RESEARCH ARTICLE

A comparison of odor plume-tracking behavior of walking and flying insects in different turbulent environments

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ABSTRACT

Many animals locate food, mates and territories by following plumes of attractive odors. There are clear differences in the structure of this plume-tracking behavior depending on whether an animal is flying, swimming, walking or crawling. These differences could arise from different control rules used by the central nervous system during these different modes of locomotion or one set of rules interacting with the different environments while walking on the surface versus flying or swimming. Flow speeds and turbulence that characterize the environments where walking and flying insects track plumes may alter the structure of odor plumes in an environment-specific way that results in the same control rules generating behaviors that appear quite different. We tested these ideas by challenging walking male cockroaches, *Periplaneta americana*, and flying male moths, *Manduca sexta*, to track plumes of their species' sex pheromones in low wind speeds characteristic of cockroach experimental environments, higher wind speeds characteristic of moth experimental environments, and conditions ranging from low to high turbulence. Introducing a turbulence-generating structure into the flow significantly improved the flying plume tracker's ability to locate the odor source, and changed the structure of the behavior of both flying and walking plume trackers. Our results support the idea that plume trackers moving slowly along the substrate may use the spatial distribution of odor, while faster moving flying plume trackers may use the timing of odor encounters to steer to locate the source.

KEY WORDS: Orientation, Pheromone, Moth, Cockroach, Turbulence

INTRODUCTION

Sexually receptive females of the American cockroach, *Periplaneta americana*, and the tobacco hornworm moth, *Manduca sexta*, attract males (Rust and Bell, 1976; Willis and Arbas, 1991) by releasing volatile sex-attractant pheromones (Abed et al., 1993; Sasaki and Riddiford, 1984) into the wind. They are distributed through the environment primarily by turbulent air flows, resulting in a plume made up of a patchy distribution of odor packets of varying size and concentration surrounded by clean air (Murlis and Jones, 1981; Murlis et al., 1990; Wright, 1958). Receptive males track these air-borne pheromones to locate females for mating (Rust and Bell, 1976; Willis and Arbas, 1991).

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Odor plume structure is determined by the speed of flowing air or water and turbulence caused by objects in those flows, i.e. flowers, bushes, tree trunks and the bodies of the animals releasing pheromone (Lugt, 1995). Larger objects generate larger turbulent eddies and a larger cross-sectional area of the odor plume (Vogel, 1994; Willis et al., 2013). Animals tracking plumes in the free-stream condition, i.e. flying or swimming, may experience very different plume structures to animals in the boundary layer, i.e. walking or crawling near the substrate (Weissburg, 2000). The boundary layer extends from the surface of a solid object (by definition at zero flow) to the point at which the flow is moving at the local free stream speed (Vogel, 1994). Walking and flying animals living in terrestrial environments may need to track plumes through open meadows, vertical cylindrical tree trunks in forests, or woodlands made of different shrub species. This variety of environments causes a broad range of turbulent conditions and odor plume structures (Griffths and Brady, 1995; Murlis et al., 1990, 2000).

The variability of plume structures across environments makes finding mates releasing pheromones a challenging task. Flying plume trackers moving through the free stream should experience a different turbulent environment from that of walkers tracking plumes in the boundary layer near the ground. These differences could produce plumes with temporal and/or spatial differences (Weissburg et al., 2002). Characterizing the flow conditions or odor plume structures of the air-borne environments in which we study plume tracking is difficult and not routinely done in air (Baker and Haynes, 1989; Connor et al., 2018; Murlis et al., 2000; Willis et al., 2013) or in aquatic environments (Ferner and Weissburg, 2005; Moore et al., 2000; Page et al., 2011a,b; Weissburg et al., 2003).

In a diverse array of animals living in air or water, odor plume tracking is thought to be generated by (1) an odor-triggered orientation to and movement into the flow (Grasso and Atema, 2002; Kennedy, 1939; Kennedy and Marsh, 1974; Page et al., 2011a; Willis and Avondet, 2005) and (2) turning maneuvers to maintain contact with the odor plume (Grasso and Atema, 2002; Kennedy and Marsh, 1974; Kuenen and Baker, 1983; Page et al., 2011b; Preiss and Kramer, 1986; Rust and Bell, 1976; Weissburg, 2000; Willis et al., 2008). In some plume trackers, the turns are thought to be triggered by encounters with the pheromone–clean air edge at the lateral boundaries of the plume (Bell and Tobin, 1982; Carton and Montgomery, 2003; Duistermars et al., 2009; Weissburg and Zimmer-Faust, 1994) and in others these turns are thought to be pre-programmed in the central nervous system and released upon detection of the attractive odor (Baker, 1990; Kanzaki et al., 1992; Kennedy et al., 1981), and modulated by the subsequent onset and offset of odor (Arbas et al., 1993; Baker, 1985b; Budick and Dickinson, 2006; Kanzaki et al., 1992; Kennedy et al., 1981; Tobin, 1981). The structure of both the flow that carries the odor and the odor plume, though not independent, can separately affect the

observed tracking behavior because insects sense these separately (Mafra-Neto and Cardé, 1994; Willis and Avondet, 2005; Willis et al., 1994).

Plume-tracking behavior is ubiquitous and used by a diversity of organisms to locate important resources in many different environments (Weissburg, 2000; Willis, 2008). However, we cannot assume they are using the same orientation mechanisms even if their behavior appears to be similar. Nor can we assume that an animal can detect and use information in tracking an odor just because it is available (Belanger and Willis, 1996). Even if the boundary layer and free-stream conditions produce similar flow and odor environments, other factors can impact the detection and processing of the odor signal, such as the structure of the antenna (Koehl, 2006; Schneider et al., 1998; Vogel, 1983), flows induced by the tracker's mode of locomotion, e.g. wing-beats draw odors across the antennae (Loudon and Koehl, 2000; Sane and Jacobson, 2006; Tripathy et al., 2010), and central nervous system mechanisms (Christensen and Hildebrand, 1997; Vickers et al., 2001; Chapman et al., 2018).

In our experiments, walking cockroaches and flying moths locate odor sources while moving at different speeds through two different environments (the boundary layer and the free stream, respectively). The antennae of *P. americana* are as long as or longer than their bodies, and can be moved both horizontally and vertically while tracking odor (Willis and Avondet, 2005). However, on average they hold their antennae at a characteristic inter-antennal angle of approximately 90 deg during plume tracking (Willis and Avondet, 2005). The antennae of the moth *M. sexta* are much shorter and also held at a relatively constant angle (143±3 deg; Sane et al., 2007) while tracking (Sane et al., 2007). The experimental environment for studying plume tracking in *M. sexta* moths has typically been low-turbulence air flowing at 100 cm s^{-1} (Arbas and Willis, 1994; Rutkowski et al., 2009), resulting in a mean moth flight speed of approximately 150 cm s^{-1}. A typical experimental environment for studying *P. americana* cockroaches is low-turbulence air flowing at a relatively low speed of 25 cm s^{-1} (Tobin, 1981; Willis and Avondet, 2005), which results in cockroaches walking upwind. As cockroaches walk upwind in the plume, their movement through the odor plumes is much slower than that of the moths. In summary, flying moths move relatively rapidly (20–30 body lengths s^{-1}) through the plume with antennae that are short relative to the plume cross-section, while cockroaches walk relatively slowly through the plume (ca. 6 body lengths s^{-1}) with antennae that in some cases can completely span its cross-section. Differences in locomotion speed, sensor structure and odor and flow environments may have important consequences for the structures of successful plume-tracking strategies.

In addition to biomechanical and environmental differences, the way the nervous systems of moths and cockroaches process the odor signal from their sensors and the decisions they make based on the sensory input are thought to be different. An animal using bilaterally symmetrical sensors can compare the odor concentration at two points in space simultaneously to determine where the odor plume is located and in which direction to turn to maintain contact with the odor. Alternatively, it can compare the odor concentration at two points in time either averaging or summing the sensory information from the two sensors. These two mechanisms have long been referred to, respectively, as spatial and temporal sampling (Frankel and Gunn, 1961). It has been thought that most animals employ the two mechanisms simultaneously to differing degrees (Schöne, 1984), but this idea has rarely been tested (Martin, 1965). For the reasons detailed above (e.g. locomotion speed and sensor structure),

moths have long been believed to use mainly temporal comparisons to track plumes (Vickers and Baker, 1991) and cockroaches mainly spatial comparisons (Rust et al., 1976). However, detailed knowledge of differences in the flow and odor environments in which moths and cockroaches are responding is needed to understand how they are using sensory information to track odor.

Our hypothesis is that animals employ tracking strategies adapted to the environment in which they live, their mode and speed of locomotion, and the structure of their sensors. The series of experiments presented here was conducted to challenge model plume trackers that appear to be adapted to perform this behavior in specific environments with an array of flow and odor environments spanning those for which each appears to be best adapted. The performance of male moths and cockroaches was compared as they tracked plumes in both slow wind speeds matching those typically used to study the walking odor orientation of cockroaches (Tobin, 1981; Willis and Avondet, 2005) and faster wind speeds typical of studies of moth flight orientation to odor (Rutkowski et al., 2009; Willis and Arbas, 1991). The level of turbulence in the environment was also altered from very low to much higher in a manner intended to model the range of flow conditions experienced by these animals in nature. The turbulent flows were quantified, characterized and reported in full elsewhere (Talley, 2010). Under our experimental conditions, the slowly walking cockroaches with long antennae performed better in lower turbulence environments where spatial comparisons should be easier, while the rapidly flying moths with short antennae performed better in higher turbulence environments where temporal comparisons may be easier.

MATERIALS AND METHODS
Insects
Male *Periplaneta americana* (Linnaeus 1758) cockroaches were removed from our colony prior to their final molt and isolated from females for at least 2 weeks prior to their use in experiments. These males were held at ca. 28°C and 50% relative humidity on a 12 h:12 h light:dark cycle in an environmental chamber in a room separated from the colony. Cockroaches were provided with Purina puppy chow and water *ad libitum*.

Male *Manduca sexta* (Linnaeus 1763) pupae were isolated from our laboratory colony the day before they eclosed. They were housed in 30×30×30 cm screened cages in an environmental chamber at ca. 28°C on a 14 h:10 h light:dark cycle.

Wind tunnel and flow manipulations
Wind tunnel
The wind tunnel used in these experiments was described in detail previously in studies of walking (Willis and Avondet, 2005) and flying (Rutkowski et al., 2009) plume-tracking behavior. All behavioral experiments were conducted in lowlight conditions (i.e. 5–7 lx) with the room lighting provided by a combination of infrared, red and white lights. The working section of the wind tunnel was 1 m×1 m×2.5 m, and two Burle TC355AC (Lancaster, PA, USA) B/W cameras each captured a 1.93 m×1 m field of view, one from overhead and the other from downwind. The two cameras were synchronized using a Peak event and video control unit (Vicon Peak, Inc.). Flow speed in the wind tunnel was set using a Testo 425 thermal anemometer. For walking cockroach experiments, a horizontal flat plate (1.52 m×0.92 m) was installed in the wind tunnel 25.4 cm above the floor of the wind tunnel. The pheromone source was held 1 cm above the center of the upwind edge of the arena. The plate was removed for flying moth experiments.

Turbulent environments

Turbulence was manipulated using (1) a custom-fabricated turbulence grid, (2) a vertically oriented 7 cm diameter cylinder or (3) a combination of these two elements (Talley, 2010) (Fig. 1A–D). The turbulence grid was custom-made of 1.3 cm wide wooden slats spaced 4.5 cm apart vertically and horizontally. It spanned the cross-section of the wind tunnel at the upwind end of the working section. A grid generates the same turbulence in all directions (isotropic) and at all locations (homogeneous) downwind (Roach, 1987). Cylinders have long been used to understand turbulence in engineering studies (Cantwell and Coles, 1983) as well as model trees to study insects that track plumes associated with tree trunks
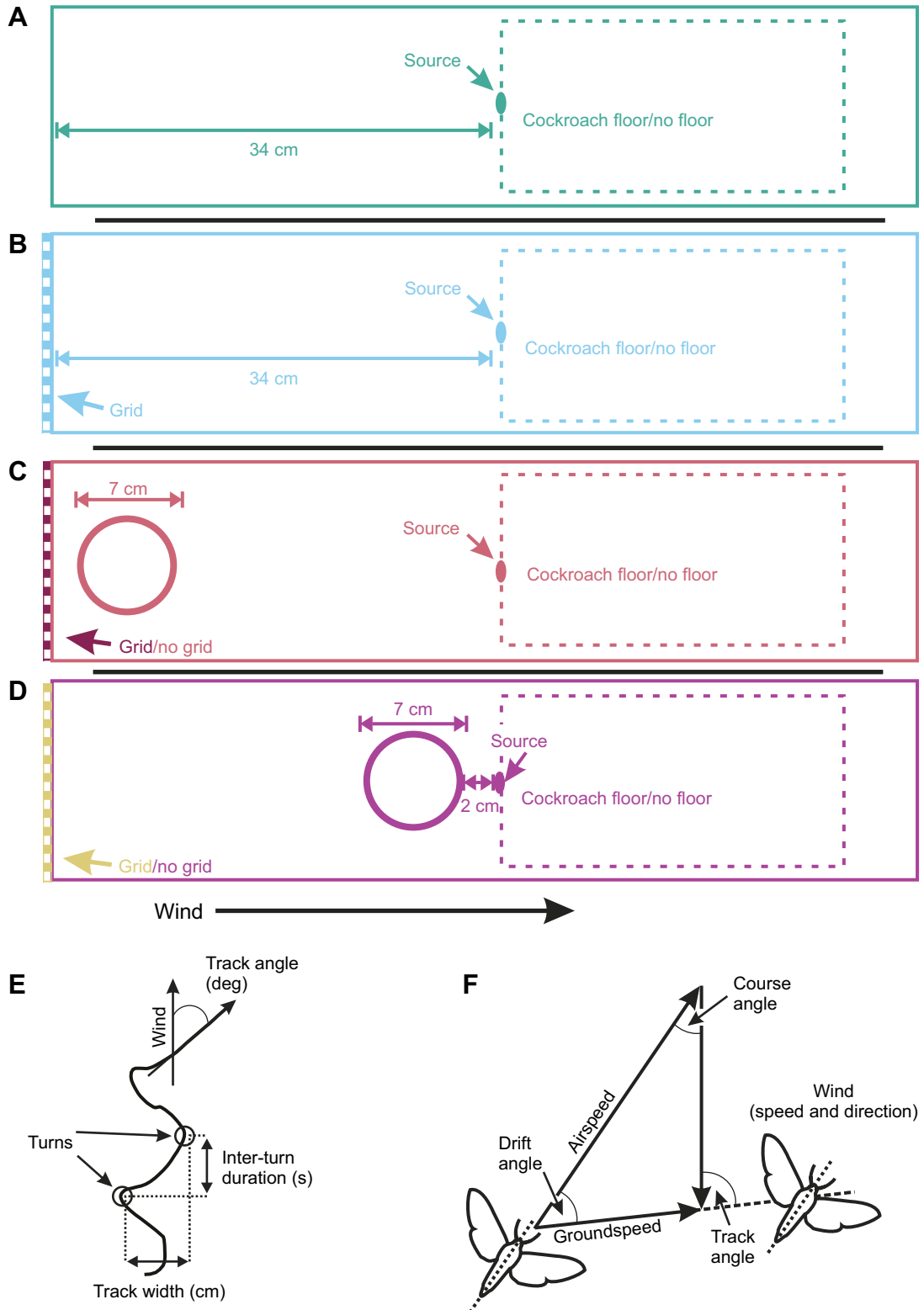

Fig. 1. See next page for legend.

Fig. 1. Diagrams of the experimental setup of the wind tunnel, and explanation of how insect trajectories were measured. (A,B) When the platform for cockroach walking experiments is in place, the source is held 1 cm above the upwind end of the cockroach floor, 50 cm from each side, 34 cm downwind of the grid. For moth flight orientation experiments, the cockroach floor is removed and the pheromone source is held 40 cm above the wind tunnel floor, 50 cm from each side, 34 cm downwind of the grid. (C,D) The cylinder is placed in each of the two positions with reference to the mean wind direction: in C, it is immediately downwind of the grid (base of the cylinder is touching the base of the grid or where the grid edge would be); in D, it is immediately upwind of the pheromone source (i.e. the side of the cylinder is within 2 cm of the pheromone source). (E) Overhead view of a walking trajectory in our wind tunnel, illustrating some of the variables measured to characterize plume-tracking behavior. Two examples of maneuvers identified as turns (open circles) are shown to illustrate how the track width and inter-turn durations are measured. Arrows show how the track angles are measured. The same measurements were taken from walking and flying trajectories. (F) Triangle of velocities in 2-dimensions adapted from Marsh et al. (1978). Plume-tracking trajectories were quantified by measuring the length and orientation of each movement vector, which provided ground speed and track angle. The wind speed and direction were set. These three known values enabled us to calculate the moth's airspeed, course angle and drift angle using the triangles of velocities method.

(Cardé, 1981; Charlton and Cardé, 1990; Fadamiro et al., 1998; Willis et al., 1994). Cylinder flow is well characterized and the diameter of our cylinder was chosen based on the estimated vortex-shedding frequency (Vogel, 1994).

All turbulence treatments were measured using hotwire anemometers (Talley, 2010). With no turbulence treatment, the wind tunnel produced about 1% freestream turbulence due to the fan. The grid produced ca. 4.9% turbulence 100 cm downwind of its location. The cylinder produced up to 25% turbulence downstream of its midline, decreasing to 10–15% at the lateral edge of the wind tunnel. The cylinder was positioned either immediately downwind of the grid (with the base of the cylinder touching the base of the grid or where the grid edge would be; Fig. 1C) or immediately upwind of the pheromone source (i.e. 2 cm upwind of the pheromone source; Fig. 1D). The two different positions enabled us to examine how the distance between the source and the cylinder (i.e. a tree trunk) affects the plume's structure and the behavior of the trackers. These treatments were termed 'upwind' (close to the grid) or 'downwind' (close to the odor source).

Pheromone
The attractant sources were either 0.1 ng of (−)-periplanone-B (cockroaches) (Kuwahara and Mori, 1990) or one female equivalent of pheromone gland extract (moths) (Willis and Arbas, 1991). Solutions were applied to a 0.7 cm diameter disk of Whatman's No. 1 filter paper. For walking cockroach trials, the filter paper was then held on the lateral midline of the wind tunnel, perpendicular to the flow ca. 32 cm above the wind tunnel floor (i.e. ca. 1 cm above the cockroach floor) on an insect pin clipped to a thin steel rod, 34 cm from the upwind end of the working section (Fig. 1A). For flying moth trials, the same size source was similarly positioned on the wind tunnel's lateral midline, 34 cm downwind from the upwind end of the working section and 40.5 cm above the wind tunnel floor (Fig. 1A–D). Fresh odor sources were loaded and used each experimental day for all experiments.

Experimental design
Walking odor tracking
The walking cockroach experiments used sexually mature virgin male cockroaches, 3–16 weeks old. No difference in pheromone-

tracking response has been observed across this age range (J. L. Avondet and M.A.W., unpublished data). Individuals were placed into cylindrical screen cages 10 cm in diameter by 3 cm in height, and then held in the wind tunnel room. At the beginning of the cockroaches' normal dark period, the lights were lowered to the conditions described above. Experiments always began 2 h after lights off, at the peak of their pheromone response, and ended no more than 2 h later (4 h after lights off). Each cockroach in its cage was placed 1.2 m downwind from the pheromone source, in the center of the plume, for 1 min prior to being allowed free access to the wind tunnel. This release position allowed freedom of movement in any direction while remaining in the arena. Once released, cockroaches ranged freely until they had either found the source or left the arena. Each male was used once.

Six different turbulence treatments at two different wind speeds were used, comprising a total of 12 treatments. The least turbulent treatment was the filter paper disc source held perpendicular to the flow (Fig. 1A). In all experiments, this was referred to as 'source only' and served as the control. The lowest level of experimentally generated turbulence resulted from introducing the grid flush to the upwind end of the working section (Fig. 1B). The highest level of turbulence developed in the wake downwind of the vertical cylinder (Fig. 1C,D) (Talley, 2010). Four treatments included positioning the cylinder in different relationships to the odor source: (1) 27 cm upwind of the source at the extreme upwind end of the working section of the wind tunnel, (2) immediately upwind of the source holder without the grid, (3) immediately downwind of the grid, 26 cm upwind of the source, and (4) immediately upwind of the source holder with the turbulence grid in place (Fig. 1C,D). A total of 266 cockroaches were used in this study resulting in 256 analyzed tracks. Males were removed from the analysis if they left the experimental arena before locating the odor source and did not return.

Flying odor tracking
Virgin male moths, 3–4 days old, were removed from their environmental chamber and placed individually into cylindrical aluminium screen cages, 15 cm in diameter by 15 cm in height. Moths were then allowed to acclimate to the environmental conditions by placing them in the wind tunnel room, under low-light conditions, from the beginning of their normal dark period for 2 h before the beginning of an experiment. Each experiment started 2 h after lights out to correspond to their peak response to female pheromone (Sasaki and Riddiford, 1984) and ended 2 h later (4 h into the light:dark cycle). Each moth in its cage was placed on the release stand in the pheromone plume for 1 min before the male was released. One minute was usually sufficient for a moth to begin wing fanning to warm its flight muscles and take off. If a moth did not wing fan or attempt to fly after an additional 2 min, it was removed from the experiment. Once released, moths were allowed to fly freely (or land) until they had either found the source or 5 min had passed. Only the paths of males that tracked the plume to the source were analyzed. Each moth was used once.

The same experimental treatments were used for flying odor trackers as described above for walking odor trackers (Fig. 1A–D). A total of 396 virgin male moths were used, and the tracks of 269 were analyzed in this experiment. Excluded from analysis were individuals that did not fly, did not exhibit plume-tracking behavior, or did not find the source as defined by approaching within two body lengths of source (ca. 10 cm).

Data analysis

Manduca sexta males fly much faster than *P. americana* walk, so the interval between digitized positions was different for the two species. The position and orientation of male cockroaches was digitized every 0.083 s by marking the position of the head and distal tip of the abdomen every 5th frame of the 60 Hz video. Moth positions were digitized every 0.033 s in both camera views by marking the head and distal tip of the abdomen. Video-recorded movement trajectories of walking (2D) and flying animals (3D) were digitized using commercial motion analysis software (Peak Motus 9, Vicon Inc.).

The groundspeed (cm s^{-1}) is the walking speed of the cockroach measured from one position to the next, the net velocity is the distance between the beginning and end of the track divided by the tracking time, and the track angle is the angle between the wind direction (upwind is 0 deg) and the movement vector drawn from one cockroach position to the next (Fig. 1E). The body axis angle is the angle between the wind direction and the longitudinal body axis, a line drawn from the head to the distal abdominal tip. The track width and inter-turn duration were measured using turns established as local minima and maxima of the cockroach head position relative to the side of the wind tunnel (Rutkowski et al., 2009) (Fig. 1E). The track width is the linear distance perpendicular to the wind direction between apices of sequential turns (Kuenen and Baker, 1982) and the inter-turn duration is the time between local maxima of lateral movement defining sequential turns (Fig. 1E). When there was no movement between sequential cockroach head positions, the event was scored as a stop and we measured both the stop duration and total number of stops per tracking performance.

Many of the variables used in the analysis of moth plume-tracking performance were the same as those used in the cockroach analysis (i.e. ground speed, track angle, track width and inter-turn duration). As the moths were flying into the wind while plume tracking, their airspeed, course angle and drift angle (Fig. 1F) were calculated according to the triangles of velocities method (Marsh et al., 1978). The airspeed is the velocity of the moth relative to the wind, the course angle is the orientation of the airspeed vector with respect to the wind direction (0 deg), and the drift angle is the difference between the track and course angles. The yaw and pitch angle of the moth's longitudinal body axis were measured at each moth position.

These experiments used a restricted randomized block design (Pilla et al., 2005), with the variables from the treatment groups being compared using a split plot analysis of variance (SAS v.9.1). All six turbulence treatments were conducted each experimental day as a block. The introduction of each insect and the order of treatment presentation were randomized within each block. The 2 h long duration of the daily peak pheromone response limited experimental time to 2 h for each species. This allowed only six treatments to be performed each day. For this reason, we broke the experiment into six treatments at each of the two wind speeds (i.e. 25 and 100 cm s^{-1}). From the perspective of an analysis of variance, this means that the six turbulence
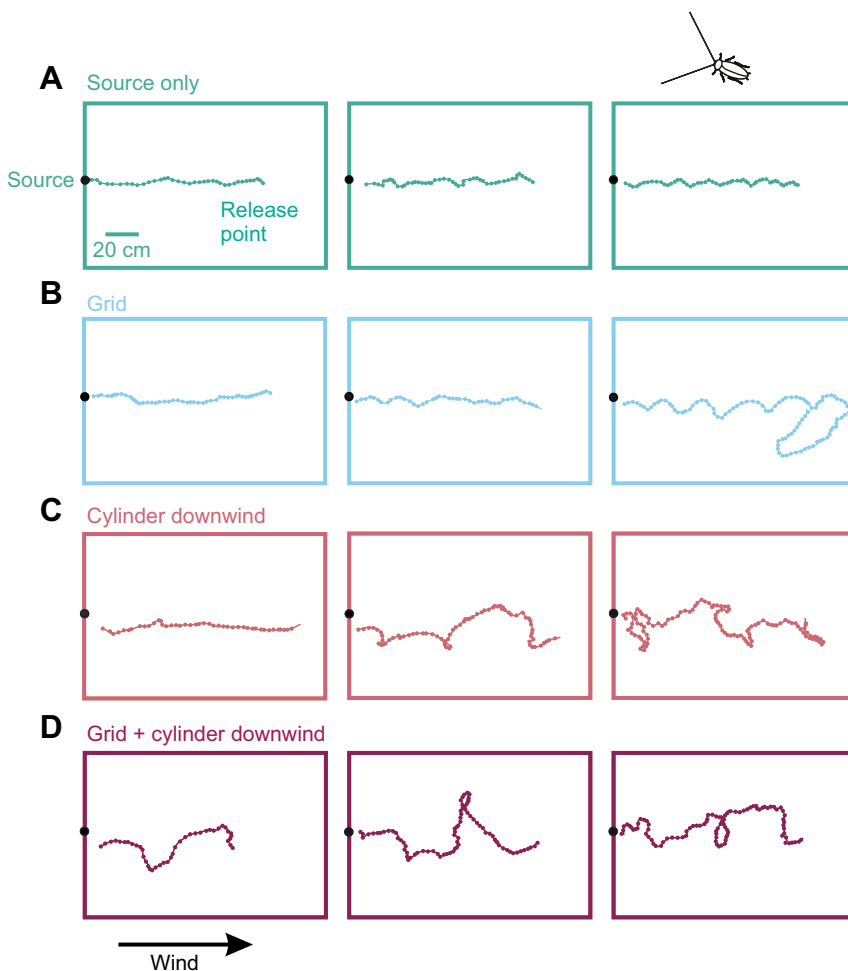

Fig. 2. Examples depicting the range of responses observed in the paths of *Periplaneta americana* cockroaches tracking plumes in different turbulent environments in 100 cm s^{-1} wind. Turbulent environments shown are: (A) source only, (B) grid, (C) cylinder downwind and (D) grid + cylinder downwind. Dots on the walking trajectory correspond to the position of the cockroach (every 0.083 s). The first plot in each row represents trajectories with ≤5 turns, the center plot has 6–9 turns, and the final plot shows trajectories with the most maneuvering (≥10 turns). Note the similarity between the movement trajectories between A and B, and how they differ from the trajectories in C and D. Cylinder upwind and grid + cylinder upwind treatments are not included because these tracks were statistically indistinguishable from the cylinder downwind and grid + cylinder downwind treatments.

treatments can be compared at each wind speed, but not between wind speeds.

The constraints of Fisher's exact test are not as restrictive as ANOVA and this enabled us to test for significant differences in the proportion of male moths tracking and locating the pheromone source across the different turbulence and wind speed treatments. We conducted Fisher's exact test using scipy.stats (v.1.9.0.dev0+1351.65d8361, Python v.3.10.1, Visual Studio v.1.63; Virtanen et al., 2020).

RESULTS

As previously reported (Willis and Avondet, 2005; Rutkowski et al., 2009), a typical level of variability was observed in tracking behavior both across each treatment group and between treatments (Figs 2 and 3). In general, the behavior of the cockroaches and moths changed depending on the kind of turbulence treatment to which they were exposed, though not always at a statistically significant level. The walking cockroaches often generated more convoluted tracks as the turbulence increased, but nearly always located the pheromone source (Fig. 4A), whereas the flying moths were as successful as the cockroaches in more turbulent environments and often ceased plume tracking in less turbulent environments (Fig. 4B).

Cockroaches almost always find the source

In our experiment, 96% of all *P. americana* males successfully tracked the plume to its source regardless of the turbulence treatment or wind speed (Fig. 4A). All males in both wind speeds left the release cage area when given the opportunity. The 4% of the entire experimental population that did not successfully track the plume were in the 100 cm s^{-1} wind speed treatment under turbulent conditions, suggesting some aspect of higher wind speed and/or turbulence is more challenging. Male cockroaches located the source so successfully in all turbulence and wind speed treatments that testing for statistical differences between them was not performed.

Cylinder turbulence alters cockroach odor tracking and is amplified at a higher wind speed

At 100 cm s^{-1}, male cockroaches generated wider tracks, moved at slower speeds and stopped more often in the environments with cylinder-generated turbulence compared with either condition without a cylinder (Fig. 5A,B,F,G). The males tracking plumes in any of the cylinder environments steered their tracks more off the wind direction, while they generally steered their tracks more directly into the wind in treatments without the cylinder (Fig. 5C). The males oriented their head to tail body axis more closely along the mean wind direction in the source only, and grid treatments than
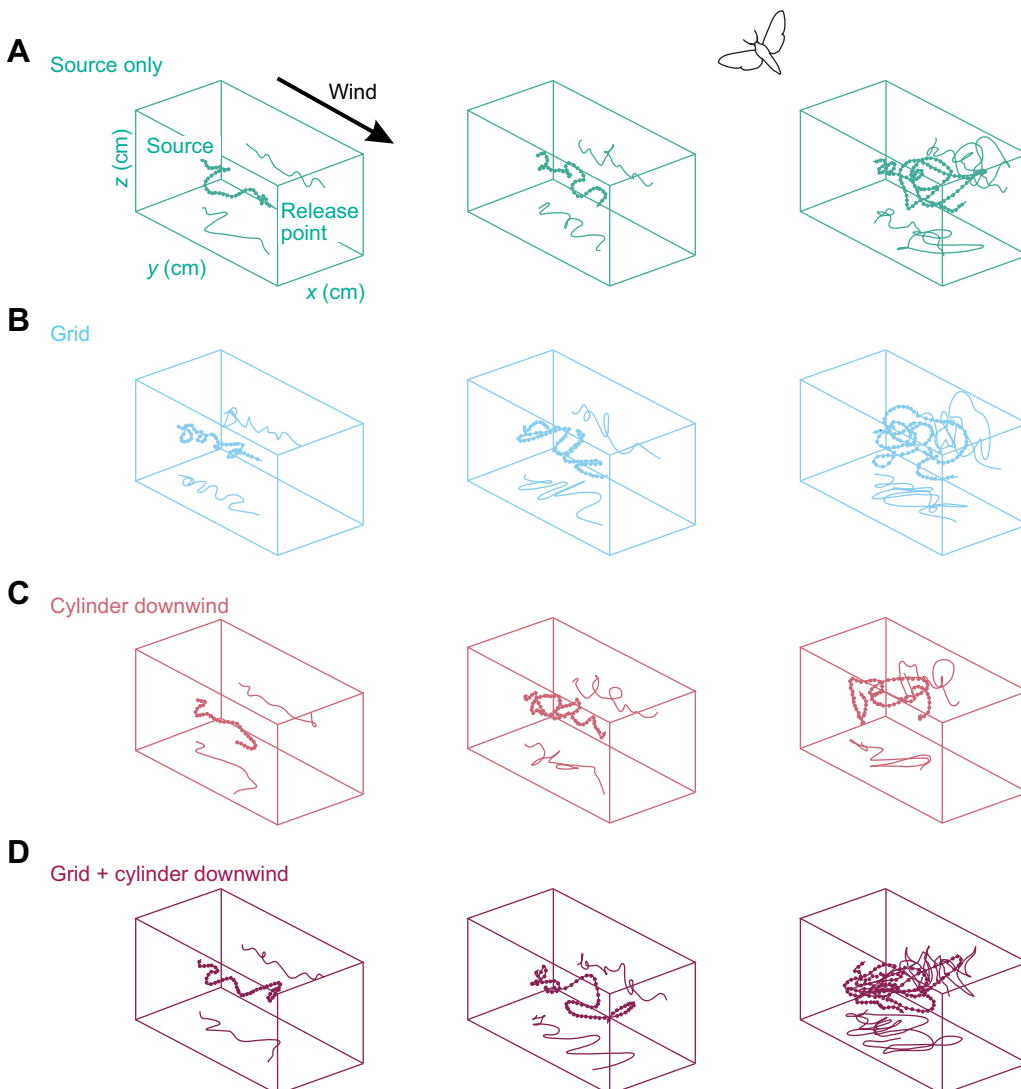

Fig. 3. Examples depicting the range of responses observed in the paths of *Manduca sexta* moths tracking plumes in different turbulent environments in 100 cm s⁻¹ wind. Turbulent environments shown are: (A) source only, (B) grid, (C) cylinder downwind and (D) grid + cylinder downwind. Dots correspond to the position of the moth every 0.033 s depicted in the 3D volume of the wind tunnel. The colored lines on the floor and wall of the wind tunnel are the projection of this 3D track onto the plan and side views. These examples show trajectories with mean course angles increasing from left to right. Cylinder upwind and grid + cylinder upwind treatments are not depicted because these tracks were statistically indistinguishable from the cylinder downwind and grid + cylinder downwind treatments.
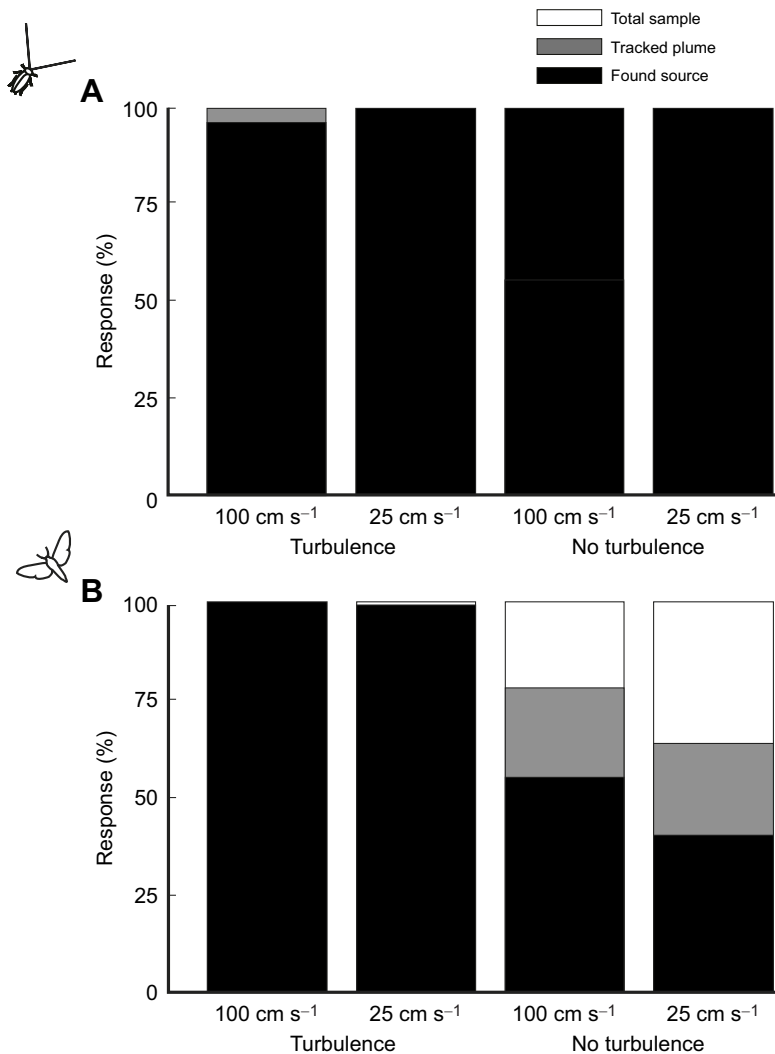
Fig. 4. Response of male *M. sexta* moths and *P. americana* cockroaches exposed to sex-attractant pheromone plumes at 25 and 100 cm s⁻¹ wind speed in different turbulence treatments. The response of (A) male cockroaches and (B) male moths. In both species, male responses to all turbulence treatments (i.e. grid, cylinder upwind, cylinder downwind and the cylinder at both positions together with the grid) were lumped for comparison with the source only treatment.

Legend: Total sample, Tracked plume, Found source

the cylinder treatments (Fig. 5C). The males in the grid and source only treatments generated narrower tracks than males in the cylinder treatments. The males in the source only and grid environments also took less time between turns than males in cylinder environments (Fig. 5E). The males in the source only and grid treatments also make fewer stops than the males in the cylinder environments (Fig. 5G). In all treatments, the males stopped for the same amount of time (Fig. 5H) ($P \geq 0.92$) as seen in previous studies with *P. americana* (Willis and Avondet, 2005). We observed only one statistically significant change in male cockroach behavior (track angle) across all of the experimental turbulence treatments in the 25 cm s⁻¹ wind speed ($P \leq 0.05$; Fig. 6D). The measured trends of the males in the cylinder treatment correspond with a previous study showing statistically significant differences between *P. americana* males tracking odor plumes in cylinder treatments (Willis and Avondet, 2005).

Moths are more successful at tracking and locating the source in more turbulent, rather than in smoother environments

Our analyses showed that the moths' ability to track the plume and locate the source was significantly dependent upon some combination of wind speed and turbulence (Fig. 4B). The presence of any turbulence-generating structure significantly improved a moth's ability to locate the source regardless of the wind speed (i.e. source only 59/160 versus turbulence 210/212, $P < 0.05$). When grouped by wind speed, moths were more successful at finding the source in 100 cm s⁻¹ wind (i.e. 133/217 in 25 cm s⁻¹ versus 136/155 in 100 cm s⁻¹, $P < 0.05$).

All male moths in the experiment, except two (in 25 cm s⁻¹), were successful at tracking the plume to the source in all treatments except the source only; 85% of the males tracked the plume (i.e. 40/47) in the source only treatment at 100 cm s⁻¹ wind speed, with only 60% (i.e. 28/47) locating the source. When the wind was reduced to 25 cm s⁻¹, only 65% (i.e.74/113) tracked the plume and 27% (i.e. 31/113) located the source (Fig. 4B).

Most moths in the experimental population responded to the introduction of the pheromone plume by becoming active and taking flight. Those that did not respond to the pheromone plume (ca. 2% at each wind speed) were distributed across the experimental design, indicating no treatment-associated bias in these early stages of plume tracking.

When the typical triangles of velocity output were measured and calculated from the reconstructed 3D flight tracks (Figs 7 and 8), we observed few statistically significant differences at 100 cm s⁻¹ wind speed. Even here, males in the lowest turbulence source only condition generated lower ground speeds and steered more into the wind than males tracking plumes in the cylinder upwind treatment
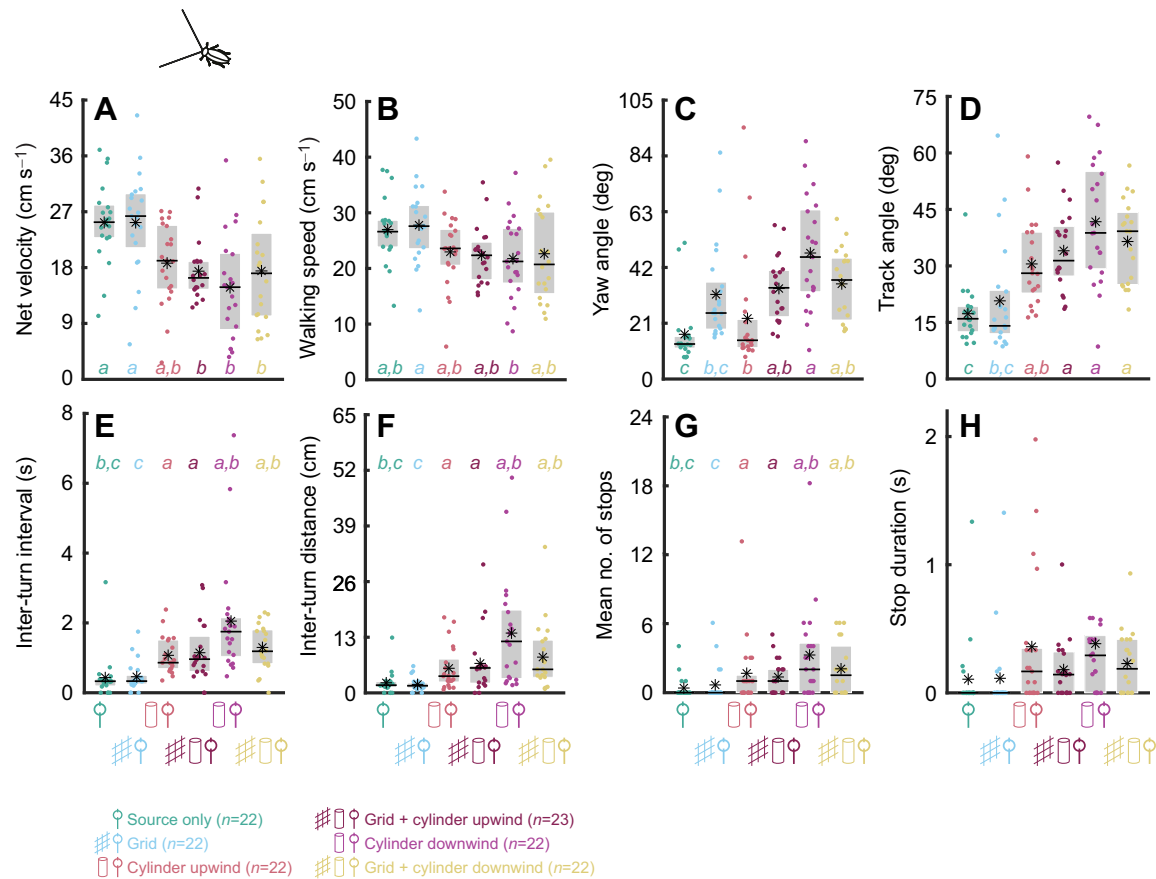
Fig. 5. Behavioral variables measured from male cockroaches and their tracks while they walked upwind in a plume of female pheromone in different turbulent environments in 100 cm s⁻¹ wind. (A) Net velocity. (B) Walking speed. (C) Absolute angle of the body yaw angle relative to upwind. (D) Mean orientation of movement vectors to due upwind (track angle). (E) Mean inter-turn interval. (F) Mean inter-turn distance. (G) Mean number of stops. (H) Mean duration of stops. The gray box is the 25–75% quartile, the horizontal black bar is the median, the asterisk is the mean, and each colored dot is an individual's mean performance plotted with a random jitter along the x-axis. Means with different letters are significantly different according to a repeated measures ANOVA($P \leq 0.05$) and *post hoc* Tukey's test. Plots of variables without lowercase letters had no statistically significant differences.

(Fig. 8A,D), with the performances of males in the other treatment groups broadly overlapping (Fig. 8). This presentation of the male moth's plume-tracking behavior is focused on their tracks in 3D because this is how they move, and because such analyses are relatively rare (Rutkowski et al., 2009; Vickers and Baker, 1996). However, when the moths' tracks were analyzed as viewed from the side or overhead, more statistically significant differences emerged (Figs S3 and S4). As with the 3D analysis, statistically significant differences were observed between lower and higher turbulence treatments, with broad overlap among the other treatments.

DISCUSSION

Male *P. americana* walking in the boundary layer and *M. sexta* flying through the free stream respond differently as they track odor plumes generated by the same turbulence. In our experiment, the moths were more successful at locating an attractive odor source in higher turbulence, while the cockroaches were almost always successful. Our results might be explained by multiple hypotheses, including: (1) the observed differences in the plume-tracking behaviors of walking cockroaches and flying moths stem from the different flow and odor information available in the boundary layer versus free-stream environments, (2) walking cockroaches and flying moths experience similar flow and odor information and employ the same tracking

mechanisms, but the different biomechanics of walking and flight produce different-looking plume-tracking behavior, or (3) flying moths and walking cockroaches use different tracking mechanisms for locating an odor source. To begin to resolve these questions, we conducted a detailed characterization of the flow and odor distribution in our experimental environments in parallel with the behavioral studies (Talley, 2010) (Figs S1 and S2). These measurements allowed us to determine both the similarities and differences in the flow and odor environments experienced by walking and flying odor trackers, and how their mode of locomotion might affect how they use the environmental information available. We can discount the impact the turbulence-generating structures (i.e. the grid and cylinder) would have on the animal's ability to use these for visual guidance. The cylinder is Plexiglas to reduce visual signatures and the grid covers the entire upwind cross-section of the wind tunnel that could provide wide-field information for flight control in addition to that already available from the wind tunnel structure itself. Vision is required for flight control and spatial orientation (Kennedy and Marsh, 1974; Copley et al., 2018), but there is little support for visual cues from the pheromone source being important in guiding pheromone plume tracking (Charlton and Cardé, 1990; but see Toshova et al., 2007).
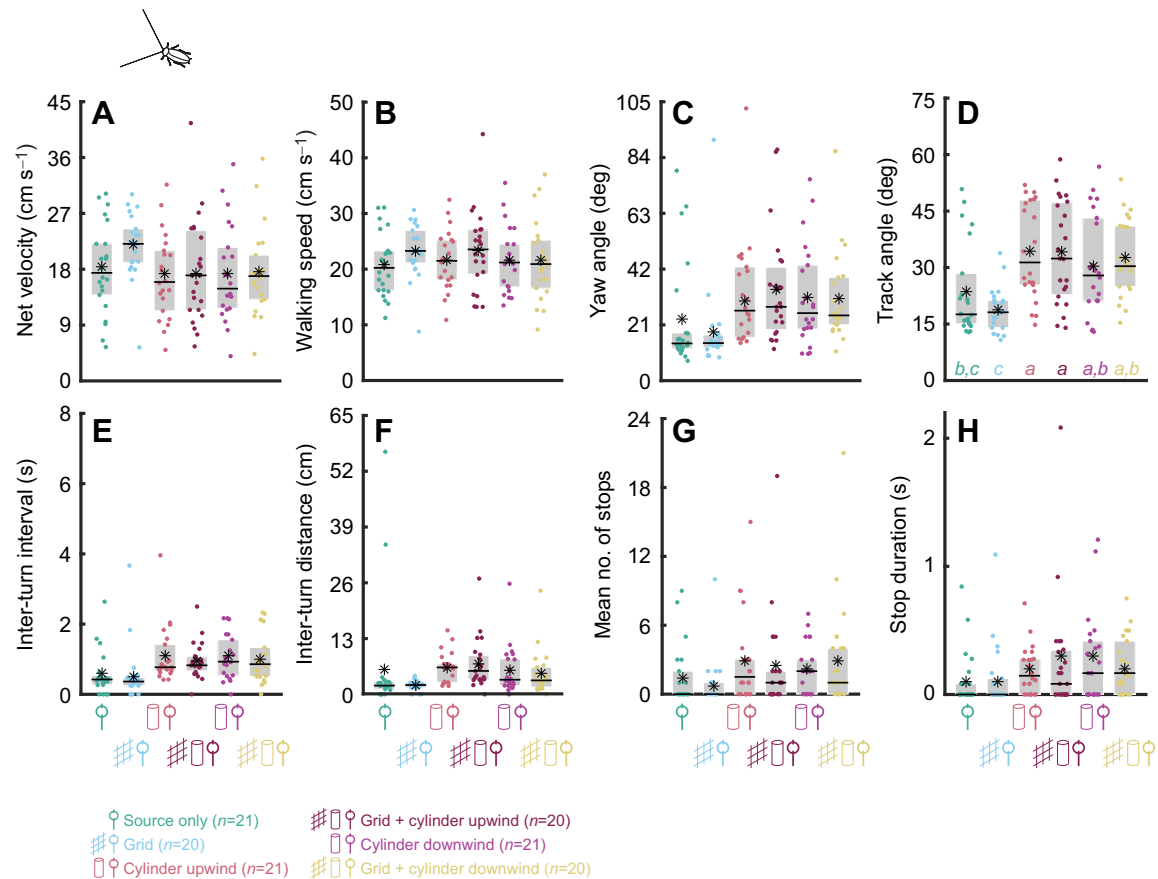
Fig. 6. Behavioral variables measured from male cockroaches and their tracks while they walked upwind in a plume of female pheromone in different turbulent environments in a 25 cm s^{-1} wind. (A) Net velocity. (B) Walking speed. (C) Absolute angle of the body yaw angle relative to upwind. (D) Mean orientation of movement vectors to due upwind (track angle). (E) Mean inter-turn interval. (F) Mean inter-turn distance. (G) Mean number of stops. (H) Mean duration of stops. Details of the plot as per Fig. 5.

Large scale eddies and boundary layer effects interact to cause differential success in walking and flying trackers

The turbulence and odor characteristics that result in our male cockroaches generating more complex tracks seem to make it easier for flying moths. A vertical cylinder of the diameter of that used in our experiment sheds vortices, alternating from side-to-side, at approximately 1 Hz according to its Strouhal number (Fig. S2), a dimensionless index characterizing the relationships of fluid speed, viscosity and dimension of the object in flow. The rotation of the vortices also alternates from clockwise to counterclockwise depending on the side of the cylinder shedding (rotation is always from the lateral margin toward the center of the cylinder) (Vogel, 1994).

Cylinders and grids both introduce turbulence into the flow, but the structure of that turbulence differs between the two structures (Talley, 2010). Grid turbulence is homogeneous and isotropic in cross-section (i.e. the same in all directions from the longitudinal centerline) (Roach, 1987). The larger turbulence intensity and large-scale turbulent eddies produced by the cylinder spread and mixed the pheromone plume across the wind tunnel. In contrast, even though the grid increased the turbulence through which the cockroaches had to track, the grid turbulence maintained a compact, albeit intermittent plume near the center of the wind tunnel (Fig. 9C,F,H) (Talley, 2010). The presence of a narrow plume in both the source only and grid treatments, which their antennae could span near the source, could account for the more directly

upwind orientation of the cockroaches tracking plumes in these environments (Fig. 2A,B).

It is well known that the primary directional cue used by plume-tracking animals (including cockroaches) is the flow direction (Kennedy, 1939; Kennedy and Marsh, 1974; Grasso and Atema, 2002; Weissburg, 2000; Willis and Avondet, 2005), which the trackers are sensitized to by an attractive odor (Kennedy, 1940). Many of the cockroaches in cylinder treatments appeared to respond to this side-to-side alternation of vortices as though the overall wind direction were changing. The increases in their average body yaw angles also indicate that they were steering more off the average wind direction (i.e. the bulk flow parallel with the long axis of the wind tunnel) in the experimental treatments that included the cylinder (Figs 5 and 6). Continuous re-orientation to this dynamically changing directional cue could have resulted in the wandering tracks we observed from male cockroaches tracking plumes in the cylinder treatments (Fig. 2C,D). Similar cockroach tracks have been observed previously in pheromone plumes downwind of a vertical cylinder (Willis and Avondet, 2005). The eddies in the cylinder flow start at roughly the diameter of the cylinder (ca. 7 cm), or almost twice the mean length of *P. americana* from our laboratory colony (3.9±0.2 cm). These vortices might be large enough to cause the cockroaches to dynamically reorient as though they were shifts in the overall flow direction. If this happened, the cockroaches would sense the local wind direction in each vortex, where the most recently detected wind direction could
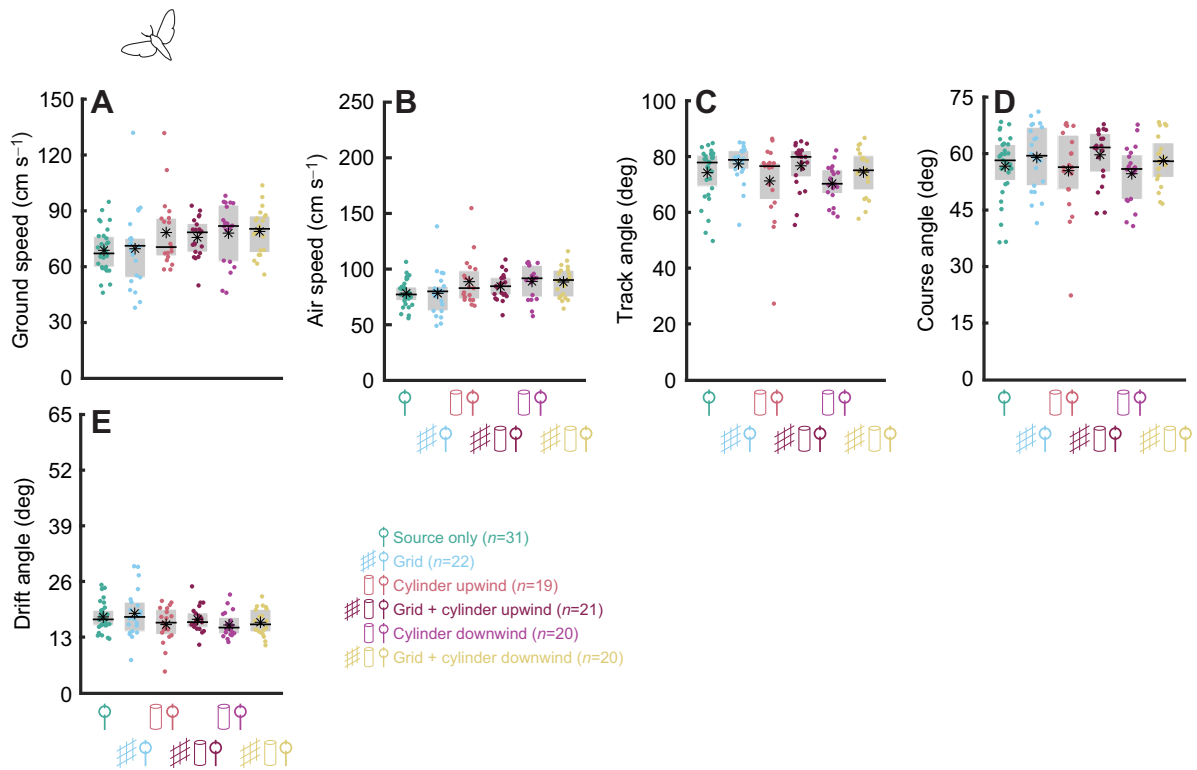
Fig. 7. Behavioral variables measured from the tracks of male moths as they flew upwind and tracked a plume of female pheromone presented in different turbulent environments in 25 cm s^{-1} wind. (A) Ground speed. (B) Air speed. (C) Track angle (mean orientation of movement vectors) measured with respect to due upwind. (D) Course angle measured with respect to due upwind. (E) Drift angle measured with respect to due upwind. Details of the plot as per Fig. 5. Variables for 3D movement only included here; for the vertical and horizontal components, see Figs S3 and S4.

be perpendicular or opposite to the bulk flow in the wind tunnel. This effect should increase as they approach the cylinder (Roach, 1987).

For flying moths, the effects of the eddies moving laterally across the wind tunnel as they are carried downwind could help explain the longer horizontal inter-turn distances between side-to-side turns generated as they tracked odor in turbulence downwind of the cylinder (Figs S3 and S4B). However, the consistent generally upwind orientation of their body yaw angles in all treatments (Figs S3 and S4) indicates that they were probably not responding to the large cylinder-generated vortices as though they were shifts in the wind direction. During plume-tracking flight, moths aim their longitudinal body axes into the wind (i.e. ±30 deg either side of due upwind) with the crosswind inter-turn legs of their zigzagging tracks resulting from wind-induced drift and laterally directed thrust (Willis and Arbas, 1998). Moths tracking an attractive odor plume alter their steering to maintain an upwind track even as the wind changes direction (Baker and Haynes, 1987; David et al., 1983). If the moths in our study treated the cylinder-generated eddies as shifts in wind direction, they should have constantly changed the direction in which their bodies were aimed as they tracked the plume upwind. No changes like this were observed.

The internal structure of the pheromone plumes in each of our turbulent treatments was measured using the physiological responses of a surgically removed antenna of an *M. sexta* male, a routine procedure known as an electroantennogram (EAG). These measurements showed the plume filaments in the higher turbulence cylinder treatments were broader (i.e. longer duration), were made up of lower concentrations of pheromone, and were further apart than pheromone filaments in the source only or grid environments

(Fig. 9) (Talley, 2010). Nearly all males successfully tracked the plume to the source in all turbulence treatments, including the grid (Fig. 4B), while our EAG measurements showed that the internal structure of the grid and source only plumes were very similar (Fig. 9) (Talley, 2010). Thus, other aspects of the sensory–motor–environment interaction must account for this difference (see 'Different locomotory behaviors produce different looking plume-tracking behavior', below).

Plume-tracking male moths have often been observed to slow and sometimes stop their upwind progress if the concentration of odorants applied to the source is very different from pheromones emitted by a live female moth (Baker et al., 1981; Charlton et al., 1993). This behavior has been termed 'arrestment' (Kennedy, 1978) and is characterized by decreasing flight speed, increased rate of counterturning, and steering more across the wind than upwind (Willis and Baker, 1988, 1994). The interaction of the moths' behavioral changes and wind-induced drift results in the moths holding station with respect to the wind tunnel while turning back and forth across the wind in the plume (Willis and Baker, 1988, 1994). It is rare for a moth to move further upwind or locate the odor source once they have arrested in the plume (Baker et al., 1981; Charlton et al., 1993).

None of the moths in our experimental population exhibited arrestment behavior in response to any of our experimental treatments, indicating that the pheromone concentrations in our experimental plumes were within the dynamic range supporting upwind plume tracking. The narrower horizontal inter-turn distances we observed in the source only treatments (Figs S3 and S4) were consistent with previously observed responses to plumes from sources of increasing concentrations or blends of
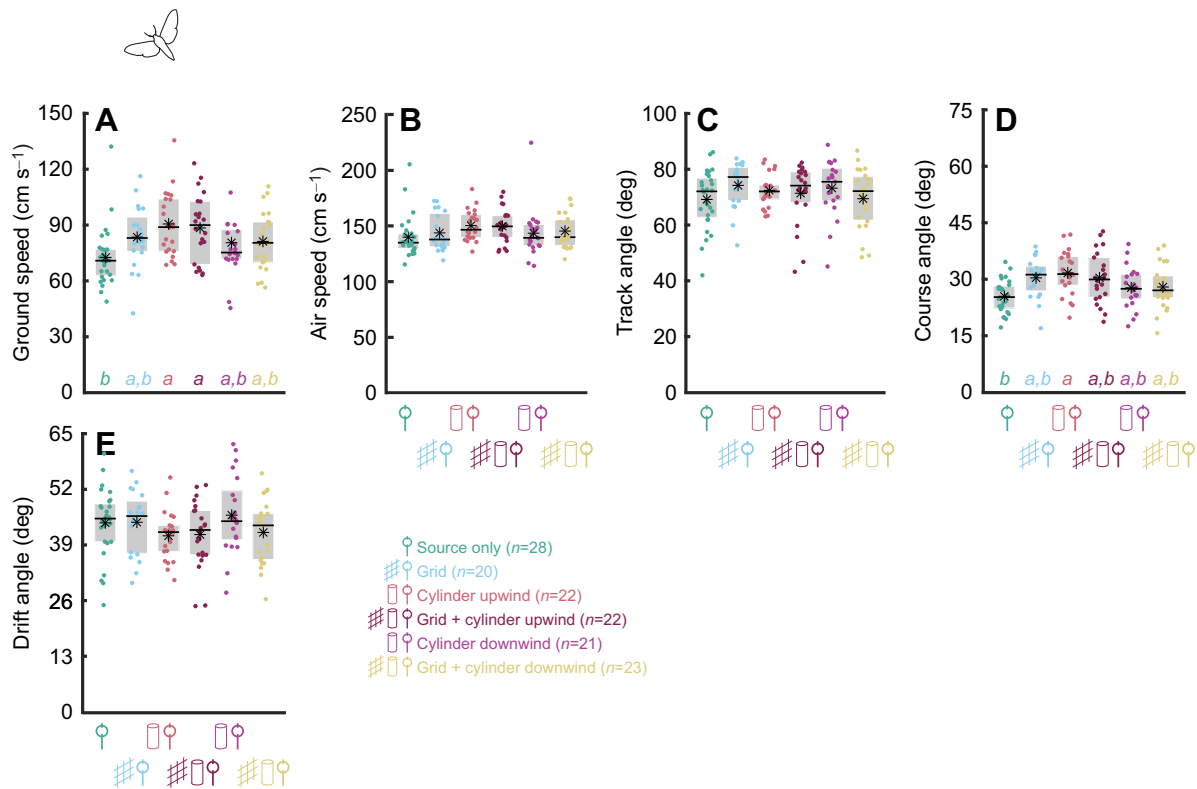
Fig. 8. Behavioral variables measured from the tracks of male moths as they flew upwind and tracked a plume of female pheromone presented in different turbulent environments in 100 cm s⁻¹ wind. (A) Ground speed. (B) Air speed. (C) Track angle (mean orientation of movement vectors) measured with respect to due upwind. (D) Course angle measured with respect to due upwind. (E) Drift angle measured with respect to due upwind. Details of the plot as per Fig. 5. Variables for 3D movement only included here; for the vertical and horizontal components, see Figs S3 and S4.

pheromone components composed of incorrect ratios (Arbas and Willis, 1994; Cardé and Hagaman, 1979; Charlton et al., 1993; Kuenen and Baker, 1982; Willis and Baker, 1988, 1994). Recent experiments with pheromone plumes of different widths but similar concentrations suggest that the cross-section (i.e. widths and heights) of the plume may also be used by *M. sexta* males to shape the width of their flight tracks (Willis et al., 2013).

In contrast to the concentration sensitivity observed in the plume-tracking behavior of some flying moths (Kuenen and Baker, 1982; Charlton et al., 1993), *P. americana* males walking in the wind tunnel have been observed to generate consistent and statistically indistinguishable plume-tracking behavior across a wide range of pheromone concentrations (10^{-6} to 10^{-3} ng) (Willis and Avondet, 2005). The direct upwind walking observed in response to plumes issuing from very low concentration sources (Willis and Avondet, 2005) suggests that the meandering tracking paths generated by males responding to plumes in the most turbulent flows, downwind of cylinders, was probably not the result of low odor concentrations. Most of the male cockroaches that left the experimental arena without tracking the plume to its source (8 individuals) were in experimental treatments that included a cylinder. These males did not display sustained orientation with the wind (i.e. negative anemotaxis), which has previously been observed when the concentration of pheromone was low (Bell and Kramer, 1979). They also did not exhibit the local search behavior that characterizes male *P. americana* responses to the loss of odor in wind (Willis et al., 2008). These eight males constituted less than 1% of the males introduced into odor plumes downwind of the cylinder, so it is possible that they may not have been motivated to track female

pheromone. It is far more typical for 100% of our experimental animals (virgin males at least 3 weeks old) to track the plume to its source (Willis and Avondet, 2005; Willis et al., 2008, 2011).

Different locomotory behaviors produce different looking plume-tracking behavior

Prior to this study, the significance of the boundary layer flow to insects tracking air-borne plumes by moving along the substrate had not been tested experimentally. In contrast, it has been relatively well studied in animals tracking plumes while walking and crawling through aquatic environments (Ferner and Weissburg, 2005; Weissburg, 2010; Weissburg et al., 2003; Weissburg and Zimmer-Faust, 1993, 1994; Zimmer-Faust et al., 1995). In most of these studies, increasing the level of turbulence in the boundary layer, typically by increasing the roughness of the substrate, degrades the tracking performance of walking or crawling animals, resulting in meandering tracks and lower proportions of the experimental population locating the source. A similar degradation in the plume-tracking performance of cockroaches was observed in our experiments as the turbulence generated by a cylinder associated with the odor source dominated the environment.

It is possible that, because *M. sexta* is a large animal flying at over 1 m s⁻¹, it may not be able to alter its flight steering fast enough to orient to the rapidly changing flow directions characterizing the vortices downwind of the cylinder. *Manduca sexta* males from our laboratory colony have mean (±s.d.) wingspans of ca. 10±0.6 cm with a mass of ca. 1.5±0.05 g (*n*=12) (M.A.W., unpublished data), and they flew at ca. 150 cm s⁻¹ (Figs 7 and 8A) when tracking plumes upwind in cylinder-generated turbulence. Their flight speed,
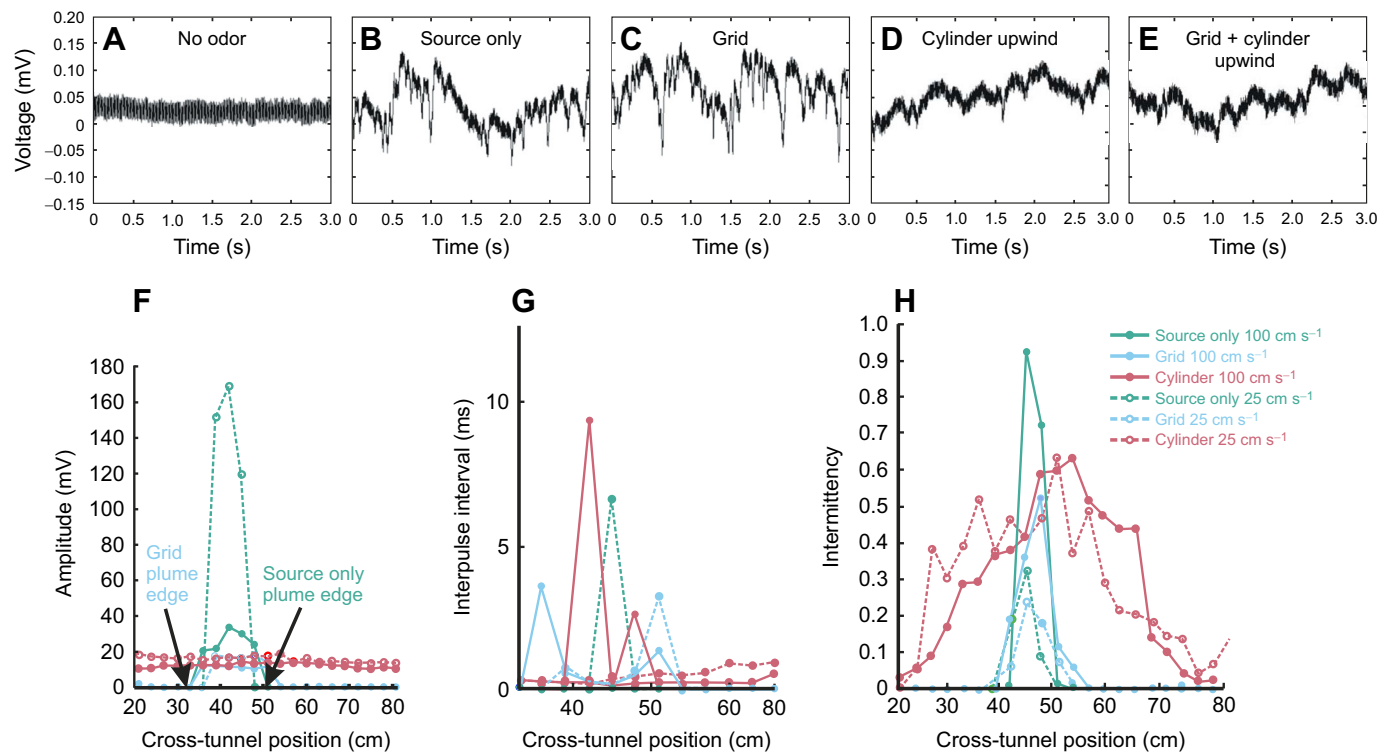
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Fig. 9. Characteristics of the odor plume measured from electroantennogram recordings of the responses of a male *M. sexta* antenna. (A) Example of electroantennogram (EAG) response to clean pheromone-free air at 100 cm s^{-1}, 2 cm above the floor of the wind tunnel. (B–E) Example of an EAG response to the source only treatment (B), grid treatment (C), cylinder upwind treatment (D) and grid+cylinder upwind treatment (E) in the same conditions as in A. EAG responses recorded in the cylinder downwind and grid+cylinder downwind treatments were similar to those depicted in D and E, respectively. (F) Peak odor amplitude values recorded 40.5 cm above the wind tunnel floor, 1 m downwind of the odor source. (G) Inter-pulse interval recorded 40.5 cm above the wind tunnel floor. (H) Intermittency recorded 40.5 cm above the wind tunnel floor. The source height for flying trackers was 40.5 cm. EAG responses depicted in F–H were measured in the free stream at 25 cm s^{-1} and 100 cm s^{-1}.

momentum and sensory-motor latency between detecting and responding to the changing directions of wind in the largest of the rotating eddies shed by the cylinder may make it difficult or impossible for them to react as though these were changes in the wind direction. The EAG recordings of pheromone plumes downwind of the cylinder show that the cylinder turbulence distributes the pheromone from wall to wall across the wind tunnel, making it certain that the moths will always be in contact with the attractive odor. This may mean that their flight speed, mass and the restrictive wind tunnel environment could combine to make it essentially impossible for them to re-orient to the changes in flow caused by the eddies downwind of the cylinder. Perhaps because the cockroaches are moving slower with less momentum, they are able to re-orient to the changes in flow caused by the same large eddies.

According to recent studies of the effects of turbulence on flight control (Coombes and Dudley, 2009), our low-turbulence environments should have been the easiest for our flying moths to maneuver through, so why is their ability to locate the source poorest there? It is possible that the moths' significantly enhanced tracking performance in environments with intermediate levels of turbulence generated by our grid can help answer this question.

The primary task of any plume-tracking animal is to maintain contact with the cross-section of the plume (Willis, 2008). The time-averaged pheromone plume 1 m downwind of the grid is approximately twice as wide as the plume in the source only environment (increase from ca. 10 cm to ca. 20 cm) (Fig. 9) (Talley, 2010). Assuming a circular cross-sectional area, a slice through the plume 1 m downwind from the source, as viewed from downwind,

should increase from approximately 113.1 cm^2 to 346.4 cm^2 because of the grid-induced turbulence. This is a roughly threefold increase in cross-sectional area. This larger cross-section may make maneuvering into the wind while maintaining contact with the odor plumes easier in the intermediate turbulence of the grid environment than in the low-turbulence source only environment. By increasing the cross-sectional area of the plume, the turbulent environment downwind of the grid makes a larger 'target' and thus a potentially easier task for the plume-tracking moths to perform. Thus, in this scenario, it is a combination of sensory and motor mechanisms that underlie the increase in the proportion of moths locating the source in the environment with grid turbulence. The only way to verify whether larger cross-sections make it easier for moths to maintain their position within the plume is to challenge them to track different-sized cross-section plumes and quantify their performance as we did here.

Coombes and Dudley (2009) studied freely flying orchid bees as they maintained contact with an attractive wind-borne odor plume by generating rapid maneuvers to compensate for turbulence-induced perturbations of their flight. We did not observe our moths making rapid corrective maneuvers during our experiments and it seems unlikely that the lower proportion of *M. sexta* males successfully tracking the plume and locating the source in our lowest turbulence environment (source only) resulted from turbulence-induced errors. In fact, *M. sexta* males were the least successful at plume tracking and source location in our experimental treatment with the lowest turbulence, the source only environment presented at the lowest wind speed. The turbulence measured in the

Environment	Turbulence	Spatial flow information	Temporal flow information	Plume cross-section	Time-averaged concentration	Intermittency
Source only	↓ Low	↑ High	↓ Low	·	↑ High	↑ High
Grid	↑ High	↑ High	↑ High	∘	↑ High	↑ High
Cylinder	↑ Highest	↓ Low	↑ High	◯	↓ Low	↓ Low

Fig. 10. Summary of environmental characterization. Turbulence, and spatial and temporal information were calculated from hot wire recordings (Talley, 2010). The plume cross-sections were measured with smoke plumes and EAG recordings, and are shown in relative scale.

source only environment was approximately 1%, which means that the peak velocity measured was ca. 1% of the mean. Consistent with the turbulence measurements, the odor plume was also narrowest in the source only treatments, regardless of the wind speed (Fig. 9) (Talley, 2010).

Flying moths and walking cockroaches might use different tracking mechanisms

Moths and cockroaches have been hypothesized to use primarily temporal and spatial mechanisms, respectively (Rust et al., 1976; Vickers and Baker, 1991; Willis, 2008). The cylinder causes eddies in the flow, and almost certainly provides less spatial information in the boundary layer, and more temporal information in the free stream (Fig. S2) (Talley, 2010). If moths compare successive sensory samples in time to determine wind direction, the cylinder (and grid) would provide more useful temporal information, and this could explain their increased success at tracking odor plumes in these environments. If cockroaches compare sensory information sampled at two points in space at the same time to orient upwind, the plumes downwind of the cylinder may have provided them with less useful spatial information, possibly explaining their poorer performance in all cylinder treatments. More available spatial cues might explain the more direct tracking performance in the grid and source only environments. The behavioral results presented here support earlier ideas for how these two odor trackers use odor information to locate import resources. However, further experiments aimed at manipulating the bilateral symmetry of the sensory inputs for these two plume trackers will be critical in resolving how they use odor to maintain contact with odors and locate their sources.

In this study, we ignored individual variability in behavior, e.g. why individual variability would differ from average measures of behavior (Arbas and Willis, 1994; Marsh et al., 1978). There is expanding interest in understanding within-individual variation leading to quantification of individuality and personality (Cleasby et al., 2015). To study this, instead of using naive un-exposed males for every test, we would have had to measure the response of each individual to each turbulence and wind treatment while controlling for age and experience.

Summary

The temporal and spatial structure of the turbulence and odor environments used in these experiments were measured (Fig. S2), making interpretations of how these changes affected the walking and flying plume trackers possible (Fig. 10). In many ways, the results substantiate previous ideas for how flying moths and walking cockroaches use odor information to track a wind-borne plume. Environments that presented the walking cockroaches with ideal conditions emphasizing odor information that could be derived from spatial comparisons between their antennae (i.e. stable high-contrast edges between odor and clean air) enabled them to track and locate the source, rapidly generating tracks almost directly upwind. The high-intensity, large-scale turbulence generated by inserting a cylinder into the flow disrupted this high-contrast olfactory 'landmark' and resulted in the cockroaches taking longer to locate the source, often generating wide meandering walking tracks. These same conditions had the opposite effect on the plume-tracking flying moths, again supporting the previously held idea that because of the faster flows in which they track odors and their higher flight speed they may emphasize primarily temporal comparisons to maintain contact with the plume.

The sensory systems and brains of these plume-tracking animals probably do not use exclusively spatial or temporal odor information to perform these tasks. The nearly 100% success of male cockroaches locating the pheromone source suggests that these animals may dynamically shift their emphasis from spatial to temporal as dictated by changing environmental conditions. Our ongoing experiments will aim to reveal whether such changes in control happen and their underlying mechanisms.

Author contributions
Conceptualization: J.T., E.W., M.A.W.; Methodology: J.T., E.W., M.A.W.; Software: J.T.; Validation: E.W.; Formal analysis: J.T., E.W.; Investigation: J.T.; Resources: E.W., M.A.W.; Data curation: J.T.; Writing - original draft: J.T.; Writing - review & editing: E.W., M.A.W.; Supervision: E.W., M.A.W.; Project administration: M.A.W.; Funding acquisition: M.A.W.

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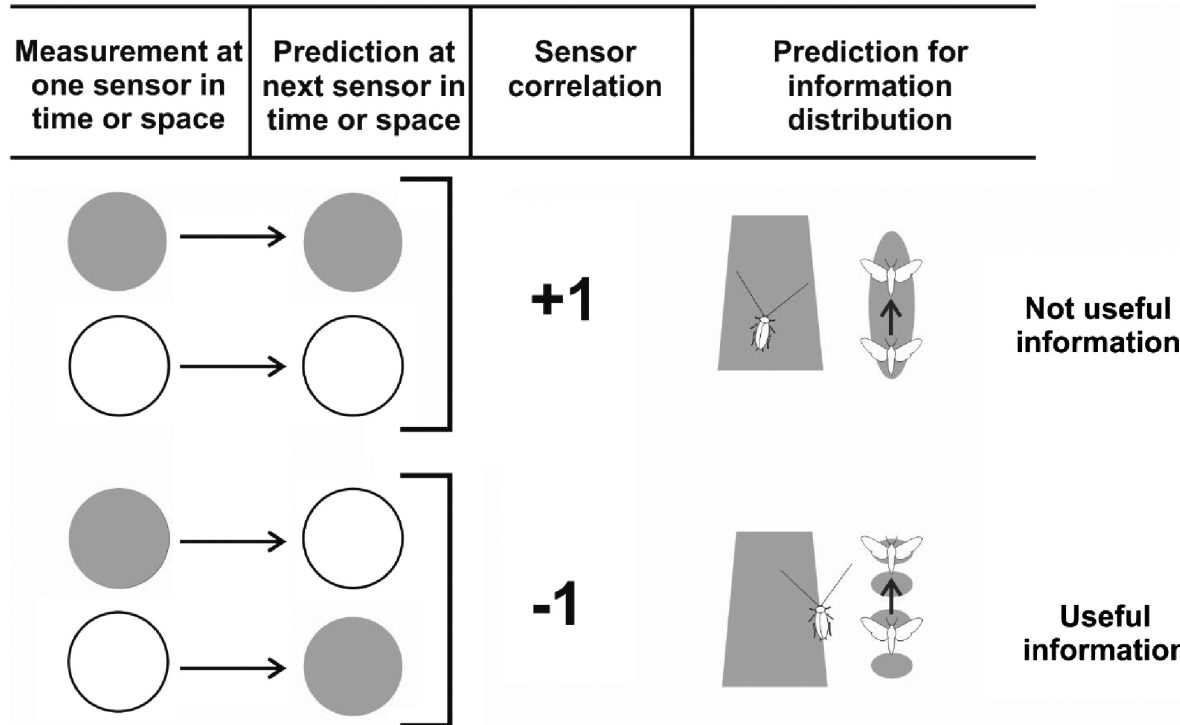
Fig. S1. Usefulness of correlation measurements for animal behavior. Odor distribution can be predicted from flow if they are highly related. For a +/- 1 correlation, the first sensor measurement reliably predicts the next so measurements taken at both does NOT provide new information. However, the positive correlation is not as useful to animals tracking a signal while the negative correlation is potentially very useful for tracking because it could indicate movement away from an attractive resource. For a zero correlation, the measurement at the first sensor cannot predict the next therefore measuring at both provides new information and it is unclear if this is useful for animals tracking an odor signal.
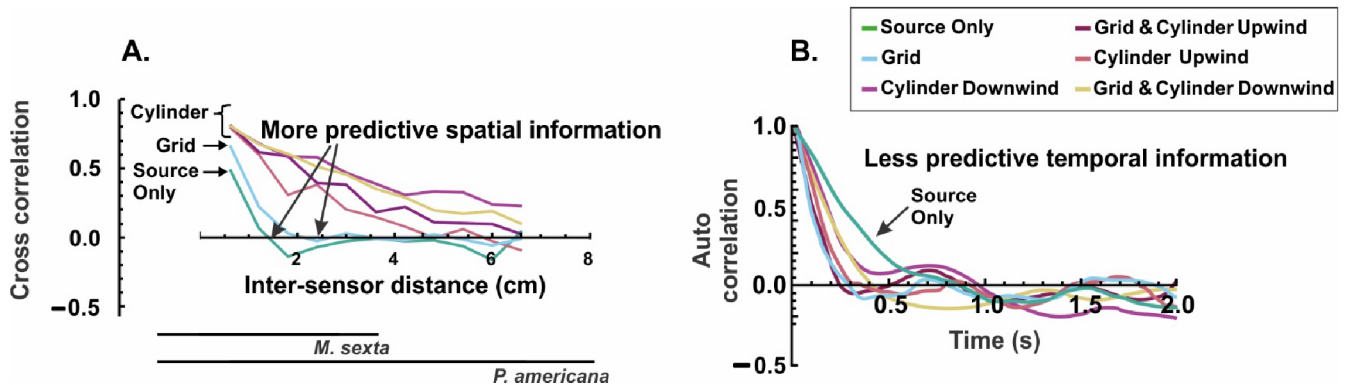
Fig. S2. Cross- and auto-correlation of turbulence using horizontal profiles measured with hotwire anemometer probes. **A)** Cross correlations of wind velocities measured from two hotwire probes held at from 0.6 to 6.6 cm apart on the centerline of the wind tunnel 2 cm above the floor. Each colored trace represents one of the turbulence treatments in our experiment. Mean flow velocity was set to 100 cm/s. Horizontal lines labeled *M. sexta* and *P. americana* illustrate the mean distances between the tips of those respective species antennae during plume tracking behavior. **B)** Auto-correlations of wind velocities measured from a hotwire probe recorded at 1 cm steps at the source height (40.5 cm above the wind tunnel floor) at the center of the cross section of the wind tunnel. Mean flow velocity was set to 100 cm/s.
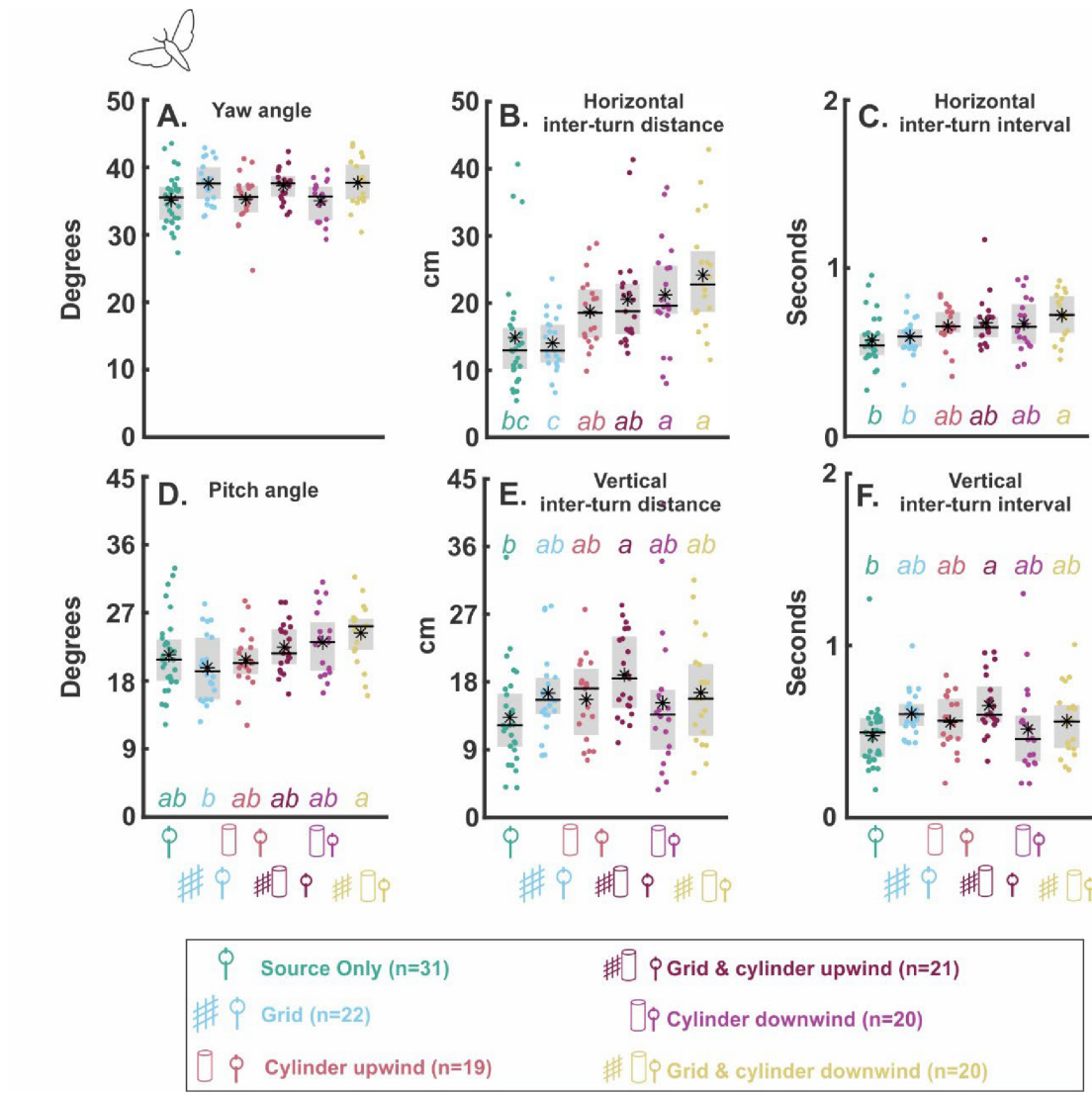
Fig. S3. Means of behavioral variables measured from the body and tracks of moths as they flew upwind and tracked a plume of female pheromone presented in different turbulent environments in 25 cm/s wind. In these plots the grey box is the 25-75% quartile, the horizontal black bar is the median, the asterisk is the mean, and each colored dot is an individual's mean performance plotted with a random jitter along the X-axis. Grand means with letters in common are not significantly different according to a repeated measures ANOVA($P \leqslant 0.05$) and *post-hoc* Tukey's test. Plots of variables without lower case letters had no statistically significant differences. (**A**) Absolute values of yaw angles of *M. sexta* males tracking female pheromone plumes. (**B**) Horizontal inter-turn distances of *M. sexta* males tracking female pheromone plumes. (**C**) Horizontal inter-turn intervals of *M. sexta* males tracking female pheromone plumes. (**D**) Pitch angles of *M. sexta* males tracking female pheromone plumes. (**E**) Vertical inter-turn distances of *M. sexta* males tracking female pheromone plumes, (**F**) Vertical inter-turn intervals of *M. sexta* males tracking female pheromone plumes.
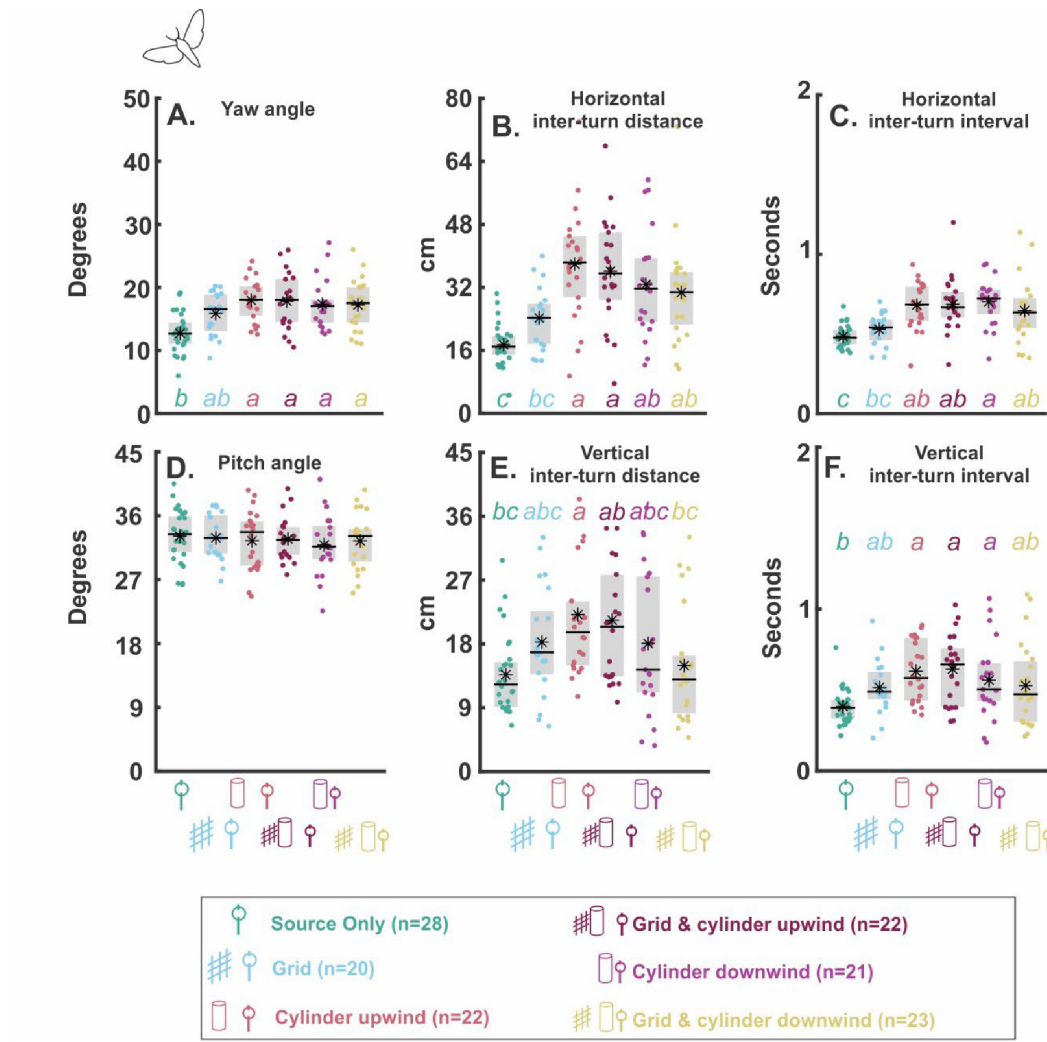
Fig. S4. Means of behavioral variables measured from the body and tracks of moths as they flew upwind and tracked a plume of female pheromone presented in different turbulent environments in 100 cm/s wind. In these plots the grey box is the 25-75% quartile, the horizontal black bar is the median, the asterisk is the mean, and each colored dot is an individual's mean performance plotted with a random jitter along the X-axis. Grand means with letters in common are not significantly different according to a repeated measures ANOVA($P \leqslant 0.05$) and *post-hoc* Tukey's test. Plots of variables without lower case letters had no statistically significant differences. (**A**) Absolute values of yaw angles of *M. sexta* males tracking female pheromone plumes. (**B**) Horizontal inter-turn distances of *M. sexta* males tracking female pheromone plumes. (**C**) Horizontal inter-turn intervals of *M. sexta* males tracking female pheromone plumes. (**D**) Pitch angles of *M. sexta* males tracking female pheromone plumes. (**E**) Vertical inter-turn distances of *M. sexta* males tracking female pheromone plumes, (**F**) Vertical inter-turn intervals of *M. sexta* males tracking female pheromone plumes.