).

The behavioral responses of males to models invested with either abdominal scales and forewings, alone or in combination, were fundamentally similar to those inherent to normal courtships (Charlton and Cardé, 1989b). The male approached the model in the wing fanning while walking mode. Following contact, the male moved forward parallel to the model, flexed his abdomen toward the model, and probed its lower and ventral surfaces with his abdominal tip (Fig. 22). After making genital contact the male's full-amplitude wing fanning changed to a low-amplitude wing quiver with the wings held in a more vertical position as the male wedged himself alongside the model. With the exception of the control, the latencies to probing were short (< 2 sec), and thus comparable to those reported for normal courtships.

Effect of Model Size and Female Abdominal Scales on Male Response to Pheromone

Exposure to the sex pheromone alone was not sufficient to release male copulatory behaviors. Males often contacted the filter paper pheromone dispenser during the course of their walking movements, whereupon they briefly paused and then continued walking; copulatory attempts were never directed at the dispenser (Table 8). Supplementing the pheromone with female abdominal scales applied on a flat surface caused a marked change in behavior. Upon establishing tarsal contact with the scale field, the males forward movement abruptly ceased and was followed almost immediately (< 1 sec) by probing behavior directed at the substrate accompanied by a decrease in wing beat amplitude (Fig. 23). In addition, the total duration of contact and probing was significantly elevated when scales were deployed. Males also remained on or near the scales for longer periods, as was reflected in the significantly higher arrestment index and lower number of excursions from the arrestment zone compared to the pheromone only situation (Table 8).

Model size also influenced the expression of male copulatory behaviors. The small model evoked responses comparable to the flat scales, whereas the normal and large models evoked the longest durations of contact and of probing. Differences in other behaviors, relatable to model size, were also noted. After contacting the small model, males frequently clambered from side to side over the top of the model, and probing attempts were directed indiscriminately over the entire surface of the model. Conversely, with the two largest models, males typically assumed a position alongside the model and directed copulatory thrusts predominately toward the model's bottom and ventral surfaces (Fig. 22), as was observed in the first experiment and in normal courtships. Males stayed in this position for comparatively extended periods but would also on occasion circle the model (with their heads directed downward at times) while maintaining constant contact. In the latter respect their behaviors deviated from the response to normal females where such maneuvers were observed only rarely; possibly the direction that scales are arrayed on a normal female provides the cue for proper male body orientation. In addition, males remained in close proximity to these models for more protracted intervals as evidenced by the significantly higher arrestment indices and the lowest mean number of exits from the arrestment zone (Table 8). The ratio of probing to contact showed the same trend as the other copulatory measures, with the lowest values for the small model or flat scales, and the highest ratios observed in response to normal and large models (Table 8).

Effect of Chemical and Structural Alteration of Scales

Extracting female abdominal scales with a range of organic solvents or strong acids and bases did not adversely affect the copulatory response of males; mean durations of contact and probing to extracted scales were comparable to those evoked by unadulterated scales but significantly higher than to bare controls (Figs. 24A, 24B, and 24C). However, the stimulatory effect of abdominal scales was eliminated by pulverization of the scales (Fig. 24D). These results indicate that mechanical attributes of the scales mediate male copulatory responses and chemical stimuli do not appreciably influence these reactions. Preliminary experiments indicated that crushing resulted in only modest decrease in activity. However, upon examination of the scales under a dissecting microscope, it was discovered that some larger scale fragments remained, and these presumably were sufficient to elicit a good male response. Thus, it apparently is critical that the scales be pulverized to a fine powder.

Male Copulatory Response to Scale Extracts

Models treated with either forewing or abdominal scale extracts elicited longer periods of contact and probing than did the solvent control model although the differences were not significant (Table 9). However, a significantly higher proportion of males exhibited at least some probing behavior (minimum 2 sec total duration) to the abdominal scale extract compared to the control. Even so, the durations of contact and probing in response to the scale extracts and the bare control were substantially lower than to models coated with abdominal scales (Table 9).

Sensory Basis of Male Response

Selective ablation of most male sensory structures did not compromise the ability of males to perform copulatory behaviors. Males whose compound eyes (Fig. 25A), tympana (Fig. 25C), tarsal chemoreceptors (Fig. 25D), labial palps (Fig. 25E), or genitalia (Fig. 25F) were removed or occluded displayed fundamentally the same behaviors as their sham-operated or normal counterparts, and the durations of contact and probing were not significantly different. Blinded males were also able to locate the model virtually as quickly as normal males (x ± SD; 11.8 ± 11.4 sec vs. 9.0 ± 10.5 sec, respectively; ($P > 0.05$): Mann Whitney *U* test, two-sided), corroborating that visual cues are not essential for mate location (Charlton and Cardé, 1989c).

Only removal of male antennae abolished the copulatory response (Fig. 25B). Antennectomized males frequently contacted the model (x ± SD = 14.8 ± 8.5) but never attempted to copulate with it. These males did not pause upon contact with the model. Rather, they appeared to be repelled by it and tended to wing fan while walking on the opposite side of the container. Conversely, all normal and sham-operated males initiated probing behavior following the first or second contact and this behavior persisted for extended periods (Fig. 25B).

Discussion

Male gypsy moth copulatory behavior is released upon contact with female scales. Optimal responses, comparable to those elicited by a live female, were recorded to models incorporating female abdominal scales alone or in combination with female forewings. Males also displayed copulatory attempts, albeit at reduced levels, to models with intact forewings or coated with forewing scales, and to scales applied to a flat surface. The role of scales as the primary releasers of copulatory behavior was first demonstrated by Ono (1974; 1977) with *P. operculella*. Additional studies on a variety of species representing several families have confirmed that this phenomenon is widespread in the Lepidoptera (Ono, 1977, 1980, 1981; Sanders, 1979; Shimizu and Tamaki, 1980; Grant, 1981, 1987; Grant et al., 1987). Moreover, this appears to be a generalized response in that males of several species will respond not only to conspecific female scales, but also to scales derived from other species as well as conspecific male scales (Shimizu and Tamaki, 1980; Ono, 1979, 1980, 1981; Grant, 1987).

Specific factors associated with the scales evoke the copulatory reactions. Solvent and base or acid extraction of female abdominal scales did not cause an appreciable diminution of male response compared to the corresponding unadulterated scale controls. Extraction with solvents representing a wide range of polarities would be expected to strip the scales of volatile and non-volatile compounds of many different chemical classes. These include the saturated and unsaturated hydrocarbons which are ubiquitous in insect cuticle, and which have been shown in some cases to play an important role in chemical communication (Howard and Blomquist, 1982; Blomquist and Dillwith, 1985; and references therein). On the other hand, pulverizing the scales which destroyed their structure while preserving associated chemical characteristics essentially reduced the copulatory response to the level elicited by a bare model. Crushing of the scales also diminished or eliminated the copulatory response of males of several other species including *O. leucostigma* (Grant, 1981; Grant et al., 1987), *P. operculella* (Ono, 1979), *Adoxophyes* sp. (Shimizu and Tamaki, 1980) and *C. fumiferana* (Grant, 1987). Thus, for *L. dispar* and other moths, the combined evidence indicates that tactile cues inherent to the scales serve to release copulatory behavior and that chemical attributes do not contribute appreciably to this response.

While the copulation-releasing effect of female moth scales appears to be due mainly to physical characteristics, other stimuli have also been implicated in this response. Grant et al. (1987) demonstrated that several straight chain alkanes extracted from *O. leucostigma* scales act as a copulation releaser pheromone. Scale extracts applied to rubber septum models (+ female pheromone gland) evoked significantly more copulation attempts than did the hexane controls. Moreover, compared to hexane controls, a greater proportion of males attempted copulation in response to septa loaded individually with n-tricosane, n-tetracosane, n-pentacosane, and n-heptacosane. Male response to scale extracts and scales was not compared directly by Grant et al. (1987); however, it appears that both treatments elicited comparable levels of copulatory behavior.

Although structural features of female scales appear to mediate male gypsy moth copulatory behaviors, the involvement of chemical cues in these reactions cannot be ruled out entirely. For example, male response to solvent, base or acid-extracted scales was diminished compared to virgin scales, although not significantly. Furthermore, male response to forewing and abdominal scale extracts was also modestly elevated over controls, and significantly more males responded with at least some probing behavior in response to abdominal scale extracts compared to controls. Nevertheless, other factors may also have contributed to the reduced response seen with extracts. First, the extraction procedures dried out the scales and thus they did not adhere to the model as well and were comparatively easily dislodged by the males' vigorous probing and wing fanning. Second, subjecting the scales to hot acid or base initiates degradation of cuticular proteins which in turn alters the structural features of the scales. Therefore, these findings suggest that mechanical attributes of the scales mediate recognition and copulatory behaviors and that chemical stimuli play at most a minor role in this process.

The presence and characteristics of models also influenced male copulatory behavior. When a model was absent, males would wingfan while walking rapidly in the vicinity of the pheromone source but the isolated source never elicited copulatory attempts. However, when a cylindrical clay model was deployed with the pheromone source, males paused upon contacting the model and usually displayed low levels of copulatory behavior. Supplementing the source with either abdominal scales applied to a flat surface or with various sized models covered with scales, prompted a marked increase in copulatory behaviors; arrestment of male locomotion immediately followed contact with the scales or models and protracted copulatory attempts ensued. Dimensional aspects of models also influenced male copulatory reactions. Both flat scales and small cylindrical models invested with scales provoked prolonged intervals of contact and copulatory behavior. However, the two larger models, which either approximated or exceeded the dimensions of females, elicited the longest durations of contact and probing and the highest arrestment indices.

The reason these larger models were more effective as releasers of copulatory behaviors may stem from the nature of the tactile stimulation they provide. Upon contact with the larger models, males generally assumed a position parallel to the long axis of the model with their head upward and probed its lower and ventral surface with their abdomen. While doing so they wedged their bodies into the narrow space between the model and the underlying substrate. Therefore, the reactions were reminiscent of those observed in normal courtships (Charlton and Cardé, 1989b). When, on the other hand, males were confronted with flat scales or small models, they were not able to establish the same degree of intimate contact and presumably did not receive the appropriate tactile feedback. Consequently, proportionately more time was spent walking over the surface of the scale field or climbing over the top of the flat scales and small model, respectively. In both situations, abdominal probing was directed randomly within the confines of the scale field or over the surface of the model.

Other studies also substantiate that the model configuration influences the expression of copulatory behaviors. Grant (1987) demonstrated that the orientation of pheromone-treated septa models affected the copulatory behavior of *C. fumiferana* males. Horizontal septa were significantly better releasers than vertical septa, because males were able to insert their head under the side of the horizontal septum only, thus providing tactile stimulation of the antennae and possibly the palps such as occurs in normal courtships. Similarly, Ono (1981) showed that copulatory reactions of three phycitid species depended on the posture of models. Models arranged flat or horizontally received no response whereas males readily attempted copulation with models whose ends were raised off the substrate. The effectiveness of the latter configuration was attributed to the ability of males to place their heads under the model; this behavior is also observed in the courtships of these phycitids (Grant and Brady, 1975). In addition, Shimizu and Tamaki (1980) assayed the copulatory responses of male *Adoxophyes orana* to models of different size to which female forewings were attached. Maximal mating values were obtained with a model that most closely approximated female dimensions; values were substantially reduced with larger or smaller models. They suggested that copulatory attempts were optimally released when males were able to align their heads with the top of the model while simultaneously establishing abdominal tip contact with the pheromone source at the opposite end of the model.

With the exception of antennae, selective ablation of various male sensory structures did not adversely affect the ability of males to perform copulatory behavior. These operations indicated that vision or hearing by themselves were not required for mate recognition. Blinded males were able to locate models as quickly as their normal counterparts and once on the model displayed similar durations of contact and probing behavior. Other studies of male walking and close-range flying orientation have demonstrated that visual cues presented by the female were not critical to either the male's choice of landing site or the efficiency with which the walking male located the female (Charlton and Cardé, 1989c). Muted males also displayed normal copulatory reactions. The use of sound as a recognition cue by gypsy moth males is unlikely because normally the only discernible movement of a 'calling' female is the slow pulsing of the abdominal tip containing the gland (Tang et al., 1987). However, Gwynne and Edwards (1986) recently reported that a male pyralid, *Syntonarchia iriastis*, stridulates using a file and scraper located on the genitalia and eighth abdominal sternite, thereby producing pulses of ultrasound that may function as a long distance mate-calling signal.

Ablation of tarsal chemoreceptors and sensilla associated with labial palps or genitalia also did not diminish the male response. The observation that male contact with models or females is principally via the tarsi, coupled with the evidence that tactile cues associated with the scales mediate copulatory behaviors, suggest that copulation-releasing stimuli are perceived through tarsal mechanoreceptors. Unfortunately, it was not possible to assay the reactions of males whose tarsal mechanoreceptors were selectively ablated because these operations prevented the males from walking. Still, perception and discrimination of scale textures to convey copulatory-releasing information remains a possibility. Such ability to detect and discriminate between textural features has been demonstrated in honeybees by Kevan and Lane (1985). They showed that honeybees use tactile cues associated with epidermal microsculptural patterns to discriminate between flower petals of different species and different ends of petals of the same species; sensilla trichodea on the tips of the antennae mediate these response.

Antennaless males failed to attempt copulation despite contacting the models numerous times. Antennectomy has also been shown to abolish the mating response of male *Trichoplusia ni* (Shorey, 1964), *Phlogophora meticulosa* (Birch, 1970), *Leucania separata* and *Mamestra brassicae* (Hirai, 1977) and *Spodoptera littoralis* (Ellis and Brimacombe, 1980). Although these results suggested that olfactory cues serve to trigger copulatory behaviors, other interpretations are possible. First, the severity of the operation may have affected the behaviors of males so drastically that other important behaviors were not expressed normally. Secondly, the fundamental importance of olfaction in male orientation behavior and the necessity of prior pheromone stimulation for evocation of copulatory behaviors renders it difficult to determine which behaviors are actually affected. In other words, if males are unable to perform certain preliminary behaviors, the copulatory response may not be expressed even though release of these behaviors depend on non-olfactory stimuli.

This is supported by other observations which demonstrate that even though perception of pheromone is necessary to 'prime' copulatory behaviors, the continued presence of pheromone is not necessary for release of copulatory behaviors. When dead, acetone-rinsed females were deployed 5 or 10 cm from a pheromone source but not in the plume, males that contacted these females displayed normal courtship and copulatory behaviors (Charlton and Cardé, 1989c). Likewise, Grant (1987) found that males *C. fumiferana* readily attempted to copulate with an untreated rubber septum model adjacent to a pheromone source but that a septum separated by only 2 cm from the source was ineffective. However, if a similarly disjunct septum was coated with female body scales, the model's ability to evoke copulatory attempts was completely restored. In addition, gypsy moth females retract their pheromone gland immediately after a male makes his initial contact so that after this point the male receives substantially no further pheromone stimulation (Charlton and Cardé, 1989b). Males will also readily copulate with freshly emerged females in the field (Cardé and Hagaman, 1984); before the wings harden, females do not call or emit detectable quantities of pheromone (Charlton and Cardé, 1982), although in the forest low ambient levels of pheromone could influence male reactions.

Males that have not had prior exposure to and stimulation by pheromone apparently will not exhibit copulatory behaviors. I have tried, without success, to elicit activation or copulatory reactions from resting males by rubbing their tarsi with female abdominal scales while they were perched on vertical screens (Charlton, unpublished). Moreover, female body odors alone are not sufficient to evoke either activation or copulatory behaviors. When quiescent males were exposed in an olfactometer tube to females whose pheromone gland was sealed with wax, none displayed an activation response. However, if the gland-occluded female was replaced with a normal calling female, all of these same males quickly activated, although none exhibited copulatory reactions.

Overall, these findings may have implications for the use of synthetic disparlure in population control strategies. Many field studies using formulated racemic disparlure as a mating disruptant have demonstrated that while mating frequencies can be greatly reduced, some mating occurs, particularly when population densities are high (Cameron, 1981; Plimmer, 1982; and references therein). The behavioral responses exhibited by males may provide at least a partial explanation for why this technique has not always proved to be an effective control strategy. Males often encounter females during the course of preprogrammed walking search maneuvers (Charlton and Cardé, 1989c; Charlton et al., 1989b). Once the female is contacted, recognition occurs and copulatory behaviors are released. Thus, even if high ambient levels of pheromone interfere with some male premating behaviors, these other behavioral responses would facilitate mating, especially in high-density populations.

The recognition cues may also find additional uses in monitoring or control schemes. For instance, applying scales around trap openings may enhance their efficiency by prolonging male exposure to the pesticide strips deployed inside many traps, thereby increasing the usefulness of traps for survey and possibly trap out strategies. Moreover, since males remain on models for extended periods when female scales are present, use of scales (or scale-like material) on attracticide lures may bolster their effectiveness or may enhance the likelihood that males could pick up and transfer microbial control agents.

Table 8. Male *L. dispar* copulatory and arrestment responses to a pheromone source alone, and in combination with female abdominal scales applied to a flat surface or models of different dimensions.

TREATMENT[1]	Total Contact Duration (sec)	Total Probing Duration (sec)	Probe/ Contact Duration	Arrestment Index[2]	No. of Excursions[3]
Pheromone Alone	2.4^{a} (2.2)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.29^{a} (0.11)	16.8^{a} (9.1)
Flat Abdominal Scales	98.0^{b} (29.4)	77.0^{b} (27.8)	0.78^{b} (0.09)	0.69^{b} (0.08)	7.7^{b} (4.0)
Small Model (5 mm x 30 mm)	93.3^{b} (41.6)	75.1^{b} (34.3)	0.80^{b} (0.08)	0.70^{b} (0.23)	7.5^{b} (3.9)
Normal Model (10 mm x 30 mm)	140.0^{c} (28.0)	130.5^{c} (29.7)	0.93^{c} (0.06)	0.84^{c} (0.13)	3.6^{c} (2.2)
Large Model (20 mm x 30 mm)	140.6^{c} (23.9)	132.9^{c} (22.8)	0.95^{c} (0.03)	0.84^{c} (0.08)	3.8^{c} (2.4)

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[1] Pheromone source for all treatments was 100 ng (+)-disparlure on filter paper. Virgin female gypsy moth abdominal scales were applied to clay models of various dimensions or flat onto a 10 mm x 30 mm area. Twenty males tested to each treatment.

[2] Arrestment Index defined as the proportion of time a male spent inside the 'arrestment zone' (a 5 cm diam. circular area with the treatment at its center) of the total time the male walked on the cylinder following initial entry into the zone.

[3] Number of excursions from the 'arrestment zone' following initial entry into zone.

Means (± SD) in the same column followed by different letters are significantly different ($P<0.05$) according to Student-Newman-Keuls' multiple range test, following log transformation.

Table 9. Copulatory response of male *L. dispar* to solvent extracts of female forewing and abdominal scales applied to models.

TREATMENT	N	% Males Displaying Probing Response	Total Contact Duration (sec)	Total Probing Duration (sec)
Control	20	50^{a}	23.5^{a} (9.2)	11.7^{a} (11.1)
Forewing Scale Extract	20	75^{ab}	26.3^{a} (6.2)	14.1^{a} (6.2)
Abdominal Scale Extract	20	90^{b}	28.1^{a} (7.0)	14.3^{a} (9.1)
Abdominal Scales	20	100^{b}	104.5^{b} (29.7)	95.1^{b} (28.0)

All models supplemented with 100 ng (+)-disparlure. Means (± SD) with no letters in common are significantly different ($P<0.05$) according to Ryan's multiple comparison test for proportions and Student-Newman-Keuls' multiple range test.

Figure 21. Male *L. dispar* copulatory response to clay models incorporating various female structural features. CON: bare control; FWS: forewings; FWAS: forewings and abdominal scales; AS: abdominal scales; FEM: normal females with wax-occluded genitalia and pheromone gland. All treatments were supplemented with 100 ng (+)-disparlure. Means (± SD) with no letters (contact) or numbers (probing) in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test. $N = 20$ males for each treatment.

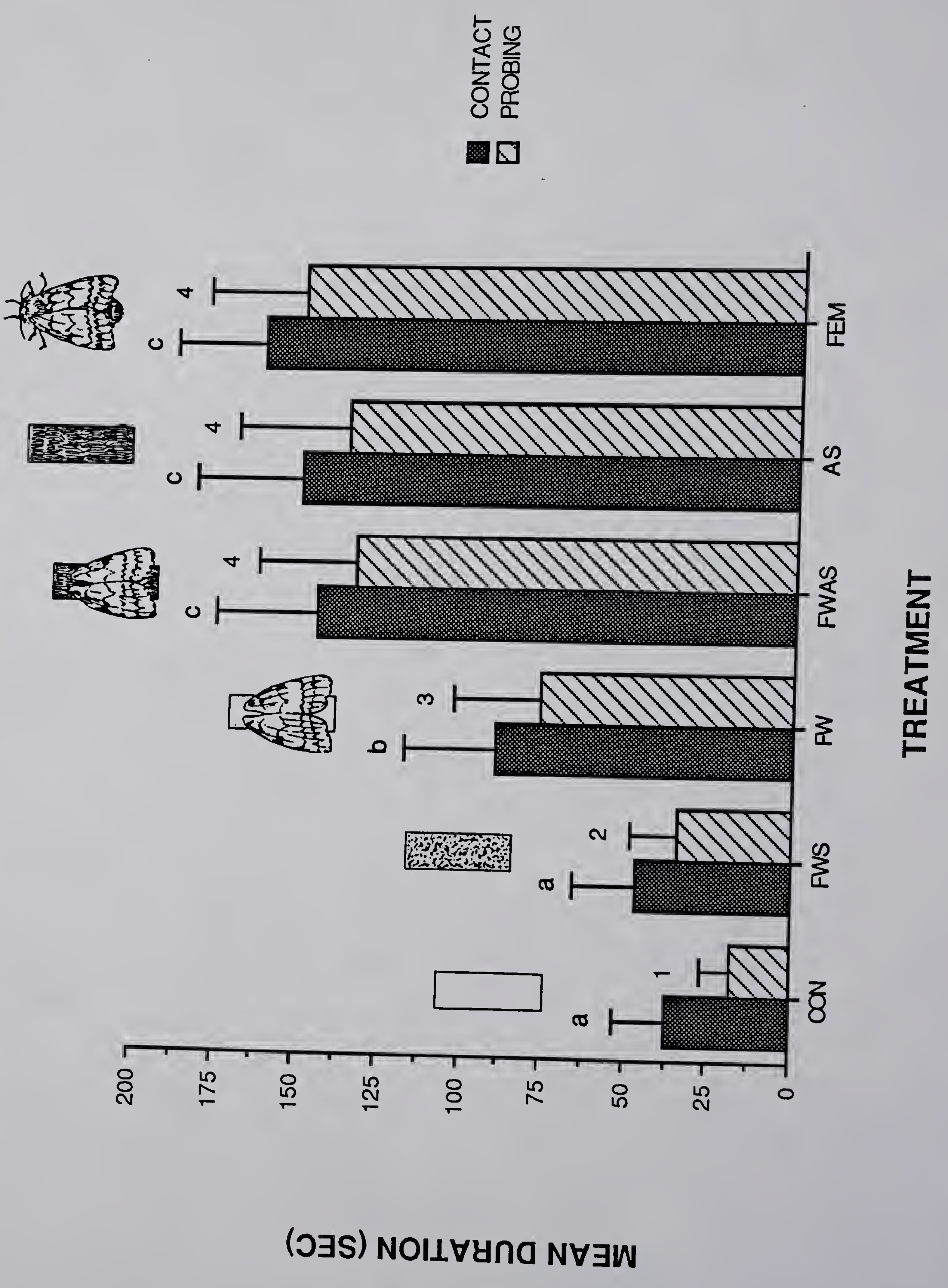

MEAN DURATION (SEC)
0
25
50
75
100
125
150
175
200
TREATMENT
CON
FWS
FW
FWAS
AS
FEM
a
1
a
2
b
3
c
4
c
4
c
4
■ CONTACT
▨ PROBING

Figure 22. Male *L. dispar* attempting to copulate with a clay model coated with female abdominal scales and supplemented with 100 ng (+)-disparlure on filter paper.

Figure 23. Male *L. dispar* directing copulatory attempt toward a flat substrate covered with female abdominal scales and supplemented with 100 ng (+)-disparlure on filter paper.

Figure 24. Effect of chemical and structural alteration of female abdominal scales on male *L. dispar* copulatory responses. Scales were applied to models combined with 100 ng (+)-disparlure. Means (± SD) with no letters (contact) or numbers (probing) in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test.

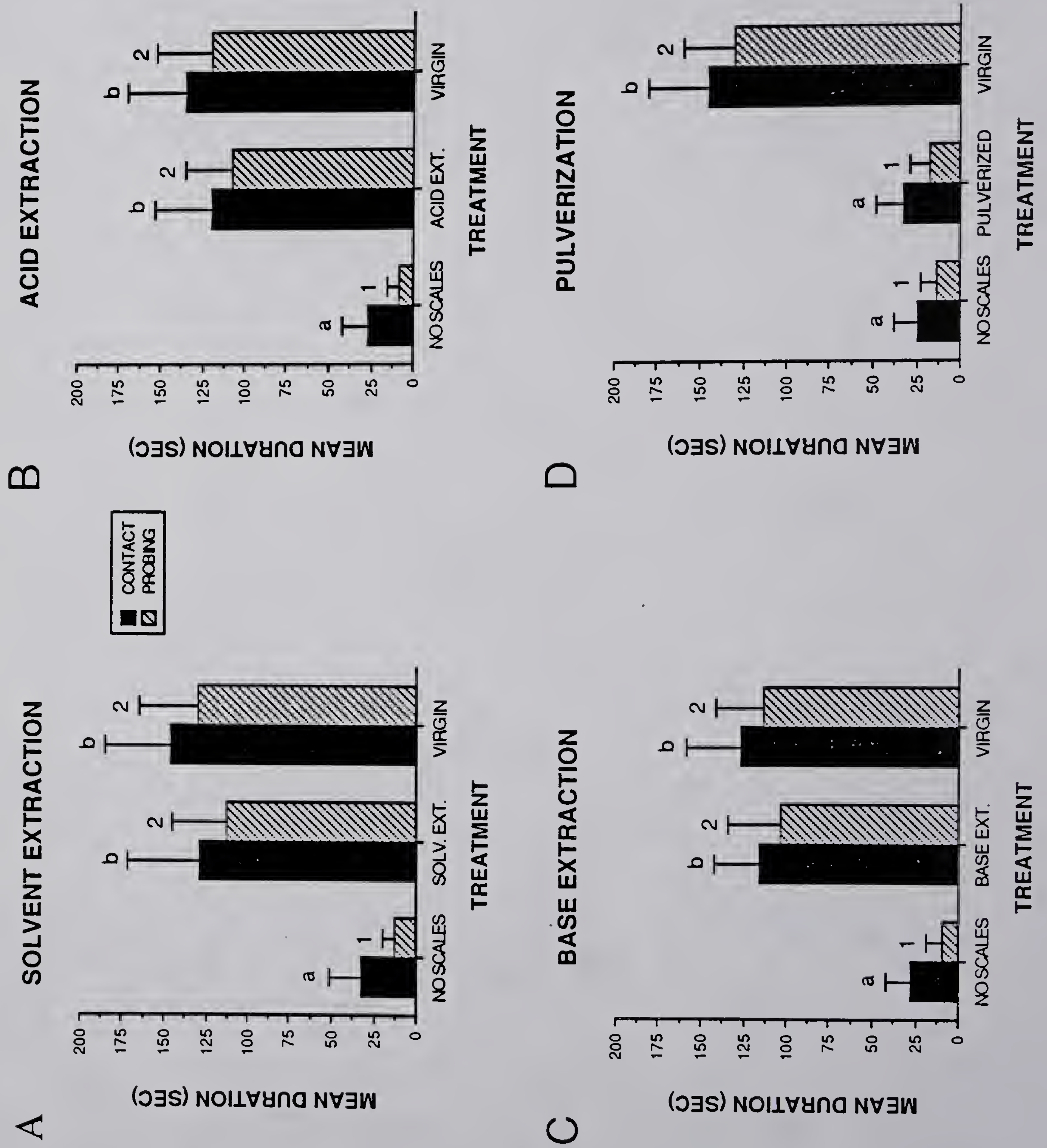
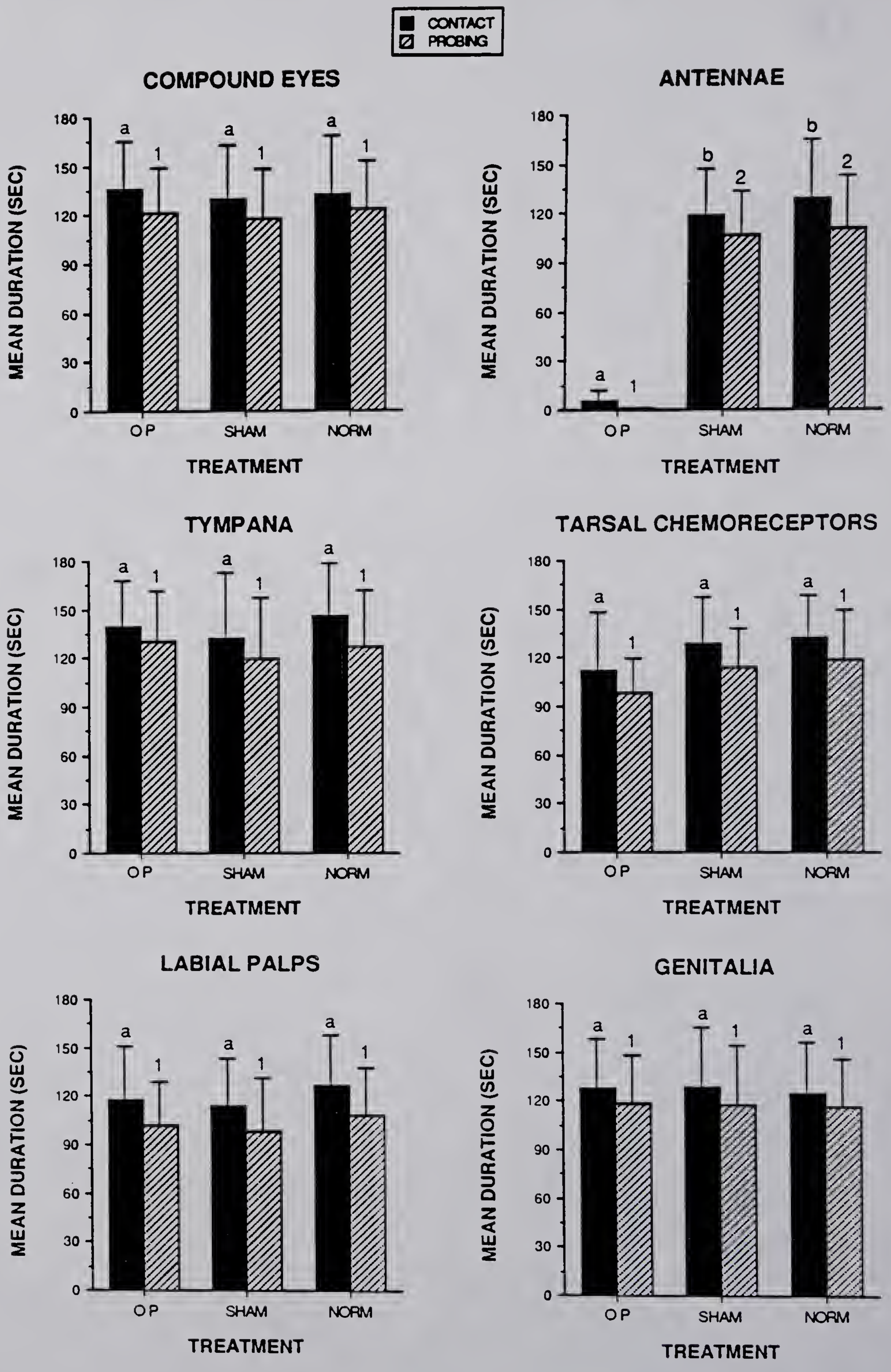

A
SOLVENT EXTRACTION
MEAN DURATION (SEC)
0
25
50
75
100
125
150
175
200
NO SCALES
SOLV. EXT.
VIRGIN
TREATMENT
a
1
b
2
b
2
CONTACT
PROBING
B
ACID EXTRACTION
MEAN DURATION (SEC)
NO SCALES
ACID EXT.
VIRGIN
TREATMENT
a
1
b
2
b
2
C
BASE EXTRACTION
MEAN DURATION (SEC)
NO SCALES
BASE EXT.
VIRGIN
TREATMENT
a
1
b
2
b
2
D
PULVERIZATION
MEAN DURATION (SEC)
NO SCALES
PULVERIZED
VIRGIN
TREATMENT
a
1
a
1
b
2

Figure 25. Effect of ablation of various male *L. dispar* sensory structures on copulatory responses to models coated with female abdominal scales. See text for details of operations. Means with no letters (contact) or numbers (probing) in common are significantly different ($P < 0.05$) according to Student-Newman-Keuls' multiple range test.



■ CONTACT
▨ PROBING
COMPOUND EYES
ANTENNAE
TYMPANA
TARSAL CHEMORECEPTORS
LABIAL PALPS
GENITALIA
MEAN DURATION (SEC)
0
30
60
90
120
150
180
O P
SHAM
NORM
TREATMENT
a
1
b
2

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