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Electroantennographic Resolution of Pulsed Pheromone Plumes in Two Species of Moths with Bipectinate Antennae

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Abstract

Trains of 20-ms-duration pulses of pheromone were delivered at rates of 1–33 Hz to antennal preparations of males of *Bombyx mori* and *Lymantria dispar*, two moth species with bipectinate antennae. Resolution of rapidly pulsed plumes of pheromone was not compromised by a complex antennal morphology or by moderate changes in wind speed (25–50 cm/s). Fourier analysis of the electroantennograms resolved the temporal structure of the signal at frequencies up to 25 Hz for *B. mori* and up to 5 Hz for *L. dispar*. The ability of these sensory structures to identify the original (unchanged) frequency of the pulse train is particularly noteworthy because air is slowed by about an order of magnitude as it passes through bipectinate antennae. Although an unchanging frequency in slowed airflow may be counterintuitive, this flow pattern, and its effects on odorant patch shape and spacing, is explained from fluid mechanical principles (i.e., the principle of continuity). An unchanging frequency suggests that as decelerating air passes through a bipectinate antenna, the slowed patches of odorant are stretched, thinned, and brought closer together by the same factor with which they are slowed.

Key words: electroantennogram, gypsy moth *Lymantria dispar*, pheromone plume, silkworm *Bombyx mori*, temporal resolution

Introduction

An insect flying in a pheromone plume generated from a point source encounters the odor as a series of bursts of variable concentration and spacing because of the plume's sinuous and filamentous structure (Murlis and Jones, 1981; Murlis *et al.*, 2000). Baker proposed that in flying moths, upwind progress is modulated by moment-to-moment encounter with the odor strands that make up the plume (Baker, 1990). This is supported by behavioral evidence showing that male moths briefly surge upwind after interception of a pheromone filament (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994) and revert to casting flight if no more filaments are encountered (Mafra-Neto and Cardé, 1996; Quero *et al.*, 2001). This mechanism is also supported by the finding that some species require fluctuating pheromone stimulation in order to maintain upwind flight, arresting their upwind progress in homogeneous clouds of pheromone (Kennedy *et al.*, 1981; Willis and Baker, 1984; Baker *et al.*, 1985). The ability of the peripheral and central nervous system to resolve rapid changes in pheromone concentration would be crucial to maintain upwind flight at high rates of filament interception. If filament encounter rate exceeds the temporal resolution abilities of the insect's neural circuitry, it is likely that the flickering signal would be perceived as a continuous or "fused" signal. However, males of the pyralid *Cadra cautella* are capable of upwind flight in homogeneous clouds of pheromone (Justus and Cardé, 2002) and along plumes pulsed at rates as high as 25 Hz (Justus *et al.*, 2002b).

Resolution of rapidly pulsed pheromone plumes has been reported at the antennal level at rates up to 33 Hz in *Spodoptera exigua* and *C. cautella* and up to 25 Hz in *Pectinophora gossypiella* (Bau *et al.*, 2002). Although it is not known if such high frequencies are preserved beyond the peripheral receptor system, some projection neurons (PNs) from the macroglomerular complex of *Manduca sexta* males discriminate both duration and interpulse intervals of pheromone arriving at the antenna at approximately 10 Hz (Christensen and Hildebrand, 1997). Furthermore, Vickers *et al.* (2001) have shown that the activity of at least some olfactory PNs of *Heliothis virescens* is time locked to the stimulus dynamics. It is possible, therefore, that high-frequency fluctuations of

pheromone concentration can be resolved at more central processing levels, but that ability is dependent on the resolution of the stimulus at its interface with the sensory apparatus.

The shape of an antenna has a strong effect on the flow dynamics through the arrays of odor-sensitive sensilla arranged on its surface. Vogel (1983) found that at wind speeds of 0.3 m/s, most of the airflow is diverted around the feathery antennae of the luna moth *Actias luna*, and only approximately 8% actually passes through its branches. At comparable airflow speeds, a similarly small fraction is expected to pass through the branches of *Bombyx mori* antennae (Loudon and Koehl, 2000). The slowing of airflow that results from this fractional processing might be expected to lead to a decrease in the rate of odor filament interception by a pectinate antenna. We investigated the response of bipectinate antennae to pulsed sex pheromone using two species of moths, *Lymantria dispar* and *B. mori*, that possess similarly plumose antennae, to determine their ability to resolve odor pulses in different wind speeds and at different orientations with respect to wind direction.

Materials and methods

Insects

Irradiated *L. dispar* males were obtained from the United States Department of Agriculture/Animal and Plant Health Inspection Service Otis Plant Protection Center. *Bombyx mori* larvae were raised on a mulberry leaf diet and sexed as pupae. Male pupae of both species were placed in cups singly or in groups of 15 in cylindrical cardboard containers 17 by 17 cm diameter and kept in growth chambers at 24°C until emergence.

Wind tunnel

Experiments were conducted in a wind tunnel constructed of a Plexiglas floor and 3-m-long by 1-m-wide Vivac sheets that were bent into a semicylindrical shape (see Justus *et al.*, 2002a,b). Except during wind-speed experiments, wind speed was set at 50 cm/s.

Electroantennogram setup

The electroantennogram (EAG) probe (PRG-2, Syntech, Hilversum, The Netherlands) was mounted on a stand inside the wind tunnel, 10 cm downwind from the dispenser pipette in all experiments, except where stated, in which *L. dispar* antennae were mounted 3 cm downwind of the dispenser pipette. No mechanoreceptor response to pulse trains of clean air was detected with antennae of *B. mori* and *L. dispar* at 10 cm or *L. dispar* at 3 cm from the pipette (no periodicity was evident). To reduce electrical interference, a layer of aluminum foil covered the inner surface of the wind tunnel 50 cm upwind and downwind of the EAG probe, and the probe stand was connected to this by wire. Pulsed trains of $TiCl_4$ "smoke" were used to visualize the plume's boundaries before positioning each antenna.

Antennae were excised with a razor blade from 2- to 3-day-old male moths. The most distal segments of the antenna were removed, and the antenna was mounted on the EAG probe using electrode gel (Spectra 360, Parker Laboratories Inc., Fairfield, NJ) with the ventral side of the antenna facing upwind. Single-branch preparations were made by excising a single lateral branch from the central part of the antenna (i.e., approximately equidistant from the base and the tip) with spring microscissors and mounting these on the probe with electrode gel. EAG signals were acquired at 10,582 samples/s through a PC interface board (IDAC-02, Syntech) and Autospike32 (Syntech) software. Low pass filters were DC coupled, and high pass filters were set at 3000 Hz.

Pheromone

Synthetic sex pheromones of *L. dispar*, (+)-disparlure [*cis*-7,9-epoxy-2-methyloctadecane], and *B. mori*, bombykol [(*E,Z*)-10,12-hexadecadien-1-ol], were formulated gravimetrically in hexane and serially diluted to obtain concentrations of 1000, 100, 10, and 1 ng/μl of (+)-disparlure and 100 and 10 ng/μl of bombykol. Purities were 96 and 99%, respectively.

The olefin precursor of (+)-disparlure, 2-methyl-*cis*-7-octadecene (Jurenka *et al.*, 2003), was obtained from Farchan (Willoughby, OH). Purity was 91%, and it was free of disparlure. It was formulated gravimetrically in hexane and diluted to 100 ng/μl. Olefin, although present in the female's pheromone gland, is a behavioral antagonist of attraction (Cardé *et al.*, 1973).

Odor-delivery system

Using a Stimulus Flow Controller (SFC-2, Syntech), sequences of 20-ms-duration pulses were delivered using an airflow of 2.5 ml/s from a Pasteur pipette containing a 7.5-mm-diameter filter paper disk impregnated with the pheromone solution. To minimize adsorption of pheromone to the inner surface of the pipette, the filter paper disk was positioned as close as possible to the pipette outlet, at a distance of approximately 1.5 cm. The tip of the pipette was bent at ~135° angle and, in pulsing and wind-speed experiments, the aperture faced upstream. This arrangement produced discrete puffs of pheromone even at high pulsing frequencies (Justus *et al.*, 2002b). Each puff was approximately 1.5 cm in diameter (large enough to engulf the antenna) at 10 cm upwind and had very low intensity internal fluctuations in concentration (Justus *et al.*, 2002a). In all other experiments, the aperture of the pipette faced downstream. In all cases, the pipette outlet was positioned 40 cm from the upwind end of the wind tunnel and 30 cm above its floor.

Experiments

Pulse trains

Pulsed stimulations of 1, 2, 5, 10, 13, 17, 25, and 33 Hz were recorded for approximately 5 s, with 30 s between recordings

of different pulsing frequencies. In all cases, each pulse was of 20-ms duration. For each species, we completed 10 replicates of antennal recordings of these frequencies in ascending order with a dose of 1 μg of the respective pheromone.

Single pulses

Single pulses were delivered to whole antennae and single branches (excised from the unused antenna of the same insect) for both species. Ten replicates were made for each species using 1 μg of the respective pheromone.

Dose response

Filter paper disks were impregnated with doses of (+)-disparlure and presented in increasing order from 10 ng to 10 μg in decade steps. The dispensing pipette was 3 cm upwind of the antenna. Stimuli were pulsed at 1, 2, 5, or 10 Hz over *L. dispar* whole antennae (from each of five insects) and single branches (excised from the unused antenna of the same insect).

Olefin

Olefin (1 μg) was applied to a filter paper disk, and pulses were delivered at 1, 2, 5, 10, 13, and 17 Hz to male *L. dispar* antennae. The dispensing pipette was 3 cm upwind of the antenna. Six replicates were made.

Antennal orientation—L. dispar

The effect of the position of the antenna relative to wind direction was tested on five antennae of *L. dispar* males presented with 100 ng of (+)-disparlure. These antennae are bipectinate, with two sets of primary branches protruding from a central stalk, which gives the antenna a concave "basket" shape. The olfactory sensilla are present only in the ventral part of the primary branches, similar to *B. mori* (Schneider *et al.*, 1977 and Figure 1). In experiments, antennae were positioned with their ventral side facing upwind (0°), the floor of the tunnel (90°), and downwind (180°) (Figure 1). Antennal angles of male *L. dispar* in flight generally are between 0° and 30° to the flow during flight, with the concavity always facing upstream (determined from detailed examination of the original photos of gypsy moth flights, reported in Baker and Cardé, 1978). However, male moth flight is rarely due upwind, and antennae may intercept filaments from an oblique angle. Here, we use three orientations to shed light on how the overall shape of the antenna affects the ability of the antenna to characterize an odor pulse. The order in which these positions were tested was randomized among five replicates.

Antennal orientation—B. mori

Three *B. mori* male adults were videotaped from an anterior direction as they initiated wing fanning, using a Panasonic WV-CL700 camera mounted on a Nikon Stemi SV6 dissecting microscope (SVHS format, 30 frames/s). Wing fanning by a male occurred in response to an adult *B. mori* female brought into his vicinity. The males were tethered ventrally (glued) to keep them from leaving the field of view of the camera.

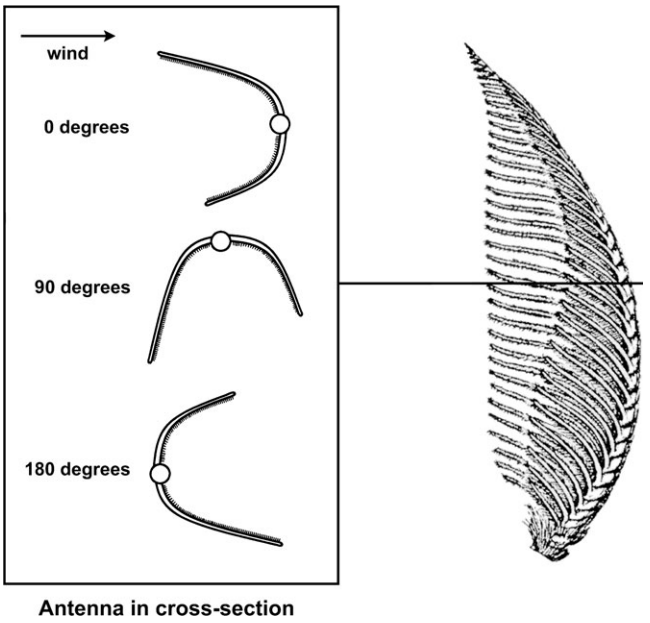

Figure 1 Position of the antenna in relation to wind direction. Drawing of a *Bombyx mori* antenna (right) reproduced with permission (Loudon and Koehl, 2000).

Wind speed

Single pulses and trains of pulses at frequencies of 2, 5, 10, 13, 17, 25, and 33 Hz of bombykol (100 ng) were delivered to each of five *B. mori* male antennae at wind speeds of 25 and 50 cm/s.

Data analysis

In-house computer software was used to analyze EAG signals. The following parameters were calculated as described in Bau *et al.* (2002): depolarization amplitude, depolarization time, repolarization time to two-thirds peak amplitude, and percent return to baseline. Percent return to baseline was calculated for each EAG peak by dividing the value of the most repolarized point following a depolarization by the value of the preceding depolarization and then subtracting this number from 1 (repolarized and depolarized points expressed relative to the baseline). Periodicity (Fourier analysis) was also calculated for pulsing regime and wind-speed experiments (see Bau *et al.*, 2002).

Results

Pulsed stimulus experiments with *L. dispar* and *B. mori*

Electroantennographic responses to trains of pheromone pulses delivered at frequencies of 1, 2, 5, 10, 13, 17, 25,

and 33 Hz were obtained for both *B. mori* and *L. dispar* (Figure 2; recording at 33 Hz not shown). Fourier analysis of EAG recordings showed a match between the response and delivery frequencies for both species, although there were clear differences between these two species. Pulsed stimulations up to 25 Hz were resolved in *B. mori*, but frequencies above 5 Hz were not resolved in *L. dispar* (Figure 3). Although pulses were discrete (Justus *et al.*, 2002b), EAG peaks did not fully return to baseline between stimulations. Antennae of *B. mori* returned almost fully to baseline between pulses at 1 Hz, but the percent return to baseline decreased at 2 Hz and dropped to approximately 50% at 5 Hz (Figure 4). *Lymantria dispar* had a much lower recovery than *B. mori* at all frequencies; even at 1 Hz, percent return to baseline was below 30% (Figure 4).

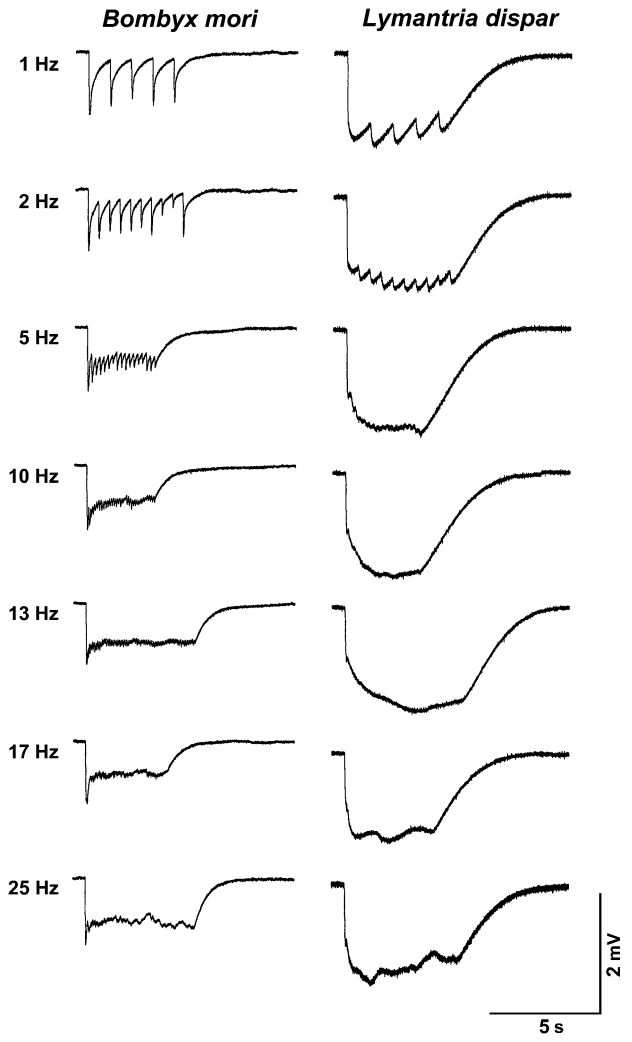

Figure 2 EAG recordings from antennae of *Bombyx mori* and *Lymantria dispar* male antennae placed 10 cm downwind from the odor source in response to 20-ms pulses of pheromone (1 μg) delivered at different rates (1, 2, 5, 10, 13, 17, and 25 pulses/s).

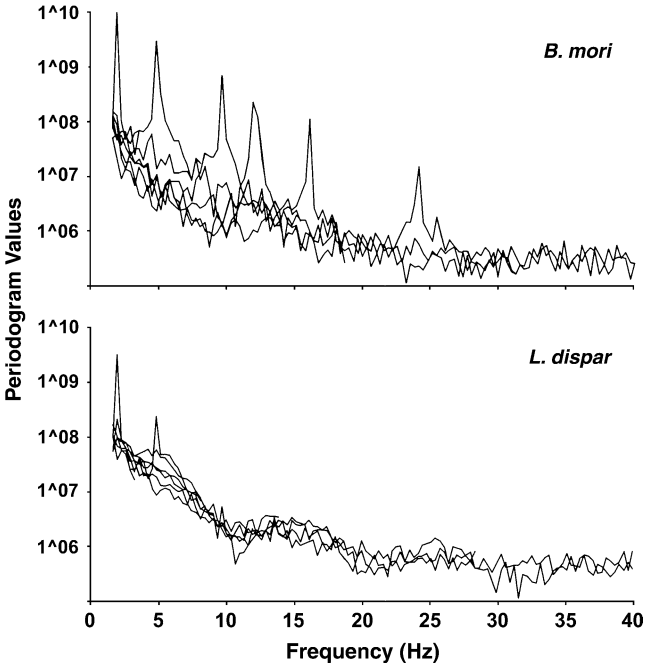

Figure 3 Periodogram plots (without harmonics) after Fourier analysis of EAG recordings on *Bombyx mori* and *Lymantria dispar*. Stimulus was mechanically pulsed at frequencies of 2, 5, 10, 13, 17, 25, and 33 Hz. Stimuli consisted of 1 μg of the respective pheromone, and the duration of every pulse was 20 ms. Each line represents analysis of 10 replicates of recordings of 3-s duration, starting 0.8 s after the initial depolarization. Lower frequency lines do not extend to the end of the plot to avoid overlap of harmonics of lower frequency settings with main peaks of higher frequency settings.

Single-pulse experiments with *L. dispar* and *B. mori*

EAG response to single pulses was recorded for both species on whole antennae and single branches (Figure 5). Depolarization and repolarization times at two-thirds peak amplitude were significantly longer in *L. dispar* than in *B. mori* for peaks of the same amplitude. This was true for recordings

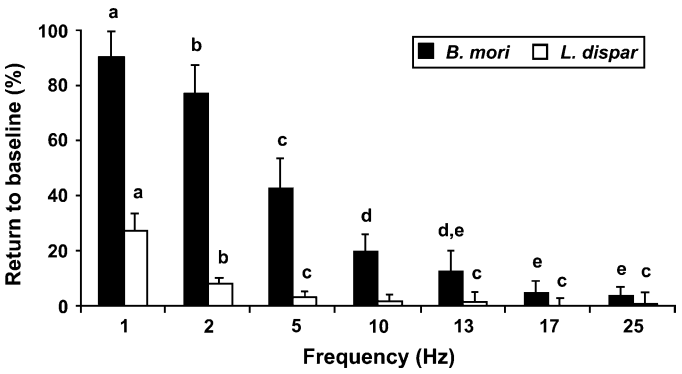

Figure 4 Return to baseline (percent + SD) of the EAG signal between pulses, at frequencies of 1–25 Hz. Different letters denote significant differences among frequencies within each species [ANOVA and Tukey honest significant difference (HSD) post hoc test; $\alpha = 0.05$, $P < 0.05$]. Significant differences were found between species at all pulsing frequencies except at 25 Hz. Ten replicates were made for each species.

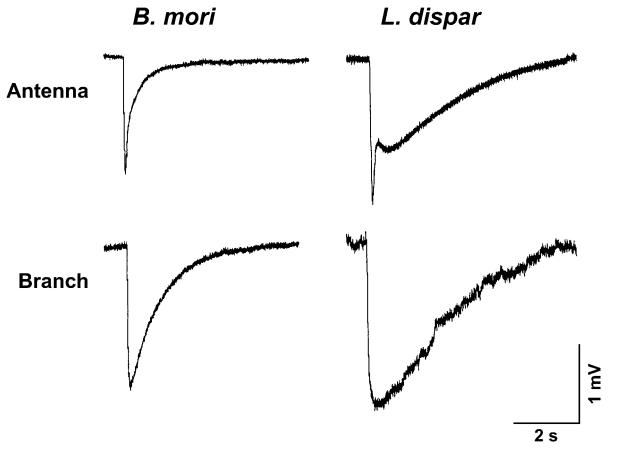

Figure 5 EAG recordings from whole antennae and single branches of *Bombyx mori* and *Lymantria dispar* male antennae placed 10 cm downwind from the odor source in response to a single 20-ms pulse of pheromone (1 μg). Note the unusual shape of the *L. dispar* whole antenna EAG. These fast recovery peaks were evident in most *L. dispar* recordings, but only in the EAG response to the first pulse (see text).

obtained from both whole antennae and single branches (Figure 6). Compared to the whole antenna, single-branch recordings showed even longer depolarization and repolarization times.

Dose-response experiments with *L. dispar*

Percent of return to baseline at frequencies of 1 and 2 Hz was significantly greater when the lowest concentration of pheromone (10 ng) was used (Figure 7). Shorter depolarization and repolarization times were also observed with lower doses, probably as a consequence of the smaller peak amplitude. Lower concentrations of pheromone were not tested because the response to 10 ng of (+)-disparlure was already very close to the sensitivity limitations of our system (peak amplitude at this dose was −0.64 mV, ±0.2 SD). However, even at the lowest dose, percent return to baseline at 1 Hz was still below 60%.

Olefin experiments with *L. dispar*

The shape of EAG peaks produced by male *L. dispar* antennae was similar to both olefin and pheromone. However, the sensitivity to olefin was much lower than to (+)-disparlure: 50 ng of pheromone elicited peaks of similar amplitude and percent of recovery values to those obtained with 1000 ng of olefin.

Antennal orientation experiments with *L. dispar*

Antennal position experiments with *L. dispar* showed significant reductions in both peak amplitude and combined depolarization and repolarization times (D + R) when the ventral side of the antenna was not facing upwind

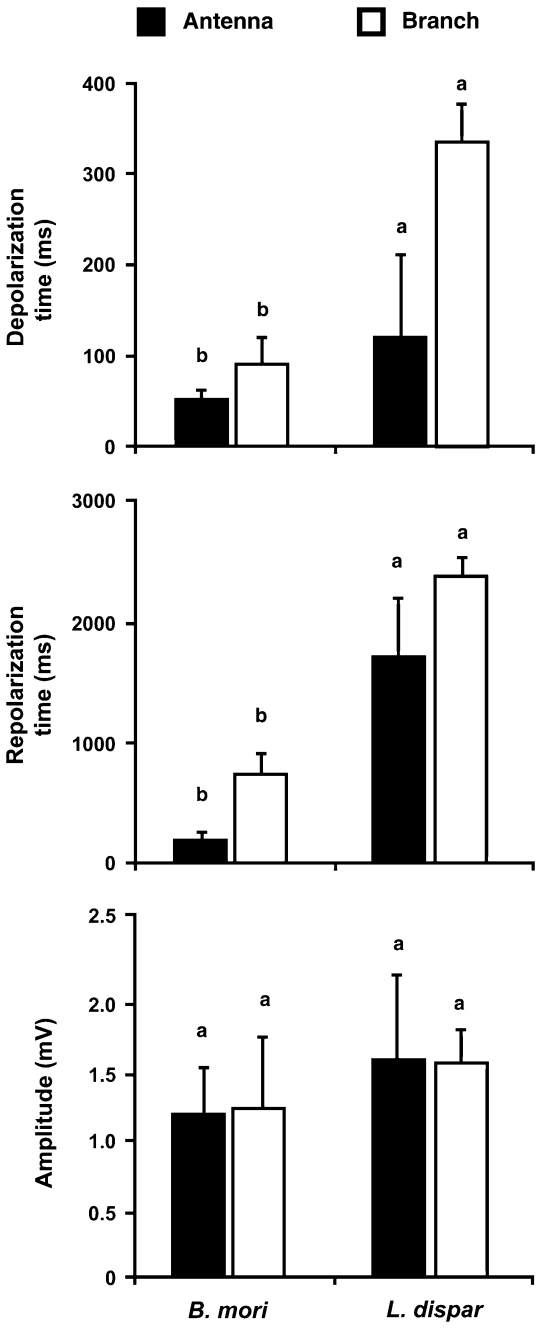

Figure 6 Mean peak characteristics (+SD) calculated from EAG recordings of whole antennae and single branches of *Bombyx mori* and *Lymantria dispar* (10 replicates) with 1 μg of the respective pheromone. Repolarization time was calculated from the highest depolarization value to two-thirds of the peak amplitude. Different lower case letters denote significant differences between species for the same treatment (*t*-test for independent samples, $P < 0.05$).

(0°). No differences were found between antennae turned 90° and 180° (Figure 8).

Antennal orientation measurements with *B. mori*

Although stationary male moths vary the orientation of their antennae, in the absence of sex pheromone the antennae are

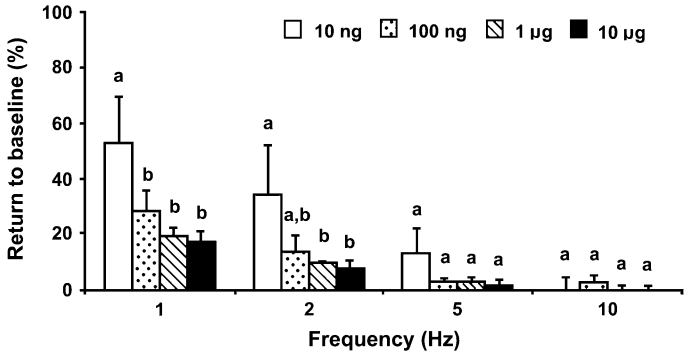

Figure 7 Return to baseline (percent + SD) of the EAG signal between pulses, at frequencies of 1–10 Hz with doses between 10 ng and 10 µg of (+)-disparlure. Different lower case letters denote significant differences among doses within each pulsing frequency (ANOVA and Tukey HSD post hoc Test; $\alpha = 0.05$, $P < 0.05$). Five male *Lymantria dispar* antennae were tested for each dose.

typically held as shown in Figure 9 (left). On stimulation with sex pheromone (identified by the onset of wing fanning), all three males rotated their antennae such that the antennae were facing more directly upwind (corresponding to the 0° treatment; Figure 9, right). This rotation occurred within 1 s for one male and was coincident (within 1/30 s) for the other two males.

Wind-speed experiments with *B. mori*

At a wind speed of 50 cm/s, *B. mori* antennae resolved pulsed stimulations at frequencies up to 25 Hz. However, with a wind speed of 25 cm/s, resolution was not observed above 17 Hz (Figure 10). No significant differences in percent return to baseline were found at any pulsing rate between these two wind speeds (data not shown). Although no differences were found in the depolarization or repolarization times, EAG amplitudes were significantly smaller (about half) at 25 cm/s than at 50 cm/s.

Discussion

A male moth flying upwind along a pheromone plume emitted by a point source will encounter pheromone as bursts of variable concentration and spacing (Murlis and Jones, 1981; Murlis *et al.*, 2000). Such an intermittent signal is required by males of some species of moths to maintain their upwind flight toward a pheromone source (Kennedy *et al.*, 1981; Willis and Baker, 1984; Baker *et al.*, 1985). Therefore, the assessment of their sensory systems' ability to resolve a rapidly flickering pheromone plume is of importance in understanding the orientation maneuvers of male moths to the fine-scale features of the plume. Of particular interest is how the shape of pectinate antennae affects the resolution of rapidly pulsed filaments of pheromone.

Vogel (1983) demonstrated that the feathery antennae of male luna moths, *A. luna*, inhibit the flow of air, such that

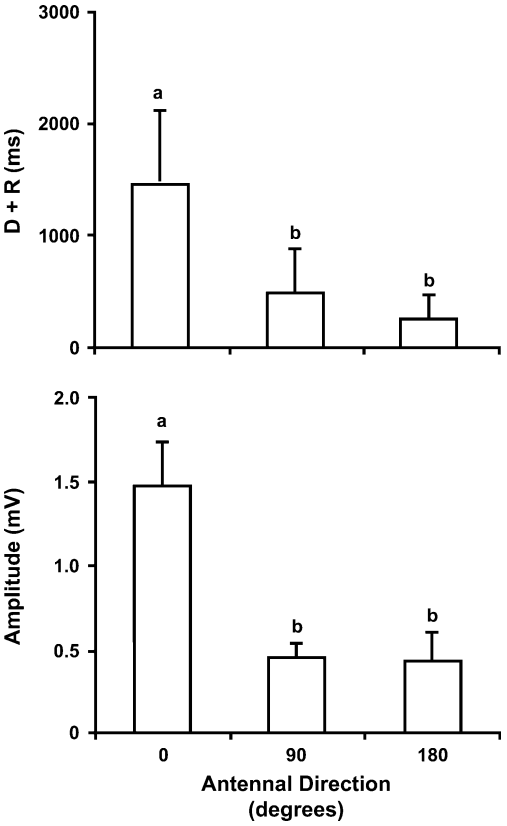

Figure 8 Mean peak characteristics (+SD) calculated from EAG recordings of *Lymantria dispar* with different antennal orientations with respect to wind direction in response to a 20-ms pulse of pheromone (100 ng). Five replicates were made with the ventral part of the antennae facing completely upwind (0°) and 90° and 180° from that position. D + R is the total time of depolarization and repolarization to two-thirds peak amplitude. Different lower case letters denote significant differences among antennal orientations (ANOVA and Tukey HSD post hoc test; $\alpha = 0.05$, $P < 0.05$).

only approximately 8% flows through its branches (at wind speeds of 0.3 m/s), and most of the air approaching the antennae is diverted around it. This "transmissivity" increases with free stream speed, because the effects of viscosity are diminished, but transmissivity appears to remain well below 20%. Similar results were found for *B. mori* (Loudon and

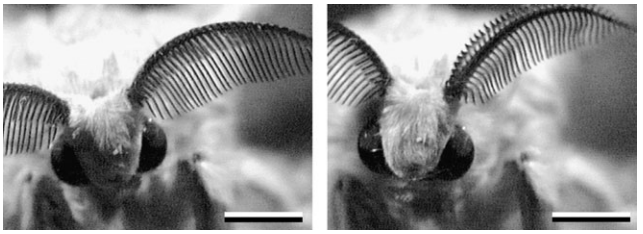

Figure 9 A *Bombyx mori* male rotates his antennae so that the concave ventral surface is facing upwind (in an anterior direction) on stimulation with female sex pheromone. Left: the video frame immediately prior to the onset of wing fanning ($t = -0.03$ s, if $t = 0.00$ s at wing fanning). Right: the same moth at $t = 1.47$ s. The scale bar is 2 mm long.

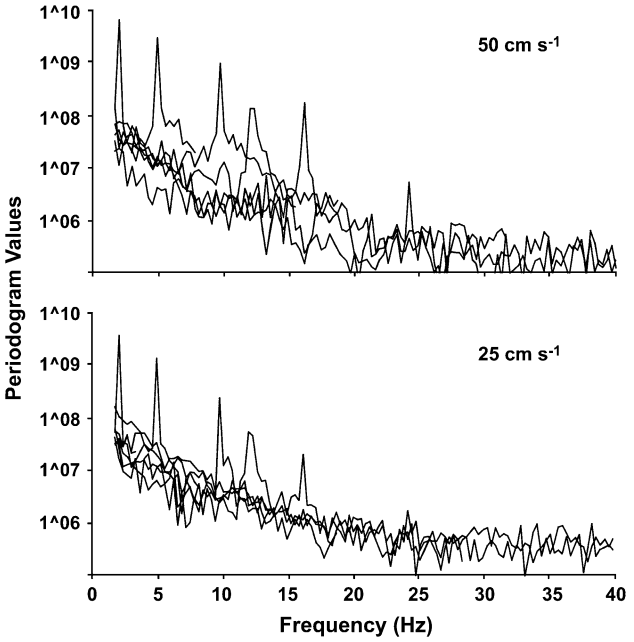

Figure 10 Combined periodogram plots after Fourier analysis of EAG recordings on *Bombyx mori* at wind speeds of 25 and 50 cm/s. Stimulus was mechanically pulsed at frequencies of 2, 5, 10, 13, 17, 25, and 33 Hz. Stimulus consisted of 1 μg of bombykol, and the duration of every pulse was 20 ms. Results are based on five replicates of recordings of 3-s duration, starting 0.8 s after the initial depolarization.

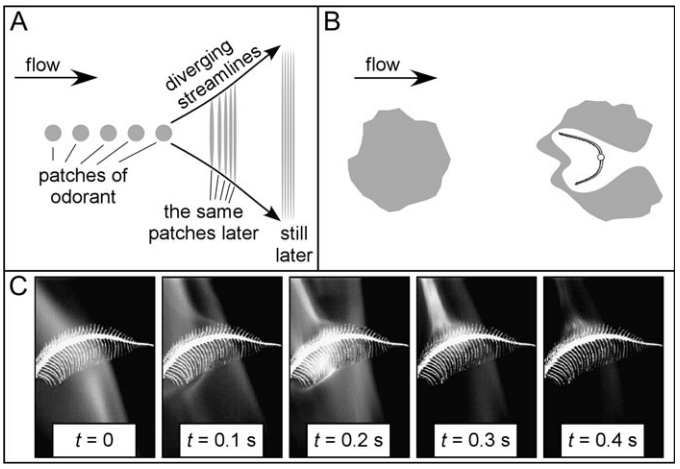

Figure 11 When flowing air slows, the streamlines diverge. **(A)** This divergence will cause any patches of odorant (shown here initially as circles) to be distorted in their shape, such that they become thinner and spaced more closely in the direction parallel to airflow. **(B)** The diverging streamlines upstream of a pectinate antenna will similarly cause elongation and closer spacing of odorant patches. In addition, the airflow pattern generated by a concave-upstream shape could cause overall bending of the odorant patches as shown. **(C)** The slowing of air as it passes through a male *Bombyx mori* antenna is made visible by marking flowing air with smoke. The view is of the downstream (convex) surface of the antenna, and the direction of flow is toward the reader and slightly to the left and top. A thin ribbon-like plume approaches the antenna ($t = 0$), the front (upstream) edge of the plume wraps around the antenna ($t = 0.1$ s), and the part of the plume that entered the concave air space enclosed by the antenna exits between the branches after the rest of the plume has passed around the outside of the antenna ($t = 0.3$ and 0.4 s). A video of smoke flowing through the antenna is posted as supplementary data (www.chemse.oxfordjournals.org).

Zhang, 1998). With only a small fraction of the approaching air passing through an antenna, the flowing air will slow down and the streamlines must diverge (principle of continuity), causing distortion of any odorant patches present in that flowing air (Figure 11A; Loudon and Davis, 2005). As the odorant patches (and the odorant-free air between them) are stretched in a plane perpendicular to the airflow by the diverging streamlines, the odorant patches will become thinner because their volume will remain approximately constant (a reasonable approximation over short time intervals and in the absence of large pressure changes). The streamlines should diverge directly upstream of a pectinate antenna (Figure 11B; Loudon and Davis, 2005). The parcel of air that enters the basket-shaped concavity of the antenna is slowed and exits between the branches later than the adjacent air that flows around the outside of the antenna (Figure 11C). Even though the flowing air is slowed by about an order of magnitude as it passes through a pectinate antenna, this could result in an unchanged perception of patch interception rate because the slower flow will contain thinner, more closely spaced patches (e.g., halving the airflow rate will also halve the thickness of the patches and the distance between patches measured in the direction of flow). Therefore, an unchanging perception of filament frequency is expected for antennae generating air distortion that is primarily two dimensional as shown in Figure 11 (the plane of distortion may be curved), although vortices or other flow patterns that cause mixing of the air could modify or obliterate any patchiness of the sampled air.

Fourier analysis of EAG recordings showed that resolution of a flickering signal was possible even at frequencies as high as 25 Hz for *B. mori* antennae and up to 5 Hz for *L. dispar* antennae (Figure 3). The ability of the pectinate antennae of these two moth species to resolve pulsed pheromone plumes, particularly at unchanged frequencies, suggests that the flow pattern of the air passing through the antennae is laminar and that the odorant plume is stretched primarily in two dimensions as shown in Figure 11. Thus, these EAG data provide empirical evidence for a flow pattern predicted from applying the "principle of continuity" to flow through pectinate antennae (Loudon and Davis, 2005). The velocity flow field is independent of the pheromone pulse rate, and therefore a match at any frequency provides such evidence.

The long depolarization and repolarization times of *L. dispar* antennae (Figure 6) are not explained by EAGs alone because EAGs indicate only the sum of electrical potentials within the antenna. We speculate that the delayed depolarization and repolarization times of *L. dispar* may be due to tonic responses of antennal pheromone receptors or due to an inability to clear pheromone from the peripheral

system quickly. Our EAG results do not show resolution of pulsed plumes at rates higher than 5 Hz in *L. dispar*, but we do not rule out the possibility that there is a population of cells with a more phasic response that, in fact, would enable *L. dispar* to resolve higher frequency pulses not observable with EAG techniques. Many of our recordings with whole *L. dispar* antennae showed a very fast rate of repolarization initially, but this was very brief, and subsequent repolarization was slowed greatly, producing unusually shaped EAG recordings (Figure 5). This phenomenon was observed only in the first repolarization in a train of EAG activity, particularly during dose-response experiments when the pipette was turned downwind and the antenna was much closer to the source. However, in pulsed stimulations, this initial fast recovery did not appear after the first depolarization and, because of this, had no effect on Fourier analysis of recordings or calculations of percent return to baseline.

Single sensillum recordings by Grant *et al.* (1997) of *Trichoplusia ni* antennae also showed an initial short phasic burst of impulse activity followed by a lower, more constant rate of tonic discharge which persisted for the duration of the whole stimulus (2 s). Although the behavior of individual neurons cannot be predicted directly from the EAG, the initially fast repolarization observed in our recordings of *L. dispar* may be due to the existence of some fast-acting phasic receptors, or it may be caused by a type of cell with a similar physiological behavior to that reported by Grant *et al.* (1997).

A reduction in sensory cell activity following the initial response to pheromone exposure has implications to male moths orienting along a pheromone source because it is the cessation of antennal activity that alters the flight path from upwind to crosswind (Vickers and Baker, 1994), a maneuver that allows flying insects to more accurately follow a pheromone plume to its source. For example, recovery of EAG responses at two-thirds peak amplitude by *C. cautella* antennae was approximately 0.12 and 0.25 s after the start of depolarization at doses of 10 and 100 ng, respectively (Bau *et al.*, 2002). Male *C. cautella* begin casting flight after plume loss within 0.3 s (Mafra-Neto and Cardé, 1995). Other species show similar latencies of casting flight after plume loss: 0.15 s in *Grapholita molesta* (Baker and Haynes, 1987), 0.3 s in *H. virescens* (Vickers and Baker, 1994), 0.48 s in *Helicoverpa zea* (Quero *et al.*, 2001), and 0.3–0.5 s in *Antheraea polyphemus* (Baker and Vogt, 1988). The first three of these species have filiform antennae, whereas *A. polyphemus* has quadripectinate antennae. In contrast, Kuenen and Cardé (1994) reported that after losing contact with the pheromone plume, male *L. dispar* progressed upwind for approximately 1 s before reverting to casting flight. Our EAG recordings of *L. dispar* antennae showed a rather slow recovery and, even with the lowest dose of 10 ng, did not recover to two-thirds peak amplitude until 0.54 s (±1.8 s SE) after the start of depolarization. The slow antennal recovery of *L. dispar* may explain its delayed onset of casting flight following loss of contact with the pheromone plume. What is clear is that *L. dispar* is able to orient toward the source of a pheromone plume, and this ability is not hindered by an apparent inability to resolve a rapidly flickering signal. We suggest that this species may not require a highly intermittent plume for oriented flight. An analogous case is the ability of *C. cautella* to orient upwind in a homogeneous plume (Justus and Cardé, 2002); although *C. cautella* antennae can resolve filaments of pheromone pulsed at high rates (Bau *et al.*, 2002), intermittency is not requisite, in this species, for upwind flight. Both *L. dispar* and *C. cautella* are exemplars of the diversity of orientation mechanisms in moths and invite further investigation.

Recordings obtained with single branches do not give any indication that the basket shape of the antennae of *L. dispar* may have contributed to their slow recovery. In both species, the recordings obtained from single branches showed a slower depolarization and repolarization time compared to whole antennae for peaks of similar amplitude (Figure 6). This slower EAG recovery by single branches may be due to damage during antennal excision or, perhaps, due to an irregular distribution of sensilla with differing temporal response patterns (phasic vs. tonic components) along the antenna. Almaas and Mustaparta (1991) found that receptor neurons on the medial side of male *H. virescens* antennae showed more pronounced phasic responses compared to receptor neurons on the lateral side. A decrease in sensitivity was also observed toward the medial and distal part of the flagellum. It is also possible that there are similar disparities across the antennae of *L. dispar* and *B. mori*, which might explain the slower temporal dynamics of single branches. All single branches tested in our study were taken from a central area on the lateral section of the antennae; we did not compare responses of medial and lateral branches.

The antennal orientation experiments revealed a strong decrease in EAG peak amplitude when the ventral part of the antenna that bears the olfactory sensilla was not directly exposed to wind (Figure 8). In EAG arrangements with the antennal branches directed at 90° and 180° with respect to wind flow, a portion of air would still pass through the branches. Indeed, Vogel (1983) reported that air passes through *A. luna* antennae front-to-back and back-to-front with equal resistance. However, the sensilla of *L. dispar* are situated mainly on the anterior surfaces of the primary branches, and, although air may pass with equal resistance at 0° and 180°, pheromone is less likely to contact as many sensilla when airflow is impeded by some directly-upwind structure, in this case, the primary branch itself. In addition, both depolarization and repolarization were slower at 90° and 180° (Figure 8). These results suggest that, unless pheromone-laden airflow is directed at the ventral part of the antenna (0°; Figure 1), pheromone perception will be suboptimal. Further evidence for this point comes from our observations that *B. mori* males rotate their antennae slightly to orient the ventral surface of their antennae directly

upwind when stimulated by sex pheromone (indicated by the onset of wing fanning, Figure 9).

Wind speed can greatly affect the airflow through a pectinate antenna. Loudon and Koehl (2000) predicted a 560-fold increase in airflow through the sensilla of *B. mori* during wing fanning, a behavior that produced a wind speed on the antenna 15 times faster than when the insect is walking at top speed. A faster airflow would affect the supply of pheromone to the sensilla. In our study, a reduction in wind speed from 50 to 25 cm/s halved the EAG peak amplitude of male *B. mori*. Pulses were generated under conditions in which wind speed does not significantly affect the shape and concentration of a pheromone pulse at short distances downstream (Justus, unpublished data). However, poorer transmissivity at the slower wind velocity (Vogel, 1983) would reduce the amount of pheromone available for interception. Therefore, the decrease in EAG amplitude is probably due to a reduction in pheromone interception by sensilla. In addition, Baker and Vickers (1994) pointed out that a stationary antenna (as in an EAG preparation) in a laminar flow will intercept odor pulses created by a pulse generator at its generated frequency regardless of wind speed; a snapshot of pulses generated at the same rate at different wind speeds would illustrate pulses as more widely spaced with greater wind velocities. Although this argument was made for filiform antennae that do not decelerate the airflow, we have shown here that the same expectation holds for pectinate antennae, albeit for a different underlying reason. In a regime of 20-ms-duration pulses generated at a rate of 25 Hz, the interpulse distance (the clean air space between odor pulses) will be 1 cm when the wind speed is 50 cm/s and 0.5 cm at a wind speed of 25 cm/s. However, as the air approaches a pectinate antenna, airflow slows, and the interpulse distances will decrease to about 1 mm and 0.5 mm as the air passes through the antenna (for wind speeds of 50 and 25 cm/s, respectively, assuming that air is slowed to 10% of its original speed as it passes through the antenna). This shortened interpulse distance may be the reason Fourier analysis does not show pulse resolution at 25 Hz at the lower wind speed (Figure 10).

We conclude that a complex morphology of bipectinate antennae, in general, does not affect pulse resolution at the antennal level. The striking differences in resolution observed between the antennae of *L. dispar* and *B. mori* are more likely due to differences in physiological dynamics of receptor response than in antennal morphology.

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