

## Pheromone-Mediated Flight in Moths

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Dedicated to the memory of Professor John S. Kennedy

### 1. Introduction

Several explanations for the existence of zigzagging upwind flight in male moths responding to sex pheromone have been given over the course of research on this topic (e.g. Kennedy 1983; Preiss and Kramer 1986b; Baker and Haynes 1987; David and Kennedy 1987; David and Birch 1989; Baker 1990; Witzgall and Am 1990; Willis and Arbas 1991a). Although a debate about the superficial shape of the flight tracks appears to be over-reductionistic, the root of the discussion involves the mechanisms that the moths use to maneuver and reach the source of pheromone in wind. As such, it is a very important debate. The behavioral mechanisms need to be precisely understood if we are to make sense of the underlying neuronal responses at the sensory, central nervous system, and motor levels and create robust neuroethological knowledge about this powerful and agriculturally important biological process called attraction.

Kennedy and Marsh (1974) and Marsh et al. (1978) convincingly demonstrated that optomotor anemotaxis was the primary mechanism used by flying male moths to locate pheromone sources. This purely anemotactic system included the wide reversals that the moths showed during casting in clean air, which were attributed to the mechanism "reversing anemotaxis" (Kennedy and Marsh 1974; Marsh et al. 1978). Importantly, no one had heretofore removed pheromone and observed what males would do in response to clean air, and these results became the first recognition that both clean air and pheromone shape the flight tracks of males. Preiss and Futschek (1985) later showed that the optomotor system is more responsive to image motion when pheromone is present than when it is absent.

The studies of Baker and Kuenen (1982) and Kuenen and Baker (1983) then revealed the existence of a nonanemotactic mechanism involved in zigzagging

upwind flight; they concluded that such a mechanism is present, since the zigzags of *Graptholia molesta* males persisted in zero wind when no anemotaxis was possible. Also, the zigzags widened in zero wind when the males flew into clean, still air, a pattern similar to the onset of casting flight in males out of pheromone into clean wind (Baker and Kuenen 1982; Kuenen and Baker 1983). Earlier reports of persistent, regular reversals by *Adoxophyes orana* males when flying within homogeneous clouds of pheromone were reinterpreted (Kennedy 1982) and pheromone was now hypothesized to switch on and modulate through its concentration a programmed, self-steered series of reversals (counterturns) as well as switching on optomotor anemotaxis (Kennedy et al. 1980, 1981; Kennedy 1982, 1983).

A plume created by a point source of pheromone is not a time-averaged homogeneous cloud. Turbulence within the moving air causes the plume to break up into strands of odor-laden air (filaments) interspersed with pockets of clean air where little or no odor is present as first articulated by Wright (1958) (Murliis this volume). The physical intermittency of these filaments was found to be a requirement for male moths to sustain their upwind progress; this insight emerged from the experiments of Kennedy et al. (1980, 1981), Willis and Baker (1984), and Baker et al. (1985), with combinations of homogeneous clouds of pheromone, side-corridor clouds of pheromone, point-source plumes placed within clouds, and clouds alternated with clean air. Baker et al. (1985) reported that the tracks of males engulfed in clouds of pheromone were similar in appearance to casting tracks, as if the males had just lost pheromone, even though they were engulfed by a cloud of pheromone. The persistent counterturns, as during casting, could be due to habituation, which would cause the equivalent of clean air to be experienced; when the same cloud was interrupted with swaths of clean air, upwind flight with smaller side-to-side oscillations occurred (Baker et al. 1985).

An important advance in knowledge further occurred when it was shown by the very fast reaction times of *G. molesta* males to both pheromone ON and pheromone OFF that these males were likely responding to individual filaments of pheromone and pockets of clean air (Baker and Haynes 1987). The frequencies of filaments in plumes had been measured using electroantennograms (EAGs) in the field and in the laboratory (Baker and Haynes 1989), and the intervals between filaments were shown to be over twice as long as the reaction latencies of the moths (Baker and Haynes 1987). These insights eventually led to a model (Baker 1990) that hypothesized that flight tracks of males were shaped by the reaction latencies to filaments and pockets of clean air, as well as by the frequency of filaments encountered in a particular type of plume. Filament contacts would evoke upwind surges, and pockets of clean air following pheromone would elicit the casting, counterturning program. A reiterative phasic system of fast reactions to contact and loss of pheromone was hypothesized to override an underlying tonic system, which, when allowed to be expressed by longer periods of clean air, would drive a counterturning oscillator, causing casting to be expressed.

The model (Baker 1990) emphasized for the first time that casting flight is pheromone-mediated flight, even though it is initiated and persists in clean air for a long time. This long-duration, tonic casting system in clean air was linked with the occurrence of large-scale turbulence that swings the wind and the plume to create large pockets of odorless air. There is a need for a system to persist for long periods when clean air is present if recontact with filaments is to occur. Conversely, the fast-acting phasic system of upwind surges was linked to the occurrence of small-scale turbulence that creates the plume's finely structured filaments. The need for the surge to stop so quickly in clean air (i.e., to be so phasic) was attributed to the fact that the next pocket of clean air following a filament may be a big one due to large-scale turbulence, and to plunge too far upwind in such a pocket is to move off-line from the source and also to move away from the shifted plume (Baker 1990).

After the experiments of Kennedy et al. (1980, 1981) and of Baker and Kuenen (1982; also Kuenen and Baker 1983) a continuum was now hypothesized to exist between (a) zigzagging triggered by pheromone and (b) casting triggered by clean air (Kennedy 1983). Both types of tracks were said to involve a program of counterturning and anemotaxis, with higher-frequency counterturning and more upwind steering evoked by pheromone contact. Now, however, we believe that the counterturning reversals are expressed only when there is sufficient clean air following pheromone relative to the response latencies of a particular species. Additionally, counterturning might be expressed when the male experiences "lean" air after pheromone as a result of habituation when flying within a homogeneous cloud (Willis and Baker 1984; Baker et al. 1985; Kennedy et al. 1980, 1981) or when flying through filaments that have excessive concentrations of pheromone and thus render antennal neurons incapable of disadapting fast enough to respond to successive filaments (Baker et al. 1988), or when flying through filaments of substandard blend quality (Witzgall and Arn 1990).

Thus we currently believe (Baker 1990) that each above-threshold filament, instead of evoking a continuum of counterturning (from casting to zigzagging depending upon the above factors), causes an upwind surge; and if repeated contacts with pheromone filaments are frequent enough, then reiterative surging will occur, resulting in straight upwind flight (Baker 1990). Flight by all moths could therefore be straight upwind if the filament quality and quantity are good enough relative to the reaction latencies of the species in question. If not, hybrid partially surging and partially casting tracks would be produced (zigzagging tracks), since clean air time interspersing filaments would allow periodic expression of the counterturning program in between surges. Although this model (Baker 1990) is superficially similar to the original idea of Kennedy and Marshall (1974; straight upwind flight in pheromone, casting in clean air), it has the distinction of incorporating the nonanemotactic component, self-steered counterturning, which previously had not been discovered (Baker and Kuenen 1982; Kuenen and Baker 1983). This model is also distinct from that of Kennedy (1983,

1986), because straight upwind flight as potentially resulting in all moths from proper pheromone filament stimulation is now included.

In a recent series of experiments by two independent pheromone research groups, this hypothesis was given substantial support (Mafra-Neto and Cardé 1994; Vickers and Baker 1994b). However, before detailing these most recent results, we would like to take some time to review the evidence for the counterturning program as an orientation mechanism, because doubts are still harbored by some groups about the existence of this mechanism (Witzgall and Arn 1990; Witzgall this volume).

## 2. Evidence for the Counterturning Program

### 2.1. Experiments in Zero Wind

Preiss and Kramer (1986b) attributed the presence of zigzags in tracks to the inability of males to steer precisely upwind when in pheromone, and they did not recognize counterturning as an element used in pheromone-mediated flight. They hypothesized that because of optomotor error in detecting transverse image drift and attaining the desired  $0^\circ$  (straight upwind) set-point, males steer through too much of an angle off to one side of the windline. They then attempt to compensate by reversing direction and overshoot  $0^\circ$  in the opposite direction. However, experiments in zero wind with *G. molesta* (Baker and Kuenen 1982; Kuenen and Baker 1983; Baker et al. 1984) and *Lymantria dispar* (David and Kennedy 1987) do not support this mechanism, but rather support the existence of counterturning. In pheromone, the zigzags of males of these two species continued *even in zero wind* and thus the Preiss and Kramer (1986b) mechanism could not be involved in the oscillations because there was no wind-induced drift to trigger any reversals (Baker and Kuenen 1982; Kuenen and Baker 1983; David and Kennedy 1987). Even more telling, when males zigzagging in a stationary pheromone plume (created by an instantaneous stopping of the wind) emerged into clean, still air, their reversals became wider and slower, with even more temporal and angular regularity than when in pheromone (Baker and Kuenen 1982; Kuenen and Baker 1983; David and Kennedy 1987). These tracks in clean zero wind, characteristic of casting flight in clean air but again occurring *without* the aid of wind-induced transverse drift, exhibited the temporally regular reversals characteristic of casting as well. Thus, pheromone exposure has also been shown to modulate the self-steered counterturning program, devoid of any possible changes in an anemotactic error function.

In another study, males took off in zigzagging flight in zero wind in pheromone, but flew with no zigzags in clean, still air (Baker et al. 1984). Thus, counterturns can *originate* in males exposed to pheromone under windless conditions without the need for wind to establish some kind of turning pattern prior to wind stoppage (Baker et al. 1984). The ability of pheromone to initiate counterturns in zero

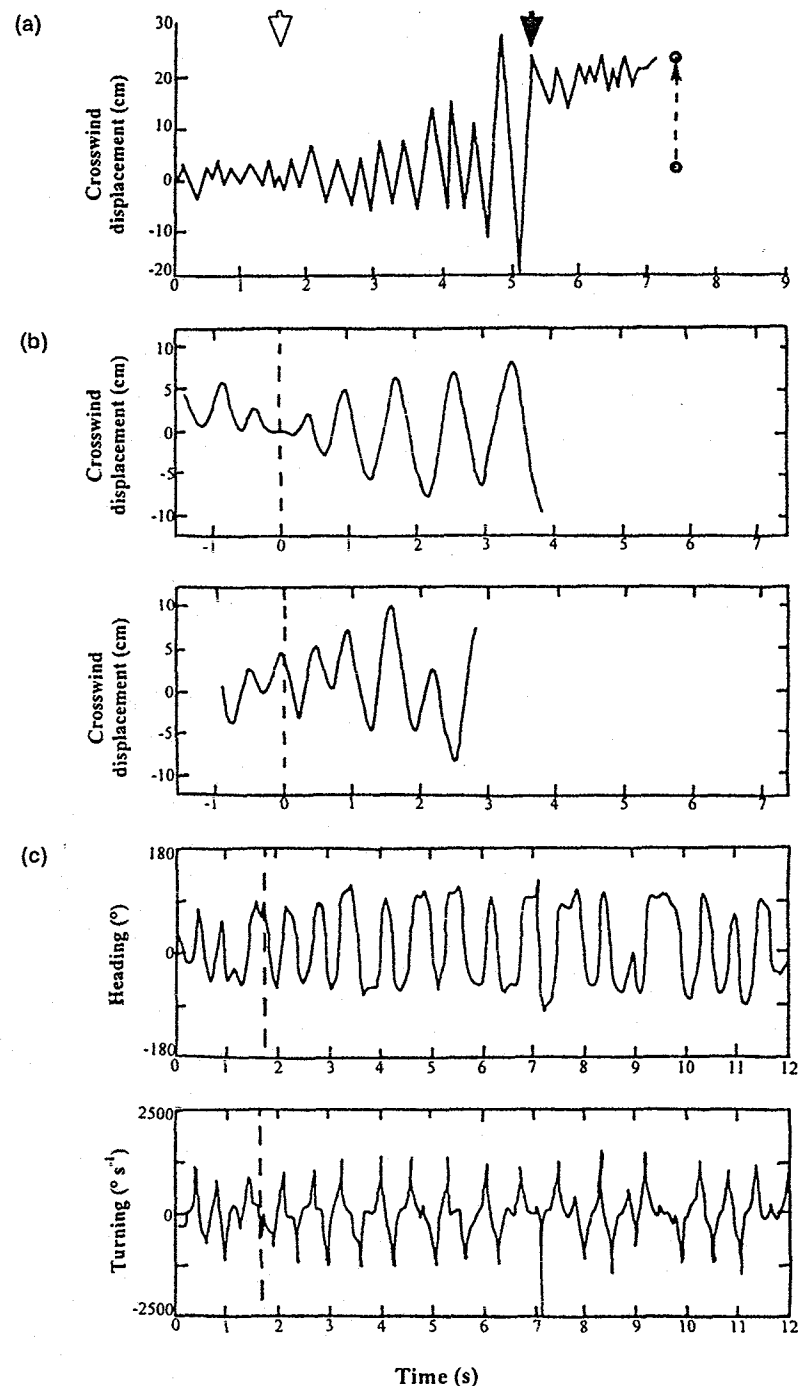
wind further establishes this self-steered system as a component distinct from anemotaxis in pheromone-mediated flight.

There is no evidence that moths that counterturn in zero wind do so with the aid of optical feedback, even though their successive turns have an angular regularity (Baker et al. 1984) in addition to a temporal regularity. If moths were using such optical feedback, perhaps it would involve a change in the *sign* of direction of rotation of their visual flow field, with males turning clockwise or anticlockwise until they receive a certain amount of rotational stimulation from their own movements and then reverse the movements. On the other hand, the regularity in the number of degrees turned may be due to the regularity of the tempo of counterturning in the two moth species in which these experiments have been performed (Baker and Kuenen 1982; Kuenen and Baker 1983; Baker et al. 1984; David and Kennedy 1987), which would fortuitously take the moth through a prescribed amount of degrees before the turn reversal generator is activated. Thus, without external feedback, such an internal program could conceivably produce an orderly succession of angular amplitudes during regular reversals in turning. When there is wind, the addition of drift may help trigger a turn back before the full number of rotational degrees has been accrued (David and Kennedy 1987) because transverse image motion may add to the degrees apparently turned through or may help trigger a reversal.

## 2.2. Regularity of the Turning Tempo

A program of counterturning with or without wind should be characterized by either an angular or a temporal regularity in the reversals; this regularity is quite obvious in clean air in the three species in which it has been analyzed; *G. molesta*, *L. dispar*, and *Amyelois transitella* (Von Keyserlingk 1984; David and Kennedy 1987; Haynes and Baker 1989). In clean wind, the temporal regularity of the reversals during casting is striking (Figs. 23.1A–C) and is evidence for a program of temporally regular oscillations that in wind is now polarized by optomotor anemotaxis to result in a spatial distribution of track angles that are centered about the windline. There was also clearly an angular and temporal regularity

**Figure 23.1.** The tempo of casting flight in clean wind as indicated by the lateral positions of males of three species plotted against time. (a) *G. molesta* male's lateral position (cm) in the wind tunnel while flying upwind in pheromone (left of clear arrow), while casting after pheromone loss (right of clear arrow), and after regaining the plume that has shifted over (right of black arrow). Note regular but diminishing tempo of turns as the exposure to clean air continues. [From Von Keyserlingk (1984).] (b) *A. transitella* female (top) and male (bottom) flight tracks during casting after losing odor plume (right of dashed line in both figures). [From Haynes and Baker (1989).] (c) *L. dispar* male's tempo of counterturning during casting flight in wind, as measured by track angle (top) and turning rate (bottom). [From David and Kennedy (1987).]



when moths flew in pheromone in zero wind (Baker and Kuenen 1982; Kuenen and Baker 1983; Baker et al. 1984) as well as out of pheromone during casting in zero wind (Baker and Kuenen 1982; Kuenen and Baker 1983; David and Kennedy 1987), the regularly repeating magnitude of successive angles but of opposite rotation providing evidence for a regularly timed reversal of direction. As the program of counterturns slows in tempo following the time since pheromone loss (Fig. 23.2), contact with pheromone can truncate the program and

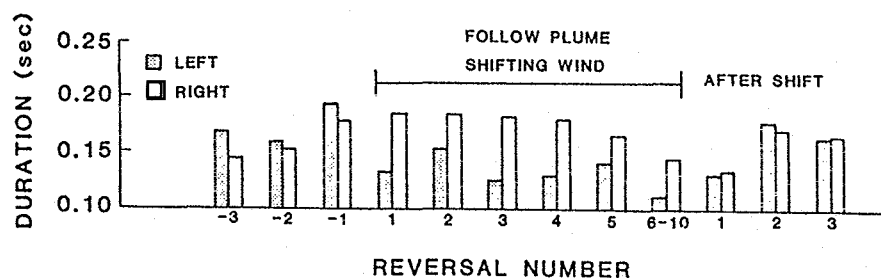
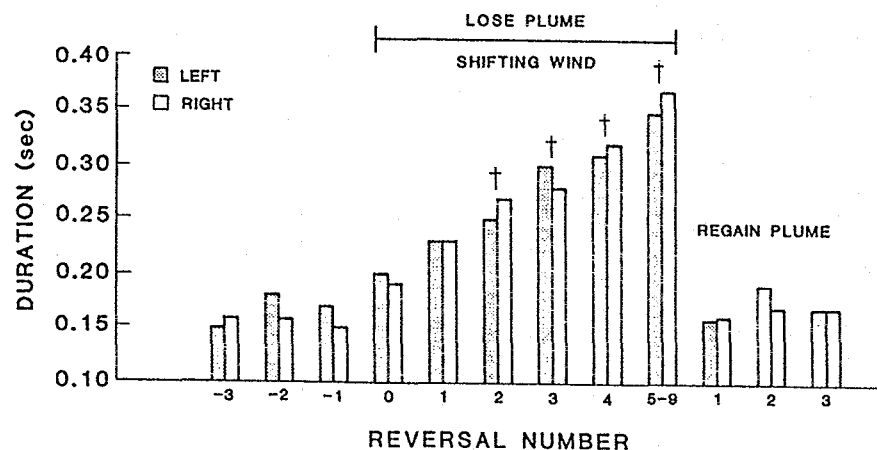


Figure 23.2. The mean inter-reversal durations of 20 *G. molesta* males that lost the plume completely and began casting (top) and of 16 males that followed the plume across and reinteractively recontacted the plume before each left-hand track leg (bottom) during an experimentally produced wind-shift of 35°. Shaded bars are during left-hand legs, while white bars are during right-hand legs, at the indicated reversal number before or after losing the plume and after regaining it (top), or before, during, and after the wind-shift (bottom). Note the regular, incremental increase in the inter-reversal duration (top) as time since pheromone contact increases, which is indicative of a retarding of the tempo of counterturning with time after pheromone contact. The tempo returns to a faster state immediately after the plume is contacted again, as well as on each leftward leg after plume contact (bottom). [From Baker and Haynes (1987).]

create shorter-duration reversals to narrow the track during an upwind surge (Baker and Haynes 1987). Exposure to clean air, beginning even on the next reversal, was shown to allow the program to begin to be expressed again and to play out with increasingly retarded reversals unless it was interrupted once more by contact with pheromone. Thus, it may be that the upwind surge is coincident with the suppression of the *expression* of casting. The results of Kanzaki et al. (1994; see also Kanzaki this volume) with walking *Bombyx mori* seem to indicate that this type of system is operating to produce reiterative straight-line rapid surges with successive pulses of pheromone that decay into increasingly greater side-to-side oscillations as the time since contact with a filament lengthens.

The reiterative interception of filaments on rightward track legs and clean air on leftward track legs revealed the rapid resetting and relaxation of the counterturning frequency, contributing to sawtooth-shaped, asymmetric tracks in males flying in an experimentally shifted plume or along a side corridor of pheromone (Baker and Haynes 1987). Thus, Baker (1990) hypothesized that it is just a question of how long it has been since the last pheromone filament was contacted and the moths' typical species-specific latency to begin casting that will determine how much casting can be expressed before a filament causes an upwind surge and resets the counterturning program. In combination with a resetting of the anemotactic system to straighter upwind, either straight-upwind or zigzagging upwind flight would occur, depending upon the above factors.

### 2.3. The Question of Bimodality of Track Angles

Much has been made lately of the question of bimodality or unimodality of track and course angles in the analysis of male flight tracks. Preiss and Kramer (1986b) considered such bimodality to be merely an extension of the anemotactic error system that is modulated by pheromone loss. Witzgall and Arn (1990) considered bimodality to be a function of the quality of pheromone used, with the poorer blend quality presumably increasing the imprecision of upwind steering as per the Preiss and Kramer model. Baker (1990) and Kaissling and Kramer (1990) viewed the amount of bimodality as being a function of the amount of casting that is expressed in a given track. Purely upwind, unimodal distributions would indicate that filaments of sufficient quality are being encountered by the male with such frequency that reiterative phasic upwind surges are evoked, to the exclusion of the counterturning program. Even brief episodes of casting thus could not occur in what little clean air is present between filaments.

All species of moths examined thus far in free flight begin casting when emerging into clean air from a pheromone- or host-odor plume. It is during casting that bimodality in track and course angle distributions are always and most obviously present (Fig. 23.3). Therefore, it is logical to conclude that the degree of bimodality in a distribution of track angles in moths flying upwind to pheromone should be an indication of the amount of casting that is being expressed

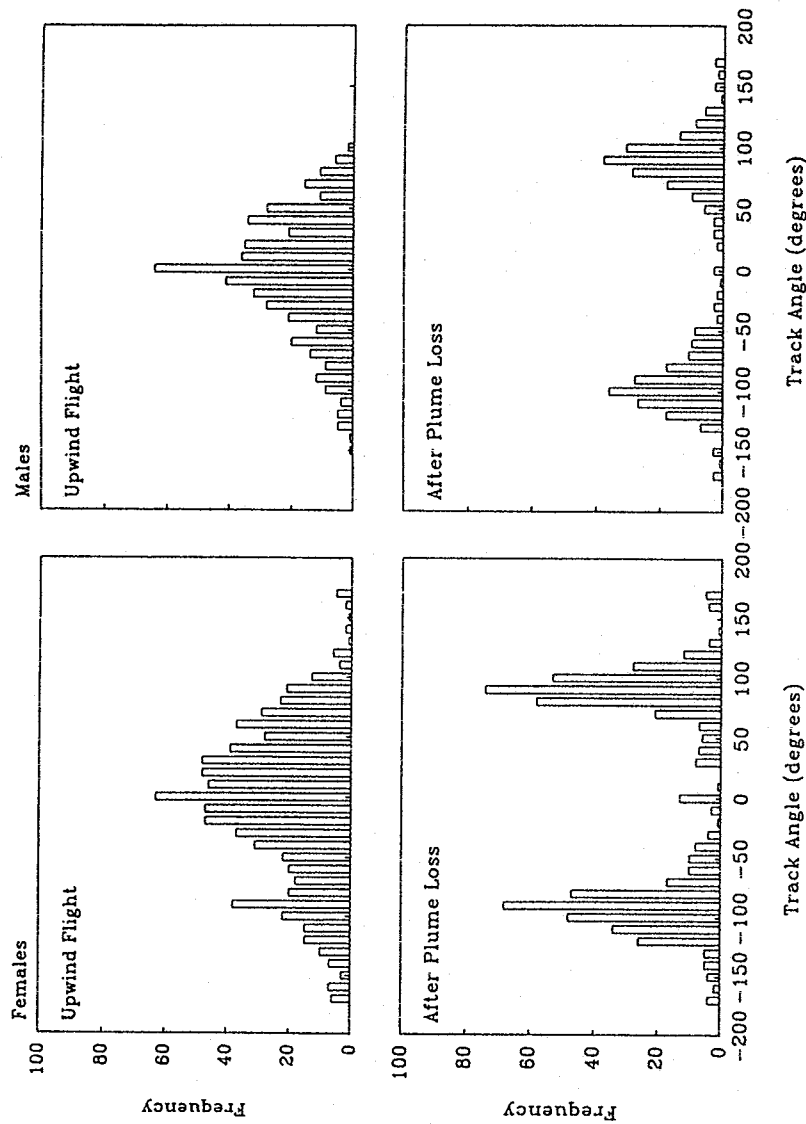


Figure 23.3. Track angles of eight female and eight male *A. transitella* moths as they flew upwind in a plume of host odor and extract from female pheromone glands, respectively (top), or engaged in casting flight following removal of the plume (bottom). [From Haynes and Baker (1989).]

in that track, or, in other words, an indication of the amount of clean air that the moth is experiencing (Fig. 23.3). Unimodal tracks of males of some species flying in pheromone plumes are not proof that a counterturning program is not used by such males, only that under the conditions used to evoke such flight, there is not enough clean air in which to observe casting as the expression of the counterturning program. As mentioned above, all moths show bimodality of course and track angles during casting. However, during flight in a pheromone plume, it is known that unimodality is dependent on the species, the wind speed (Willis and Cardé 1990; Willis and Arbas 1991a; Willis et al. 1991), and other conditions such as pheromone blend quality (Witzgall and Arn 1990), which may or may not result in bimodality of the distributions of track and course angles.

The work of Preiss and Kramer (1986b) in which removal of pheromone caused no obvious counterturning-based casting flight is the only exception to clean-air casting in moths. However, the moths in their experiments were not in free flight. Rather, they were tethered and could only rotate in the yaw plane; their ability to roll or change their pitch, both known to occur in free flight as part of normal maneuvering, was restricted. As outlined by David and Kennedy (1987), there are many problems with the tethering technique used by Preiss and Kramer (1986b) that restricted normal male maneuvers, as well as with the pheromone delivery technique, all of which could explain the unusual behavior seen in these experiments. In *Lobesia botrana*, the other species for which it is claimed that no counterturning occurs (when natural pheromone is used), casting flight in clean air has never been attempted to be evoked by removing pheromone (Witzgall and Arn 1990), and so the story is incomplete for this species.

#### 2.4. The Question of Blend Quality

Witzgall and Arn (1990), who, similar to Preiss and Kramer (1986), questioned the need to invoke the counterturning program as a mechanism in pheromone source location, stated, "It is worth noting that most hypotheses on an internal counterturning program have been derived from experiments with synthetic compounds." This statement is incorrect, however, because in actuality the hypotheses supporting a counterturning program [or "reversing anemomenotaxis" of Kennedy and Marsh (1974)] were derived by using *clean air following pheromone* to evoke casting flight. A self-steered counterturning program, in addition to anemotaxis, is an important and most obvious underlying element to casting flight in clean wind (see above). In all species that have been examined, clean air following pheromone exposure reliably turns on casting flight, regardless of which blends of pheromone, synthetic or natural, are used.

Interestingly, the experiments of Haynes and Baker (1989), cited by Witzgall and Arn (1990) as an example of the value of using natural pheromone to produce nearly straight upwind flight in plumes, also included the evocation of casting flight in clean air following removal of these plumes of pheromone for males,

or of natural host odor for females. Thus, even with optimal natural pheromone, it is only a matter of time before males will express counterturning-based casting after pheromone is removed and clean air is experienced [see also Willis and Arbas (1991a) for comparisons of male and female casting after gland-derived pheromone and host-odor loss, respectively]. It is not difficult to imagine then that even in such plumes of natural odors, the latency of the transformation of upwind flight into casting flight and the amount of time that elapses between contact with filaments will determine how frequently casting will be expressed and therefore how zigzaggy the track will be (how bimodally distributed the track angles will be) (Baker 1990). If filament contact is just frequent enough to prevent casting from being expressed (the interval between filament contacts is just shorter than the postfilament casting latency), then the missing of a filament will cause the beginning of counterturning to be expressed. Some short episodes of casting can be seen even in these flight tracks to natural odor (see Fig. 1 of Baker and Haynes 1987). The absence of filaments, such as in a large pocket of clean air due to large-scale turbulence, will allow the casting program of counterturns to be expressed even more fully.

Poorer-quality filaments will be less likely to be above threshold (less likely to cause upwind surges) than better-quality filaments. Such relationships between pheromone blend quality and upwind flight thresholds have been demonstrated repeatedly (Baker et al. 1981; Linn et al. 1984, 1986a), with off-blends being able to evoke sustained upwind flight, but at higher concentrations than optimal blends. When projected to the level of individual filaments, blend threshold differences of this type should translate into a lower *effective* frequency of filament contact by males flying in suboptimal blends and result in more clean-air time for casting to be expressed. Such is probably the case in the zigzagging tracks of male *L. botrana* (Witzgall and Arn 1990) when the (imperfect) synthetic blend was used compared to straighter tracks in response to the natural blend. More casting will be initiated and fewer upwind surges will occur in such plumes, and the tracks will thus exhibit more bimodality of course and track angles than flight in the natural plume, even though the physical structure (filament frequency) will be on average the same in both. The effective filament frequency will be greater in the optimal, natural plume of pheromone to which the male's threshold for responding to filaments is lower. It is thus not surprising that an optimal blend and emission rate, as afforded by calling females, should produce more direct and less zigzaggy upwind flight than will a synthetic blend.

Synthetic blends are not per se the cause of zigzagging flight tracks, since, as mentioned above, the interaction of filament encounter rate, moth reaction latencies, and filament blend quality and concentration all shape the track, and a synthetic blend at one filament frequency that causes zigzagging tracks can cause straight upwind flight when the filament frequency is increased to produce only successive surges (see below) (Mafra-Neto and Cardé 1994; Vickers and Baker 1994b). Con-

versely, the use of natural pheromone or an optimal synthetic pheromone blend will not ensure straight upwind flight if the plume structure is suboptimal.

### 3. Recent Advances

It is clear from past data and from recent experiments that the amount of bimodality of track or course angles in moth flight tracks is an indication of how much casting is occurring in these tracks, and the amount of casting is a measure of how frequently filaments of sufficient quality and concentration are being encountered by antennae to evoke upwind surges. This is quite apparent in the recent results of Mafra-Neto and Cardé (1994) and Vickers and Baker (1994b) in two different species of moths. The following is a summary of the findings of Vickers and Baker (1994b). A summary of Mafra-Neto and Cardé's (1994) results can be found in Cardé and Mafra-Neto (this volume).

Vickers and Baker (1994b) characterized single upwind surges of flying male *Heliothis virescens* moths in response to individual strands of pheromone generated experimentally in a wind tunnel, using a pulsing device. All males flying upwind in a 10-filament  $\text{sec}^{-1}$  pulsed plume responded to the truncation of the plume by casting. Males that intercepted one of the last filaments produced by the pulsing device prior to plume truncation began casting within an average of 0.27 sec. Of these casting males, 32% ( $n = 192$ ) subsequently responded to a single filament by making a short upwind surge followed by a reversion to casting. In many cases the complete sequence of casting following plume truncation, interception of a single pulse, ensuing surge, and lapse back to casting flight was not captured entirely within the camera's field of view. Hence, only 13 (or 7%) of the responding males' tracks could be used. No casting males exhibited any upwind displacement in the control where no filament was generated.

The latency between filament interception and the surge was found to average 0.30 sec, while the surge itself had an average duration of 0.38 sec. The males covered an average of 13.4 cm in the upwind direction during this time. By calculating average track angles and average ground speeds for each 1/30th of a second, synchronized by the passage of the filament past each of the 13 responding moths, we produced an average cast-surge-cast track that then served as a template for later comparisons (Vickers and Baker 1994b). Counterturning frequencies, higher during ( $3.33 \text{ counterturns sec}^{-1}$ ) and lower before and after ( $2.38 \text{ counterturns sec}^{-1}$ ) the surge, were also integrated into the cast-surge-cast template (Vickers and Baker 1994b).

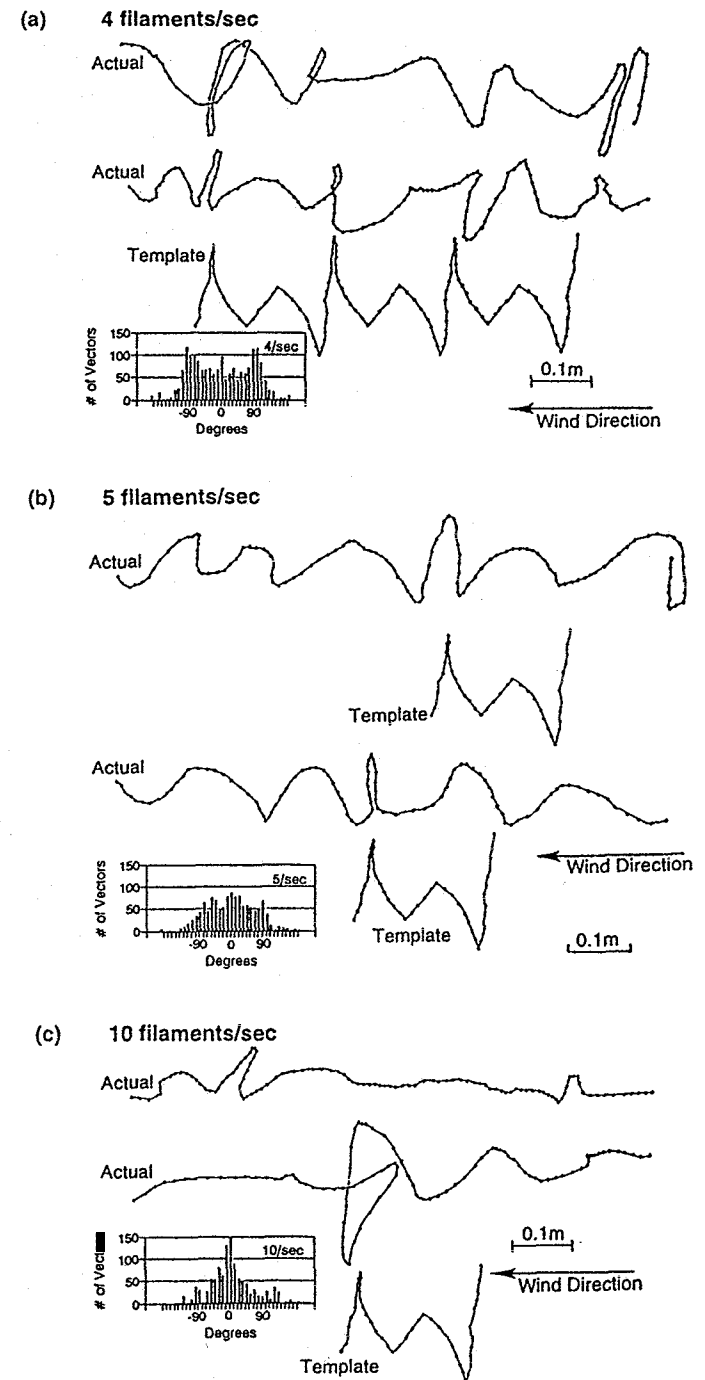
When flown upwind to pulsed plumes generated at varying frequencies, males exhibited the template for a single iteration, complete with cross-wind casting both before and after the straighter upwind surging portion, at the lowest frequency capable of sustaining successful upwind flight to the source, as hypothesized by

an earlier models (Baker 1990; Kaissling and Kramer 1990). Also as predicted, when the pulse generation rate was increased and filament contact by males became more frequent, only the straightest upwind portions of the surges were reiterated, producing direct upwind flight with little cross-wind casting (Fig. 23.4).

Males were not able to fly upwind in plumes consisting of fewer than 4 filaments per second, although they did become activated and cast for short periods of time above the take-off point. This is in close agreement with our previous observational accounts detailing males' ability to fly upwind in pulsed plumes generated at various frequencies (Vickers 1992; Vickers and Baker 1992). The tracks in response to 4 filaments  $\text{sec}^{-1}$  (Fig. 23.4) were tortuous in shape with a bimodal distribution of track angle vectors around  $\pm 90^\circ$  indicative of more cross-wind casting flight. In contrast, at 10 filaments  $\text{sec}^{-1}$ , where many tracks had extended periods of nearly straight upwind flight and fewer periods of cross-wind casting, track angles were unimodally distributed about  $0^\circ$ . At 5 filaments  $\text{sec}^{-1}$  (Fig. 23.4) the shape of the flight tracks and the distribution of track angles were intermediate in appearance between those of the 4-filament  $\text{sec}^{-1}$  and 10-filament  $\text{sec}^{-1}$  frequencies. Since only filament frequency was varied in these experiments, the more straight upwind distribution of track angles cannot be attributed to a compensatory response to changes in wind velocity.

Many of the actual flight tracks produced in response to 4 filaments  $\text{sec}^{-1}$ , a sufficient frequency to sustain upwind flight, exhibit identifiable templates of an average moth responding to a single pulse (surge) followed by clean air (cast) strung together in succession (Fig. 23.4). Occasionally males ceased upwind progress at the higher pulse frequencies. Importantly, the initial upwind movement following interception of the pulses anew (since upwind progress was never observed in males casting in clean air) resembled the surge part of the cast–surge–

**Figure 23.4.** (a) Flight tracks (Actual) of two male *H. virescens* in response to a pulsed plume generated at 4 filaments  $\text{sec}^{-1}$ . The cast–surge–cast template (Template) (Vickers and Baker 1994b) laid end to end indicates that the upwind progress of these males is due to the repetitive evocation of single surges interspersed by casting. (b) Two flight tracks of males responding to a pulsed plume generated at a rate of 5 filaments  $\text{sec}^{-1}$ . The tracks are more upwind than tracks of males flying to 4 filaments  $\text{sec}^{-1}$  and less upwind than those of males responding to plumes generated at 10 filaments  $\text{sec}^{-1}$ . This is reflected by the intermediate distribution of track angle vectors. The tracks are now not obviously composed entirely of the cast–surge–cast template laid end to end (as at 4 filaments  $\text{sec}^{-1}$ ), but there are instances when the template does reveal itself within the flight track. (c) Flight tracks of two males in response to a plume generated at a rate of 10 filaments  $\text{sec}^{-1}$ . The middle, upwindmost part of the surge is reiteratively evoked by fast filament contact resulting in straighter upwind tracks compared to 4 and 5 filaments  $\text{sec}^{-1}$ , respectively. When males did lose the train of pulses at these higher production rates (lower Actual track), the initial part of the recovery following pulse recontact resembled the first part of the cast–surge–cast template, before becoming canalized more upwind once again.





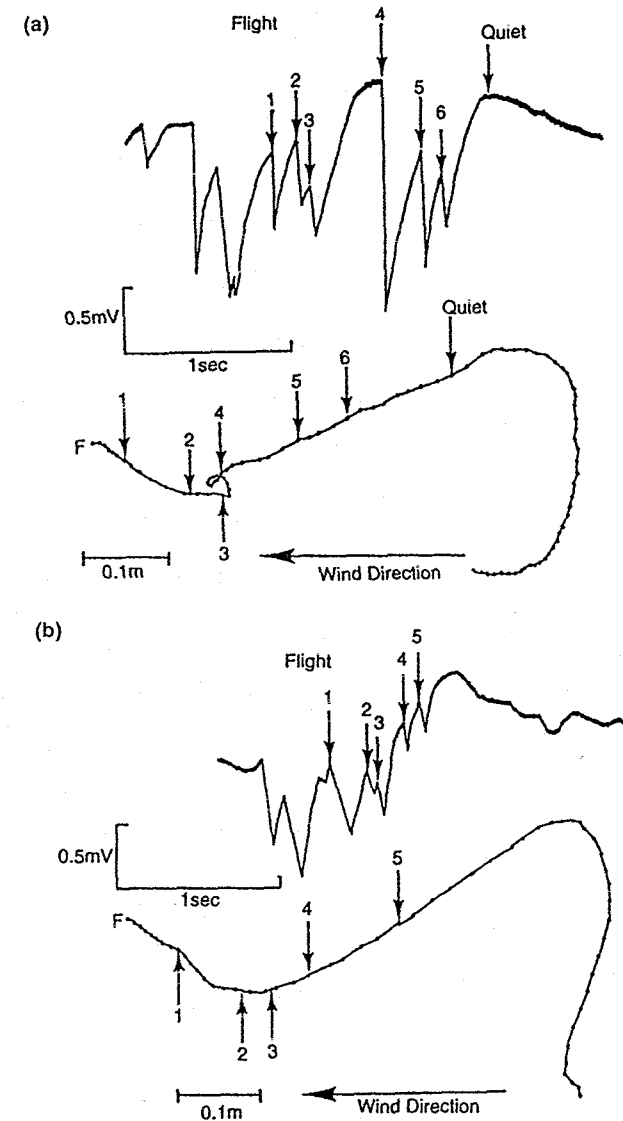
cast template (Fig. 23.4). Thereafter the tracks became canalized directly upwind because faster contact with filaments resulted in the repeated evocation of only the middle, upwindmost section of the surge without time for casting to be expressed within the shorter time-span pockets of clean air.

In-flight EAGs made from males in free flight upwind in a pheromone plume further support the idea that reiterated straight surges occur when a high frequency of filaments is encountered under the usual point-source pheromone plume conditions and results in more direct upwind flight (Vickers and Baker 1994b). Intervals between successive hits with pheromone, as indicated by EAG activity, showed that males making relatively steady upwind progress with no overt casting intercepted more than 5 filaments  $\text{sec}^{-1}$  (mean interval: 0.19 sec), which was greater than for males flying crosswind (less than 4 filaments  $\text{sec}^{-1}$ ; mean interval: 0.28 sec) or for antennae held stationary in the plume before flight (3.4 filaments  $\text{sec}^{-1}$ ) (Vickers and Baker 1994b).

In-flight EAGs also showed that when filament contacts cease, the casting, counterturning program begins to be expressed after a latency period of 0.30 sec. Males that were flying crosswind upon encountering a filament turned their tracks upwind in an average of 0.23 sec following contact. These response latencies, this time measured in a typical pheromone plume emitted by a standard dispenser, correspond closely to the results from the single-pulse experiment confirming that the surge duration in response to pheromone was 0.38 sec before reverting to casting flight, and then casting males responded to a filament itself within 0.30 sec.

With the recent realization of the importance of measuring reaction times to odor onset and loss as being critical to understanding odor-mediated orientation by moths (Baker 1989b, 1990), it is not surprising that the reaction times to odor loss have only been estimated for a few species (Marsh et al. 1978; Baker and Haynes 1987; Baker and Vogt 1988; Haynes and Baker 1989; Willis and Arbas 1991a; Vickers 1992). Moreover, only in *G. molesta* (Baker and Haynes 1987), and *H. virescens* (Vickers 1992; Vickers and Baker 1994b) have the response

**Figure 23.5.** Flight tracks of two males viewed from above together with the simultaneously recorded electroantennogram activity of a third antenna transported by each flying male. The first male (a) is hit by one filament just before, and two filaments just as, it takes flight, producing an upwind trajectory. During this surge, the male is hit by three filaments (gaps between filaments are F-#1, 0.18 sec; #1-#2, 0.18 sec; and #2-#3, 0.08 sec). Filament #1 appears to have no effect on the behavior because after the average 0.23-sec latency period, now 0.26 sec later (just after #3), the moth begins to go into casting flight across the windline to the left. Filaments #2 and #3 occurred too late in the surge to prevent this cast, but 0.27 sec later (and before casting is fully established) the moth does begin another upwind surge. The male then contacts three more filaments in quick succession—filaments #4 (0.17 sec after #3), #5 (0.23 sec after #4), and #6 (0.13 sec after #5)—and these are apparently sufficiently frequent to sustain the upwind movement.



**Figure 23.5. (Con't.)** Following filament #6, the moth turns its track across the windline after 0.5 sec without filament contact. In (b) the male initiates flight in the upwind direction. Almost at take-off he receives a filament and then again shortly after taking flight (0.27 sec #1). Upwind flight appears to be sustained owing to the sequential arrival of filaments with short gaps between them (0.20 sec #1-#2; 0.07 sec #2-#3; 0.08 sec #3-#4; 0.17 sec #4-#5). After filament #5, there is a latency period of 0.43 sec without further contact with pheromone filaments before the male turns its track crosswind on a casting leg. [From Vickers and Baker (1994b).]



times (latencies and durations) to pheromone onset been measured. Our findings that *H. virescens* males react more slowly to clean air and hence have longer-duration surges than *G. molesta* males support the phasic-tonic model for upwind flight and explain why *H. virescens* males can have much straighter tracks than *G. molesta*, whose tracks are nearly always zigzag-shaped. *H. virescens* males will be less likely to go into casting flight and zigzag like *G. molesta* in plumes of similar structure (and filament frequency) because *H. virescens*' longer-duration surges carry them into filaments more often before casting can be fully expressed, as evidenced by their straight upwind flight tracks in the laboratory and field (Vickers 1992; Vickers and Baker 1994b). As alluded to earlier, recent results from another species, *Cadra cautella*, show relationships between track straightness and response to single filaments (Mafra-Neto, 1993; Mafra-Neto and Cardé 1994; Cardé and Mafra-Neto this volume) remarkably similar to those found in *H. virescens* (Vickers 1992; Vickers and Baker 1994b). Results from both studies support the filament-frequency-based surging-casting model of pheromone source location (Baker 1990).

They also support the existence of two mechanisms that create the surging-casting interplay: a self-steered counterturning program integrated with optomotor anemotaxis (Kennedy 1983; Kuenen and Baker 1983; Kennedy 1986).

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## 24

### Modulation of Pheromone-Mediated Flight in Male Moths

Peter Witzgall

#### 1. Pheromone-Mediated Optomotor Anemotaxis

Male moths find sources of sex pheromone by flying into the scented wind. The upwind flight response is triggered immediately upon perception of pheromone, up to many meters downwind from a source releasing nanogram amounts per hour. This behavior has fascinated naturalists for centuries and has inspired studies on the chemistry of the female signal, the male receptor physiology, and the underlying orientation mechanism. The strength of the male response has even encouraged speculations and research toward the use of pheromones to control harmful insects.

In flight, the moth cannot sense the wind directly, but visually detects the wind direction through its displacement over the ground. While flying due upwind, the image flow is in line with the body axis; during lateral excursions, the male experiences visual drift (Kennedy 1940; Kennedy and Marsh 1974; Marsh et al. 1978; David et al. 1983; Cardé 1984; David 1986; Preiss and Kramer 1986a,b).

The upwind orientation behavior has been termed "zigzagging," due to a meandering flight path with irregular excursions across the wind line. This sequence of track reversals is thought to be the result of visually steered upwind flight (optomotor anemotaxis) integrated with an internal program of counterturns (Kennedy et al. 1980, 1981; Baker and Kuenen 1982; Kuenen and Baker 1983; Kennedy 1983, 1986; Baker et al. 1984; Baker 1990). However, experiments with tethered moths in a flight simulator have failed to confirm self-steered counterturning: In regard to "the male's inability to fly precisely upwind," optomotor anemotaxis alone suffices to explain the observed flight pattern (Preiss and Kramer 1986a,b).

The anemotactic response is modulated by pheromone; most studies on male orientation behavior have relied on synthetic chemicals, released from rubber septa