

RESEARCH ARTICLE

Wind and obstacle motion affect honeybee flight strategies in cluttered environments

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ABSTRACT

Bees often forage in habitats with cluttered vegetation and unpredictable winds. Navigating obstacles in wind presents a challenge that may be exacerbated by wind-induced motions of vegetation. Although wind-blown vegetation is common in natural habitats, we know little about how the strategies of bees for flying through clutter are affected by obstacle motion and wind. We filmed honeybees *Apis mellifera* flying through obstacles in a flight tunnel with still air, headwinds or tailwinds. We tested how their ground speeds and centering behavior (trajectory relative to the midline between obstacles) changed when obstacles were moving versus stationary, and how their approach strategies affected flight outcome (successful transit versus collision). We found that obstacle motion affects ground speed: bees flew slower when approaching moving versus stationary obstacles in still air but tended to fly faster when approaching moving obstacles in headwinds or tailwinds. Bees in still air reduced their chances of colliding with obstacles (whether moving or stationary) by reducing ground speed, whereas flight outcomes in wind were not associated with ground speed, but rather with improvement in centering behavior during the approach. We hypothesize that in challenging flight situations (e.g. navigating moving obstacles in wind), bees may speed up to reduce the number of wing collisions that occur if they pass too close to an obstacle. Our results show that wind and obstacle motion can interact to affect flight strategies in unexpected ways, suggesting that wind-blown vegetation may have important effects on foraging behaviors and flight performance of bees in natural habitats.

KEY WORDS: *Apis mellifera*, Flight behavior, Clutter, Headwind, Tailwind

INTRODUCTION

Bees (Hymenoptera: Apoidea) provide vital pollination services to plants, enhancing biodiversity in many places around the world (Klein et al., 2007; Potts et al., 2010). To provide these ecosystem services, bees must leave their nests and fly through habitats that are often cluttered with vegetation and buffeted by unpredictable winds – features that pose challenges to flight performance. Bees flying through obstacles in still air fly more slowly and perform braking maneuvers (Crall et al., 2015), but collisions with vegetation still occur frequently in natural habitats (Foster and Cartar, 2011). These

collisions can cause irreversible wing damage, which reduces the capacity of bees to accelerate during sudden maneuvers (Mountcastle et al., 2016) and increases mortality (Cartar, 1992). The unsteady, turbulent winds that characterize most natural habitats reduce the flight stability of bees (Crall et al., 2017), leading to stabilizing, but energetically costly, changes in flight kinematics (Combes and Dudley, 2009; Crall et al., 2017). Vegetation and wind are components of habitat complexity that are likely to change in the future owing to anthropogenic land use (e.g. habitat fragmentation, agriculture) and climate change (e.g. increasing frequency and magnitude of windstorms) (Breitbach et al., 2012; Seidl et al., 2017). Thus, gaining a more complete understanding of how clutter and wind affect flying bees may help us predict how bees will be impacted by future environmental changes.


Bees in natural habitats encounter vegetation with varied spatial characteristics (e.g. leaf size, gaps between branches), and they use several strategies to minimize the risk of collision while negotiating these obstacles. When bees fly through a landscape, they can estimate their own flight speed based on the optic flow of landscape features moving from front to back past their eyes; closer obstacles produce a higher optic flow, and bees reduce their flight speed when flying through smaller spaces (Srinivasan et al., 1991, 1996). Bees center themselves when flying between landscape features such as obstacles by balancing optic flow across the left and right sides of their eyes, helping them to avoid collisions (Baird and Dacke, 2012; Linander et al., 2017; Lecoœur et al., 2019). When bees approach an obstacle directly in front of them, they can decelerate smoothly to land on the obstacle by maintaining a constant apparent rate of image expansion (Baird et al., 2013), although this strategy is not always observed in the presence of wind (Chang et al., 2016). Additionally, bees may cast from side to side when approaching obstacles, presumably to gather visual information about the obstacle (Ravi et al., 2019). Bees also perform braking maneuvers, in which they increase their body pitch angle and reverse direction to avoid collisions with obstacles (Crall et al., 2015). Bees flying through moving obstacles sometimes perform extreme corrective maneuvers, accelerating at near maximum capacity to avoid unexpected collisions (Mountcastle et al., 2016). When multiple routes around an obstacle exist, bees tend to choose the route that provides the greatest distance between their wings and the obstacles (Kirchner and Srinivasan, 1989; Baird and Dacke, 2016; Ong et al., 2017).

Bees in natural habitats also encounter wind that varies widely in velocity, direction and flow structure (e.g. periodic vortices, fully mixed turbulence), and different types of environmental flows affect flight behavior and performance in different ways. Bees typically adjust their flight kinematics in order to maintain a constant ground speed (motion relative to the ground), regardless of whether they are flying through still air or into a headwind (Barron and Srinivasan, 2006), and their preferred ground speed (and resulting optic flow rate) depends on the width of the flight tunnel (i.e. proximity of lateral obstacles) (Srinivasan et al., 1996). Bees can also gauge their

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own flight speed using mechanosensory feedback from the velocity of air moving past their bodies, although this may be limited to certain wind conditions (e.g. laminar headwinds) and a narrow range of wind and flight speeds (i.e. between 1.5 and 3.0 m s⁻¹) (Roy Khurana and Sane, 2016). Bees exposed to isolated gusts display initial, passive disturbances in body orientation and heading followed by an active recovery response, and they are more severely affected by gusts in some directions (e.g. sideways, downwards) than others (e.g. upwards) (Jakobi et al., 2018). Bees flying in fully mixed turbulence display greater asymmetries in stroke amplitude between wings and an elevated flapping frequency, which may enhance stability (Crall et al., 2017). When flying downstream of wind-blown vegetation, bees often encounter periodic vortices shed by the vegetation. These vortices reduce stability, decrease flight speed and increase potentially damaging impact forces when landing on flowers (Ravi et al., 2013; Chang et al., 2016). In addition to implications for flight control and an increased risk of injury, most studies on flight in wind point to an increased energetic cost owing to kinematic changes such as elevated flapping frequency (Combes and Dudley, 2009; Crall et al., 2017) and/or reduced flight speeds that prolong the total flight time (Ravi et al., 2013; Jakobi et al., 2018).

Wind can further affect bee flight by causing vegetation to move passively (e.g. flutter or sway), creating dynamic, unpredictable obstacles that flying bees must avoid. Although many studies have investigated how bees fly in wind and how they traverse stationary obstacles, we know little about how bees negotiate dynamically moving obstacles (Mountcastle et al., 2016), and nothing about how the combination of wind and moving obstacles affects flight performance. One previous experiment examined the separate effects of controlled flower motion and wind on foraging behavior (e.g. flower visitation rate) in honeybees, and found that the most notable effects were due to wind, although the combination of wind and flower motion was not examined (Hennessy et al., 2020).

Here, we examined how wind and obstacle motion (separately and in combination) affect flight strategy and performance of the honeybee *Apis mellifera*, an important pollinator. Because previous work has shown that bees fly more slowly when negotiating stationary obstacles (in still air) than when flying in the open (Crall et al., 2015), we hypothesized that the additional challenge of flying through moving obstacles would cause bees to slow down even further, and impair their ability to fly towards the exact center between obstacles (i.e. impair centering behavior, *sensu* Baird and Dacke, 2012; Lecoeur et al., 2019). We expected to see similar flight speeds (relative to the ground) and approach strategies in still air and in mild headwinds or tailwinds, as bees typically display consistent ground speeds that depend on tunnel size, but not wind speed (Srinivasan et al., 1996; Barron and Srinivasan, 2006).

To test these hypotheses, we filmed bees flying through a tunnel with still air, or with mild (~0.5 m s⁻¹) headwinds or tailwinds, as they approached a single row of vertical obstacles that was either stationary or oscillating laterally. We determined whether obstacle motion affected the ground speed and/or centering behavior of approaching bees, and we examined whether these responses differed when bees were flying in still air, headwinds or tailwinds. We also tested whether flight outcomes (successful transit versus collision with obstacles) were associated with differences observed in the flight behaviour of bees.

MATERIALS AND METHODS

Experimental set-up

Experiments were conducted in a flight tunnel (20×20×115 cm) with fans (AC Infinity) at each end that produced wind speeds of

~0.5 m s⁻¹ in the tunnel. At the tunnel's midpoint, a row of four vertical obstacles spaced 4 cm apart spanned the tunnel's width (Fig. 1A). Each obstacle was a green, translucent post (7 mm diameter) extending to the tunnel's ceiling (Fig. 1B), which bees were able to detect (as demonstrated by their active avoidance of obstacles during trial flights; see Results). Obstacles were positioned on a platform that could oscillate laterally relative to the tunnel's long axis with a peak-to-peak amplitude of 2.1 cm (Fig. 1A) and a frequency of 3 Hz, which is within the range of fluttering motions observed for individual leaves and plant stems in wind (Py et al., 2006; Kothari and Burnett, 2017; de Langre et al., 2019). The oscillation amplitude was smaller than the spacing between obstacles, resulting in a 1.9-cm space between adjacent obstacles through which moving obstacles never passed (i.e. a 'safe zone') – a distance that is approximately equal to the total wingspan of the bees (Altshuler et al., 2005). The maximum speed of the obstacles was <0.35 m s⁻¹, which was within the range of flight speeds observed in bees (see Results). The tunnel's walls were covered with a black and white speckled pattern, and the ceiling was overlaid with translucent circles to provide a visual reference for bees (as preliminary trials without this patterning yielded a high frequency of ceiling collisions).

Honeybees (*Apis mellifera* Linnaeus 1758) were collected outdoors on the campus of the University of California, Davis. Single bees were flown in the tunnel with either moving ($n=13$ bees; 73 flights) or stationary obstacles ($n=14$ bees; 77 flights). Full spectrum lights (26 W; Hagen) at each end of the tunnel were alternately turned on and off to motivate bees to fly back and forth through the obstacles. We filmed between 1 and 10 flights per bee (approximately half in still air, half in wind), randomly choosing whether bees started their flights in wind or in still air. Bees flew in both headwinds (into the wind) and tailwinds (with the wind), depending on their flight direction relative to the air flow. A random number generator was used to select whether a bee flew with moving or stationary obstacles. Only one bee was in the tunnel at a time, bees were not trained beforehand, and each bee spent <45 min in the tunnel. Flights were discarded if the bee came within 1 cm of the ceiling or walls.

Flights were filmed with two synchronized Phantom v611 high-speed video cameras (Vision Research, Inc.) sampling at 1500 frames s⁻¹ (exposure time=300 μs), each positioned 30 deg from the vertical on opposing sides of the obstacles and viewing down the length of the tunnel (Fig. 1B). Cameras were calibrated using a black and white printed checkerboard and built-in MATLAB functions (Heikkila and Silven, 1997; Zhang, 2000).

Kinematic analysis

We digitized the positions of the obstacles and of the bee's thorax in each frame using DLTdv6 (Hedrick, 2008), and trajectories were smoothed with quintic spline curves (Walker, 1998). We analyzed the final 50 mm of each approach (longitudinal distance along the axis of the tunnel) because this is the region in which bees were expected to display strong behavioral responses to the obstacles, based on previous studies (e.g. Ravi et al., 2019). When bees were approaching obstacles in a headwind, the filmed approach region would lie entirely within the wake produced by the obstacles, as vortices shed from obstacles in wind at this Reynolds number (~240) create a turbulent wake downstream for distances >50 mm (e.g. Kumar et al., 2019). We calculated the median ground speed (speed relative to the ground, not incorporating flow velocity) of each approach, based on the velocity of the bees within the horizontal plane (i.e. incorporating movement along the lateral and

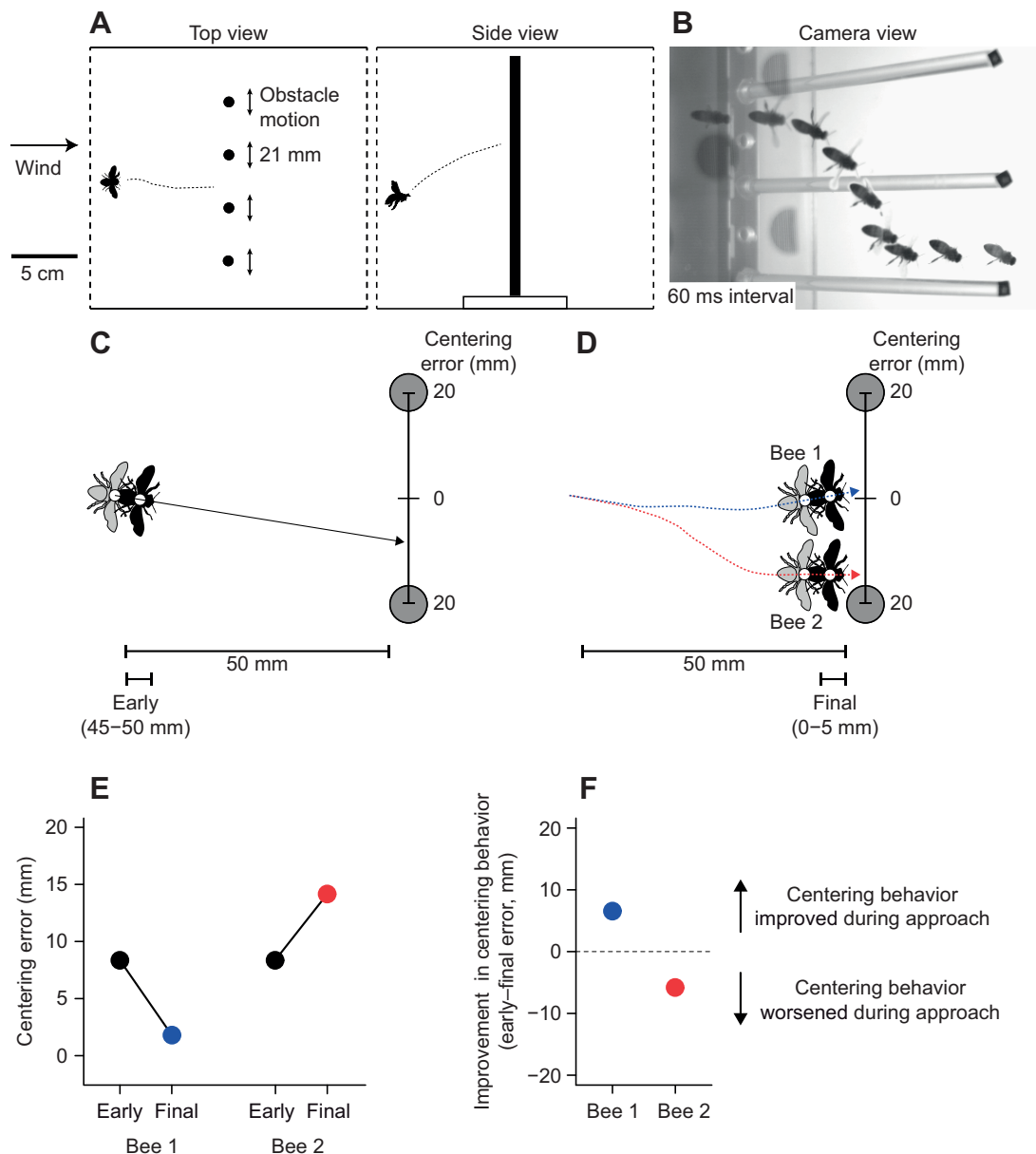


Fig. 1. Wind tunnel setup and calculation of centering error. (A) Schematic of tunnel, showing lateral spacing of obstacles and range of motion of obstacles when moving. When wind was present, bees flying back and forth experienced both headwinds and tailwinds during alternate flights. (B) Overlaid video frames showing a bee transiting obstacles. Camera view is an oblique angle from above (i.e. obstacles are completely vertical). (C,D) Closer view of bees approaching two obstacles, illustrating the method of calculating centering error. Average heading vectors of bees over (C) the early approach (45–50 mm from obstacles) and (D) the final approach (0–5 mm from obstacles) were used to calculate their centering error during each period, as the difference between the projected path of the bee when crossing the obstacles and the midline between obstacles. Examples of final approaches of two bees are shown in D, one that displayed low centering error (bee 1, blue) and one that displayed high centering error (bee 2, red) during the final approach. For each flight, (E) centering errors during the early and final approach periods were used to calculate (F) the improvement in centering behavior over the approach, by subtracting centering error during the final approach from centering error during the early approach.

longitudinal axes of the tunnel, but ignoring vertical motion; Baird and Dacke, 2012; Mountcastle et al., 2016; Ravi et al., 2019).

We also analyzed the centering behavior of bees during two different portions of the approach – the first 5 mm (‘early’ approach) and the final 5 mm (‘final’ approach) – based on the longitudinal position of the bee relative to the obstacles. Over each of these periods, we calculated an average heading vector for the bee (based on its changes in position over the period) and determined the bee’s projected position at the point where it would cross the obstacles, based on its average heading (Fig 1C,D). We then compared

the bee’s projected lateral position at the point where it would cross the obstacles with the exact center between adjacent obstacles over the same time period (Fig 1C,D). We call the lateral difference between these two positions the ‘centering error’ – thus, if a bee were aiming perfectly for the midpoint between obstacles, the centering error would be zero. After calculating the median centering error over both the early and final approach periods (Fig 1C–E), we calculated the improvement in centering behavior over each approach, as centering error during the early approach minus centering error during the final approach (Fig. 1F). Finally,

we categorized the outcome of each flight, noting whether bees successfully transited or collided with obstacles. Note that the kinematic analysis of the approach ended once bees reached the longitudinal position within the tunnel corresponding to the leading edge of the obstacles – thus, their behavior while safely transiting (or colliding with) the obstacles is not included.

Statistical analysis

We tested whether ground speed and centering error during bees' approaches were associated with obstacle motion (moving versus stationary) and flight outcome (transit versus collision) using a linear mixed-effects ANOVA. Bee identity was included as a random factor to account for multiple observations per individual (Bates et al., 2015), and our model allowed for interaction between obstacle motion and flight outcome. A separate model was used for each wind condition because sample sizes varied between wind conditions, and we hoped to help detect unique effects of each wind condition on ground speed and centering behavior that might be obscured in a single, large analysis. Assumptions of normality and homogeneity of variance were checked for each statistical model using Shapiro–Wilk and Levene's tests. Statistical analyses were performed in R (<https://www.r-project.org/>). For multiple pairwise comparisons of model terms, we used the lsmeans package (Lenth, 2016) with Tukey's tests and a critical *P*-value of 0.05 for significance.

RESULTS

Obstacle motion

We analyzed 77 flights of 13 individual bees approaching moving obstacles (30 in still air, 19 in headwinds and 28 in tailwinds) and compared these with 73 flights of 14 bees approaching stationary obstacles (30 in still air, 15 in headwinds and 28 in tailwinds). Means (\pm s.d.) of variables are given for each obstacle motion (stationary versus moving) and wind condition in Table 1. During the average time period of bees' 50-mm approach to moving obstacles (161 ± 79 ms; mean \pm s.d.; $n=77$), the oscillating obstacles moved a total distance of 2.5 ± 1.2 cm (mean \pm s.d.; $n=77$), which is just over one-half of the total distance traveled during a full oscillation cycle (4.2 cm total, starting and returning to the same obstacle position; Fig. 1A). Thus, during the observed portion of their approach, bees were not tracking the obstacles (or the gaps between obstacles) through multiple oscillations, but rather responding to obstacles moving slowly in one (or at most two) directions.

We found that obstacle motion significantly affected the ground speeds of bees as they flew towards obstacles, but this effect differed in still air versus wind. In still air, bees approaching moving obstacles displayed lower ground speeds than bees approaching stationary obstacles, but the opposite was generally true in headwinds and tailwinds. In still air, ground speeds were 22% lower for bees approaching moving obstacles than for bees approaching stationary ones ($P=0.010$; Fig. 2A). However, in tailwinds, ground speeds were 59% higher for bees approaching moving obstacles than for bees approaching stationary obstacles ($P=0.048$; Fig. 2C) and in headwinds, ground speeds were 50% higher for bees approaching moving obstacles than for those approaching stationary ones (although not significantly different, $P=0.218$; Fig. 2B; all statistical results are presented in Tables S1–S4). Generally, bees displayed consistent differences in ground speed between their approaches to stationary versus moving obstacles throughout the entire approach period (Fig. 2D–F). However, in headwinds, bees initially flew $>50\%$ faster when approaching moving (as opposed to stationary) obstacles, but this difference decreased as they reduced their ground speed at the end of the approach (Fig. 2E), resulting in no significant difference in median ground speed over the entire approach period.

In contrast to its clear effects on ground speeds, obstacle motion had few effects on the centering behavior of bees. In still air, centering error during the early approach was larger for bees approaching stationary obstacles than for those approaching moving obstacles across all flight outcomes ($P=0.013$), whereas centering error was not affected by obstacle motion in either headwinds ($P=0.614$) or tailwinds ($P=0.473$). Improvement in centering behavior between the early and final approaches was unaffected by obstacle motion in every wind condition ($P>0.05$). Centering error during the final approach period was also unaffected by obstacle motion in still air ($P=0.058$) and tailwinds ($P=0.310$). In headwinds, centering error during the final approach was larger for bees approaching stationary obstacles than for those approaching moving obstacles across all flight outcomes, although this difference was only marginally statistically significant ($P=0.047$).

Flight outcomes

We analyzed 79 flights in which 25 out of 27 bees safely transited through obstacles (34 flights in still air, 16 in headwinds and 29 in tailwinds) and 71 flights in which 26 out of 27 bees collided with obstacles (26 flights in still air, 18 in headwinds and 27 in

Table 1. Summary of approach behavior for flights grouped by obstacle motion

Flights by obstacle motion	Stationary obstacles	Moving obstacles	<i>P</i> -value for main effect of obstacle motion
Still air	$n=30$ flights, 11 bees	$n=30$ flights, 9 bees	
Ground speed ($m\ s^{-1}$)	-0.44 ± 0.23	-0.56 ± 0.11	0.010
Early centering error (mm)	11.0 ± 5.8	10.1 ± 4.7	0.013
Final centering error (mm)	10.0 ± 5.4	5.8 ± 8.1	0.058
Centering improvement (mm)	1.0 ± 7.3	2.0 ± 6.9	0.736
Headwind	$n=15$ flights, 9 bees	$n=19$ flights, 9 bees	
Ground speed ($m\ s^{-1}$)	-0.58 ± 0.17	-0.41 ± 0.25	0.218
Early centering error (mm)	12.1 ± 4.6	9.2 ± 5.2	0.614
Final centering error (mm)	11.0 ± 5.7	7.4 ± 1	0.047
Centering improvement (mm)	1.1 ± 9.2	1.7 ± 7.2	0.080
Tailwind	$n=28$ flights, 9 bees	$n=28$ flights, 13 bees	
Ground speed ($m\ s^{-1}$)	-0.41 ± 0.24	-0.21 ± 0.28	0.048
Early centering error (mm)	9.4 ± 5.6	10.3 ± 6.4	0.473
Final centering error (mm)	8.5 ± 6.1	10.3 ± 6.1	0.310
Centering improvement (mm)	1.0 ± 7.8	0.0 ± 7.7	0.983

Values are means \pm s.d. Ground speed was \log_{10} transformed. Bold *P*-values indicate statistically significant effects of obstacle motion ($P<0.05$).

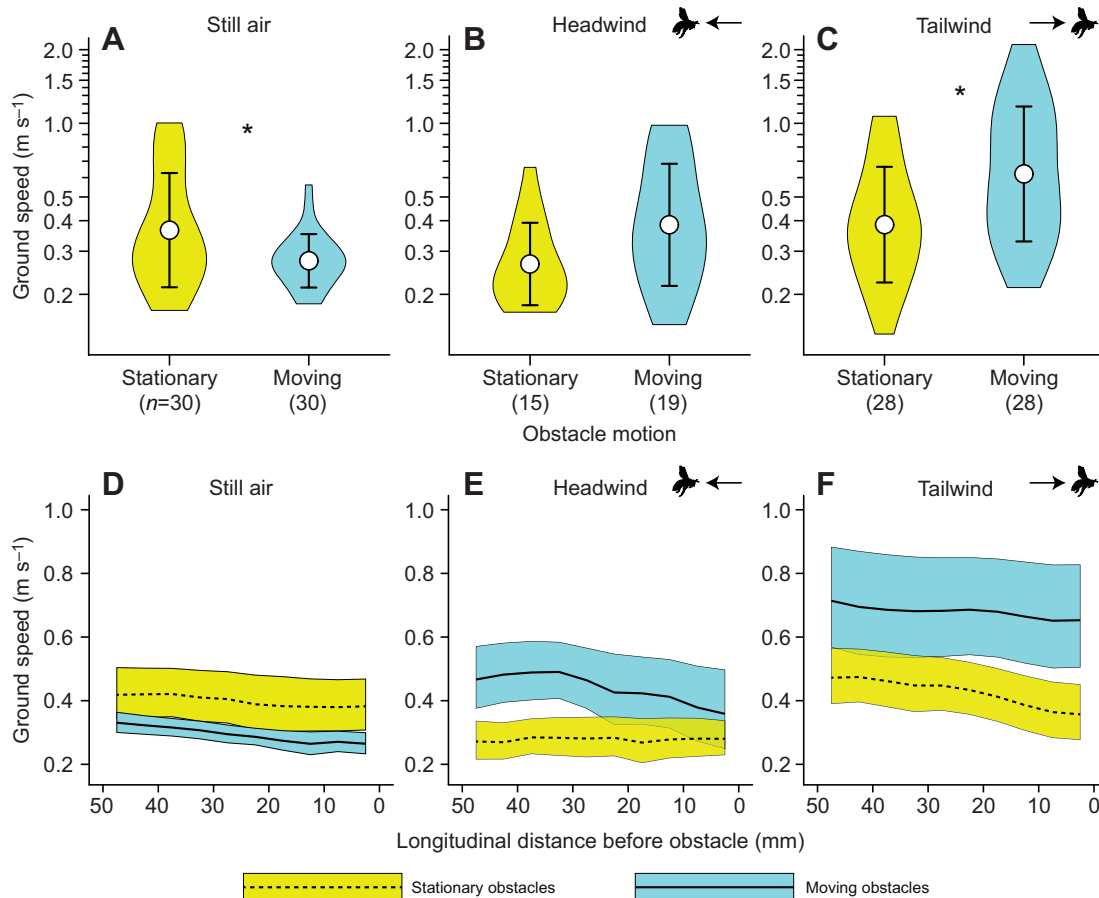


Fig. 2. Obstacle motion affects bees' ground speeds differently in still air versus wind. (A–C) Median ground speeds over the entire 50-mm approach toward stationary versus moving obstacles in (A) still air, (B) headwinds and (C) tailwinds. Bees flew more slowly when approaching moving versus stationary obstacles in still air ($P=0.010$), but more quickly when approaching moving obstacles in tailwinds ($P=0.048$) or headwinds (although not statistically different, $P=0.218$). Violin plots show the kernel density-smoothed representations of the frequency distributions for flights approaching stationary (yellow) and moving (blue) obstacles. White circles and error bars show means \pm 1 s.d. of all individuals, and asterisks show significant differences between groups. (D–F) Mean ground speeds (calculated in 5-mm intervals) as bees approached stationary and moving obstacles in (D) still air, (E) headwinds and (F) tailwinds. Lines show means of all individuals, and shaded areas show 95% confidence intervals.

tailwinds). Means (\pm s.d.) of variables are given for each flight outcome and wind condition in Table 2. We found that flight outcomes (i.e. successful transits versus collisions with obstacles) were significantly associated with the approach strategies of bees,

but whether outcomes depended on median ground speed or centering behavior depended on the wind condition. In still air, bees that successfully transited through obstacles flew 17% slower on average during their approach than bees that collided

Table 2. Summary of approach behavior for flights grouped by outcome

Flights by outcome	Successful transits	Collisions	<i>P</i> -value for main effect of flight outcome
Still air	$n=34$ flights, 17 bees	$n=26$ flights, 16 bees	
Ground speed (m s ⁻¹)	-0.53 ± 0.18	-0.46 ± 0.21	0.014
Early centering error (mm)	9.7 ± 5.2	11.7 ± 5.2	0.003
Final centering error (mm)	7.3 ± 5.0	11.4 ± 5.6	0.003
Centering improvement (mm)	2.5 ± 7.7	0.3 ± 6.1	0.875
Headwind	$n=16$ flights, 12 bees	$n=18$ flights, 12 bees	
Ground speed (m s ⁻¹)	-0.48 ± 0.22	-0.49 ± 0.24	0.483
Early centering error (mm)	11.6 ± 4.9	9.5 ± 5.2	0.021
Final centering error (mm)	6.5 ± 5.3	11.2 ± 6.0	0.025
Centering improvement (mm)	5.1 ± 6.6	-1.7 ± 7.9	0.002
Tailwind	$n=29$ flights, 16 bees	$n=27$ flights, 18 bees	
Ground speed (m s ⁻¹)	-0.36 ± 0.24	-0.26 ± 0.31	0.218
Early centering error (mm)	8.2 ± 6.0	11.6 ± 5.4	0.225
Final centering error (mm)	5.1 ± 4.1	13.9 ± 4.5	<0.001
Centering improvement (mm)	3.1 ± 7.9	-2.4 ± 6.5	0.021

Values are means \pm s.d. Ground speed was log₁₀ transformed. Bold *P*-values indicate statistically significant effects of flight outcome ($P < 0.05$).

with obstacles, whether moving or stationary ($P=0.014$; Fig. 3C). Improvement in centering behavior over the course of the approach (change in centering error from early to final approach) had no effect on flight outcome in still air ($P=0.875$; Fig. 3B). However, centering errors during the final approach were higher for bees that collided with either type of obstacle than for bees that successfully transited ($P=0.003$; Fig. 3A). In addition, bees that collided with stationary obstacles in still air displayed larger centering errors during the early approach period than bees that safely transited ($P=0.003$).

In headwinds, flight outcomes were unaffected by median ground speed ($P=0.483$; Fig. 3F), but bees that successfully transited obstacles (whether moving or stationary) showed a significant improvement in centering behavior over the approach as compared with bees that collided with obstacles, whose centering performance

declined over the approach ($P=0.002$; Fig. 3E). In addition, bees that collided with either type of obstacle had larger centering errors during the final approach period than bees that safely transited ($P=0.025$; Fig. 3D).

Results for flights in tailwinds were similar to those for flights in headwinds: flight outcomes were unaffected by median ground speed ($P=0.218$; Fig. 3I), but bees that successfully transited obstacles showed a significant improvement in their centering behavior over the approach as compared with bees that collided with obstacles, whose centering performance declined over the approach ($P=0.021$; Fig. 3H). In addition, bees that collided with either type of obstacle in tailwinds had larger centering errors during the final approach period than bees that successfully transited ($P<0.001$; Fig. 3G).

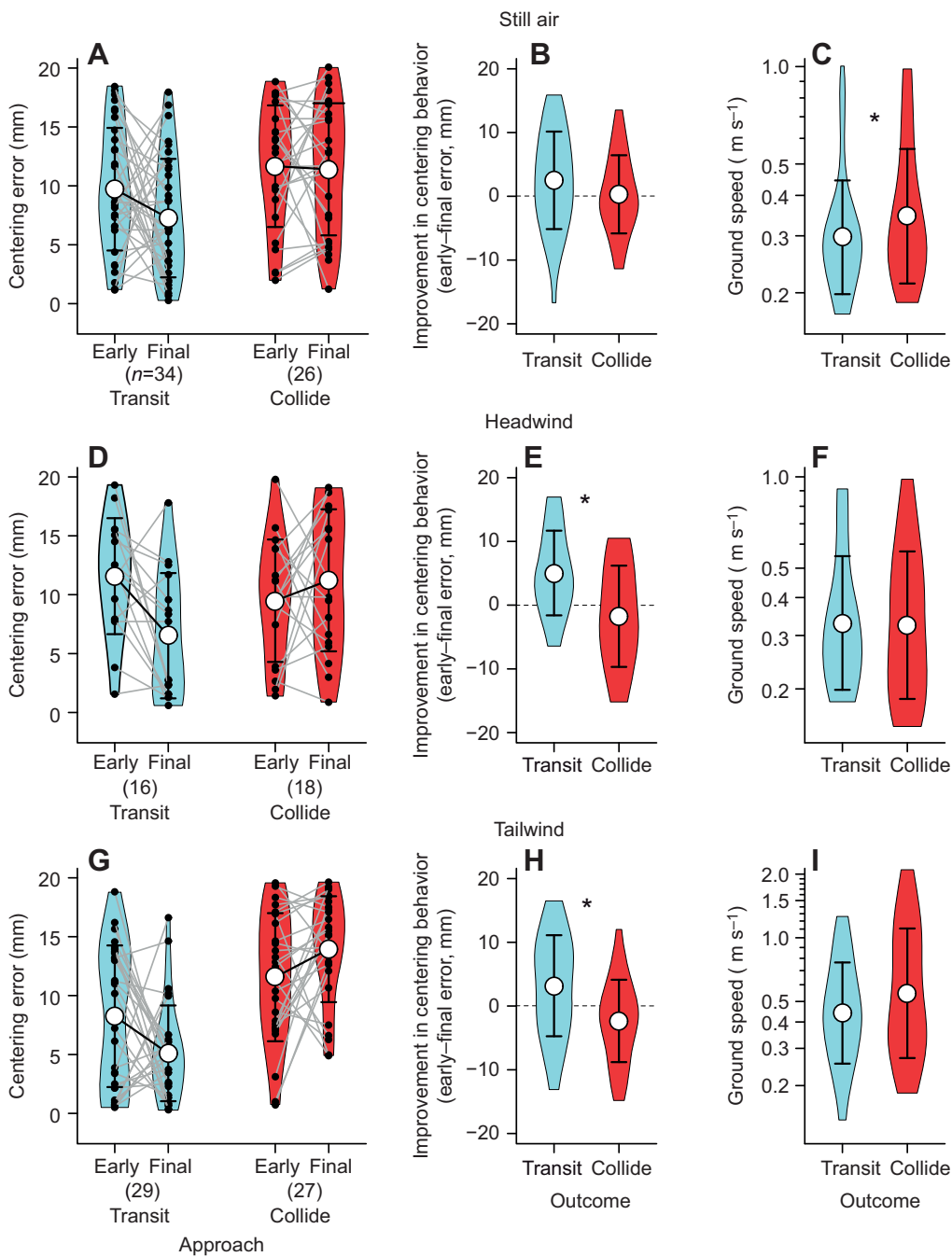


Fig. 3. Flight strategies for avoiding collisions differ in still air versus wind. Flights in still air, headwinds and tailwinds are shown on the top, middle and bottom rows, respectively, with flights grouped by outcome (successful transit in blue, collision in red); trials with stationary obstacles and those with moving obstacles are shown together. (A,D,G) Centering error during the early and final approaches for bees that successfully transited versus collided with obstacles, with individual flights joined by a line. In all wind conditions, bees that successfully passed through obstacles displayed lower centering error during the final approach than those that collided with obstacles. (B,E,H) Improvement in centering behavior over the approach (early–final centering error). In both headwinds and tailwinds (but not still air), bees that successfully transited displayed a greater improvement in centering behavior over the approach than those that collided with obstacles ($P=0.002$ for headwinds, $P=0.021$ for tailwinds). (C,F,I) Median ground speed of bees during approaches. In still air (but not in wind), bees that successfully transited displayed lower ground speeds than those that collided with obstacles ($P=0.014$ for still air). In all graphs, violin plots show the kernel density-smoothed representations of the frequency distributions, white circles and error bars show means ± 1 s.d., and asterisks show significant differences between groups.

DISCUSSION

We found that obstacle motion had a significant effect on the flight strategies of bees when approaching obstacles – particularly on ground speed – but surprisingly, the effect of obstacle motion on ground speed was reversed in still air versus wind. In still air, bees reduced their ground speed when approaching moving (as opposed to stationary) obstacles, as predicted (Fig. 2A). This is consistent with the behavior of other flying insects, such as house flies and fruit flies, that slow down when approaching obstacles (Wagner, 1982; van Breugel and Dickinson, 2012), and suggests that moving obstacles pose an even more difficult navigational challenge than stationary ones. Our results also show that reducing ground speeds when flying in still air significantly reduced the likelihood of bees colliding with obstacles, whether moving or stationary (Fig. 3C).

Although obstacle motion in still air led to slower, more cautious approaches, obstacle motion in wind tended to prompt – on average – faster ground speeds during approaches. This average difference was statistically significant only in tailwinds, where bees approaching moving (as opposed to stationary) obstacles had faster ground speeds throughout their approaches, whereas in headwinds, bees approaching moving obstacles initially had faster ground speeds but eventually slowed down as they neared the obstacles.

The observed increase in ground speeds for bees approaching moving obstacles in wind is unexpected because bees, as well as many other insects, are known to regulate their ground speeds based on optic flow from the surrounding visual environment, allowing them to maintain a preferred speed despite any ambient wind (Kennedy, 1951; Willis and Arbas, 1991; Barron and Srinivasan, 2006; Fuller et al., 2014; Copley et al., 2018). In our experiment, bees did display fairly consistent ground speeds when approaching stationary obstacles, regardless of flow condition: the averages [and 95% confidence intervals: (lower, upper limits)] of median ground speeds when bees approached stationary obstacles were 0.36 m s^{-1} (0.30, 0.43) in still air and 0.39 m s^{-1} (0.32, 0.47) in tailwinds, and declined slightly to 0.26 m s^{-1} (0.22, 0.32) in headwinds (slight declines in ground speed were also seen in headwinds by Barron and Srinivasan, 2006). However, this similarity in ground speed across flow conditions was not upheld when bees were approaching moving obstacles; in this case, average ground speed increased from 0.28 m s^{-1} (0.25, 0.30) in still air to 0.39 m s^{-1} (0.30, 0.51) in headwinds and 0.62 m s^{-1} (0.49, 0.77) in tailwinds. The fact that bees are clearly capable of reducing their ground speed to the lower values observed when approaching stationary obstacles in headwinds and tailwinds suggests that this difference reflects a change in behavioral strategy, rather than a limitation in their ability to regulate ground speed in these flow conditions.

Our results in still air demonstrate that flying more slowly can be an effective strategy for reducing the likelihood of collisions (Fig. 3C) when bees must contend with a complex flight challenge such as navigating through moving obstacles. Why then did bees not reduce their ground speed as much (or even further) when approaching moving obstacles in wind? One potential hypothesis for why bees, on average, flew more quickly when approaching moving obstacles in wind is that the obstacles' lateral movement provides enhanced visual information (e.g. Kirchner and Srinivasan, 1989; Srinivasan et al., 1996) that bees would normally gather by slowing down and performing lateral casting motions (Ravi et al., 2019), and this allowed the bees to traverse moving obstacles without slowing to gather this information. However, if this were true, we would expect to have seen the same result in still air (i.e. faster ground speeds when approaching moving obstacles). Instead, bees reduced their ground speeds when

approaching moving obstacles in still air, which argues against the idea that ground speeds when approaching obstacles are regulated solely by the need to gather sufficient visual information.

Obstacle motion could potentially affect bees differently in wind versus still air because air flowing through moving obstacles would produce a more complex, mixed wake, as compared with the predictable, periodic vortices shed by stationary obstacles in wind. Complex, unpredictable wakes could lead bees to increase their ground speed in order to minimize the amount of time they are exposed to an unfavorable flow environment [as suggested by Jakobi et al. (2018) to explain the increased ground speeds of bees when passing through areas with wind gusts]. However, this explanation would only account for the increased ground speeds observed during approaches in headwinds (when bees encounter the wake before reaching the obstacles), not for the increased speeds observed during approaches in tailwinds (when bees reach the obstacles before encountering the wake).

Another possible explanation for the apparent lack of caution that bees displayed when approaching moving obstacles in wind [building on Jakobi et al.'s (2018) idea of minimizing time in unfavorable flow environments] is that when bees are faced with two flight challenges simultaneously – passing through moving obstacles and contending with headwinds or tailwinds – they adopt an alternative strategy of speeding up in order to reduce the amount of time they are subject to the challenging flight conditions. Although speeding up would not help mitigate the danger of a bee's body colliding with an obstacle, wing collisions with obstacles are known to be a much more frequent occurrence among bumblebees navigating through cluttered, natural environments (Foster and Cartar, 2011). Wing collisions cause cumulative damage to wings (Mountcastle and Combes, 2014), and this damage has several negative effects on bees, including reduced acceleration capacity (Mountcastle et al., 2016) and increased mortality (Cartar, 1992).

Wing collisions typically occur in bursts of tens (or even hundreds) of rapid collisions, because when a bee's wing comes too close to an obstacle, it continues hitting the obstacle repeatedly as the wing flaps back and forth at high frequencies, until the bee passes beyond the obstacle or is able to actively adjust its lateral position. By increasing their ground speed, bees reduce the amount of time that they are close enough to obstacles for wing collisions to occur, and thus lower the total number of wing collisions that they will experience if they are unable to navigate through the obstacles without making contact. Based on the diameter of our obstacles (7 mm), the average flapping frequency of honeybees (~230 Hz; Altshuler et al., 2005) and the average ground speeds measured here, we estimate that bees whose wings come into contact with moving obstacles would experience a minimum of ~12 wing collisions in still air, 8–9 in headwinds and 5–6 in tailwinds (assuming contact only when wings are at mid-stroke), and as many as 35 wing collisions in still air, 25 in headwinds and 15 in tailwinds if considering the movement of the wing over its entire 90 deg fore–aft flapping envelope (Altshuler et al., 2005). Thus, by increasing their ground speed in this challenging flight situation (navigating through moving obstacles in wind) – during which the likelihood of passing through obstacles with no contact at all may be relatively low – bees can reduce the number of potential wing collisions they will experience if they do come too close to obstacles by approximately 30% in headwinds and 55% in tailwinds, as compared with what they would experience if flying at the lower ground speeds observed in still air.

Despite its strong effects on ground speed, obstacle motion had no effect on centering behavior in either headwinds or tailwinds, and

only minor effects on centering behavior in still air. However, centering behavior did play an important role in bees navigating through obstacles, particularly in wind. For bees flying in either headwinds or tailwinds, the most important feature of their approach (in terms of safely transiting versus colliding with obstacles) was how much their centering behavior improved from the beginning to the end of the approach (Fig 3D,H). Bees that safely transited obstacles in either wind condition generally showed an improvement in centering from the early to the final approach periods, whereas those that collided with obstacles typically showed a decline in centering behavior (i.e. larger centering error when they reached the obstacles as compared with early in their approach; Fig 3D,H). These changes in centering behavior played a significant role in determining the outcome of flights in wind (whether obstacles were stationary or moving), whereas changes in median ground speed did not (Fig 3E, I). That is, bees in wind relied on changing their trajectories to safely transit obstacles rather than modifying their ground speeds (Fig 3D–I), and these results are consistent with the behavior of other insects, such as fruit flies and locusts, that primarily avoid obstacles by altering their flight paths (Robertson and Johnson, 1993; Tammero and Dickinson, 2002; van Breugel and Dickinson, 2012; Muijres et al., 2014).

In contrast, bees in still air generally displayed smaller changes in centering behavior over the course of their approach than those flying in wind, and improvement in centering behavior during the approach did not play a significant role in determining the outcome of flights in still air (Fig. 3B). Centering behavior during the final approach was still important, though, and bees flying in still air (as in all other wind conditions) that were well aligned with the midpoint between obstacles just before passing through them (during the final 5 mm of their approach) were more likely to successfully transit obstacles (Fig 3A,D,G). However, unlike flights in wind, the outcomes of flights in still air were not determined by improvement in the centering behavior of bees over the approach, but rather by their ground speeds, with bees that slowed down being more likely to avoid collisions (Fig 3B,C).

Overall, our results show that bees approach moving obstacles in a significantly different way than they approach stationary ones. Previous studies have shown that when bees and other insects are in immediate danger of colliding with an obstacle, they brake suddenly and perform corrective maneuvers in an attempt to avoid a collision (Robertson and Johnson, 1993; Tammero and Dickinson, 2002; Muijres et al., 2014; Crall et al., 2015; Mountcastle et al., 2016). Here, we show that even during the approach to obstacles (when there is no imminent danger of collision), bees change their flight behavior, altering their ground speeds in unique ways depending on the combination of obstacle motion and wind: bees slow down and approach moving obstacles more cautiously than stationary ones in still air, but speed up when approaching moving obstacles in wind, perhaps in an attempt to minimize the number of wing collisions they may experience in these more challenging flight conditions.

In addition, our results show that to safely transit obstacles, bees employ different strategies depending on whether they are in still air or wind: in still air, bees safely transit obstacles by reducing their ground speeds and ensuring that they are well centered at the point where they pass through obstacles, whereas in wind, bees safely transit obstacles by altering their trajectories throughout their approach, markedly improving their centering behavior while maintaining or even increasing their speed. Given that obstacle motion and wind, both separately and in combination, are common in natural habitats, and that changes in ground speed and trajectory can affect the energetic cost, duration and distance of foraging trips

(Schmid-Hempel et al., 1985; Comba, 1999), our findings suggest that these environmental challenges could affect overall pollination activity. Additional studies on the flight behavior of bees in wind-blown clutter could shed light on the specific mechanisms (e.g. visual versus mechanical stimuli) driving the responses reported here and provide further insight into the question of how future changes in vegetation and wind may affect bees and the pollination services they provide.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.P.B.; Methodology: N.P.B., M.B., S.A.C.; Software: M.B.; Validation: N.P.B.; Formal analysis: N.P.B., M.B., S.A.C.; Investigation: N.P.B., M.B., S.A.C.; Data curation: N.P.B.; Writing - original draft: N.P.B.; Writing - review & editing: N.P.B., M.B., S.A.C.; Visualization: N.P.B., S.A.C.; Supervision: S.A.C.; Project administration: N.P.B., S.A.C.; Funding acquisition: N.P.B., S.A.C.

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Supplementary information

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