

Odor-modulated orientation in walking male cockroaches *Periplaneta americana*, and the effects of odor plumes of different structure

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Accepted 29 November 2004

Summary

The location of distant resources using odor information usually also requires information on the flow of air (or water) through the environment together with the expression of internally programmed steering responses. The orientation responses of virgin male *Periplaneta americana* L. to wind and the female sex-pheromone component (–)-periplanone-B were video-recorded in a laboratory wind tunnel and quantified. *P. americana* males showed no preferred walking orientation when released in zero wind in the absence of pheromone. When introduced into 25 cm s⁻¹ wind in the absence of pheromone, 79% of males walked downwind. Upon introduction to a plume of (–)-periplanone-B in wind, 100% of males walked upwind in the pheromone plume to the source. Males were then challenged with wind-borne plumes of (–)-periplanone-B of four different temporal/spatial structures. In nearly all cases, the only statistically significant changes in behavioral parameters measured from their walking

tracks were observed from males tracking the treatment consisting of the most turbulent plume. The plume-tracking performances of males challenged with the other three less turbulent plumes were visually and quantitatively similar, regardless of the width. Males tracking all four plumes showed evidence of turns resulting from multiple mechanisms. Some of the observed maneuvers were temporally regular counterturns, suggesting steering according to an ongoing internal program, while others could have been triggered by encountering the change in odor concentration between pheromone and clean air at the lateral boundary of the plume or chemotactically upon the detection of changes in odor concentration.

Key words: olfaction, orientation, cockroach, *Periplaneta americana*, (–)-periplanone-B, pheromone.

Introduction

Multicellular organisms using chemical information to locate resources in their environment rarely respond to concentration gradients set up *via* diffusion. Rather, they typically combine chemical cues issuing from the source with other sensory information to orient and steer their locomotion. Specifically, the direction and speed of the fluid (i.e. either water or air) that is carrying the odor molecules are used to orient to and navigate toward the source (Bell, 1983; Bell and Tobin, 1982; Arbas et al., 1993). In most known environments, odors are borne away from their source and distributed in the environment by fluid flows of varying turbulence. The direction and speed of this flow are typically the most reliable and useful information indicating direction to the source location and are incorporated with information about the odor stimulus during odor-tracking behavior (Arbas et al., 1993; Belanger and Willis, 1996; Weissburg, 2000; Grasso and Basil, 2002). However, under certain conditions, in certain forest and woodland environments, the wind directions may change so continuously that at any given moment an animal experiencing odor and orienting to the wind may be aimed away from the source (Elkinton et al., 1987; Brady et al., 1989).

Insects tracking plumes of airborne odors in flight (Arbas et al., 1993; Willis and Arbas, 1997) or while walking (Kennedy and Moorehouse, 1969; Tobin and Bell, 1986; Bell and Kramer, 1980; Rust and Bell, 1976; Wolf and Wehner, 2000) orient their movement into the wind, while in the absence of odor they typically show no preferred orientation direction or orient their movement with the wind. Similar behavior has been observed in swimming fish (Baker et al., 2002; Johnsen and Teeter, 1985), walking crustaceans (Grasso and Basil, 2002; Weissburg and Zimmer-Faust, 1994), crawling sea stars (Moore and Lepper, 1997) and crawling snails (Susswein et al., 1982; Brown and Rittschoff, 1984) orienting to odors in flow in marine environments. Thus, an orientation to environmental flow that is modulated by the level of odor in the environment appears to be a generic component of the mechanisms used by animals to locate resources using chemical cues. Animals using this mechanism are not determining the direction of their locomotion according to the chemical signal *directly*; rather, the chemical information is modulating their orientation to flow information (Bell and Tobin, 1982; Schöne, 1984). As long as the chemical signal is detected simultaneously with

flow, the organism will continue to orient and move counter to the direction of flow and toward the source of the chemical (Baker et al., 1984; David et al., 1983; Baker and Haynes, 1987; Willis and Cardé, 1990).

Periplaneta americana, the American cockroach, has been studied extensively as a model for odor-modulated behavior (Rust and Bell, 1976; Rust et al., 1976; Bell and Kramer, 1980; Bell and Tobin, 1981; Seelinger, 1984; Tobin, 1981) and olfactory processing (Boeckh et al., 1987). The majority of this previous work was performed on tethered walking preparations (Rust and Bell, 1976; Rust et al., 1976) and animals free to walk but restricted to computer-controlled locomotion compensators (Bell and Kramer, 1979, 1980), two similar but unnatural conditions. Relatively little of the published work on the odor-modulated orientation and navigation behavior of cockroaches reflects research performed on animals moving freely through an environment in either the laboratory (Bell and Tobin, 1981; Tobin, 1981) or the field (Seelinger, 1984).

These earlier experiments were aimed at determining the specific sensory information and orientation mechanisms that could be used by walking cockroaches to locate sources of odor in wind, or using odor information alone. Specifically, these studies examined whether the changes in steering observed during orientation to odor were caused: (1) by changes in the walking speed or rate of turning in direct response to changes in odor concentration (i.e. chemotactic orientation), or (2) by odor stimuli initiating the production of pre-programmed internally stored steering maneuvers with no direct relationship to the odor concentration (i.e. turning rate and direction determined by internally stored information rather than directly correlated with the environment; Bell and Tobin, 1982; Schöne, 1984). Although it is easy to state this as a dichotomy, it is probable that individual turning maneuvers executed by an organism during its response to a specific environmental condition may result from either one of these mechanisms or a combination (Schöne, 1984). Therefore the orientation tracks observed may not be the result of a single orientation mechanism, but rather the result of an individual switching amongst an array of different alternative mechanisms, depending on the local environmental conditions and the individual's internal state.

One of the few experiments in which freely walking *P. americana* males oriented to wind and odor (i.e. female sex-attractant pheromone) was aimed at determining whether the turns observed resulted from an ongoing internal program of counterturning initiated upon odor contact or were triggered by the decrease in concentration or loss of contact with pheromone when the male encountered the clean air-pheromone boundary at the lateral edge of the plume (Tobin, 1981). Internally programmed counterturning is thought to be a primary mechanism underling the zigzagging tracks generated during upwind orientation to odor in flying moths (Arbas et al., 1993; Vickers and Baker, 1994; Mafra-Neto and Cardé, 1994; Willis and Arbas, 1997). The results of Tobin's experiment (Tobin, 1981) showed that when the distance between the plume's edges increased, the width of the

males' tracks increased in concert, with many of the observed turns occurring at the edges of the time-averaged plume boundaries. These turns were interpreted as resulting from a pre-programmed turning response to the loss of odor (Tobin, 1981; Bell and Tobin, 1982), in contrast to an internal counterturn generator similar to that hypothesized for moths (Kennedy, 1983). However, not all turns observed occurred at the time-averaged plume boundaries and it was suggested that internally generated turns of various origins also contributed to the final structure of the orientation tracks observed (Tobin, 1981).

The results presented here address the mechanisms underlying the olfactory orientation behavior of *P. americana* males by challenging freely walking cockroaches to orient and track different combinations of wind and odor through an experimental arena in our laboratory wind tunnel. We use the earlier experiments of Tobin (1981) as a starting point. To determine whether the walking male cockroaches tracking a plume of wind-borne female pheromone were orienting directly to the olfactory stimuli, or whether the olfactory information initiated and modulated orientation to the wind, we observed the preferred orientation direction of males released in different combinations of wind and the female pheromone component (–)-periplanone-B. Once we had established the preferred orientation responses to wind and odor, we tested whether males tracking pheromone plumes (1) turned in response to a decrease in odor concentration or the loss of contact with odor caused by encounters with the lateral margin of the time-averaged pheromone plume, or (2) executed turns according to an internal counterturning generator. Our working hypothesis was that the large concentration differences experienced at the time-averaged edges of the odor plumes would be the primary stimulus accounting for turns observed during plume tracking. Consequently, as the plumes increased in width we expected the overall widths of the tracks to increase in concert, as has been observed previously (Tobin, 1981). The goal of the work presented here is to form the foundation of a series of experiments examining the orientation mechanisms used by walking animals to track odor in terrestrial environments, and to compare them explicitly to the more extensively studied examples of odor plume tracking by flying insects, especially moths.

Materials and methods

Insects

We used virgin adult male *Periplaneta americana* L. from our laboratory colony for this study. Ultimate instar nymphs were separated from the colony according to sex and males were held until they eclosed as adults. Upon day 1 of adulthood, male cockroaches were separated and held in groups of 15 or less in 2.8 l plastic bins with water and chicken feed *ad libitum*. Each bin contained cardboard egg cartons to provide sheltered hiding places. We applied petroleum jelly around the upper rim of each bin to prevent cockroaches from escaping. The bins were held in an environmental chamber at a range of 27–29°C,

approximately 50% RH, on a 12 h:12 h L:D cycle (Rust et al., 1976; Seelinger, 1984; Abed et al., 1993).

Wind tunnel

We conducted experiments in low-light conditions with light levels of approximately 7 lux provided by four voltage-regulated 25 W red incandescent bulbs and one separately adjustable 25 W white incandescent bulb. There were also seven 40–60 W infrared lights distributed around the working area to provide illumination for the video cameras. Wind speed was set at 25 cm s⁻¹ (Tobin, 1981). We released *P. americana* males onto our experimental arena, a flat aluminum platform (1.52 m × 0.92 m) held 25.4 cm above the floor of a Plexiglas wind tunnel with a 1 m × 1 m × 2.5 m working section. The pheromone source holder was centered at the upwind end of the aluminum experimental arena and it held the pheromone ca. 1 cm above the aluminum platform. The odor plume was removed from the wind tunnel and the building *via* an exhaust duct attached to the downwind end of the tunnel.

We recorded the responses of cockroaches to the different pheromone plume structures using 4 Burtle TC355AC (Lancaster, PA, USA) B/W cameras: one positioned directly overhead with a field of view of 1.93 m long × 1 m wide; a second with a zoom lens positioned overhead with a field of view of 0.67 m long × 0.86 m wide; a third positioned on one side of the wind tunnel just above the level of the aluminum floor with a field of view of 0.44 m wide × 0.59 m tall; and a fourth placed at the downwind end of the wind tunnel with a field of view of 0.68 m wide × 0.20 m tall. These latter three cameras with smaller fields of view were all aimed and focused on a volume approximately 40 cm³ beginning 45.1 cm downwind of the pheromone source and ending 111.8 cm downwind of the source. In all cases, we recorded the cockroaches' behavior at the standard frame rate for NTSC video of 30 frames s⁻¹.

We used a Peak event and video control unit (Peak Performance Technologies, Inc., Englewood, CO, USA) to synchronize the three cameras with smaller fields of view to enable the 3D reconstruction of individual antennal movements associated with plume tracking.

To determine the nature of the cockroaches' orientation movements with respect to the odor plume, we videorecorded titanium tetrachloride (TiCl₄) smoke plumes issuing from the same size and shape filter paper sources used to disperse pheromone during odor-tracking experiments. The time-averaged plume boundaries were determined by digitizing and tracking the paths of smoke packets using our motion analysis system. Using this method we determined both the time-averaged crosswind width of the plume and the maximum time-averaged height above the floor of the experimental arena at five sampling points at different distances from the source: 0 cm from the source, 38 cm downwind, 76 cm downwind, 114 cm downwind and 152 cm downwind. Since the smoke plumes and the cockroach orientation behavior were recorded in the same conditions, we were able to overlay the time-averaged plume outlines and the animals' tracks to determine

the association between the clean air–pheromone boundary and the cockroaches' tracking behavior.

Experimental design

We determined the characteristics of orientation with respect to the wind in walking *P. americana* males by recording their behavior when challenged with an experimental environment characterized by (1) no wind or odor, (2) 25 cm s⁻¹ wind with no odor, and (3) 25 cm s⁻¹ wind carrying a plume issuing from a point-source bearing 0.1 ng of (–)-periplanone-B. We released males from the center point of the experimental arena to make any orientation direction available to them. For the purpose of this analysis, we defined the orientation direction as the direction in which the males were walking when they left the field of view of the camera. We used 3–18 week old (Seelinger, 1985; Abed et al., 1993) sexually mature virgin male *P. americana* for this study, and placed them individually in cylindrical (3 cm tall × 10 cm diameter) aluminum screen release cages in the darkened wind tunnel room at the beginning of their scotophase, and left them to acclimate to room conditions for 2 h prior to beginning experiments. The release cage dimensions were important because males exhibited a reduced propensity to walk in the treatments in which (–)-periplanone-B was absent; pilot studies showed that shorter cages made it more likely that the males would leave the release point.

We generated four different plume structures by varying the size, shape and orientation of the pheromone source (Fig. 1). We generated the first plume structure by using a 0.7 cm diameter circular filter paper disk (Whatman No.1, Eastbourne, East Sussex, UK) held perpendicular to the airflow with an insect pin (Fig. 1A). We used this source size and shape to enable easier comparison to earlier studies of plume tracking behavior in flying moths (Cardé and Minks, 1997). [The majority of studies of moth flight orientation behavior have used plumes issuing from point-sources of pheromone that are either identical to (Willis and Cardé, 1990; Willis and Arbas, 1991; Charlton et al., 1993; Willis et al., 1994) or similar in size and shape to the point-source in these experiments.] The structure of the second plume treatment was generated by rotating the 0.7 filter paper disk 90°, so that the disk shape was parallel to air flow in the wind tunnel, resulting in a very narrow plume (modified after the 'ribbon plume' of Mafra-Neto and Cardé, 1994) (Fig. 1B). The third plume was meant to significantly increase the width of the plume while nearly maintaining its concentration; this was achieved by increasing the surface area of the source by ca. 25 times while also proportionally increasing the dosage of pheromone solution applied to the source. The source of this wide plume treatment was 14.3 cm wide × 0.7 cm tall (Fig. 1C). The fourth type of plume structure was generated by placing a Plexiglas cylinder (81.28 cm tall × 7.62 cm diameter) 5 cm upwind of the 0.7 cm diameter circular filter paper disk held perpendicular to airflow. This induces turbulence through vortex shedding, which dramatically alters the plume structure (Fig. 1D). We will refer to the treatments as point-source plume, ribbon plume, wide plume and cylinder plume, respectively, throughout this paper.

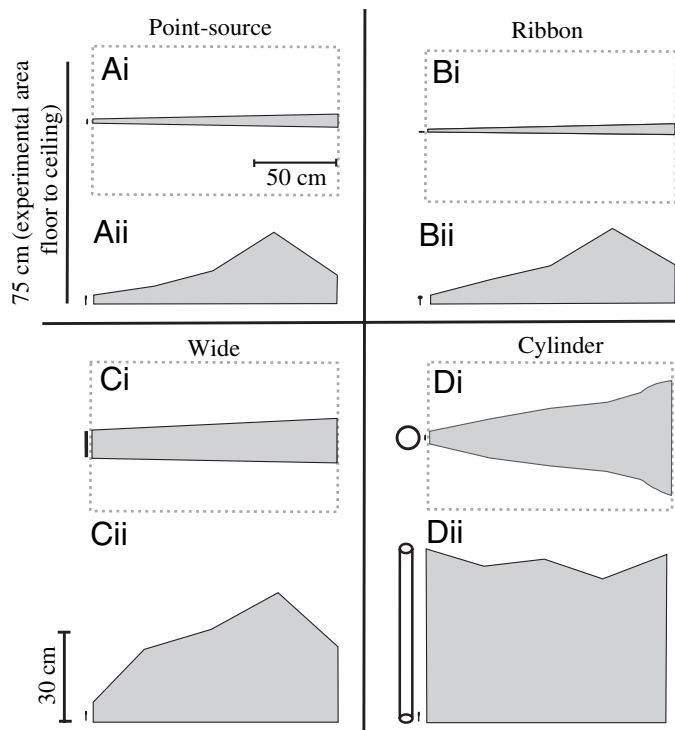


Fig. 1. (Ai–Di) Time-averaged plume boundaries of titanium tetrachloride smoke plume in 25 cm s^{-1} wind as viewed from above. (Ai) Point-source plume, 2.4 cm wide at the source, spreads to 7.7 cm wide at the downwind end. (Bi) Ribbon plume, 1.5 cm wide at the source, spreads to 6.1 cm wide at the downwind end. (Ci) Wide plume, 17 cm wide at the source, spreads to 26.5 cm at the downwind end. (Di) Cylinder plume, 7.6 cm wide at the source, spreads to 68.1 cm at the downwind end. In this figure, wind flows from left to right. Schematic illustrations of the pheromone sources are depicted at the upwind end of each plume envelope. Broken rectangles represent the boundaries of the experimental arena. (Aii–Dii) Vertical cross-section of time-averaged plume boundaries of titanium tetrachloride smoke plume in 25 cm s^{-1} wind. (Aii) Point-source plume, 2.8 cm high at the source, spreads to maximum of 22.4 cm and decreases to 8.4 cm at the downwind end. (Bii) Ribbon plume, 2.2 cm high at the source, spreads to maximum of 22.2 cm and decreases to 10.1 cm at the downwind end. (Cii) Wide plume, 5.6 cm high at the source, spreads to maximum of 38.4 cm and decreases to 22.4 cm high at the downwind end. (Dii) Cylinder plume, 56 cm high at the source (maximum), spreads to 54.3 cm at the downwind end.

In this experiment we placed the virgin *P. americana* males in larger release cages (13.5 cm tall \times 5.7 cm diameter) to hold them at the downwind end of the pheromone plume for a 1 min pre-release period. The larger cages allowed the males more range of movement during the acclimation period and while waiting to be released during an experimental trial. For each experimental day, we randomized the animals used according to their age and each trial according to the order of treatment presentation (each animal was used only once). We introduced *P. americana* males to the respective plumes beginning 2 h into their scotophase and challenged the cockroaches to respond to the appropriate experimental conditions.

Pheromone

We used the same filter paper loaded with 0.1 ng synthetic (–)-periplanone-B (Kitahara et al., 1987; Kuwahara and Mori 1990) for the point-source, ribbon and cylinder treatments. The wide plume treatment was dosed ten times with this same amount pipetted evenly across the surface of the filter paper, resulting in a source that bore 1.0 ng of pheromone on ca. 25 times the area of the filter paper disks used for the other treatments. This combination of size, shape and pheromone dosage resulted in filter paper disk sources that were 0.25 ng cm^{-2} and a wide filter paper source that was 0.1 ng cm^{-2} . Earlier observations by Tobin (1981) and pilot studies by us suggest that there is no observable difference in the pheromone response of *P. americana* males in the range of 10^{-6} – $10^{-3} \mu\text{g}$ of (–)-periplanone-B [(±)-periplanone-B in the case of Tobin, 1981].

Data analysis

We digitized the walking paths of male cockroaches tracking pheromone plumes every 83 ms using a computerized motion analysis system (Peak Motus 7.1™, Peak Performance Technologies, Inc.). The Motus software separates each 1/30th s video frame into the two 1/60th s video fields that it comprises. Therefore, the maximum temporal resolution from an NTSC video recording is 1/60th s or 16.7 ms per sample. Thus, we digitized each 5th position of the cockroach during its response to the experimental treatments.

In the large field of view camera we digitized two points on the cockroach: the center of the head and the distal tip of the abdomen. In the three smaller field-of-view cameras we digitized four points on each animal: the distal tip of each antenna, the center of the head and the distal tip of the abdomen. In all cases the movement tracks that were quantified and presented in the figures were the path of the digitized point on the head.

The response variables we measured from the video-recorded cockroach tracks were: track angle (orientation of the movement vector from one cockroach position to the next with respect to the wind direction; due upwind is 0° by convention), track width (distance between turn apices measured perpendicular to the wind direction, as per Kuenen and Baker, 1982), groundspeed (cockroaches' walking speed measured from point to point along its track), inter-turn duration (time between the apices of sequential turns), and net velocity (net speed from the beginning to the end of each walking track). For the purposes of our analysis a turn was the location at which the head reached a local maximum or minimum value with respect to the lateral frame of reference of the wind tunnel.

We also quantified the stopping behavior of males in different environmental conditions and the four plumes used in these experiments. The number and duration of stops made by each individual was recorded and grand means for each treatment group were compared in a manner similar to the rest of the experimental variables. For the purposes of this study an animal was said to be stopped if there was no movement between two sequential positions of the head point.

In addition we measured finer-scale kinematic variables including: body yaw angle (angle of the longitudinal body axis as defined by a line drawn between the head and the distal tip of the abdomen, with respect to the wind direction), body pitch angle (angle of the body axis with respect to the floor), inter-antennal distance (distance between the distal tips of the antennae in three dimensions), and the height of the antenna above the floor (measured from the distal tips of the antennae to the floor). Most of these parameters, aside from those describing the kinematics of the antennae, have been routinely measured from the performances of flying moths tracking pheromone plumes (Kuenen and Baker, 1982; Willis and Arbas, 1991; Arbas et al., 1993).

The experiment was designed as a randomized complete block design, with each experimental manipulation as a treatment, and a complete group of treatments was performed as a block each day. These data were analyzed using a general linear model (i.e. proc GLM) in SAS (ver. 8.2) to perform an analysis of variance (ANOVA) for a randomized complete block design. Treatments and individuals were randomized daily. Approximately five individuals were exposed to the first treatment, the treatment was then switched and the next five individuals were introduced sequentially, and so on. When an ANOVA revealed significant effects in the experiment, we applied a Tukey multiple comparison test to determine which track and kinematic variables differed significantly among the experimental treatments at the 0.05 ($P < 0.05$) level.

According to our analysis there was an unexpected significant day effect associated with certain response variables in our model. This day effect was associated with only some of the track parameters measured (in the 'wind plus pheromone' experiment: track angle, body axis and inter-turn duration; in the 'plume structure' experiment: net velocity, groundspeed and inter-turn duration) and the effect was observed on different days for each parameter, therefore a single day could not be eliminated from the experimental design. Because of this unexpected effect we plotted each track parameter by day and treatment to determine any obvious patterns associated with days or order of treatment presentation. The means of the variables associated with the cylinder treatment stood out on all days and for this reason we removed it from the analysis and repeated the analysis with only the ribbon, point-source and wide treatments. The result of this analysis was that there is no effect of the order of treatment presentation, and no predictable pattern in day differences among variables. Thus, the cylinder plume treatment appeared to be the main source of the treatment effect in the ANOVA results and we concluded that the statistically significant day and day-by-treatment effects that we observed were caused by incomplete randomization in the treatment-individual groupings (Pilla et al., in press).

Results

P. americana males responded to the conditions in our experimental arena by exhibiting odor-modulated anemotaxis. When released in the experimental arena in zero wind and zero

odor, the males showed no preferred walking orientation (Fig. 2A). The mean direction of walking orientation was 67.5° , and this orientation was not significantly different from a random distribution according to Rayleigh's test ($z=0.46$, $P > 0.05$; Fisher, 2000). When presented with clean air moving at 25 cm s^{-1} , 79% of male cockroaches showed a negative anemotactic response by turning and walking away from the source of the wind (Fig. 2B). The mean direction of orientation for this group was 169° and this orientation was significantly different from a random distribution according to Rayleigh's test ($z=8.52$, $P < 0.05$; Fisher, 2000). The mean track angles measured from the cockroaches' walking paths were significantly greater when walking in wind with no pheromone compared to those walking in no wind and no pheromone,

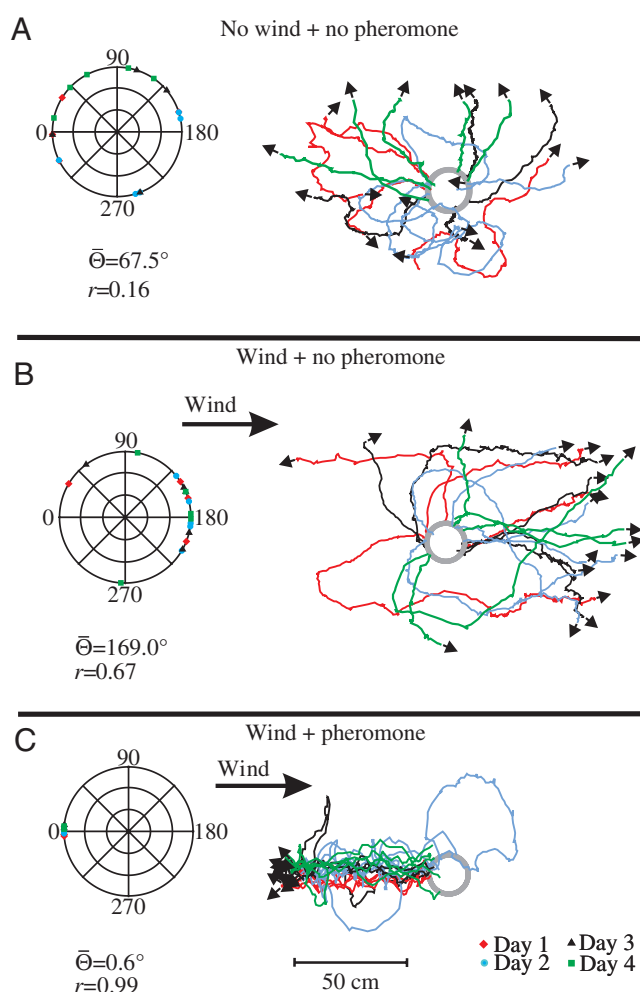


Fig. 2. Walking orientation of *P. americana* males in (A) No wind + no pheromone, (B) wind + no pheromone and (C) wind + point-source pheromone. Arrowheads indicate the orientation of the cockroach at the end of the track in laboratory wind tunnel. The circle represents the release cage. Vanishing direction (angle from release point to point where the cockroach leaves the field of view of the camera) is displayed in the angular histogram to the left with the mean resultant length (r) associated with the mean direction ($\bar{\Theta}$). Day 1, 17/4/03; Day 2, 22/4/03; Day 3, 22/4/03; Day 4, 7/5/03.

Table 1. Track parameters measured from *P. americana* males walking in a laboratory wind tunnel while exposed to (1) no wind + no pheromone, (2) wind + no pheromone and (3) wind + pheromone plume from a 0.1 ng point source

Treatment	N	Net velocity (cm s ⁻¹)	Groundspeed (cm s ⁻¹)	Track angle* (degrees)	Body axis* (degrees)	Track width (cm)	Inter-turn duration (s)	Stop duration (s)	Number of stops [†]
NoW+NoP	18	4.3±4.7 ^b	11.5±6.8 ^a	86.0±26.2 ^b	84.5±43.7 ^b	6.6±3.7 ^a	1.69±1.88 ^a	0.15±0.05 ^a	5.0±4.3 ^a (16)
W+NoP	19	7.0±3.9 ^{a,b}	11.1±4.2 ^a	118.1±29.5 ^a	132.1±36.2 ^a	5.6±8.5 ^a	1.53±2.45 ^a	0.16±0.10 ^a	4.2±3.9 ^a (19)
W+P	18	9.6±4.0 ^a	13.5±3.2 ^a	44.0±15.2 ^c	39.0±45.7 ^c	4.5±2.4 ^a	0.74±0.31 ^b	0.13±0.06 ^a	2.2±2.4 ^a (13)

NoW+NoP, no wind + no pheromone; W+NoP, 25 cm s⁻¹ wind + no pheromone; W+P, 25 cm s⁻¹ wind + pheromone plume from a 0.1 ng point source.

Values (mean ± s.d.) in the same column with the same letters do not differ significantly according to a two-way ANOVA ($P < 0.05$) and a *post hoc* Tukey test.

*All angles depicted are absolute values of the distributions measured.

[†]The number in parentheses indicates the subset of individuals that stopped while tracking.

indicating downwind movement (Table 1). Conversely, when presented with identical wind conditions and an olfactory stimulus consisting of a plume of (–)-periplanone-B, 100% of the males turned into the wind and walked upwind while tracking the plume to its source (Fig. 1C). The mean direction of orientation was 0.6° and this orientation was significantly different from a random distribution according to Rayleigh's test ($z = 17.64$, $P < 0.05$; Fisher, 2000). The mean of track angles measured from the movement paths of males in wind and pheromone was significantly smaller than the other two treatments (Table 1). Thus, the presence of an attractive odor caused most of these males to reverse their preferred orientation to the wind direction.

The yaw angle of the cockroaches' body showed similar changes in the three treatments of this experiment and were similarly significantly different according to the changes from no wind, to wind, to wind plus pheromone (Table 1). The addition of wind and pheromone to the environment did not significantly affect the groundspeed at which the cockroaches walked, nor the overall width of their tracks (Table 1). However, the net velocity or speed from the point of release to the point at which the cockroach left the field of view of the camera increased significantly and incrementally as wind, and then wind and pheromone, were added to the environment (Table 1). By referring to the movement tracks (Fig. 2), it is clear that this increase in net velocity is a reflection of the increased 'directedness' or polarization of the cockroaches' orientation once the wind and then the pheromone were added to the environment. The duration of the inter-turn intervals was significantly affected by the addition of pheromone but not by the addition of wind to the environment (Table 1). The inter-turn duration of males tracking a pheromone plume upwind was significantly less than males walking in no wind or wind of 25 cm s⁻¹. There were no statistically significant differences in the average number of stops made by males in either wind, no wind or wind plus pheromone conditions (Table 1). Likewise, there were no significant differences in the durations of stops in any of the experimental conditions. The similarity in the stopping durations suggests that the stops we observed during walking orientation may be internally controlled sensory sampling or information processing periods.

The time-averaged boundaries of smoke plume visualizations of our four experimental plumes, as viewed from overhead, are illustrated in Fig. 1. Since the dimensions, shape and material making up the source of the point-source plume is the same as used in many previously published studies of odor-tracking behavior in flying moths, it is particularly important for comparison (Fig. 1A; Charlton et al., 1993). In these experiments the point-source TiCl₄ smoke plume was 2.4 cm wide at the source and reached 7.7 cm wide at the downwind end of the experimental arena. (Smoke plume measurements show that the same point-source generates a plume 1.7 cm wide at the source and 6.2 cm at the downwind end of the wind tunnel at the 100 cm s⁻¹ wind speed often used in moth flight experiments; Willis and Arbas, 1991.) The time-averaged dimensions of the smoke plume issuing from the ribbon source started narrower, at 1.5 cm, and remained narrower, at 6.1 cm, for the length of the experimental arena, than all other plumes tested (Fig. 1B). At 17 cm, the time-averaged dimensions of the plume issuing from the wide-source was ca. 7 times as wide as the point-source plume at the source and expanded to 26.5 cm by the time it reached the downwind end of the experimental arena (Fig. 1C). As expected, the widest and most turbulent smoke plume issued from the point-source positioned immediately downwind from a 7.6 cm diameter Plexiglas cylinder (Fig. 1D). The plume downwind of the cylinder was a turbulent wake of alternating vortices known as a vortex street (Vogel, 1994). These vortices typically extended 81 cm from the floor of the experimental arena to the top of the cylinder. According to Reynolds number and Strouhal numbers determined for the experimental conditions in our wind tunnel, the vortex shedding frequency for the cylinder should be ca. 0.6 vortices s⁻¹ (Vogel, 1994). The time-averaged heights above the floor of the experimental arena that each plume treatment extended to are also illustrated in Fig. 1. The plume downwind of the cylinder extended the furthest above the floor because the low pressure area immediately downwind of the cylinder drew the smoke from the floor to the top of the cylinder, and thus generated the widest and tallest plume used in this experiment. The smoke plumes issuing from the other sources increased in width and height as they moved downwind away from the source. In each

of the point-source, ribbon and wide plumes the height above the arena floor decreased at the downwind end of the arena. This decrease was the result of the close proximity of the exhaust, which removed the plumes from the wind tunnel room.

The behavioral responses of *P. americana* males to these four different types of plumes are illustrated in Fig. 3. The three tracks in each panel of Fig. 3 illustrate the range of variability observed from our experimental population: one track with the least turns (top track in each panel), one track with the most turns (bottom track in each panel) and one track representing a typical response by males to that treatment (central track in each panel). In general, the males tracking the most turbulent plume, generated downwind of the cylinder (Fig. 3D), had tracks whose response variables were statistically different from the other three plume treatments, and males tracking the point-source (Fig. 3A), ribbon (Fig. 3B) and wide plumes (Fig. 3C) generated tracks whose response variables were statistically the same (Table 2).

Males that tracked the plumes generated downwind of the cylinder walked significantly slower, on average, than males tracking any of the other plume treatments (Table 2). The groundspeeds at which males walked up the other three pheromone plume treatments were nearly the same (Table 2). Average net velocity measured from the males' performances showed a similar pattern (Table 2). The average track angles of males walking up the cylinder plume were significantly greater than those steered by males in any of the other plume treatments (Table 2). The explanation for these large track angles is illustrated in Fig. 3, which reveals that males tracking the cylinder plume generated many more turns and steered their tracks at angles much further off of the wind direction than most males in the other treatments. Track angles steered by males in ribbon plumes were significantly smaller, on average, than males tracking point-source plumes, with males tracking wide plumes generating mean track angles that were not significantly different from either (Table 2). Males walking upwind in the plumes generated downwind of the cylinder steered the yaw angles of their bodies significantly more off of the wind direction, on average, than males tracking the other plume treatments (Table 2). There were no statistically significant differences in the orientation of the yaw angle of the body amongst males tracking any of the other plumes (Table 2). The mean orientations of the yaw angles of males tracking the four plume treatments in our experiment were not equal to their mean track angle (Table 2); that is, on average, the direction that the males aimed their bodies was not the same

direction as the vector along which they moved (Fig. 3). In all cases the orientation of the body's yaw angle was, on average, oriented more directly into the wind than the resulting walking track. This sort of steering is also typical of male moths tracking female pheromones in flight (Arbas et al., 1993; Willis and Arbas, 1997).

Males tracking the four different plumes showed a similar pattern in their stopping behavior as observed in the other response variables (Table 2). There were no statistically significant differences in the number of stops observed from males tracking point-source, ribbon or wide plumes while those tracking the cylinder plumes stopped significantly more than males in any of the other treatments. There were no statistically significant differences in the duration of stops in any of the different plume treatments or in the treatments of the wind plus pheromone experiment (Tables 1, 2).

The temporal measurements of the pheromone tracking response showed trends similar to the parameters quantifying

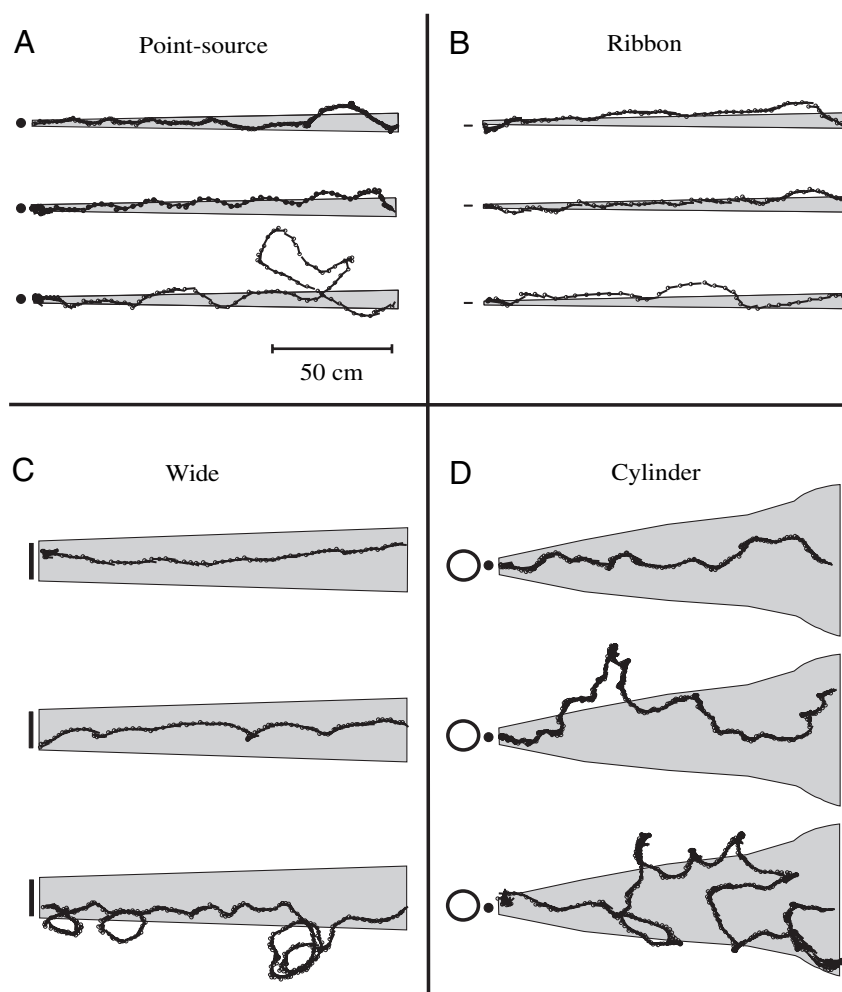


Fig. 3. Examples of *P. americana* males tracking female pheromone upwind (direction right to left) in a wind tunnel illustrated with the time-averaged plume boundaries of titanium tetrachloride smoke visualization of the plumes as they track in 25 cm s^{-1} wind. Each circle represents the body position every 0.083 s. Details of the time-averaged plume envelopes as per Fig. 1.

Table 2. Track parameters measured from *P. americana* males walking upwind in plumes of female sex pheromone issuing from sources of different sizes and shapes in a laboratory wind tunnel

Treatment	<i>N</i>	Net velocity (cm s ⁻¹)	Groundspeed (cm s ⁻¹)	Track angle* (degrees)	Body axis* (degrees)	Track width (cm)	Inter-turn duration (s)	Stop duration (s)	Number of stops†
Point source	20	19.0±6.3 ^a	23.1±6.9 ^a	36.7±17.5 ^b	24.1±11.8 ^b	4.1±2.0 ^{b,c}	0.58±0.22 ^{b,c}	0.10±0.03 ^a	1.3±3.9 ^b (8)
Ribbon	21	19.5±8.2 ^a	23.0±6.6 ^a	27.3±10.6 ^c	18.2±10.7 ^b	2.9±1.5 ^c	0.49±0.18 ^c	0.12±0.06 ^a	0.5±1.0 ^b (12)
Wide	23	20.3±5.7 ^a	24.0±5.0 ^a	30.7±10.9 ^{b,c}	21.3±9.9 ^b	4.8±1.9 ^b	0.68±0.25 ^b	0.12±0.01 ^a	1.0±1.3 ^b (6)
Cylinder	22	6.6±3.3 ^b	13.5±4.5 ^b	63.2±11.4 ^a	57.7±28.3 ^a	8.0±3.2 ^a	1.22±0.53 ^a	0.15±0.09 ^a	9.8±8.1 ^a (22)

Values (mean ± s.d.) in the same column with the same letters do not differ significantly according to a two-way ANOVA ($P < 0.05$) and *post hoc* Tukey test.

*All angles depicted are absolute values of the distributions measured.

†The number in parentheses indicates the subset of individuals that stopped while tracking.

their speed and steering. The inter-turn durations measured from the responses of males tracking cylinder plumes were significantly longer than males tracking any of the other plume treatments (Table 2). The next longest were those measured from males tracking wide plumes, but these were ca. half the duration of males tracking plumes issuing from the cylinder (Table 2). Inter-turn durations of males tracking the wide plume were significantly greater than males tracking the ribbon plume, and the inter-turn durations of males tracking the point-source plume were not significantly different from either (Table 2).

It should be noted that the average groundspeeds exhibited by males tracking point-source plumes in the plume structure experiment and those of males tracking the point-source plume in the wind plus pheromone experiment were not as similar as one might expect. Even though the wind speed, pheromone source and plume structure were the same across these two experiments we observed the performance of the cockroaches in two different parts of the experimental arena and for different total distances and times. The male responses in the plume structure experiment were measured and averaged over the length of the entire experimental arena. In the wind and pheromone experiment the males were released at the lateral and longitudinal mid-point of the arena. These differences in the males' environment result in changes in their behavior. Males in the wind plus pheromone experiment were released ca. 75 cm closer to the pheromone source than those in the plume structure experiment. This would result in these males experiencing both different plume structure and a higher mean pheromone concentration than that present at the downwind end of the arena.

When we broke down the longer plume structure tracks lengthwise into three ca. 50 cm long sections (e.g. downwind, middle and upwind) and compared the average groundspeeds, we found that the males walked upwind more slowly in the area near the release point and as they approached the pheromone source, while they walked faster inbetween these two areas (data not shown). Thus, in the wind plus pheromone experiment we focused on the area near the release point where the males were walking more slowly.

On average, the antennae of *P. americana* are longer than the body length from head to the tip of the abdomen (antenna

length, 4.8±0.3 cm; body length, 3.9±0.1 cm; means ± s.d.; $N=5$ individuals, i.e. 10 antennae). Since the antennae are the cockroaches' primary olfactory structure it was critical for the interpretation of the turning maneuvers observed to know where the antennae were during the odor-tracking performances. Our video recordings sub-sampled the plume tracking arena at higher spatial resolution to measure the position and angular orientation of the antennae with respect to the environment and the cockroaches' body in three dimensions. Fig. 4 shows examples of *P. americana* males tracking plumes from a point-source and a wide-source, with 'exploded' views depicting the tracks of the tips of their antennae along with the body. This figure suggests at least two possible types of steering maneuvers executed by males during odor tracking: broad, gradual turns with continuous upwind progress (Fig. 4A), and temporally brief sharp turns, during which little or no upwind progress is made (Fig. 4B). By tracking the positions of the tips of the antennae (broken lines in Fig. 4) it can be seen that an 'envelope' of space much wider than the body is scanned for odor information. Furthermore, the distance between the tips of the antennae is held in a narrow range between 5 and 6 cm apart during pheromone tracking behavior, and this same limited range of inter-antennal distances is held by males tracking plumes from all four sources ($P > 0.05$; Table 3). Thus, males maintaining their inter-antennal distances at or near the observed average values could theoretically walk straight upwind in point-source and ribbon plumes with the tips of their antennae protruding beyond the time-averaged plume boundaries into clean air for almost the entire length of the experimental arena. Such a scenario would have been impossible in the wide plume. Unless the cockroach approached close to the edge of the plume boundary (e.g. Fig. 4B), it could have tracked the full length of the wide plume while its body and antennae were completely imbedded in the pheromone plume. Likewise, in any of the four plume types, the height of the time-averaged plume envelope above the floor was always great enough that the probability of the tips of the antennae encountering the clean air-pheromone boundaries above them would have been very low (Table 3). In addition, there were no statistically significant differences in the average height at which the males held their antennae across the four different plume treatments (Table 3). Another way that males

tracking the plumes could change the height of their antennae above the floor, while maintaining a stereotyped antennal posture, would be to alter the pitch angle of their body with respect to the floor (Fig. 5). By walking upwind while holding their heads higher above the floor, their antennae would consequently also be held further above the floor. However, the males tracking the plume downwind from the cylinder were the only individuals that showed behavior statistically different from the rest (Table 3).

Similar measurements were made describing the antennal behavior of males orienting in different wind and pheromone conditions, with similar results (Table 4). As with males orienting to different plumes, males in the different wind and

pheromone conditions showed essentially the same inter-antennal distances, with almost the same average maximum and minimum angles displayed by the males tracking the four different plumes (Tables 3, 4). The small differences between the mean inter-antennal distances of the males in wind and pheromone (Table 4), and those generated by males tracking plumes (Table 3), could be the result of the shorter length of track measured in the orientation experiment (due to the smaller field of view recorded) and the position of the recording area in the larger experimental arena (see discussion of differences between these experiments, above). We expected to observe greater differences between males in zero wind and zero pheromone and males experiencing environmental conditions including wind and odor, but instead found their antennal behaviors to be broadly similar both in inter-antennal distance and height above the floor (Table 4). The mean pitch angles of the body adopted by males orienting to wind alone were significantly less compared to males orienting to wind and pheromone and zero wind and zero pheromone (Table 4). Further fine-scaled analysis of the association of specific antennal postures and movements, tracking behavior and plume structure from this data set is in preparation and will be published separately.

Discussion

Odor-modulated orientation to wind

The results presented here show that freely walking *P. americana* males orient to wind-borne odors by modulating their orientation to wind while in contact with an attractive odor. In zero wind, males show no preferred walking orientation. When the wind speed was increased from zero to 25 cm s^{-1} , most males exhibited a negative anemotaxis, by turning and walking with the wind. Upon addition of (–)-periplanone-B, all of the males changed their orientation with respect to the wind by ca. 180° and exhibited a positive anemotaxis, walking upwind in the pheromone plume (Fig. 2).

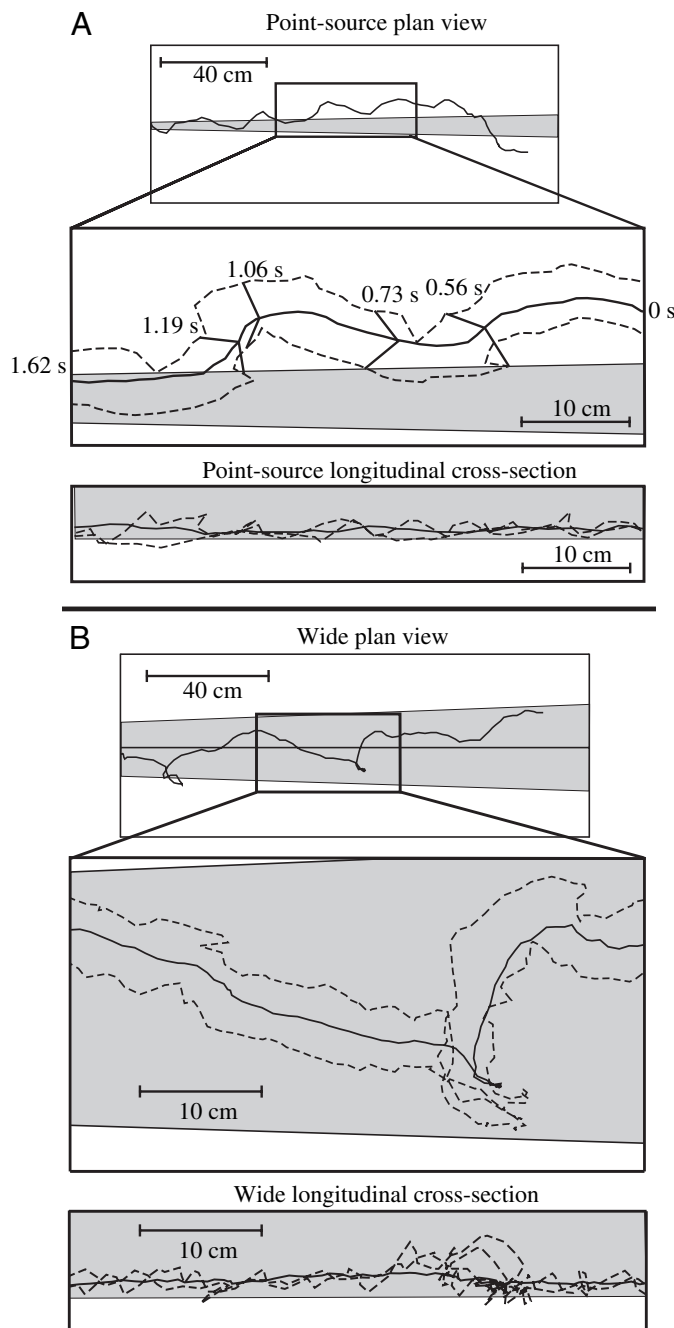


Fig. 4. Time-averaged plume boundaries of point-source and wide plumes with the track of the cockroach head (solid line) and the tips of the left and right antennae (broken lines). (A) Upwind track of a *P. americana* male in a point-source plume. Shallow v-shaped lines indicate the position of the left and right antennae and the head position at important points along the track. The timing of these points, with respect to the zero time at which the male entered the field of view of the camera, is indicated in association with each antennal position. The time signatures represent: 0.56 s, contact of the left antenna with the time-averaged plume boundaries; 0.73 s, loss of contact between plume and the left antenna; 1.06 s, initiation of left turn; 1.19 s, re-contact between the left antenna and the time-averaged plume. (B) Upwind track of a *P. americana* male in a wide plume. In each example the upper box represents the overhead view of the track. An exploded view of the indicated short section of the track shows the cockroach head (solid line) and tips of the left and right antennae (broken lines). Below is the exploded view from the side. Cockroaches are traveling upwind from right to left. Details as per Figs 1 and 3.

Table 3. Kinematic variables depicting the angle of the body and the position of the antennae with respect to each other and the environment of male *P. americana* tracking female sex pheromone from four different sources in a wind tunnel at 25 cm s⁻¹

Treatment	N	Descriptive statistic	Interantennal distance (cm)	Antennal height* (cm)	Pitch angle (degrees)
Point source	10	Mean	5.7±0.5 ^a	1.2±0.2 ^a	4.3±2.7 ^a
		Max	8.4	3.5	20.4
		Min	1.4	0.2	-9.5
Ribbon	11	Mean	5.1±0.7 ^a	1.3±0.2 ^a	4.1±2.2 ^{a,b}
		Max	7.7	3.6	44.1
		Min	2.2	0.1	-8.2
Wide	10	Mean	5.3±0.5 ^a	1.3±0.2 ^a	3.9±1.5 ^{a,b}
		Max	8.1	4.0	45.0
		Min	2.0	0.1	-7.8
Cylinder	10	Mean	5.4±0.7 ^a	1.1±0.2 ^a	1.1±1.9 ^b
		Max	10.0	4.4	45.8
		Min	1.4	0.1	-25.2

Values (mean ± s.d., maxima and minima) in the same column with the same letters do not differ significantly according to a two-way ANOVA ($P < 0.05$) followed by a *post hoc* Tukey test.

*Antennal height is measured with respect to the surface of experimental area.

A similar mechanism accounts for much of the upwind response of flying male moths tracking plumes of female sex pheromone (Kennedy and Marsh, 1974; Kennedy, 1983; Baker et al., 1984).

Previous work on the orientation of walking with respect to the wind direction showed that *P. americana* males walking on a locomotion compensator preferentially turned into the wind and walked upwind at wind speeds below 20 cm s⁻¹ (Bell and Kramer, 1979). When the wind speed increased above 20 cm s⁻¹ they turned and walked with the wind direction, downwind (Bell and Kramer, 1979). These results demonstrated that walking cockroaches show a positive or negative anemotactic response depending on the wind speed. Our experimental design did not include exposure to wind speeds between zero and 25 cm s⁻¹, so our results cannot speak to this earlier observation. As in our experiments, when female sex pheromone was added to the air stream the male cockroaches exhibited an odor-mediated change in their orientation preferences, changing from orienting with the wind direction to orienting into the wind (Bell and Kramer, 1980). Thus, the results of our experiments with freely walking cockroaches support and extend the results of the earlier work using restrained preparations.

One important difference between our results and the earlier experiments studying cockroaches and the small beetle *Trogoderma variable*, is that insects in these earlier experiments oriented to wind and odor on a servosphere apparatus by walking upwind at an angle to the wind (Bell and Kramer, 1980; Tobin and Bell, 1986). In neither case was a stereotyped orientation angle steered with respect to the wind. However, each individual's mean steering angle was consistent across trials. *P. americana* males in our experiments showed no such tendency for off-axis orientation to the wind, and in most cases showed an overall trend to walk directly upwind

while in the pheromone plume (Figs 2, 3). One obvious explanation for this difference in behavior is the different arenas used in these experiments. Males walking in our laboratory wind tunnel move upwind through a visual environment consisting of a variety of objects (e.g. camera mounts, infra-red lights, structural components of the wind tunnel, etc.) oriented at variable angles with respect to their horizon. Most diagrams and photographs of servosphere locomotion compensators show what appears to be a uniformly sparse visual environment (Tobin and Bell, 1986; Kramer, 1976). In addition, these experiments are typically described as being performed in total darkness (Tobin and Bell, 1986).

It has been demonstrated that *P. americana* orient their walking orientation with respect to visual landmarks (Mizunami et al., 1998) and that the addition of fixed visual cues to studies of the walking orientation of female crickets to auditory stimuli (male calling song) on servosphere locomotion compensators resulted in tracks that were oriented more directly toward the sound source (Weber, 1990; Böhm et al., 1991). Thus, steering with respect to the visual surroundings may explain some of the differences in behavioral observations of odor-modulated upwind walking in *P. americana* between studies using locomotion compensators and our wind tunnel, but this awaits future experimentation.

Effects of plume structure on olfactory orientation behavior

The responses of *P. americana* males to the different plume structures presented in our experiments can be placed in two general categories: one comprising the performances of males responding to the ribbon, point- and wide-source and the other comprising those responding to the cylinder plume (Table 2, Fig. 3). Some individuals responding to the ribbon, point- and wide-source plumes walked upwind in the plume, generating tracks that often had segments aimed nearly directly upwind in

the plume, while the tracks of others showed ‘moth-like’ examples of temporally regular zigzagging counterturns (Fig. 3). A few individuals showed examples of relatively sharp ‘turns-back’ at or near the time-averaged boundaries of the odor plume that could be interpreted as being caused by encounters with the abrupt clean air–pheromone edge at the lateral margins of the plume (Figs 3, 4). The males responding to plumes in the turbulent wake of the cylinder also generated tracks with segments of zigzagging turning made up of temporally regular counterturns together with irregular sharp rapid turns, suggesting turning back at a distinct clean air–pheromone edge. However, because of the highly turbulent nature of the cylinder’s wake and the possibility that the males responded to the large turbulent eddies as apparent shifts in wind direction, the responses of males tracking the cylinder plumes were significantly more variable than those tracking any of the other plume treatments (Fig. 3).

If our *P. americana* males had behaved according to our working hypothesis, their plume tracking paths should have increased in overall width as the plumes that they were tracking increased in width. In a limited sense that is what we found (Table 2), but our results are not consistent with those published previously (Tobin, 1981). By adding the ribbon and cylinder plume treatments to our experiment we challenged our males to track plumes that were both narrower and wider than those used by Tobin (1981). The ribbon plume could have elicited narrower orientation tracks for at least two reasons. First, the source of the ribbon plume was so narrow (0.5 mm) that no turbulent plume was generated (our TiCl_4 smoke plume observations confirmed this – no turbulent eddies were generated downwind of the ribbon plume source). This lack of turbulence meant less mixing between the pheromone-bearing ribbon and clean air, resulting in a plume that was at least slightly more concentrated. The plume’s narrow cross section also means that, if the same number of molecules evaporated off the ribbon source as evaporated off of the point-source, those molecules were now occupying a smaller volume, resulting in a higher concentration. Increased odor concentration has been shown to cause male moths tracking plumes in flight to generate narrower tracks than those tracking plumes of relatively lower concentrations (Kuenen and Baker, 1982; Charlton et al., 1993). Thus, it is possible that the higher pheromone concentration experienced by the males tracking the ribbon plume could have affected the overall width of their walking tracks. In only one case have different source concentrations of pheromone been presented to walking cockroaches, and in that study *P. americana* males generated straighter tracks while walking upwind in plumes issuing from higher concentration sources on a locomotion compensator (Bell and Kramer, 1980). The second point to consider is that the width of the time-averaged plume envelope of the ribbon plume would have allowed a male positioned near the plume’s centerline to walk upwind with at least the distal parts of both antennae extending through the lateral margins of the plume into clean air (Fig. 3, Table 3). Thus, if *P. americana* males can make spatial comparisons between their two antennae,

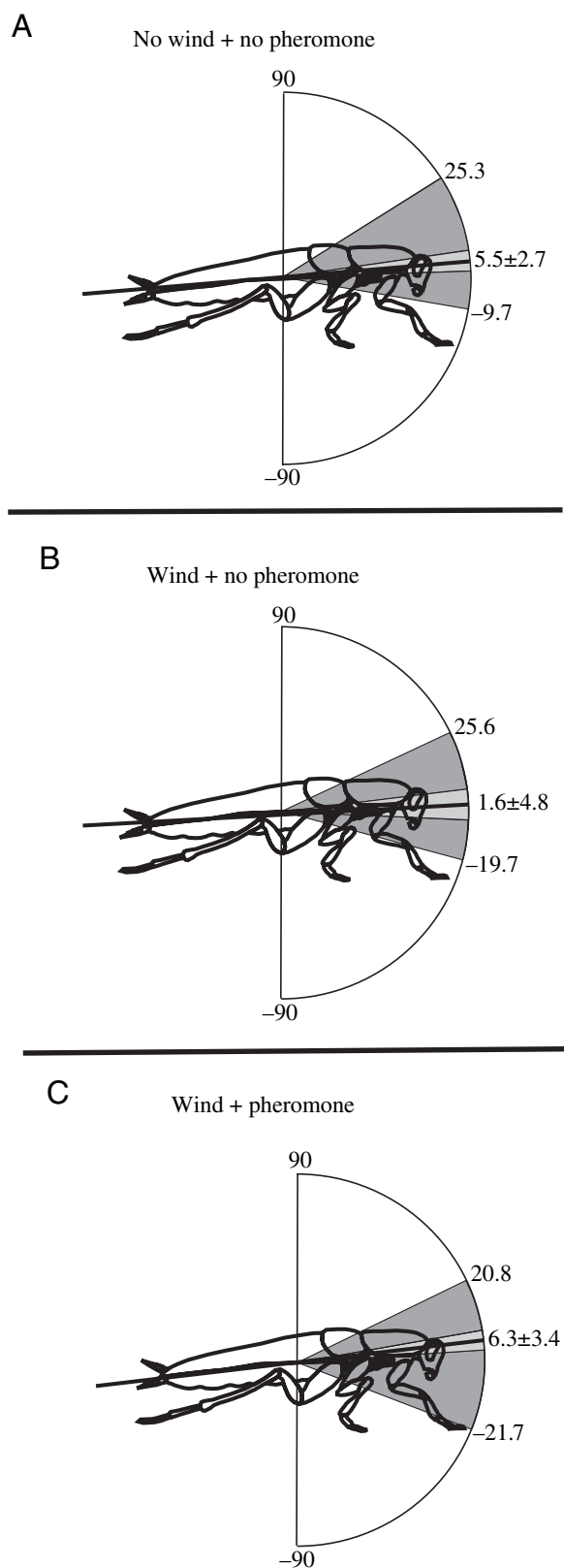


Fig. 5. Body pitch angle of *P. americana* walking in (A) no wind + no pheromone, (B) wind + no pheromone and (C) wind + pheromone. The black line and light gray shaded area indicate the mean \pm S.D. The dark gray shaded area represents the maximum and minimum pitch angles reached out of the entire experimental population.

Table 4. Kinematic variables depicting the angle of the body and the position of the antennae with respect to each other and the environment of male *P. americana* in (1) no wind and no pheromone, (2) wind and no pheromone and (3) wind and pheromone

Treatment*	N	Descriptive statistic	Interantennal distance (cm)	Antennal height [†] (cm)	Pitch angle (degrees)
NoW+NoP	10	Mean	4.6±0.6 ^a	1.9±0.6 ^a	5.5±2.7 ^a
		Max	7.6	4.6	25.3
		Min	1.7	0.1	-9.7
W+NoP	11	Mean	4.5±0.8 ^a	1.7±0.7 ^a	1.6±4.8 ^b
		Max	7.9	5.9	25.6
		Min	1.3	0.2	-19.7
W+P	10	Mean	4.9±0.6 ^a	2.7±1.2 ^a	6.3±3.4 ^a
		Max	8.0	7.9	20.8
		Min	1.2	0.4	-21.7

NoW+NoP, no wind + no pheromone; W+NoP, wind + no pheromone; W+P, wind + pheromone.
 Values (mean ± s.d., maxima and minima) in the same column with the same letters do not differ significantly according to a two-way ANOVA ($P < 0.05$) and *post hoc* Tukey test.
 *Pheromone source is 0.7 cm diameter filter paper held perpendicular to the wind.
 †Antennal height is measured with respect to the surface of experimental area.

these males could have been able to position themselves in the plume and maintain upwind progress with few or only minor turning maneuvers. Visual inspection of the significantly narrower movement paths (Fig. 3), and inter-antennal distances that span the time-averaged plume envelope (Tables 2, 4) support this idea. Of course both of the above mechanisms, or others, could have influenced the cockroaches' steering maneuvers simultaneously.

The biggest difference between the results presented here and the earlier results (Tobin, 1981) was observed in the comparison between the male responses to our point-source and wide plumes. The turbulent structure of these two plumes should have been very similar because the wind speed in these experiments was the same, and the diameter of the point-source was equal to the height of the wide-source. The combination of these facts should have resulted in the shedding of turbulent vortices in the same range of sizes and frequencies. Our direct visual observations of TiCl_4 smoke plumes support this. Thus, with the turbulent structure and the amounts of pheromone/area of these two sources being very similar, the main difference between these two treatments was the width of the plume. According to our working hypothesis and the previously published data (Tobin, 1981), this should have resulted in males generating orientation tracks with substantially different structures. However, this was not the case. Whether comparing the quantitative measurements (Table 2) or visually assessing the qualitative aspects of the tracking performance (Fig. 3), it is clear that the responses of *P. americana* males to the point-source and wide plumes under our experimental conditions were essentially the same. Thus, we must conclude that the abrupt spatial and rapid temporal decrease in pheromone concentration at the lateral time-averaged plume boundaries did not account for the width of the walking tracks we observed from males tracking the wide plume to the source. However, it is certainly possible that males responding to the point-

source plume could have tracked the plume in a manner similar to that proposed above for the ribbon plume, by spatial comparison of the distal tips of the antenna projecting beyond the time-averaged plume boundaries.

One must also remember that the turbulent nature of the pheromone plume means that males tracking all but the ribbon plume would have been experiencing a rapid and continuous stream of pheromone onsets and offsets; truly an intermittent pheromone stimulation. Furthermore, the time-averaged plume boundaries are an artifact of sampling and plume statistics and a fixed line between pheromone and clean air does not exist. However, the relative difference, both spatially and temporally, between offset and onset of pheromone at the plume boundaries would be expected to be greater than those experienced within the plume envelope.

Antennal structure and behavior

The ability of *P. americana* to utilize the spatial distribution of odors to control its steering maneuvers and orientation is critical to our understanding of this behavior. It is clear that the long, maneuverable antennae of these animals could provide information on the spatial distribution of odor as much as a body length away from the head on both sides. In earlier work it was not known whether *P. americana* males averaged the chemical information detected across the whole antennae, or if they could detect odor stimuli impinging on specific segments along the length of the antennae (Tobin, 1981).

While it is still not known whether the sensory processing centers in the brain of *P. americana* average odor information across the entire length of its antennae, it is known that there is a substrate in the central nervous system that could enable the cockroaches to determine where along the length of their antenna an odor stimulus exists. The antennal lobes of male cockroaches *P. americana*, and moths *Manduca sexta*, have been shown to possess projection neurons that receive inputs

from olfactory receptor cells from spatially distinct locations along the length of the antennae (Hösl, 1990; Heinbockel and Hildebrand, 1998). The response properties of these neurons indicate that the higher centers of the brains of male cockroaches receive information that should enable them to discriminate between odor contacts made at the proximal end, the middle and the distal tip of their antennae (Hösl, 1990). This system could have provided the information on the spatial distribution of the pheromone plume necessary to enable the males in our experiment to walk directly upwind along a plume that was narrow enough for part of their antennae to be in the plume while part projected into clean air. Similar types of projection neurons with spatially distinct receptive fields have also been identified in the antennal lobes of male moths (Heinbockel and Hildebrand, 1998), but their potential role in supporting olfactory orientation in flight or walking is much less clear.

The distribution of pheromone-sensitive sensilla along the length of male cockroach antennae has also been studied (Schaller, 1978). Based on mainly morphological data, the study showed that putative pheromone-sensitive hairs composed different proportions of the population of sensilla in different parts of the male antenna. Pheromone-sensitive sensilla made up ca. 80, 50 and 30% of the sensillar population in the proximal, medial and distal segments of the male antenna respectively (Schaller, 1978).

Pre-programmed behavior during olfactory orientation

What adaptive advantage might the execution of an internally generated program of counterturns provide to a *P. americana* male walking upwind in a plume of female pheromone? It has been argued repeatedly that the temporally regular counterturning observed in flying male moths tracking pheromone plumes is an active sampling behavior serving to expose the moths to wind-induced drift of the visual flow-field information necessary to detect any differences between their intended flight path and the path that they actually fly along. This exposure to being drifted off course thus enables rapid and more precise adaptation to changes in wind speed and shifting wind directions (Kennedy, 1983; Cardé, 1984; Baker, 1985). However, its primary function has always been thought to increase the volume of air scanned by the moth in order to remain in contact with, or reacquire, an elusive olfactory stimulus (Kennedy, 1983). Walking male cockroaches, like any walking insect, should not require the sort of active scanning for wind information argued for the flying moths. Since they are in constant contact with the ground they should have constant and perfect information on the direction and magnitude of air flow, as provided by mechanosensory hairs and specialized wind sensing organs on the antennae (Bell and Kramer, 1979; Gewecke, 1977). In fact, when moths that normally fly upwind to female pheromone are forced to walk upwind to the same source, the temporally regular counterturns characterizing flight tracks are not observed (Willis and Baker, 1987). Alternatively, intermittent expression of an internal program of counterturns could serve the scanning function

proposed by Kennedy (1983). A modified version of Kennedy's idea has been proposed by Tobin and Bell (1986) to explain the zigzagging walking tracks of a small beetle walking upwind to their female pheromone. Tobin and Bell (1986) suggest that males executing internally generated counterturns would be limiting the lateral extent of their walking tracks, and thus maintain contact with the odor plume. This might be especially important while tracking a pheromone plume in the shifting wind and complex environments encountered by male cockroaches in their natural environment.

Acquisition of olfactory information *via* specific behavioral adaptations may also be occurring during odor-tracking behavior in *P. americana* males. At some point in the responses of many of the males, we observed individuals that stopped during their orientation tracks (Tables 1, 2). There are many possible explanations for stopping during upwind odor-tracking behavior. Males could be stopping to sample or actively scan the environment to update their information on wind, odor or other variables. What we observed as a stop could have been the male experiencing a decrease or increase in odor concentration large enough to trigger the beginning of a shift in behavior from tracking to searching behavior. Another explanation could be that stopping or slowing down is a requirement for executing sharp turning maneuvers, i.e. the turn to be executed is large enough and the duration short enough that the male must slow down in order to successfully perform the maneuver. While the latter explanation is almost certainly true, we observed stopping behavior that was not associated with turns (data not shown). The number of males that stopped during odor tracking in different plumes might shed light on the role of stopping during odor tracking. Few of the males tracking the point-source and wide plumes stopped (Table 2), while more than half of the males tracking the ribbon plume and all of the males tracking the cylinder plume stopped sometime during their performance (Table 2). One possible interpretation of this result is that more individuals stopped in the two treatments in which males might have most easily experienced a loss of contact with the plume. The loss of plume contact in males tracking the ribbon plume could have been caused by their own maneuvering i.e. the plume was narrow enough so that relatively small magnitude turning could have taken an individual out of contact with the plume or resulted in bilaterally asymmetrical stimulation of the antennae. It is easy to imagine the turbulent vortex street downwind of the cylinder resulting in repeated loss of odor contact during tracking. Interestingly, males tracking the two plume treatments with the most similar temporal/spatial filamentous structure (i.e. the point-source and wide plumes) had similar mean numbers of stops, and the lowest numbers of individuals stopping during odor tracking (Table 2). Thus, the relatively rapid and continuous pattern of pheromone onset and offset experienced during contact with the point-source and wide plumes may be a 'better' stimulus resulting in the ongoing expression of upwind tracking and walking. Taken together, these two results suggest that rapid, large scale changes in olfactory environment may trigger or contribute to the

expression of stopping during upwind odor-tracking behavior in *P. americana*. In our study, more *P. americana* males released into an environment in the absence of wind and pheromone stopped than most of the males in the plume structure experiment (Table 1), suggesting that stopping while walking may be a generic sampling behavior, or response to large or rapid environmental changes of any kind.

It is also interesting to note that in both experiments reported here there were no significant differences in the duration of the stops produced by individuals in any of the experimental treatments. The homogeneous nature of the stop durations in these different environmental conditions suggests that stopping during walking may be a preprogrammed behavioral response. Similar stereotyped stopping behavior has been observed in female frogs using their ears to orient and navigate toward male calling songs (Rheinlaender et al., 1979). However, while stopped, these female frogs actively scan their heads from side to side, adjust their steering direction, and continue walking. A detailed analysis of the stopping behavior of our male cockroaches and associated active scanning behavior is ongoing and will be published in the future.

Temporal structure of olfactory orientation behavior

We measured the timing of the turning behavior observed from walking *P. americana* males in the same manner used to analyze the upwind flights of male moths tracking plumes of pheromone (Willis and Baker, 1987; Willis and Arbas, 1991). In most cases the average inter-turn durations measured from walking male cockroaches in this experiment were similar to those measured from flying *Manduca sexta* males tracking pheromone plumes in a laboratory wind tunnel (Table 2; see table 1 in Willis and Arbas, 1991). The inter-turn durations in *M. sexta* are the most stereotyped aspect of their odor-tracking behavior known. So far the only experimental manipulation known to cause flying *M. sexta* males to change their inter-turn durations is removing the odor source during upwind orientation, thus forcing the males to change from tracking an odor plume to flight in clean air (Willis and Arbas, 1991). The stereotyped nature of the turn timing in pheromone-tracking flying moths has led researchers to hypothesize the existence of a central nervous system turn timer that is activated during pheromone-tracking behavior (Kennedy, 1983; Willis and Arbas, 1997). Such a timer has also been hypothesized to underlie at least some of the turning maneuvers executed by plume tracking *P. americana* males walking upwind to a source of female pheromone (Tobin, 1981). Males tracking pheromone plumes in our experiments generated sequential series of counterturns that appeared very similar to the plume following tracks of flying moths (Fig. 3). However, the variability of the timing of the turns in our walking tracks is an order of magnitude larger than that measured from the tracks of male moths flying upwind in plumes of pheromone (Table 2; see table 1 in Willis and Arbas, 1991). This difference in the variability in turn timing may reflect the differences in the two modes of locomotion, specifically the

lower speed of walking and thus the increased ability to respond to fine scale changes in the olfactory stimulus.

By making explicit comparisons between the relatively well-studied odor-tracking behaviors of male moths, and walking odor trackers, we expect to gain insights into both systems. Clearly the intermittent plumes encountered by both walkers and fliers are shaped by the same sort of environmentally determined turbulence, and they must perform similar odor-tracking tasks to locate the source. By comparing how these different odor trackers have been shaped by similar environmental constraints, and the effects of different locomotory and sensory behaviors, we may reveal core similarities and unique adaptive solutions to the complex task of locating distant unseen odor sources.

We are very grateful to K. Mori and S. Kuwahara for their kind donation of the synthetic (–)-periplanone-B. Without this contribution these experiments would have been nearly impossible. We thank Angela Ridgel and Joe Koonce for contributing to the quality of the final paper. We especially thank Katherine Louder and David Kitska for their suggestions and assistance in the analysis of our data. We also wish to thank Andy Finnell, Amanda Goodwin, Elizabeth Morrison, and Adam Rutkowski for their skilled assistance and support in many tasks from insect rearing to data analysis.

References

- Abed, D., Cheviet, P., Farine, J. P., Bonnard, O., Le Quééré, J. L. and Brossut, R. (1993). Calling behavior of female *Periplaneta americana*: Behavioral analysis and identification of the pheromone source. *J. Insect Physiol.* **39**, 709-720.
- Arbas, E. A., Willis, M. A. and Kanzaki, R. (1993). Organization of goal-oriented locomotion: Pheromone-modulated flight behavior of moths. In *Biological Neural Networks in Invertebrate Neuroethology and Robotics* (ed. R. D. Beer, R. E. Ritzmann and T. McKenna), pp. 159-193. San Diego, CA: Academic Press.
- Baker, C. F., Montgomery, J. C. and Dennis, T. E. (2002). The sensory basis of olfactory search behavior in banded kokopu (*Galaxias fasciatus*). *J. Comp. Physiol. A* **188**, 553-560.
- Baker, T. C. (1985). Chemical control of behavior. In *Behaviour*, vol. 9, *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. G. A. Kerkut and L. I. Gilbert), pp. 621-672. Oxford: Pergamon Press.
- Baker, T. C. and Haynes, K. F. (1987). Manoeuvres used by flying male oriental fruit moths to relocate a sex pheromone plume in an experimentally shifted wind-field. *Physiol. Entomol.* **12**, 263-279.
- Baker, T. C., Willis, M. A. and Phelan, P. L. (1984). Optomotor anemotaxis polarizes self-steered zigzagging in flying moths. *Physiol. Entomol.* **9**, 365-376.
- Belanger, J. H. and Willis, M. A. (1996). Adaptive control of odor-guided locomotion: Behavioral flexibility as an antidote to environmental unpredictability. *Adapt. Behav.* **4**, 217-253.
- Bell, W. J. (1983). Chemo-orientation in walking insects. In *Chemical Ecology of Insects* (ed. W. J. Bell and R. T. Cardé), pp. 93-106. London: Chapman and Hall.
- Bell, W. J. and Kramer, E. (1979). Search and anemotactic orientation of cockroaches. *J. Insect Physiol.* **25**, 631-640.
- Bell, W. J. and Kramer, E. (1980). Sex pheromone-stimulated orientation of the American cockroach on a servosphere apparatus. *J. Chem. Ecol.* **6**, 287-295.
- Bell, W. J. and Tobin, T. R. (1981). Orientation to sex pheromone in the American cockroach: Analysis of chemo-orientation mechanisms. *J. Insect Physiol.* **27**, 501-508.
- Bell, W. J. and Tobin, T. R. (1982). Chemo-orientation. *Biol. Rev.* **57**, 219-260.

- Boeckh, J. Ernst, K. and Selsam, P. (1987). Neurophysiology and neuroanatomy of the olfactory pathway in the cockroach. *Ann. NY Acad. Sci.* **510**, 39-43.
- Böhm, H., Schildberger, K. and Huber, F. (1991). Visual and acoustic course control in the cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **159**, 235-248.
- Brady, J., Gibson, G. and Packer, M. J. (1989). Odour movement, wind direction, and the problem of host-finding by tsetse flies. *Physiol. Entomol.* **14**, 369-380.
- Brown, B. and Rittschoff, D. (1984). Effects of flow and concentration of attractant on newly hatched oyster drills *Urosalpinx cinerea* (Say). *Mar. Behav. Physiol.* **1**, 75-93.
- Cardé, R. T. (1984). Chemo-orientation in flying insects. In *Chemical Ecology of Insects* (ed. W. J. Bell and R. T. Cardé), pp. 111-124. London: Chapman and Hall.
- Cardé, R. T. and Minks, A. K. (1997). *Pheromone Research: New Directions*. New York: Chapman and Hall.
- Charlton, R. E., Kanno, H., Collins, R. D. and Cardé, R. T. (1993). Influence of pheromone concentration and ambient temperature on flight of the gypsy moth, *Lymantria dispar* (L), in a sustained-flight wind tunnel. *Physiol. Entomol.* **18**, 349-362.
- David, C. T., Kennedy, J. S. and Ludlow, A. R. (1983). Finding a sex pheromone source by gypsy moths, *Lymantria dispar*, released in the field. *Nature* **303**, 804-806.
- Elkinton, J. S., Schal, C., Ono, T. and Cardé, R. T. (1987). Pheromone puff trajectory and upwind flight of male gypsy moths in a forest. *Physiol. Entomol.* **12**, 399-406.
- Fisher, N. I. (2000). *Statistical Analysis Of Circular Data*. Cambridge, UK: Cambridge University Press.
- Gewecke, M. (1977). Control of flight in relation to the air in *Locusta migratoria* (Insecta, Orthoptera). *J. Physiol. Paris* **73**, 581-592.
- Grasso, F. W. and Basil, J. A. (2002). How lobsters, crayfishes, and crabs locate sources of odor: current perspectives and future directions. *Curr. Opinion Neurobiol.* **12**, 721-727.
- Heinbockel, T. and Hildebrand, J. G. (1998). Antennal receptive fields of pheromone-responsive projection neurons in the antennal lobes of the male sphinx moth *Manduca sexta*. *J. Comp. Physiol. A* **183**, 121-133.
- Hösl, M. (1990). Pheromone-sensitive neurons in the deutocerebrum of *Periplaneta americana*: Receptive fields on the antenna. *J. Comp. Physiol. A* **167**, 321-327.
- Johnsen, P. B. and Teeter, J. H. (1985). Behavioral responses of bonnethead sharks to controlled olfactory stimulation. *Mar. Behav. Physiol.* **11**, 283-291.
- Kennedy, J. S. (1983). Zigzagging and casting as a programmed response to wind-borne odour: a review. *Physiol. Entomol.* **8**, 109-120.
- Kennedy, J. S. and Marsh, D. (1974). Pheromone regulated anemotaxis in flying moths. *Science* **184**, 999-1001.
- Kennedy, J. S. and Moorehouse, J. E. (1969). Laboratory observations on locust responses to wind-borne grass odour. *Ent. Exp. Appl.* **12**, 487-503.
- Kitahara, T., Mori, M. and Mori, K. (1987). Pheromone synthesis, Part 122. Total synthesis of (-)-periplanone-B, natural major sex-excitant pheromone of the American cockroach, *Periplaneta americana*. *Tetrahedron* **43**, 2689-2699.
- Kramer, E. (1976). The orientation of walking honeybees in odour fields with small concentration gradients. *Physiol. Entomol.* **1**, 27-37.
- Kuenen, L. P. S. and Baker, T. C. (1982). The effects of pheromone concentration on the flight behavior of the oriental fruit moth. *Physiol. Entomol.* **7**, 423-434.
- Kuwahara, S. and Mori, K. (1990). Pheromone synthesis Part 123. Synthesis of (-)-periplanone-B, a sex pheromone of the American cockroach (*Periplaneta americana*). *Tetrahedron* **46**, 8075-8082.
- Mafra-Neto, A. and Cardé, R. T. (1994). Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature* **369**, 142.
- Mizunami, M., Weibrecht, J. M. and Strausfeld, N. J. (1998). Mushroom bodies of the cockroach: Their participation in place memory. *J. Comp. Neurol.* **402**, 520-537.
- Moore, P. A. and Lepper, D. M. E. (1997). Role of chemical signals in the orientation behavior of the sea star *Asterias forbsii*. *Biol. Bull.* **192**, 410-417.
- Pilla, R. S., Kitska, D. J. and Loader, C. (2005). Statistical analysis of modified complete randomized designs: applications to chemo-orientation studies. *J. Exp. Biol.* (in press).
- Rheinlaender, J., Gerhardt, H. C., Yager, D. O. and Capranica, R. R. (1979). Accuracy of phonotaxis by the green treefrog (*Hyla cinerea*). *J. Comp. Physiol. A* **133**, 247-255.
- Rust, M. K. and Bell, W. J. (1976). Chemo-anemotaxis: A behavioral response to sex pheromone in nonflying insects. *Proc. Natl. Acad. Sci. USA* **73**, 2524-2526.
- Rust, M. K., Burk, T. and Bell, W. J. (1976). Pheromone-stimulated locomotory and orientation responses in the American cockroach. *Anim. Behav.* **24**, 52-67.
- Schaller, D. (1978). Antennal sensory system of *Periplaneta americana* L.: Distribution and frequency of morphologic types of sensilla and their sex-specific changes during post-embryonic development. *Cell Tiss. Res.* **191**, 121-139.
- Schöne, H. (1984). *Spatial Orientation*. Princeton, NJ: Princeton University Press.
- Seelinger, G. (1984). Sex-specific activity patterns in *Periplaneta americana* and their relation to mate-finding. *Z. Tierpsychol.* **65**, 309-326.
- Seelinger, G. (1985). Behavioral responses to female sex pheromone components in *Periplaneta americana*. *Anim. Behav.* **33**, 591-598.
- Susswein, A. J., Cappell, M. S. and Bennett, M. V. L. (1982). Distance chemoreception in *Navanax inermis*. *Mar. Behav. Physiol.* **8**, 231-241.
- Tobin, T. R. (1981). Pheromone orientation: Role of internal control mechanisms. *Science* **214**, 1147-1149.
- Tobin, T. R. and Bell, W. J. (1986). Chemo-orientation of male *Trogoderma variable* (Coleoptera: Dermestidae) in a simulated corridor of female sex pheromone. *J. Comp. Physiol. A* **158**, 729-739.
- Vickers, N. J. and Baker, T. C. (1994). Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proc. Natl. Acad. Sci. USA* **91**, 5756-5760.
- Vogel, S. (1994). *Life in Moving Fluids*. Princeton, NJ: Princeton University Press.
- Weber, T. (1990). Phonotaxis and visual orientation in *Gryllus campestris* L.: Behavioural experiments. In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. V. Popov), pp. 377-386. Basel: Birkhäuser Verlag.
- Weissburg, M. J. (2000). The fluid dynamical context of chemosensory behavior. *Biol. Bull.* **198**, 188-200.
- Weissburg, M. J. and Zimmer-Faust, R. K. (1994). Odor plumes and how blue crabs use them in finding prey. *J. Exp. Biol.* **197**, 349-375.
- Willis, M. A. and Arbas, E. A. (1991). Odor-modulated upwind flight of the sphinx moth, *Manduca sexta* L. *J. Comp. Physiol. A* **169**, 427-440.
- Willis, M. A. and Arbas, E. A. (1997). Active behavior and reflexive responses: Another perspective on odor-modulated locomotion. In *Pheromone Research: New Directions* (ed. R. T. Cardé and A. K. Minks), pp. 304-319. New York: Chapman and Hall.
- Willis, M. A. and Baker, T. C. (1987). Comparison of maneuvers used by walking versus flying *Grapholita molesta* males during pheromone-mediated upwind movement. *J. Insect Physiol.* **33**, 875-883.
- Willis, M. A. and Cardé, R. T. (1990). Pheromone-modulated optomotor response in male gypsy moths, *Lymantria dispar* L.: Upwind flight in a pheromone plume in different wind velocities. *J. Comp. Physiol. A* **167**, 699-706.
- Willis, M. A., David, C. T., Murlis, J. and Cardé, R. T. (1994). Effects of pheromone plume structure and visual stimuli on the pheromone-modulated upwind flight of male gypsy moths (*Lymantria dispar* L.), in a forest. *J. Insect Behav.* **7**, 385-409.
- Wolf, H. and Wehner, R. (2000). Pinpointing food sources: Olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. *J. Exp. Biol.* **203**, 857-868.