Effects of altering flow and odor information on plume tracking behavior in walking cockroaches, *Periplaneta americana* (L.)

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SUMMARY

Animals using odor plumes to locate resources are activated to track these plumes by the presence of an attractive odor, and typically steer toward the source using directional cues from the flowing air or water bearing the odor. We challenged freely walking virgin male cockroaches, *Periplaneta americana*, to track plumes of airborne female pheromone and then video-recorded and analyzed their responses as the odor plume and wind were independently manipulated. Plume tracking males that experienced the total loss of directional air flow halfway to the odor source showed little change in their performance, and 100% were able to quickly locate the pheromone source. By contrast, males experiencing a sudden loss of odor while tracking a plume rapidly changed their behavior; often turning downwind and retracing their steps to the release point, or walking in loops, but rarely moving upwind to the previous location of the source. In a subsequent experiment, in order to determine whether a memory of the previously experienced wind direction could provide the directional information necessary to locate an odor source, we challenged males to track plumes in *zero wind* after pre-exposing them to: (1) wind and pheromone, (2) wind only, and (3) neither wind nor pheromone. These were compared to males tracking a wind-borne pheromone plume, in which case, all males were able to locate the pheromone source. Our results show that males require the detection of wind and pheromone simultaneously *during* plume tracking in order to quickly and efficiently locate the odor source. These results are consistent with those reported from flying moths tracking wind-borne pheromone plumes, and suggest that the control system underlying this behavior requires ongoing simultaneous experience with wind and odor information during the performance of the behavior to operate efficiently.

Key words: orientation, pheromone, wind, behavior, cockroach.

INTRODUCTION

Odor molecules released from objects are distributed through the environment by flowing fluid (either water or air). In nature, most flows are turbulent and this combination of turbulent flow and evaporating chemicals generates an intermittent odor plume, a structure that many animals use to locate important resources from relatively long distances (Arbas et al., 1993; Cardé and Minks, 1997; Weissburg, 2000; Vickers, 2000). The structure of plumes means that animals tracking them are not exposed to smooth concentration gradients. Therefore, they cannot rely on the simple orientation rules of chemokinesis or chemotaxis (Frankel and Gunn, 1961; Schöne, 1984) to orient toward and efficiently locate the odor source. In fact, it is not clear that the intermittent structure of an odor plume *can* provide the directional information necessary to locate its source unless an organism can develop a statistical impression of the plume (Webster and Weissburg, 2001).

If no chemical gradient exists, how do plume tracking organisms orient and steer toward the source? To perform this task most animals require two pieces of information: (1) the presence of attractive chemicals *and* (2) the direction of the flow carrying the attractants (Arbas et al., 1993; Weissburg, 2000; Webb et al., 2004; Willis, 2008). The preferred orientation with respect to flowing air or water steered by plume tracking organisms appears to be modulated by the presence of an attractive odor (Baker et al., 1984; Johnsen and Teeter, 1985; Emmanuel and Dodson, 1979; Grasso and Atema, 2002; Kennedy and Moorehouse, 1969; Kennedy and Marsh, 1974; Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994; Willis and

Avondet, 2005). For example: male cockroaches and immature locusts prefer to walk downwind (i.e. with the flow) in the absence of an attractive odor, but reverse this preference and turn and walk upwind when an attractive odor is present (Kennedy and Moorehouse, 1969; Rust and Bell, 1976; Rust et al., 1976; Willis and Avondet, 2005).

Whether tested in the laboratory (Baker et al., 1984) or observed in the field (Elkinton and Cardé, 1983), flying male moths seem to have no preferred orientation to the wind direction prior to detecting their sex-attractant pheromone. After encountering a wind-borne plume of their species' sex pheromone, responsive males all turn into the wind and fly upwind while in contact with the plume (Baker and Haynes, 1987; Baker et al., 1984; Kennedy and Marsh, 1974). In addition, insects tracking attractive odors upwind, whether walking or flying, continuously re-orient to the dynamically changing wind direction as long as they are in contact with, or have only recently lost contact with the odor (Baker and Haynes, 1987; David et al., 1983; David and Kennedy, 1987; Wolf and Wehner, 2000; Vickers and Baker, 1997).

To further understand how walking organisms use information on flow direction and odor to track odor plumes, we recorded the responses of virgin male American cockroaches, *Periplaneta americana*, to the loss of either odor or wind information while they were tracking a plume of their species' sex pheromone upwind.

The goal of the work presented here is to expand our knowledge of the orientation mechanisms used by walking animals to track odor in terrestrial environments, and to explicitly compare them to

the more extensively studied examples of odor plume tracking by flying insects, especially moths. By comparing the performance of the same task by similar organisms using different modes of locomotion we hope to better understand the environmental constraints and biological adaptations that underlie odor plume tracking.

MATERIALS AND METHODS
Insects

We used virgin male *Periplaneta americana* for all trials. Prior to their final molt, we removed males from the main laboratory colony and held them in an environmental chamber isolated from females at 27–29°C and ~50% relative humidity on a 12 h:12 h light:dark cycle.

Wind tunnel

The experimental arena was a flat aluminum platform (1.52 m×0.92 m) held 25.4 cm above the floor of the working section of our laboratory wind tunnel (1 m×1 m×2.5 m). Light levels were ~3–5 lux. The pheromone source was held at the upwind end of the aluminum platform, 1 cm above the surface. An exhaust duct at the downwind end of the wind tunnel removed the pheromone plume from the building. For a more detailed description please see Willis and Avondet (Willis and Avondet, 2005).

We recorded the cockroaches' movements with a Burle TC355AC B/W video camera (Lancaster, PA, USA) positioned above the wind tunnel. The field of view of this camera encompassed the entire experimental arena. Trials were recorded at 30 frames s^{-1}.

In all of the experiments described here the pheromone source was a 0.7 cm diameter disk of filter paper (Whatman No.1, Eastbourne, East Sussex, UK) containing 0.10 ng of (–)-periplanone-B (Kitahara et al., 1987; Kuwahara and Mori, 1990). All solutions of (–)-periplanone-B were made with 95% *n*-hexane (Acros Organics, Geel, Belgium). To generate a turbulent plume, the source was oriented perpendicular to the airflow in the wind tunnel. In all experiments in which wind was present, the fan generated a constant 25 cm s^{-1} flow.

Experimental procedures
Series 1 experiments

To determine the effect of the sudden loss of either odor or wind information on plume tracking behavior we compared the responses of males to the experimental treatments detailed below. Treatment order and the introduction of individual animals were randomized and each animal was used only once.

Wind and plume (*N*=14)

In this treatment, the wind and the pheromone source remained constant and therefore the plume was stable and continuous. We placed individual cockroaches into the plume on the downwind edge of the experimental arena in coarse meshed aluminum window screen (holes=2 mm) cages (3 cm×10 cm; height × diameter) and allowed them to acclimate for 1 min before releasing them into the plume by manually removing the top of the cage from the arena. [Prior to experimentation, smoke plume flow visualization (titanium tetrachloride) showed that a windborne plume flowed through the cage unhindered.] This treatment served as a control for the two following experimental trials, 'wind stop' and 'plume pull'.

Wind stop (*N*=16)

Individual *P. americana* males were presented with the same initial conditions as described above. However, once they had initiated

tracking behavior, we abruptly stopped the wind in the tunnel, leaving only a slowly expanding plume hanging in a zero wind environment. The cockroach's behavior was recorded until he reached the odor source. During practice sessions prior to data recording, we used a piece of filter paper of the same size and shape dosed with titanium tetrachloride (TiCl$_4$), a source of dense white smoke, to visualize the wind stopping event. The wind stopping procedure involved three parts performed in rapid sequence: (1) the fan generating the flow through the wind tunnel was turned off, (2) a sliding door rapidly closed the air intake of the wind tunnel, and (3) the exhaust fan was turned off. The three steps were coordinated to produce a consistent, abrupt and complete cessation of the wind. The duration of the wind stopping procedure from initiation to total wind stop (as measured from TiCl$_4$ smoke) was approximately 1 s. We found that the expanding plume reached the lateral edges of the aluminum platform roughly 45 s after the wind stopped. The moment of completion of the wind stopping procedure was marked by illumination of an infra-red light emitting diode (LED) in the field of view of the camera. The LED was triggered automatically as the sliding door at the tunnel's air intake closed.

Plume pull (*N*=17)

For this treatment the pheromone source was mounted on nylon fishing line that formed a continuous loop from the floor to the ceiling and connected outside the wind tunnel. As a cockroach approached the center of the arena, the source was removed from the arena by pulling it rapidly to the ceiling of the wind tunnel. Rapidly removing the pheromone source truncated the plume, and its remains were carried down the wind tunnel and out the exhaust, leaving the cockroach walking upwind in clean air (Kuenen and Baker, 1983). We tested this by using TiCl$_4$ to visualize the smoke plume in the same manner that we used it to test the wind stop as described above. We recorded each individual's response to odor loss until it left the experimental arena. Knowing the wind speed and the average walking speed of a cockroach enabled us to time the removal of the pheromone source so that the cockroach lost the plume in the field of view of the camera. The exact moment of odor loss was determined for each individual later. We calculated the plume to move downwind 2.075 cm in the interval between each digitized cockroach position. When the truncated end of the pheromone plume passed the male cockroach as he walked upwind, we declared that to be the moment of odor loss.

Series 2 experiments

To determine whether prior exposure to wind, with or without odor, could support plume tracking in zero wind, we exposed the cockroaches to the following four different combinations of wind and odor prior to release into the pheromone plume: (1) continuous pheromone plume in wind, (2) pre-exposure to pheromone plume and wind, (3) pre-exposure to wind only, and (4) no exposure to wind or pheromone plume (see Table 1 for more details). After being exposed to an experimental treatment for 1 min, the cockroaches were released from screen cages in the center of the experimental arena, rather than at the downwind end as above. This allowed them to orient to the experimental environment and leave the release point in any direction. When applicable, we stopped the wind in the same manner as detailed above, prior to releasing the cockroach. In all pre-exposure experiments the males were placed into the plume with their longitudinal body axes aimed in random directions to eliminate bias in the individual's walking direction. Pilot studies showed that males detect and orient to air flow while in the screen release cages

Table 1. Description of experimental manipulations of the flow and odor environment for *P. americana* males walking upwind in a pheromone plume in a laboratory wind tunnel (Series 2 experiment)

Experimental treatment	Stimulus exposure prior to release	Explanation
Continuous odor plume in wind	Odor and Wind	Placed in center of experimental arena. Continuous exposure to wind and odor throughout performance.
Pre-exposure to odor and wind	Odor and Wind	Placed in center of experimental arena. Wind stopped just prior to release into stationary odor plume.
Pre-exposure to wind only	Wind	Placed in arena, outside of odor plume to ensure no exposure to odor. Wind stopped just prior to release into stationary odor plume.
No exposure to wind or odor	None	Placed in a sealed container in arena outside of plume to ensure no exposure to wind or odor. Wind stopped just prior to release into stationary odor plume.

(M.A.W. and J.L.A., unpublished). Each individual's response was recorded until it located the odor source.

Continuous exposure to wind and odor (N=15)

Each individual was placed in the plume at the longitudinal midpoint of the experimental arena, providing a continuous exposure to wind and odor. In this treatment the wind was not stopped, serving as the control.

Pre-exposure to odor and wind (N=16)

Each individual was placed into a wind-borne pheromone plume in the center of the experimental arena for 1 min, the wind was then stopped and the cockroach was released from the screen cage into the stationary plume.

Pre-exposure to wind only (N=16)

The cockroach was placed at lateral edge of the experimental arena, exposed to wind, but ~40 cm outside the time-averaged plume boundary. After 1 min, the wind was stopped and the cockroach was slowly released from the screen cage into the now stationary pheromone plume.

No exposure to wind or odor (N=15)

The cockroach was placed at the same point as those in the pre-exposure to wind only treatment, but in a sealed plastic container approximately the same dimensions as the screen cages used for the other treatments. Then, the wind was stopped and the cockroach was released.

Data analysis

We manually digitized the walking paths of male cockroaches using a computerized motion analysis system (Motus 7.1™, Vicon Inc., Centennial, CO, USA). We then digitized the position of the cockroaches' heads every 83 ms. The response variables measured from the video-recorded 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°), body yaw angle (angle of the longitudinal body axis with respect to the wind), track width [distance between sequential turn apices measured perpendicular to the wind direction, as per Kuenen and Baker (Kuenen and Baker, 1982)], groundspeed (walking speed of cockroaches measured from point to point along its track), inter-turn duration (time between the apices of sequential turns), net velocity (speed in the upwind direction from the beginning to the end of each walking track), the number of times each individual stopped during an odor-tracking performance, the duration of each stop, and the linearity index (degree of straightness of the walking track).

In order to examine different sections of the cockroach's tracking behavior, each individual's response to the plume pull, wind stop and wind and plume treatments were split into two parts: (1) prior to the experimental manipulation, known hereafter as pre-plume pull and pre-wind stop, and (2) after experimental manipulation, known hereafter as post-plume pull and post-wind stop (Fig. 1). To control for any bias in position in the wind tunnel, the responses of males tracking the point-source plume were divided into two segments (split after 3 s. which was the mean pre-plume pull and pre-wind stop duration) termed wind and plume beginning half and end half (Fig. 1). These sub-divisions yielded a total of six different treatments for analysis.

Both experiments were designed as a randomized complete block design, with each experimental manipulation and subset as a treatment, and a complete group of treatments was performed as a block each day. These data were analyzed using a split-plot ANOVA in SAS (ver. 8.2) (Pilla et al., 2005) to test for differences among treatments (plume pull and wind stop: N=6, wind and odor orientation: N=4), day (plume pull and wind stop: N=4, wind and odor orientation: N=5), and day by treatment interactions. Treatments and individuals were randomized daily. Each day the number of individuals available for experimentation was divided evenly across the treatments. Individuals from the first group were introduced sequentially to the first treatment, the next group to the second treatment, and so on. When an ANOVA revealed significant effects in the experiment, we applied Tukey's multiple comparison test to determine which variables differed significantly among the experimental treatments. We considered test statistics with a probability of less than 0.05 ($P<0.05$) unlikely to be obtained by chance, and thus statistically significant.

RESULTS

P. americana males responded in characteristically different ways to either the loss of wind, or odor information, while tracking a pheromone plume. For both Series 1 and 2 experiments, if female pheromone was present, whether in the controls or experimental treatments, 100% of the males located the source. However, note that it was impossible for the cockroaches to find the source in the plume pull treatment (Fig. 1Ci–iii) because it was removed from the arena. Stopping the wind ('wind stop' treatment in Series 1) when the males were half way to the source had little effect on the tracking behavior or success at locating the pheromone source (Fig. 1Bi–iii). Even in the experiments where exposure to wind and odor was manipulated prior to release into a pheromone plume in zero wind (Series 2 experiments), 100% of males located the source, although it took much longer in some cases (Fig. 2). Thus, the directional cue provided by the wind enabled the cockroaches to locate the source much faster (Fig. 2).

Series 1

Wind and plume

When the cockroaches were given continuous wind and pheromone odor information, they all were able to track the odor to its source; some more quickly than others (Fig. 1Ai–iii). These data are consistent with results found in previous experiments (Willis and Avondet, 2005).

Wind stop

After the wind was stopped, the cockroaches continued to track the now stationary plume with few changes in behavior detectable by eye (Fig. 1Bi–iii). Eighty-seven percent of the males walked directly to the source (Fig. 1Bi,ii), whereas 13% briefly lost and then found the plume and located the source (Fig. 1Biii; average total tracking time: 9.64±8.38 s, N=16). Few quantifiable changes in the behavior of males were detected from before to after the wind stopped (Fig. 1Bi–iii, Table 2). However, they did increase the width of their tracks and steered their track angles more across the previous wind direction after the wind stopped (Table 2). The behavior of the males tracking the continuous plume in continuous wind was not different from the males that experienced the wind stop. (Average total tracking time: 9.52±5.93 s, N=14.)

Plume pull

Males dramatically changed their plume tracking behavior when they walked upwind through the truncated end of the pheromone plume after the pheromone was pulled (Fig. 1Ci–iii). Upon loss of pheromone contact the cockroaches ceased upwind progress and began to perform behaviors that we interpret as search strategies aimed at locating the source of the pheromone, or relocating the plume. The duration of the response to loss of pheromone (from plume loss until they encountered an edge of the arena) was significantly longer (P<0.05) than the responses to the wind stopping treatment (average total tracking time: 30.67±11.04 s, N=17). The average interval between encountering the truncated upwind end of the plume and their initial behavioral response [i.e. turning at an angle greater than 90° with respect to the wind direction (N=9) or stopping for longer than one sampling period and then executing a turn greater than 90° (N=8)] was 1.1±0.4 s. When the cockroaches stopped upon plume loss, they waited for an average of 0.3±0.2 s before they re-initiated walking and began turning. In 41% of the cases, upon losing the pheromone plume the individuals looped across and downwind (Fig. 1Ci,iii). In 59% of the cases, the males stopped moving upwind and rather than looping around the point of behavioral response to odor loss, they retraced their steps downwind, sometimes walking all the way back to the release point (Fig. 1Cii). Males apparently switched their preferred orientation with respect to the wind from upwind to downwind, and while walking downwind typically turned back-and-forth across the area of the arena where the plume had been.

In most cases no statistically significant differences were observed between the average movement parameters measured from the pre-plume pull walking patterns and the walking tracks generated by males tracking the standardized point-source plume. However, there were several statistically significant differences between the males' behavior during the pre-plume pull and post-plume pull condition. The looping maneuvers initiated upon plume loss caused the linearity index to decrease significantly (i.e. the tracks become less linear) (P≤0.05), and the body yaw angle and track angle also
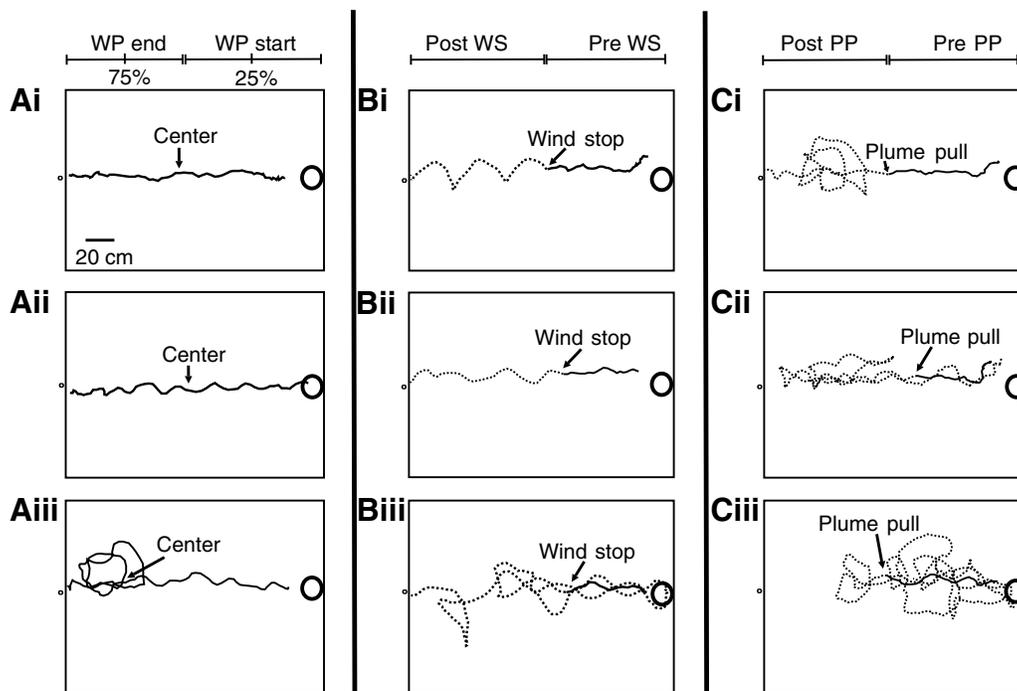

Fig. 1. Examples of the range of variability in walking tracks of *P. americana* males tracking responses to (Ai–iii) continuous windborne plumes of female pheromone, (Bi–iii) loss of directional wind while tracking a plume, and (Ci–iii) loss of the pheromone plume while tracking it in wind in Series 1 experiments. The first panel (i) shows a response with the fewest turns, the second panel (ii) an example of a typical number of turns, and the last panel (iii) the responses with the largest number of turns. Continuous lines represent tracking while wind and pheromone were both present. Dotted lines represent behavior after the loss of wind or pheromone. Black circle at right side of plots represents the point of release of the cockroaches. Note that the first position was digitized when we could clearly resolve the cockroach and its walking path. When present, wind direction was from left to right and the pheromone source was on the left-hand edge of the arena (the arena is depicted by the black boxes in which the tracks are plotted).

Table 2. Track parameters measured from *P. americana* males walking in a laboratory wind tunnel from the Series 1 experiment

Treatment	N	Net velocity (cm s⁻¹)	Ground speed (cm s⁻¹)	Track angle* (deg.)	Body yaw angle* (deg.)	Track width (cm)	Inter-turn duration (s)	Number of stops	Stop duration (s)	Linearity index†
WP beg	14	18.9±11.0[a,b]	21.2±9.9[a]	30.9±23.9[b,c]	23.1±25.8[b]	2.8±2.2[b,c]	0.4±0.3[b]	0.4±0.6[a]	0.04±0.06[a]	0.7±0.3[a,b]
WP end	14	17.6±7.2[a,b]	24.9±8.1[a]	37.0±20.4[b]	24.1±15.2[b]	3.9±3.3[b,c]	0.5±0.4[b]	0.8±1.6[a]	0.04±0.08[a]	0.7±0.2[b]
Pre-WS	16	21.4±10.0[a,b]	21.7±8.6[a]	21.3±9.3[b,c]	15.7±7.9[b]	2.1±1.3[c]	0.3±0.1[b]	0.2±0.5[a]	0.01±0.04[a]	0.8±0.1[a,b]
Post-WS	16	23.4±19.3[a]	24.8±6.5[a]	36.8±19.5[b]	26.3±21.0[b]	5.8±4.6[b]	0.6±0.4[b]	0.9±1.8[a]	0.05±0.07[a]	0.8±0.2[a,b]
Pre-PP	17	21.1±5.4[a,b]	22.8±5.4[a]	20.2±8.1[c]	15.3±3.9[b]	2.4±2.6[b,c]	0.4±0.5[b]	0.6±0.2[a]	0.02±0.08[a]	0.9±0.0[a]
Post-PP	17	8.6±19.9[b]	27.7±6.1[a]	95.1±11.9[a]	92.8±27.3[a]	14.9±5.9[a]	1.2±0.4[a]	0.8±1.1[a]	0.04±0.05[a]	0.2±0.1[c]

Each treatment was split into two sections: wind and plume tracking beginning and end (WP beg, WP end), pre- and post-plume pull (Pre-PP, Post-PP), and pre- and post-wind stop (Pre-WS, Post-WS).

Values are means ± s.d. Values in the same column with the same letters do not differ significantly according to a repeated measures ANOVA ($P \leq 0.05$) and *post-hoc* Tukey's test.

*All angles are absolute values of the distributions measured.

†Linearity index range = 0.0–1.0, with 1.0 = straight line from release point to source.

increased significantly (i.e. oriented more perpendicular to the wind direction or downwind; Fig. 1Ci–iii). This contrasts dramatically with the typical straight inter-turn track legs aimed mainly upwind observed prior to the plume pull (Fig. 1Ci–iii, Table 2). The track width also increases, which in turn causes the inter-turn duration to increase and the net velocity to decrease (Table 2).

To identify behavioral changes at the transitions from wind to no-wind, or pheromone to no-pheromone, and if any observed changes persisted, we then divided each track into three sections labeled as: tracking, event, and post-event. Our analysis was based on a 1.0 s sub-sample of data from the center of each of these three sections. The control tracks (wind and plume) were also divided into three track sections in a similar way (0–25% to source, 26–74% to source, and 75% to source; Fig. 1). To make a matched comparison of the three track segments across the treatments, we divided the pre-plume pull and pre-wind stop track sections described above (Fig. 1, Table 2), as well as the section where the cockroach, responding to the wind and plume, was approximately 25% of the way down the wind tunnel floor. We then took a 1 s sample of data from the event (initiation of the plume pull odor loss ±500 ms, initiation point where the wind was stopped ±500 ms), and from the center section of the wind and plume track. We compared these to a 1 s sample of data from the center section of the post-plume pull and post-wind stop data sets, as well as from the section where the cockroach was approximately 75% of the way down the wind tunnel floor.

In this experiment, the only aspect of the cockroaches' behavior that changed significantly when tracking an odor plume in wind was the groundspeed (Table 3). Cockroaches tracking wind and plumes walked significantly faster through the center section of their tracks than the beginning (Table 3). In the plume pull trials the only aspect of the animals' performance that changed significantly was their track angles. Once they lost contact with the plume, the cockroaches began to circle, resulting in a significant increase in the track angles measured (Table 3). The only aspect of the cockroaches' behavior that changed significantly in the wind stop experiment was the track width. During the event period in the wind stop treatment, the track width increased slightly and then continued to increase, becoming significantly wider than the pre-wind stop tracks, as the time post-wind stop increased (Table 3). At the spatial and temporal resolution of the video that we used we were unable to detect an identifiable transient change in behavior associated with the rapid cessation of wind.

Series 2
Orientation to wind and odor after pre-exposure treatments

We observed a wide variety of behavior from the males exposed to the different combinations of wind and odor before they were released into a plume in *zero wind* (Fig. 2). All cockroaches eventually found the source, some more rapidly than others.

In the treatment where cockroaches were pre-exposed to wind and pheromone *prior* to odor tracking, 40% walked relatively directly to the source (Fig. 2Ai), 33% walked in looping maneuvers covering much of the area of the arena, eventually making it to the source (Fig. 2Aiii), whereas 27% displayed an intermediate amount of looping before locating the source (Fig. 2Aii). For the cockroaches with no prior wind or odor information before being released into the plume, 25% walked relatively directly to the source (Fig. 2Bi), 50% generated looping tracks that, in many cases covered much of the arena before finally locating the source (Fig. 2Biii), and 25% showed an intermediate level of searching before locating the pheromone source (Fig. 2Bii). In the treatment where cockroaches were exposed to *wind only* before the wind was stopped, 31% went

Table 3. A one-second sub-sample of track parameters measured from *P. americana* males tracking pheromone (track), in the period of a plume pull, wind stop or middle of wind and plume tracking (event), and post-event responses (post event) in a laboratory wind tunnel from the Series 1 experiment

Treatment	N	Event	Net velocity (cm s^{-1})	Ground speed (cm s^{-1})	Track angle* (deg.)	Body yaw* (deg.)	Track width (cm)	Inter-turn duration (s)	Number of stops	Stop duration (s)
Wind + plume	14	Track	23.1±9.5[a]	21.4±5.9[b]	21.4±11.5[a]	16.1±7.4[a]	3.6±2.8[a]	0.6±0.5[a]	0.15±0.4	0.13±0.06
	14	Event	23.3±9.6[a]	30.4±9.1[a]	17.3±14.4[a]	15.1±9.7[a]	3.6±5.0[a]	0.5±0.7[a]	0	
	14	Post event	23.2±9.6[a]	25.5±5.0[a,b]	27.6±23.0[a]	23.2±18.3[a]	4.1±3.9[a]	0.4±0.4[a]	0	
Wind stop	16	Track	20.7±21.6[a]	21.5±9.7[a]	21.5±18.9[a]	17.4±18.0[a]	1.9±0.9[b]	0.3±0.1[a]	0	
	16	Event	21.1±21.4[a]	27.9±10.1[a]	19.0±8.8[a]	16.1±8.5[a]	2.5±2.1[a,b]	0.4±0.3[a]	0	
	16	Post event	22.2±22.6[a]	28.2±10.0[a]	20.5±11.4[a]	20.3±11.9[a]	4.7±2.9[a]	0.5±0.2[a]	0.06±0.3	0.167
Plume pull	17	Track	21.9±15.0[a]	24.3±5.8[a]	21.9±18.3[b]	17.9±11.1[a]	2.7±1.9[a]	0.5±0.2[a]	0	
	17	Event	21.9±15.0[a]	29.0±9.3[a]	20.9±17.1[b]	14.1±7.1[a]	4.3±2.0[a]	0.6±0.3[a]	0	
	17	Post event	22.4±16.2[a]	23.9±9.2[a]	96.1±47.8[a]	44.0±23.2[a]	11.1±9.7[a]	0.9±0.5[a]	0	

Values are means ± s.d. Values in the same column with the same letters do not differ significantly according to a split-plot ANOVA ($P \leq 0.05$) and *post-hoc* Tukey's test.
*All angles are absolute values of the distributions measured.

relatively directly to the source (Fig. 2Ci), 38% looped searching across both ends of the floor until finally reaching the source (Fig. 2Ciii), and 31% displayed some looping before finding the source (Fig. 2Cii). In the standardized treatment where odor and wind were *continuously* present, 80% of the cockroaches walked directly to the source (Fig. 2Di), whereas 20% of them looped before reaching the source (Fig. 2Dii).

Cockroaches responding to the continuous wind and odor treatment, in which the wind and pheromone stimulus was continuously present, behaved in a significantly different way from those exposed to the other treatments (Table 4). The cockroaches that had no pre-exposure to either wind or odor had longer inter-turn durations than those in the other three treatments, but the cockroaches pre-exposed to odor and wind did not have significantly shorter times between their turns (Table 4).

Did exposure to odor or wind information, in any combination, prior to release in the pheromone plume without wind affect the initial walking direction of the cockroaches? The mean leaving direction of cockroaches that were not pre-exposed to wind or pheromone was 91.3° and was not significantly different from a random distribution according to Rayleigh's test ($z=0.34$, $P>0.05$; Fig. 3) (Fisher, 2000). Cockroaches that were pre-exposed to wind and pheromone prior to release in the plume with no wind showed no preference in the direction they left the release point (mean direction of leaving=151.7°). This orientation was not significantly different from a random distribution ($z=2.56$, $0.05<P<0.10$) (Fisher, 2000). The cockroaches pre-exposed to wind alone had a mean leaving direction that was 24.9°, on average, but the distribution of leaving directions was not significantly different from random ($z=0.16$, $P>0.05$) (Fisher, 2000). Only the cockroaches that were continuously exposed to wind and odor generated a distribution of leaving directions that was significantly different from random ($z=3.75$, $P<0.05$) (Fisher, 2000), with a mean direction of orientation of 37.7°.

DISCUSSION

The results of our experiments show that *P. americana* males require simultaneous wind and odor information to efficiently orient to and track an odor plume. Both forms of information must be detected at the same time for most individuals to initiate movement toward the pheromone source. However, once tracking the plume, most males were able to successfully continue and locate its source after the wind was stopped. By contrast, loss of odor information resulted in rapid cessation of upwind movement, followed by what appears to be different forms of local search (Bell, 1991). Under these conditions few males moved further upwind than the point at which they lost the plume.

When we removed the plume most of the cockroaches turned and walked downwind, while maintaining their tracks roughly within the boundaries of the previously existing time-averaged plume. Upon reaching their starting point or the downwind end of the arena, they turned and walked back upwind and often repeated this behavior even though the odor plume had been gone for many seconds (Fig. 1Cii). Two possible explanations for this behavior are: (1) that they were orienting to and tracking residual pheromone molecules deposited on the floor of the 'plume area' of the arena, and (2) they had associated the pheromone plume, and then pheromone loss, with specific locations in the experimental arena. (According to our TiCl$_4$ smoke visualizations, the plume was in contact with the floor from a few centimeters downwind of the source to the downwind end of the arena.)

Orientation to residual pheromone deposited on the floor would require the cockroaches to switch from pheromone-modulated orientation to wind direction, to a mechanism used by insects like

Pre-exposure to wind and pheromone

Ai   N=6 to source, 11.1±4.2 s

Aii   N=4 intermediate, 45.1±15.4 s

Aiii   N=5 searching, 78.2±14.3 s

No pre-exposure to wind or odor

Bi   N=4 to source, 11.3±3.8 s

Bii   N=4 intermediate, 29.3±10.7 s

Biii   N=7 searching, 99.1±23.8 s

Pre-exposure to wind only

Ci   N=5 to source, 5.5±1.6 s

Cii   N=5 intermediate, 34.2±8.5 s

Ciii   N=6 searching, 75.6±21.8 s

Continuous exposure to wind and odor

Di   N=12 standard tracking, 4.4±0.9 s
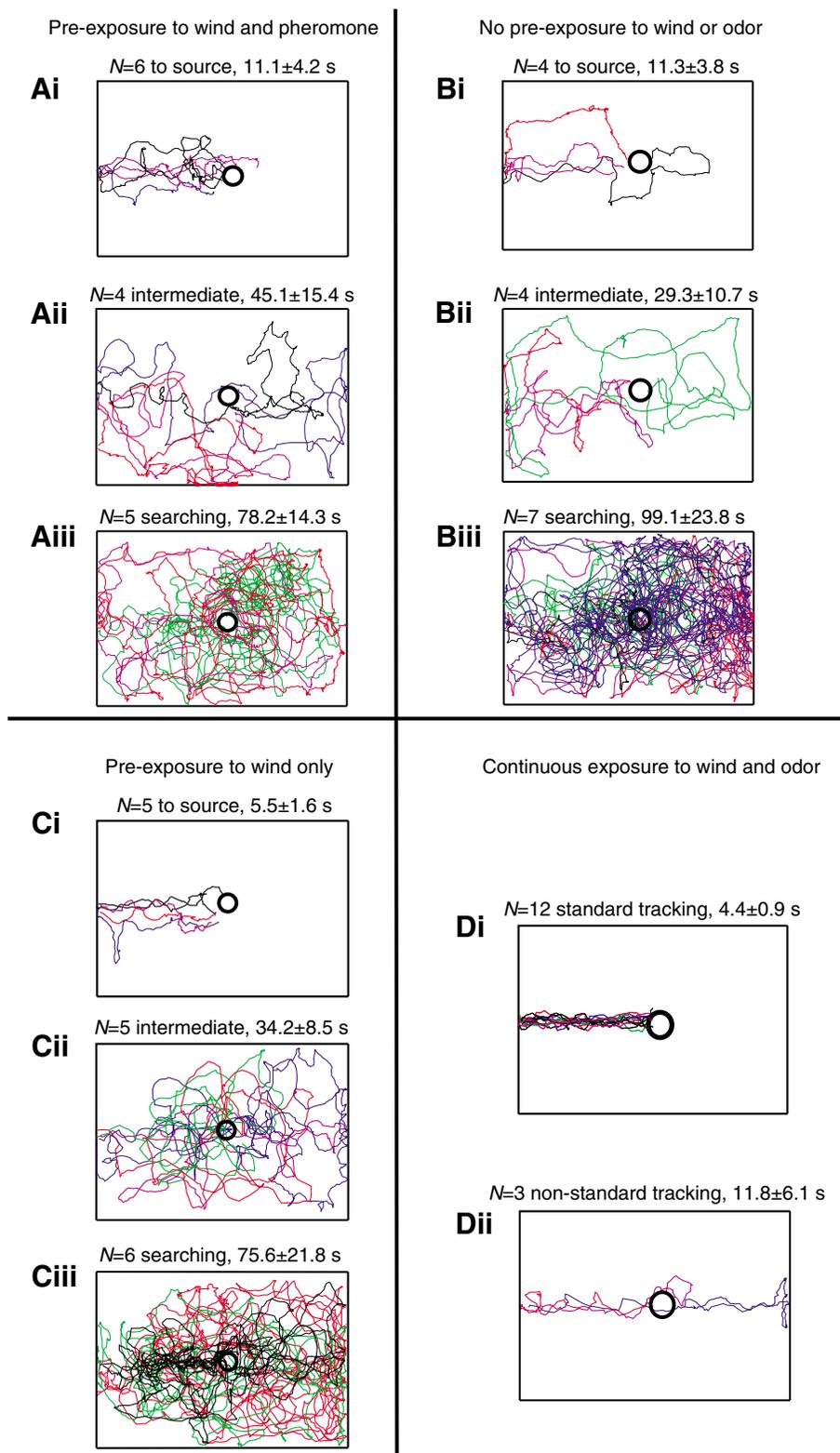
Dii   N=3 non-standard tracking, 11.8±6.1 s

Fig. 2. Full range of variation in the responses of *P. americana* males as they tracked a pheromone plume in zero wind after being pre-exposed to (A) wind + pheromone, (B) no wind + no pheromone and (C) wind only in the Series 2 experiments. Controls tracked plumes while being exposed to wind and pheromone continuously (D). Black circle in center of plots represents the point of release of the cockroaches. Cockroach tracks on each experimental day are denoted by a different color. Wind direction was from left to right prior to cessation, and the pheromone source was on the left-hand edge of the arena (the arena is depicted by the black boxes in which the tracks are plotted). Mean tracking times (and s.d.) are given.

and Kramer, 1980; Kennedy and Moorehouse, 1969; Rust and Bell, 1976; Rust et al., 1976; VanVorhis Key and Baker, 1982; Willis and Avondet, 2005). If our males are using chemical cues deposited on the floor of the arena when they turn down wind, they seem to ignore the wind direction. Thus, they have apparently 'disconnected' the typical linkage between the pheromone inputs and directional cues provided by the wind direction (Rust and Bell, 1976; Rust et al., 1976; Willis and Avondet, 2005). If they are following a trail of pheromone deposited on the floor of the arena, then the link between pheromone detection and preferred orientation to wind direction may be context dependent. Loss of contact with a source of female pheromone is an ecological context that may demand the chemical signal receive increased weight, and wind direction decreased. Detection of the pheromone on the local substrate may be a more relevant stimulus.

Male cockroaches that generated looping tracks after loss of pheromone increased the area of the arena that they encountered, presumably in an attempt to re-contact the pheromone plume. In addition, this looping local search seemed to be focused on the location where odor contact was lost, suggesting that they may be incorporating landmark cues into their working knowledge of their environment. Landmark learning has been demonstrated previously in *P. americana* (Mizunami et al., 1998). This looping behavior after odor loss may be functionally equivalent to the side-to-side turning behavior, known as 'casting', executed by flying moths when they lose contact with a pheromone plume (Kennedy and Marsh, 1974; Kennedy,

ants that track trails deposited on the ground [i.e. turning toward the antenna that detects the highest odor concentration (Hangartner, 1967)]. It is clear from other observations that their antennae do contact the floor during antennal movements performed continuously during plume tracking (M.A.W., unpublished data).

Previous experiments have all shown that attractive chemical cues can alter the preferred orientation to flow in walking insects (Bell

1983). In contrast to the irregular looping search performed by walking cockroach males, moths performing casting flight execute temporally regular left–right turns with the inter-turn legs oriented roughly 90° to the wind (Kennedy and Marsh, 1974). These side-to-side turns are continuously re-oriented as the wind direction changes (Baker and Haynes, 1987), whereas there is no obvious preferred orientation to the looping search of walking cockroaches (Fig. 1).

Table 4. Track parameters measured from *P. americana* males walking in a laboratory wind tunnel for wind and odor orientation (Series 2) experiment

Treatment*	N	Net velocity (cm s⁻¹)	Groundspeed (cm s⁻¹)	Track angle† (deg.)	Body yaw† (deg.)	Track width (cm)	Inter-turn duration (s)	Stop duration (s)	Number of stops	Linearity index
Continuous odor + wind	15	15.9±5.2[a]	19.5±4.6[a]	33.5±19.3[b]	26.2±23.8[b]	2.8±1.9[b]	0.4±0.3[b]	0.1±0.1[b]	0.5±0.6[b]	0.8±0.2[a]
Odor + wind	16	4.5±4.1[b]	20.6±15.1[a]	64.5±18.1[a]	69.5±36.4[a]	16.1±9.0[a]	2.1±1.1[a,b]	0.2±0.1[a]	12.6±11.6[a]	0.3±0.2[b]
No odor + wind	16	5.8±6.5[b]	25.2±9.6[a]	68.2±22.3[a]	67.4±28.7[a]	17.3±11.0[a]	1.6±0.9[b]	0.1±0.1[a,b]	6.1±7.4[a,b]	0.3±0.3[b]
No odor, no wind	15	7.5±18.8[a,b]	20.5±12.9[a]	72.1±15.1[a]	75.0±20.1[a]	22.6±10.6[a]	3.3±3.1[a]	0.2±0.2[a]	13.4±20.2[a]	0.2±0.2[b]

Values are means ± s.d. Values in the same column with the same letters do not differ significantly according to a split-plot ANOVA ($P \leq 0.05$) and *post-hoc* Tukey's test.

*Treatment prior to the wind being stopped (the wind was not stopped for the continuous odor + wind).

†All angles are absolute values of the distributions measured.

At wind-stop, the loss of directional information provided by the wind will require our cockroaches to switch and use only chemo-orientation mechanisms to follow the plume. Results from our work (Willis and Avondet, 2005) and others (Tobin, 1981), suggest that the steering maneuvers of plume tracking *P. americana* males may result from multiple mechanisms. These include: (1) a temporally regular internal turn timer, similar to that proposed for plume tracking flying male moths (Kennedy, 1983; Tobin, 1981), (2) turns triggered by bilateral spatial comparisons of concentration detected by the two antennae (i.e. turn toward the higher concentration) (Tobin, 1981; Willis and Avondet, 2005), and (3) internally generated so-called stochastic turns, or turns not clearly associated with any obvious external stimulus (Tobin, 1981). Until we can know when an individual detects odor and how it subsequently responds, we will be unable to completely discriminate between these alternatives. However, results of other work in our lab (Willis and Avondet, 2005) suggest that bilateral comparisons of concentrations detected by each antenna are likely to support the successful plume tracking behavior we observed after wind-stop.

Measurements of time-averaged plume boundaries and antennal locations during plume tracking by *P. americana* males suggest that many of the side-to-side turns are triggered when the cockroach encounters the high contrast edge between clean air and the plume (Willis and Avondet, 2005). In our experiments, the 5 cm average span between the tips of the cockroaches' antennae (Willis and Avondet, 2005) allows males near the lateral boundary of the point-source plume to have one antenna near the plume boundary or outside the plume in clean air while the other is near the zone of highest concentration, near the midline of the plume. Observations of TiCl₄ smoke visualizations of the plume during practice prior to performing these experiments show that when the wind stops, the two most obvious changes in the structure of the pheromone plume are: (1) the high contrast boundaries between pheromone and clean air move outward laterally, away from the centerline of the arena, and 2) as the stationary plume expands away from its centerline the space between filaments increases and the size of each filament also increases. Because the plume continues to expand after the wind stops, the high-contrast signal provided by the lateral boundary of the plume recedes away from the tracking cockroach. Thus, the significant increase in the width of walking tracks after wind stop may be the result of the males 'feeling for the edge' of the plume and using it as a cue to turn back into the plume.

It is well known that the tracking behavior of flying male moths is significantly affected by plumes issuing from sources of different concentrations (Cardé and Hagaman, 1979; Charlton et al., 1993; Kuenen and Baker, 1982) or with different turbulent structures (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994). Therefore it is possible that some of the changes we observed from our cockroaches could have been caused by the changes to the structure of the plume after the wind stopped. The fact that it was often impossible to discriminate between pre- and post-wind stop tracking behavior suggests that if the changes in plume structure are affecting the cockroaches' performance, these effects are subtle.

It has been proposed that flying male moths are able to determine the correct direction to track pheromone plumes in still air, and possibly in wind, by comparing the concentration of sequential odor samples (i.e. increasing concentration across sequential samples indicates that the tracker is approaching the source, decreasing concentration indicates that the tracker is moving away from the source) (Baker and Kuenen, 1982; Kuenen and Baker, 1983). This information may be available from the plume, since the probability of encountering odor filaments, and the concentration in these
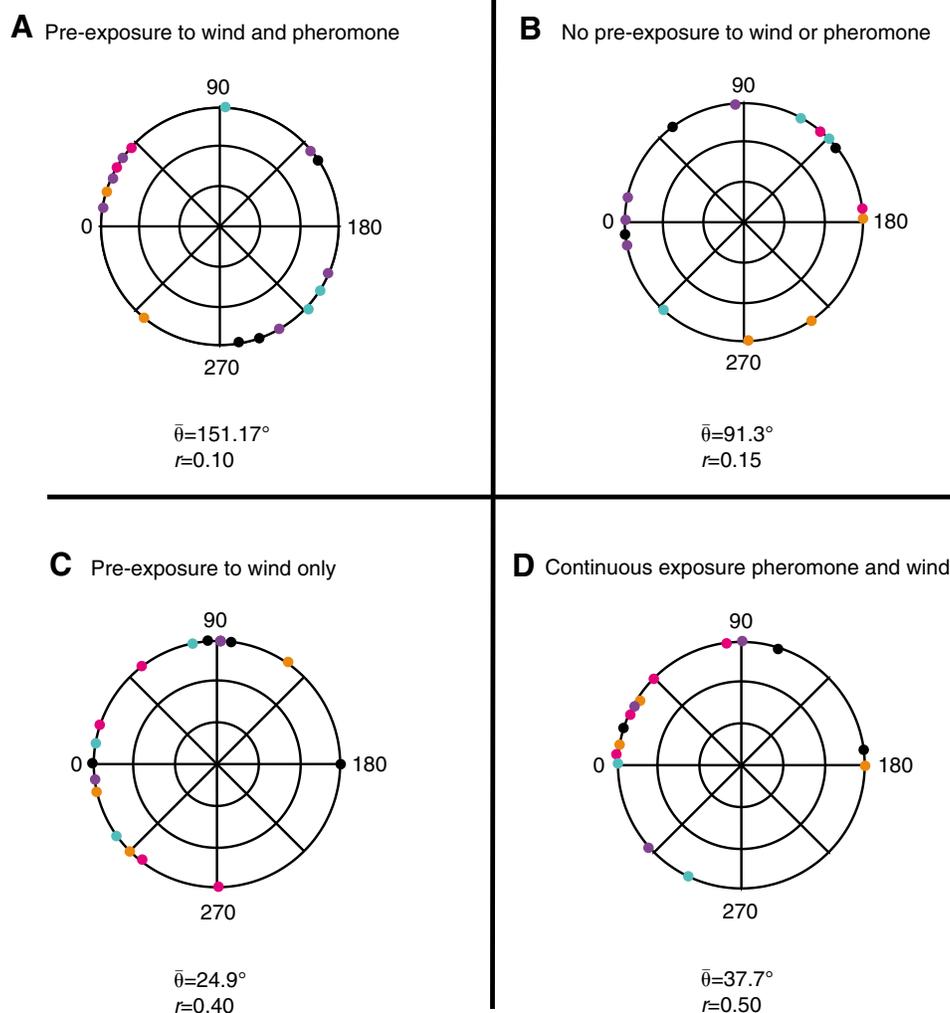
Fig. 3. Orientation of the walking track as *P. americana* males left the release point to track a pheromone plume in zero wind after being pre-exposed to (A) wind + pheromone, (B) no wind + no pheromone, (C) wind only and (D) continuous wind and pheromone from the Series 2 experiments. Controls tracked plumes while being exposed to wind and pheromone continuously (D). On these circular plots due upwind=0° and downwind=180°. θ, angle of mean leaving direction; *r*, length of mean vector. *r* is distributed between 0 and 1, with 0 indicating no movement in the mean direction, and 1 indicating all movements were in the mean direction.

filaments, decrease predictably with both distance downwind from the source, and distance from centerline across the plume (Moore and Atema, 1991; Murlis and Jones, 1981; Murlis et al., 2000; Vickers et al., 2001). Comparison of sequential samples could enable any plume tracking animal to continue toward and possibly successfully locate the source during a lull in wind, especially if it moved fast enough to arrive near the source before the plume dissipated. Such temporal comparisons along the plume, together with bilateral spatial comparisons between the antennae, may explain the performances of our cockroaches that successfully tracked the stationary plume with no directional information from the wind.

The results of the 'Series 2' experiment suggest that the chemo-orientation capabilities of *P. americana* males vary in different individuals (Fig. 3). In each treatment group where the wind was stopped, between 25–40% of the individuals were able to quickly and efficiently track the stationary plume to the source, whether or not they had been exposed to wind prior to their release into the plume. The proportions of males able to rapidly track the stationary

plume to the source decreased from those that had experienced wind and pheromone (40%), to those that had experienced wind alone (31%), to those that had experienced neither (25%), prior to introduction to a stationary plume in still air. Thus, there is some indication that experience with the wind direction immediately prior to release in the plume may have increased the number of individuals able to subsequently track the plume with no directional cues from wind. However, since the number of individuals accounting for this increase is only one or two, this effect seems small. When released into a stationary plume in still air, the majority of our experimental population (60–75% depending on the treatment group) had considerable difficulty locating the pheromone source. Thus, unless the cockroach is already tracking a plume upwind when odor is lost, the directional information available to them from chemical information alone enables only slow, inefficient location of the pheromone source.

Even in the treatment groups that did not experience wind and pheromone together while tracking, all of the individuals located

the pheromone source when given enough time. It could be argued that by leaving the males to walk around the surface of our experimental arena we are assuring that they will eventually locate the source. However, during the course of previous experiments we have observed males routinely leaving the experimental arena, sometimes not to return. We interpret the males' persistent search of the experimental arena, and the fact that they never left the arena when they could have, as an indication of continued orientation to the pheromone in the still air above the arena.

The behavior of our cockroaches is broadly similar to that reported for plume tracking moths challenged with similar experimental conditions (Baker and Kuenen, 1982; Baker et al., 1984; David and Kennedy, 1987; Farkas and Shorey, 1972; Kuenen and Baker, 1983; Willis and Cardé, 1990). Like our cockroaches, male moths that had begun to track plumes of pheromone before wind was stopped, continued to track the stationary plume to the source with success rates similar to those in wind (Baker et al., 1984; Farkas and Shorey, 1972; Willis and Cardé, 1990). Furthermore, whether tracking a plume in flight (Baker et al., 1984) or while walking (this study), these animals must experience the odor and wind cues while locomoting in order to orient toward the odor source and locate it quickly and efficiently. Both moths and cockroaches can track a stationary plume in still air, but they take much longer to locate the source and fewer may be able to locate the source (Baker et al., 1984).

The variety of apparent searching behaviors expressed by our cockroaches upon loss of the plume is somewhat at odds with the consistent descriptions of crosswind casting flight reported for several species of plume-tracking moths upon plume loss. We suggest that the cockroaches may be incorporating a memory of the visual landmarks in its environment during plume tracking to support this olfactory searching, and that the addition of these visual cues may allow the cockroaches to re-prioritize the directional information provided by the wind. Further experiments aimed at characterizing the interplay amongst the various sensory modalities supporting this behavior are necessary before we can draw any conclusions. However, as we compare and contrast the plume tracking task as performed by similar organisms using different modes of locomotion, we expect the differences and similarities to reveal underlying organizational principles critical to understanding control rules and how they are adapted in different environmental and biological contexts.

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