

The mechanism of flight guidance in honeybee swarms: subtle guides or streaker bees?

Kevin M. Schultz^{1,*}, Kevin M. Passino¹ and Thomas D. Seeley²

¹Department of Electrical and Computer Engineering, The Ohio State University, Columbus, OH 43210, USA and ²Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

*Author for correspondence (e-mail: schultzk@ece.osu.edu)

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SUMMARY

When a honeybee swarm takes off to fly to its new home site, less than 5% of the bees in the swarm have visited the site and thereby know in what direction the swarm must fly. How does the small minority of informed bees indicate the swarm's flight direction to the large majority of uninformed bees? Previous simulation studies have suggested two possible mechanisms of visual flight guidance: the informed bees guide by flying in the preferred direction but without an elevated speed (subtle guide hypothesis) or they guide by flying in the preferred direction and with an elevated speed (streaker bee hypothesis). We tested these hypotheses by performing a video analysis that enabled us to measure the flight directions and flight speeds of individual bees in a flying swarm. The distributions of flight speed as a function of flight direction have conspicuous peaks for bees flying toward the swarm's new home, especially for bees in the top of the swarm. This is strong support for the streaker bee hypothesis.

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Key words: *Apis mellifera*, honeybee, honey bee, swarm, flying swarm, in-transit swarm.

INTRODUCTION

In many animal species, individuals move about in groups as they perform seasonal migrations, travel to food sources and return from safe havens (Boinski and Garber, 2000; Krause and Ruxton, 2002). An enduring mystery about such group movements is how they are steered. In some species, all the individuals in a group share a genetically determined propensity to travel in a certain direction (Berthold and Querner, 1981; Berthold et al., 1992), or are involved in determining the travel direction (Neill, 1979; Grünbaum, 1998). In other species, only some of the group's members possess information about the group's travel destination, usually because of differences in age or experience, and these informed individuals guide the rest. In this study, we investigated a striking form of group movement that relies on guidance by a small subset of informed individuals: the flight of a honeybee (*Apis mellifera* Linnaeus) swarm.

A swarm of honeybees consists of one queen and several thousand workers. Swarms are produced in spring when large colonies divide for reproduction. During colony fissioning, the mother queen and approximately two-thirds of the worker bees leave the parental nest to establish a new colony, while a daughter queen and the balance of the workers stay behind to perpetuate the old colony at the original nest (reviewed by Winston, 1987). The swarm bees quickly leave their nest and form a spectacular cloud of some 10,000 flying bees, but they do not travel far. Usually they coalesce into a beard-like cluster on a tree branch less than 50 m from the parental nest (Ambrose, 1976). Over the next day or so, the clustered swarm bees conduct a sophisticated process of group decision making to choose their future home site (reviewed by Seeley et al., 2006; Passino et al., 2008). Once they have made their choice, the swarm bees launch again into flight and fly together to their new dwelling place, generally a tree cavity a kilometer or more away (Seeley and Morse, 1977; Villa, 2004). A curious feature of the

home-site selection process is that it involves only 3–5% of the bees in a swarm, the so-called 'scout bees' (Seeley et al., 1979; Seeley and Visscher, 2007). We know, therefore, that fewer than 5% of a swarm's members have visited (and hence know the location of) the swarm's new home site as the swarm makes its cross-country flight to its new domicile.

How does the small minority of informed bees provide guidance to the rest of the bees in an airborne swarm? Avitabile and colleagues hypothesized that the informed bees guide the other bees chemically, by releasing an assembly pheromone on the front of the cloud of flying bees, thereby creating an odor gradient that indicates the desired flight direction (Avitabile et al., 1975). However, this hypothesis has been falsified by Beekman and colleagues, who found that swarms composed of bees whose assembly pheromone glands were sealed shut were perfectly capable of flying directly to a new nest site (Beekman et al., 2006). There are currently two other hypotheses, both suggesting that the informed bees provide guidance information to the other bees visually. The first, which we call the 'subtle guide' hypothesis, suggests that the informed bees do not conspicuously signal the correct travel direction but instead steer the swarm by tending to move in the direction of the new home. Simulation work has shown that if each individual in the swarm attempts to avoid collisions by turning away from neighbors within a critical distance, and tends to be attracted towards and aligned with neighbors outside the critical distance, and flies either with a preferred movement direction (informed individuals) or without a preferred movement direction (naive individuals), then the swarm will be steered toward its new home even if the proportion of informed individuals is small (<10%) (Couzin et al., 2005). The second hypothesis, which was originally proposed by Lindauer (Lindauer, 1955) and which we call the 'streaker bee' hypothesis, suggests that the informed bees conspicuously signal the correct travel direction by repeatedly

making high-speed flights through the airborne swarm in the direction of the new home followed by low-speed flights along the edge of the swarm to return to the back of the swarm. The non-leader dynamics are similar to those of the subtle guide case, they obey attraction and repulsion rules, but at least part of the alignment rule favors alignment with fast-flying agents. Here again, simulation work has shown that the streaker bee hypothesis is a plausible mechanism of flight guidance (Janson et al., 2005). The key difference between the two strategies is whether or not naive individuals favor alignment with only the fastest flying individuals, and whether the informed leaders are acting on this tendency. A photographic analysis has recently shown that there are fast-flying, streaker bees in swarms, especially in the upper half of the cloud of flying bees (Beekman et al., 2006), but this analysis could not determine the direction of flight of the fast-flying bees. Here, we report a video analysis that tested whether or not these streakers are preferentially traveling in the direction of a swarm's new home.

Ascertaining whether or not the fast-flying bees in a swarm are flying in the direction of the swarm's destination is important because it enables us to resolve the subtle guide and streaker bee hypotheses. A swarm of bees is sparse and the bees inside appear to be flying in every direction. The subtle guide hypothesis does not predict that the bees flying toward the new home site will have higher speeds than the bees traveling in other directions, whereas the streaker bee hypothesis does predict the presence of high-speed bees flying toward the new home site. Such bees, if they exist, are likely to include not only the informed streaker bees but also uninformed bees reacting to the streaker bees.

Several features of a flying swarm of honeybees make it difficult to track the movements of individual bees in a swarm. First, a flying swarm is a large cloud composed of small bees. A typical swarm stretches 8–12 m from front to rear, 6–8 m from side to side and 3–4 m from top to bottom, but each bee is only 14 mm long. Thus it is difficult to both film an entire swarm and obtain detailed information on the individuals within it. Second, a flying swarm contains thousands of bees, so tracking a sizable fraction of them is a mammoth task. Third, a flying swarm traverses hundreds or thousands of meters, and so its entire flight cannot be filmed from any one site. These three features of honeybee swarms make the present study substantially different from previous video analyses of schools of fish or swarms of other insects (reviewed by Parrish and Hamner, 1997). For example, Okubo and his colleagues studied swarms of midges (*Anarete pritchardii*), which are much smaller than swarms of bees, both in volume and number of individuals, and which do not travel far (Okubo and Chiang, 1974; Okubo et al., 1981). These investigators accomplished things that are not yet feasible with swarms of bees, such as filming the midge swarms close up and extracting information on the individual midges' movements in three dimensions by exploiting the shadows cast by the midges on a white backdrop. Also, small schools of fish have been analyzed in an observation tank where stereovision techniques could be used to reconstruct the trajectories of individual fish in three dimensions (Grünbaum et al., 2004). Here the small group size, large individual size and limited movement area made possible methods that cannot yet be used with swarms of bees.

MATERIALS AND METHODS

Video data collection

To have a swarm fly directly over the video camera along a flight axis that parallels the left and right sides of the camera's field of view, we needed control over the nest site that the swarm would choose. To achieve this control, we offered an attractive nest box

to swarms of bees that we took to Appledore Island, a 39 ha island off the coast of Maine (42°58'N, 70°37'W) where there are no trees large enough to contain potential nest cavities and where few other suitable nest sites can be found by bees.

The swarms we used were artificial swarms, prepared by shaking 1.0 kg of bees, about 8000 individuals, from the combs of a hive into a screen cage with their queen in a smaller cage among them, then feeding this cage of bees with sucrose solution (1:1 sucrose:water by volume) for 3 days. The production of abundant wax scales signified that these bees had shifted into a condition like that of a natural swarm, and we then set them up on a stand for observation. The swarm stand was that described by Seeley and Buhrman (Seeley and Buhrman, 1999). We set up each swarm, one at a time, in a clearing beside the old Coast Guard building, and placed a 40 l nest box with a 12.5 cm² entrance hole, like that described by Seeley and Morse (Seeley and Morse, 1978), at a distance of 255 m from the swarm. The nest box, which has characteristics favored as a home site by bees, was sheltered from wind, sun and rain in a small hut. Each swarm chose the nest box that we provided for its future home. Before each swarm took off to fly to the nest box, we released the queen from her small cage (except in one case discussed below) so that the swarm could perform a normal flight to the nest box.

Between 15 June and 3 July, 2006, we recorded the flights of several swarms. However, because the video data processing for even just one swarm required several hundreds of hours of exacting work, we fully analyzed only the recording of the swarm that flew to the nest box on 2 July. In this case, the camera was positioned 15 m from the swarm stand, so the recording was made of the swarm when it was just starting to move away from the swarm stand but was already clearly showing its flight direction: straight toward the nest box. During this swarm's fly-over of the camera, there was a moderately bright background of blue sky and little or no wind. We also present data from two additional swarm flights where we analyzed only a fraction of the video of each fly-by. The swarm fly-by of 29 June had a caged queen and was filmed 9 m from the swarm stand, and the fly-by of 3 July had a queen free to fly with the swarm and was filmed 8 m from the swarm stand. Again, there was little to no wind, and in both cases the swarms had begun to fly in the direction of the nest box. However, in these latter two cases, the sun was high in the sky and there were many clouds. The bright sun and clouds present some difficulties in data extraction and for this reason these two cases were not analyzed as fully as the more ideal 2 July fly-by.

Video camera and experimental setup

After filming several flying swarms with different cameras in pilot studies, we chose a Sony HDR-HC1 high-definition miniDV camcorder, mainly because it has interlaced scanning, high resolution and variable shutter speed (see below). We filmed flying swarms from below, to minimize the camera-to-swarm separation, to maximize bee-to-background contrast, and to determine each bee's flight direction relative to the swarm's axis of travel, i.e. the direction to the new home. The camera was mounted on a tripod located along the swarm's flight path (see Fig. 1) and aimed straight up, with the bottom of the camera facing the swarm's cluster site and perpendicular to the line of the swarm's flight. This layout caused the leading edge of the swarm cloud to cross the bottom of the camera's field of view first, and it allowed the widescreen aspect of the camera to capture a wide, vertical 'slice' of the swarm cloud. We used a wide-angle lens with minimal zoom (0.7) to maximize the portion of the swarm cloud recorded at any one time. The

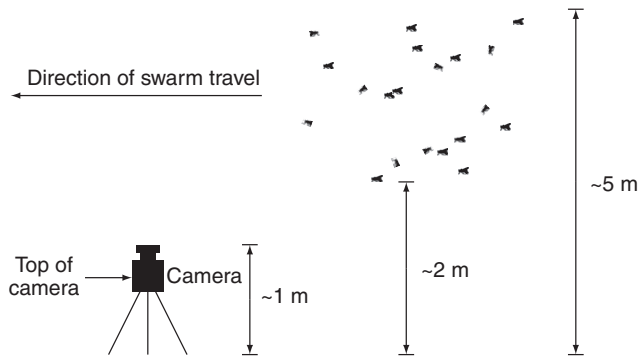


Fig. 1. Camera setup for swarm fly-by filming.

camera's focus was set manually to 3 m, which put its focal plane at a vertical height that roughly corresponded to the center of the swarm cloud. The camera's shutter speed was adjusted to its fastest setting, $1/10,000$ of a second, to minimize blurring of the flying bees. Because we were filming under an open sky, the camera received sufficient light to give excellent recordings despite the extremely high shutter speed.

The Sony HDR-HC1 camera captures 1440×1080 rectangular pixels in a 16:9 ratio (so the output video is 1920×1080 square pixels) at a frame rate of 60 interlaced (60i) frames per second. The interlacing of frames means that 540 of the 1080 horizontal lines are updated each $1/60$ of a second, in such a way that every other line is updated at once. This contrasts with a progressive frame rate where the entire field is updated at once, typically 24 or 30 frames s^{-1} (denoted 24p or 30p). A frame rate of 60i frames s^{-1} results in the entire field being updated at 30 frames s^{-1} , but when objects are traveling rapidly there is some displacement between where the object appears on one set of horizontal lines and the other set in sequential interlaced half-frames. Typically, interlaced video is fed through a de-interlacing algorithm to resolve this discrepancy, thus producing a video image that appears smooth and correct to the human eye. However, because we could use the displacement information between successive 60i half-frames to determine each bee's direction of movement, we did not de-interlace our video recordings. Supplementary material Fig. S1 shows a screen capture of one frame of the interlaced video. It shows several 'pairs' of bees, each of which represents just one bee that is moving so fast that the interlacing causes it to appear twice, once for each scan. The image also shows how for each bee we could determine a line of travel. We could also compare the speeds of bees, by comparing the distances between interlaced images of different bees; comparisons were made only between bees that were approximately the same size in the video image, and hence were at roughly the same altitude. We had to ignore the complication that not all bees were traveling precisely in a plane perpendicular to the axis of the camera.

Video data processing

Video processing was done with the Max/MSP software using the Jitter package, developed and distributed by Cycling '74 (San Francisco, CA, USA). In addition to the base packages ('patches') included in Max/MSP and Jitter, we also used a set of computer vision patches for Jitter: cv.jit (Pelletier, 2006). Jitter provides a library of computer vision routines, including morphological erosion and dilatation patches that were especially helpful in this study. The main attraction of this software is that it allows one to process video streams

using standard building blocks. Additionally, all of the data are stored in matrix format, and can be manipulated as one complete matrix or as individual cells. Furthermore, there is built-in support for mouse, keyboard, and file input/output, which simplifies constructing a user interface that easily handles a variety of image types.

Our video processing algorithm served to split the input video stream into two concurrent streams, one containing the bees in the top region of the swarm and another containing the bees below the top region, hereafter called the bottom region. Under the ideal conditions of the 2 July fly-by, our top region contained 10–20% of the bees in the swarm and the bottom region contained the rest (see sample sizes in Fig. 3). For the other two cases, the variability of brightness in the sky made it more difficult to isolate only the bees flying in the top of the swarm, and the bright sun washed out bees flying near it, resulting in those bees appearing smaller (and thus higher) than they actually were, or erasing them entirely. As a result of this, the percentages of bees gathered in the top of the swarm are higher than in the 2 July case (supplementary material Table S1).

The first step in the video processing was to convert the color video to grayscale, and then to find the pixels with intensity above a threshold. At this point, we had a video stream of black, interlaced blobs on a white field, which correspond to the bees on the original video stream. Next, we used the morphological dilatation operation followed by an erosion to fill in the gaps from interlacing, and to eliminate noise. We then applied multiple erosion operations to eliminate the small bees, i.e. those in the top of the swarm. Multiple dilatations were then performed to bring the remaining bees (those that were relatively large in the original stream) back to their original size. We then used the video stream of the larger bees as a mask on the stream with all the bees in it, thereby producing a stream with only the smaller bees in it. At this point, we had two streams of video, one with large blobs and one with small blobs representing bees in the bottom and top portions of the swarm, respectively. The final processing step was to run each video stream through a filter that held the black blobs on the screen, but slowly faded them to gray as the video was advanced, thereby producing a fading 'trail' of each bee's flight path. We then clicked on the trails of bees, one by one, to obtain the velocity vectors (*via* the difference in position along trails between interlaced frames) of individual bees for each set of 10 consecutive frames (see Fig. 2). Due to memory and computational delays, we could gather at most 500 blob positions in each set of 10 consecutive frames. When there were more than 500 blobs on the screen, data were collected (by clicking on blobs, in pairs) such that each bee's trail had the same length but was less than 10 blobs long. At this point, if fewer than 500 blobs total had been clicked on, then trails were chosen at random to receive an extra pair of blobs. In the end, all 500 blobs were distributed among the trails so that the trails were matched in length. Blobs were gathered in pairs so that we could extract both position and velocity information on the bees. For the swarm fly-bys of 29 June and 3 July, the decay rate of the filter was increased, and data were gathered only from the most recent frame, so that these two swarms were essentially sampled every 10 frames, instead of nearly the entire swarm (subject to computational limits) that was gathered in the 2 July case.

Throughout the data collection, the camera was oriented so the top of the recorded image pointed toward the nest box and therefore represents the direction of swarm travel (see Fig. 1). During the data processing, however, the origin falls in the top-left corner of the screen in Fig. 2 [pixel (0, 0)]. Thus, in representing the angles of the bees' flight trajectories, 0 rad corresponds to the right of the

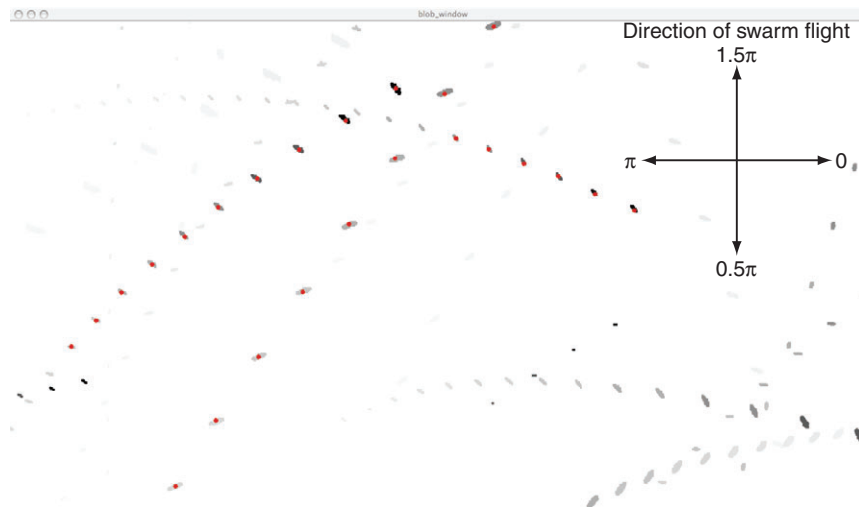


Fig. 2. Screenshot of data acquisition process. Direction of swarm flight is given in π rad.

screen, 0.5π rad corresponds to the bottom of the screen (flight rearward toward the swarm stand), 1.0π rad corresponds to the left of the screen and 1.5π rad corresponds to the top of the screen (flight forward toward the nest box). We expect, therefore, the most common flight trajectory to be near 1.5π rad, which is the direction of the nest box.

RESULTS

Distributions of bee flight angles

In our analyses, each swarm fly-by is divided into the front, the middle and the rear of the swarm, and further separated into bees in the top and bees in the bottom, giving a total of six overall divisions for each swarm. The 2 July fly-by was split into frames 1–1000, frames 1001–2500 and frames 2501–3480; the 3 July case into frames 1–800, frames 801–1400 and frames 1401–2009; and the 29 June case into frames 1–1000, frames 1001–2500 and frames 2501–3590. Fig. 3 shows the 2 July angular distributions of the bee flight movements for these three sections of the video, with separate sets of distributions for bees in the top and bottom regions of the swarm. From these three pairs of distributions, it can be seen that in the middle of the swarm there is a marked difference in tendency to fly toward the nest box (aligned with the flight axis of the whole swarm) between bees in the top *vs* bottom of the swarm. The bees in the top were clearly more likely to be traveling toward the nest box than were the bees in the bottom. The plots representing data from the rear of the swarm show little difference in directionality between top and bottom bees, while those from the front of the swarm show an intermediate level of directionality difference between top and bottom bees. Fig. 3 also reveals a bimodality in the distributions of flight angles for bees in the rear of the swarm, in both top and bottom regions. Fig. 3E and F both show two peaks, one near 1.25π rad and one near 1.75π rad, indicating that bees in the rear of the swarm were not tending to fly parallel with the axis of the swarm's flight. Similar, although not as distinct, results hold for the 3 July and 29 June cases, which are included in the supplementary files (supplementary material Fig. S2 and Fig. S4, respectively). We provide below a more quantitative analysis of the differences in flight directionality between top and bottom bees.

To determine whether the directedness observed in the angular plots is significant we used the circular statistics as presented by Batschelet (Batschelet, 1981). The principle statistic used is the notion of the mean vector $\boldsymbol{\mu} \in \mathbf{R}^2$. If the set of N angular

measurements of a given sample are denoted by θ_i , $i=1, \dots, N$, then:

$$\boldsymbol{\mu} = \frac{1}{N} \sum_{i=1}^N \begin{bmatrix} \cos(\theta_i) \\ \sin(\theta_i) \end{bmatrix}^T, \quad (1)$$

and we denote $r = \|\boldsymbol{\mu}\| \in [0, 1]$ and $\bar{\phi} = \angle \boldsymbol{\mu} \in [0, 2\pi)$, where $\|\cdot\|$ is the Euclidean norm and \angle is the angle in the plane. Supplementary material Table S1 shows the sample size of each swarm portion for each fly-by, along with $\bar{\phi}$, r and the circular variance $s^2 = 2(1-r)$ (Batschelet, 1981).

The Rayleigh test is one of the classic tests for orientedness in angular observations *vs* the null hypothesis of a uniform angular distribution. It is known that the statistic $2Nr^2$ is distributed as a chi-square with two degrees of freedom (Mardia and Jupp, 2000). All of the Rayleigh tests reject the null hypothesis with high confidence (<0.002), so no additional, more powerful tests for orientedness or corrections for bimodality were performed (see supplementary material Table S2).

Top–bottom comparisons of scatter in flight angles, from front to rear of swarm

To compare the scatter in flight angle distributions between top and bottom bees, we used the notion of angular variance defined as $s^2 = 2(1-r)$ presented by Batschelet (Batschelet, 1981). We denote the angular variances of the top and bottom by s_{top}^2 and s_{bottom}^2 , respectively. For each of the three swarms, across all three time divisions, all of the top distributions were more concentrated (i.e. had less angular variance) than the corresponding distribution in the bottom. To test whether the sample angular variances differed significantly from top to bottom we used a non-parametric test for dispersion which tests the angular deviation from the samples' respective mean angles, i.e. differences in mean angle do not affect the test, only the concentration of angles about the mean. For each portion of the swarm, the angular distances between each measured angle and that portion's mean angle were calculated. Then, a univariate rank test (Wilcoxon–Mann–Whitney U -test) was applied to the calculated distances for each top–bottom pair (see Table 1).

Using a similar idea to that above to ascertain the significance of the dispersion of the flight trajectories to the direction of the new nest-site, 1.5π rad, we calculated the angular distance between each measured angle and 1.5π rad, and repeated the above rank test. For this test, we present the average angular distance from 1.5π rad of

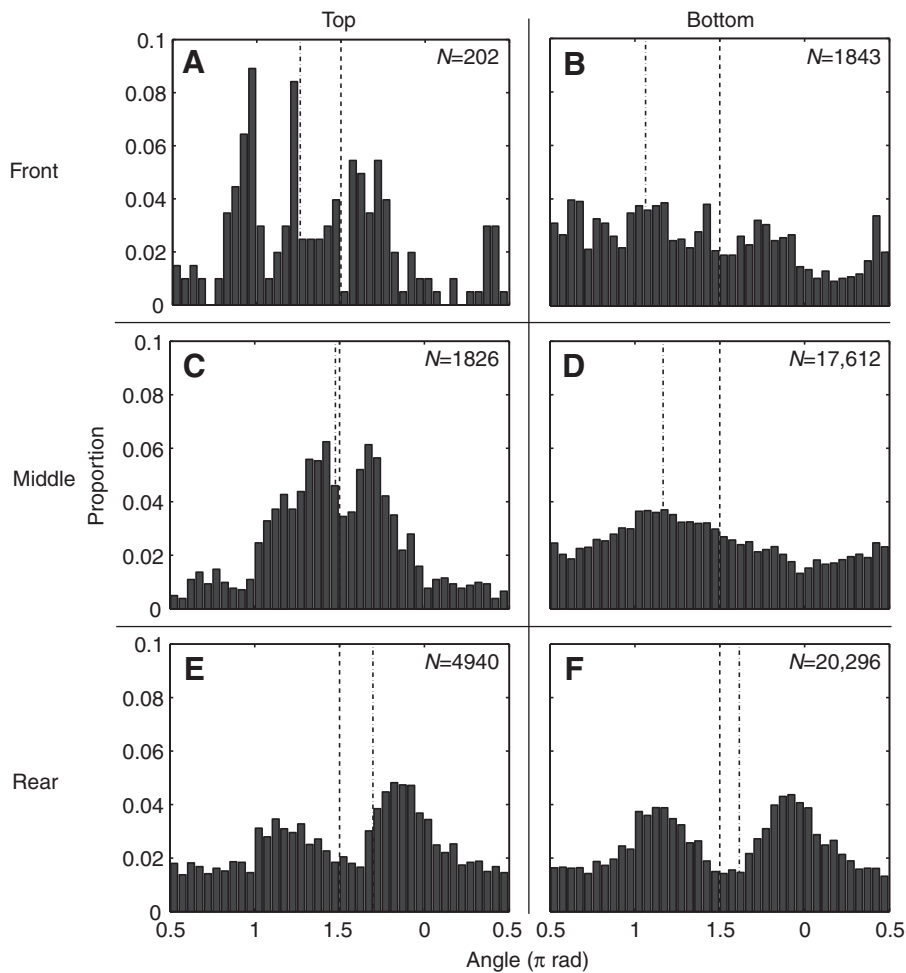


Fig. 3. Distribution of flight angles, for the swarm fly-by of 2 July. The dashed line indicates the direction of the new nest site (1.5π rad), and the dash-dot line indicates the angle of the mean vector, $\bar{\phi}$. N , sample size.

each swarm portion as a measure of concentration (denoted by $\bar{\Delta}_{\text{top}}$ and $\bar{\Delta}_{\text{bottom}}$ for the top and bottom, respectively), as well as the significance level of the U -test (see Table 2).

Quartile plots of bee flight speeds

Fig. 4 shows the distribution of the bees' flight speeds (in pixels per half-frame) grouped by flight angle, for the fly-by of 2 July. Note that the flight speeds that we measured are not the true velocity vector for each bee, but instead are the projection of each bee's velocity vector onto the plane of the camera's field of view. Furthermore, the greater the distance between bee and camera, the smaller the projection of any given velocity vector, so the higher average flight speeds measured for the bottom of the swarm than for the top are probably an artifact of our measurement system. Comparisons between bees in the top or between bees in the bottom, however, are less subject to this artifact problem.

In general, plots of the middle of the swarm show marked peaks in flight speed associated with bees flying toward the nest box (1.5π rad) in both the top and the bottom of the swarm, for all three fly-bys. Evidently, in the middle of the swarm, both in the top and the bottom, the bees flying along the axis of swarm flight were flying faster than those flying in other directions. A similar, but less striking, pattern exists for the bees in the front of the swarm, especially among bees in the top of the swarm. For bees in the rear of the swarm, the flight speed distributions are nearly flat, for both top and bottom bees. The data from the 3 July fly-by exhibit this peaking trend in the middle and the rear, although to a lesser extent

in the rear. The 29 June case shows only the slightest peaking in middle cases. Both of these figures are included in the supplementary data (supplementary material Fig. S3 and Fig. S5).

In order to assess the significance of these peaks, several statistical tests were performed. The first test performed on the peaks was to use an analysis of variance to see whether the peaked region varied significantly from the rest of the angular ranges. First, the velocities of each swarm portion were divided into four ranges of size $\pi/2$ rad centered on 0.5π , π , 1.5π and 2π , based on their angle. The magnitudes of these four angular ranges were then processed using a one-way ANOVA to test the null hypothesis that all four were drawn from the same population. Table 3 shows the results of this analysis, indicating that in nearly every swarm portion the observed peaking is significant. In some instances, the results of the ANOVA reject the null hypothesis, but the peaking trend is not as clear for that swarm portion. Due to this, we present two correlation based quantifications for this peaking behavior, with corresponding P -values against the alternative hypothesis that the correlation is greater than or equal to zero.

The first correlation-based analysis was to use Spearman's rank correlation coefficient (denoted ρ) between the magnitude of the velocity vs the angular distance between the angle of the velocity vector and 1.5π rad. Spearman's correlation coefficient is a non-parametric analog to Pearson's correlation coefficient [in fact, it is Pearson's correlation coefficient calculated for the ranks of the observations (Conover, 1971)]. The second analysis used the same angular groupings as the quartile plots, and merged the groups with

Table 1. Table of top–bottom comparison of angular concentration

	S_{top}^2	S_{bottom}^2	$S_{bottom}^2 - S_{top}^2$	U	z	$P(S_{top}^2 > S_{bottom}^2)$
2 July – front	1.33	1.67	-0.34	182,445	-3.04	0.0023
2 July – middle	1.04	1.66	-0.62	13,865,497	-17.01	6.99e-65
2 July – rear	1.64	1.78	-0.14	58,176,392	-9.06	1.34e-19
3 July – front	1.29	1.62	-0.32	13,774	-2.53	0.011
3 July – middle	1.46	1.79	-0.33	1,476,329	-3.07	0.0022
3 July – rear	1.68	1.88	-0.20	3,932,729	4.61	3.91e-06
29 June – front	1.32	1.44	-0.11	31,251	-0.99	0.32
29 June – middle	1.50	1.73	-0.23	8,885,640	-6.62	3.70e-11
29 June – rear	1.27	1.76	-0.49	1,719,749	-7.72	1.12e-14

S_{top}^2 and S_{bottom}^2 , angular variances for the top and bottom of the swarm, respectively. U , rank sum of the Wilcoxon–Mann–Whitney U -test; z , normal approximation to U .

the bin-centers that are the same angular distance from 1.5π . Then, Spearman's correlation coefficient was calculated for the median magnitudes of the merged groups vs the angular distance of the bin-centers from 1.5π rad (denoted by ρ_{med}). The results for both of these calculations are shown in Table 3, along with the large-sample approximation to the permutation distribution of the probability of the null hypothesis that the actual correlation is greater than or equal to zero. In nearly every swarm portion, the correlation-based analyses yielded significant indications of velocity peaking by the bees flying toward the nest box.

DISCUSSION

Our video analyses of honeybee swarms flying directly over a camera have revealed several striking features of the flight patterns of the bees in the swarms. Regarding flight direction (Fig. 3; Tables 1 and 2; supplementary material Table S2), we found that bees in the top–middle portion of a swarm showed the strongest tendency to fly toward the nest box. This is evident both in $\bar{\phi}$, which is nearest to 1.5π rad at this portion of the swarm for all three cases, and by the lower values for $\bar{\Delta}_{top}$ for these cases. Regarding flight speed, we found that bees flying toward the nest box had average speeds noticeably higher than bees not flying toward the nest box (Fig. 4; Table 3). These trends are summarized schematically by Fig. 5. The top of the swarm has fast-flying bees and high alignment in the middle portion, and slower bees with lower alignment in the other portions. The bottom of the swarm is much less aligned than the top, but there are fast bees flying in the direction of the nest site. In general, the swarm is very chaotic with bees flying in all directions with a wide range of speeds.

What do these patterns tell us about how the informed bees provide flight guidance to the others? Specifically, do the flight patterns support more strongly the subtle guide or the streaker bee hypothesis? The flight direction data support the notion of leaders

flying in the top of the swarm, but do not favor one hypothesis over the other. The flight speed data, however, do strongly favor the streaker bee hypothesis. Consider first the flight direction data. Both the subtle guide and the streaker bee hypotheses predict that the informed bees will fly preferentially toward the nest box and so induce other bees to do likewise. Thus, the finding that bees in the top–middle region of the swarm flew preferentially toward the nest box supports both hypotheses. At this point, we are not certain that the pattern of coherent flight toward the nest box in the top of the swarm represents the actions of the informed bees, but this seems likely, especially when one considers that the percentage of bees in the top–middle of the swarm was small, <10% (Fig. 3, compare the sample sizes for top and bottom data), and it is known that the percentage of informed bees in a swarm is also small, <5% (Seeley et al., 1979; Seeley and Visscher, 2007). Due to the aforementioned brightness issues, the other two fly-bys do not have this same distribution of bees between the top and bottom, but there were generally at least twice as many bees in the bottom as the top for most of the swarm portions.

Consider now the flight speed data. The subtle guide hypothesis predicts that the informed bees will differ from the uninformed bees only by having a preferred direction of flight, but the streaker bee hypothesis predicts that the informed bees will differ from the others by having not only a preferred direction of flight but also a higher speed of flight. Thus, only the streaker bee hypothesis predicts the finding that bees flying toward the nest box had higher speeds than those flying in other directions. Similarly, since we have these fast-flying bees traveling at rates significantly faster than the overall motion of the swarm, we can conclude that these fast-flying bees must have some method for making repeated streaks through the swarm, e.g. by returning along the bottom and edges or by stopping completely and allowing the swarm to pass by. If this were not the case, then these fast-flying bees would continue to fly rapidly

Table 2. Table of top–bottom comparison of angular concentration about 1.5π rad

	$\bar{\Delta}_{top}$	$\bar{\Delta}_{bottom}$	$\bar{\Delta}_{bottom} - \bar{\Delta}_{top}$	U	z	$P(\bar{\Delta}_{top} > \bar{\Delta}_{bottom})$
2 July – front	1.27	1.54	-0.28	-4.01	174,668	5.98e-05
2 July – middle	0.97	1.47	-0.49	-22.45	12,624,453.5	1.38e-111
2 July – rear	1.40	1.46	-0.06	-5.30	59,903,343.5	1.18e-07
3 July – front	1.29	1.48	-0.19	-1.49	14,596	0.14
3 July – middle	1.23	1.44	-0.21	-4.50	1,440,515.5	6.70e-06
3 July – rear	1.38	1.49	-0.11	2.87	3,871,539	0.0042
29 June – front	1.24	1.55	-0.31	-3.29	27,571	0.0010
29 June – middle	1.27	1.52	-0.26	-11.60	8,427,936.5	4.18e-31
29 June – rear	1.27	1.42	-0.15	-3.95	1,812,390.5	7.75e-05

$\bar{\Delta}_{top}$ and $\bar{\Delta}_{bottom}$, angular concentration around direction of the nest for the top and bottom of the swarm, respectively.

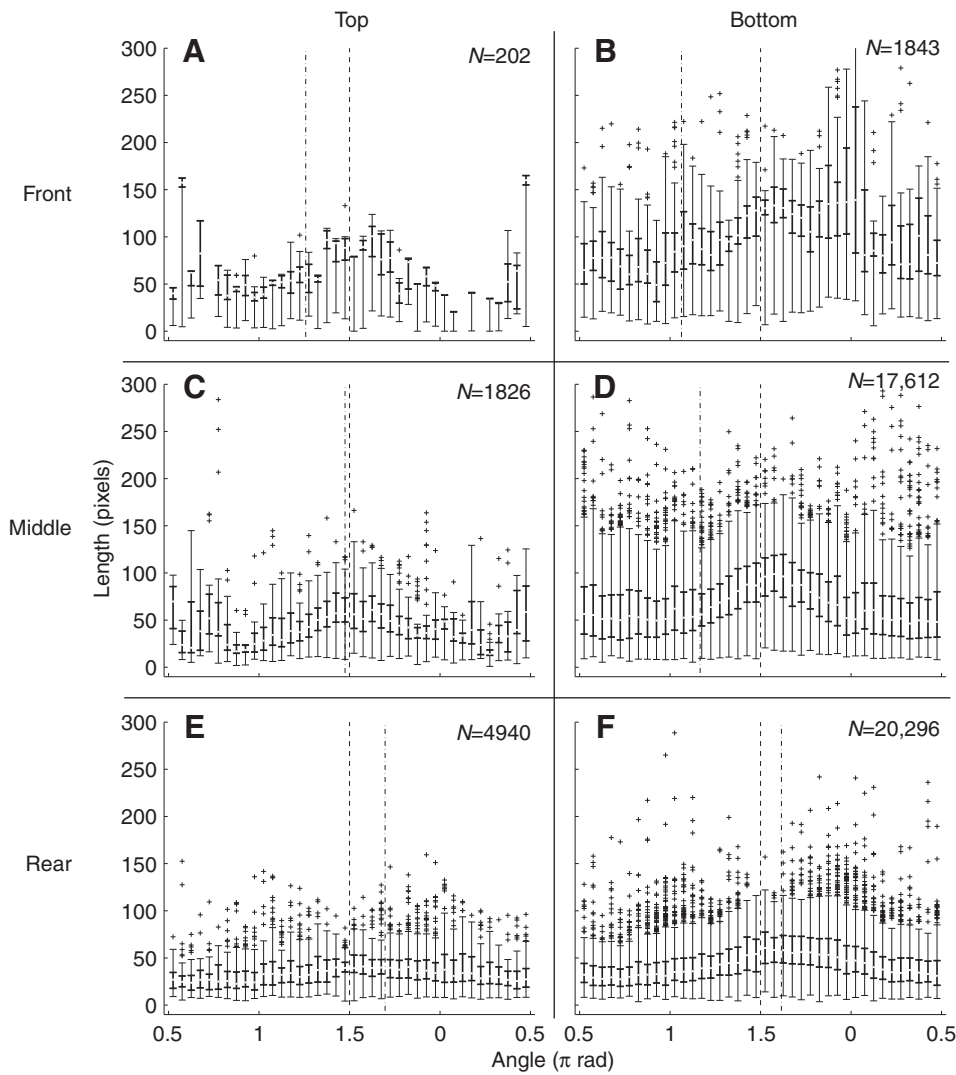


Fig. 4. Flight speed vs flight angle for the swarm fly-by of 2 July. The dashed line indicates the direction of the new nest site (1.5π rad), and the dash-dot line indicates the angle of the mean vector, ϕ . In these plots, the inner quartile range is denoted by bold lines with a gap for the median, the outer quartile range by thin lines, and outliers by a +.

forward and thus leave the swarm. If the swarm were using subtle guides, we would not see this higher velocity towards the nest site, as the subtle guides would reach the front of the swarm and stay there, not accounting for the persistent pattern of fast-flying bees through the swarm. We conclude, therefore, that our flight speed data strongly favor the streaker bee hypothesis over the subtle guide hypothesis for the mechanism of flight guidance in honeybee swarms.

There is a complication to our above conclusions that needs to be addressed. It is the possibility that the observed peaks in the velocity plots are artifacts of projection. Since we recorded the projections of the bees onto the focal plane of the camera, the calculated velocities are actually the real velocities multiplied by the cosine of the (unknown) azimuthal deviation from the focal plane. If the bees flying in the direction of the nest site flew more parallel to the ground than the bees flying in other directions, then it would appear that the bees flying toward the nest site were faster than the other bees (whose velocities would be projected shorter on the focal plane). However, it was shown by Beekman and colleagues that the azimuthal angles for bees in both the top and the bottom are heavily concentrated around the focal plane (Beekman et al., 2006). In this case, if flight speed were truly independent from flight angle, we would expect the maximum flight speeds to be uniform across planar angular ranges as the large number of samples should

produce fast-flying bees that are flying nearly flat with the camera, giving similar maxima between planar flight angles. This is not what is seen for the velocity plots of the bees in the top (Fig. 4), and would most probably hold for bees in the bottom if it accounted for bees flying very close to the camera.

There are additional observations to note that do not directly relate to the subtle guide vs streaker bee hypothesis. As noted above, individual bees are traveling much faster than the swarm as a whole is traveling to the new nest site, and through the data collection process it was found that most bees are traveling in fairly straight lines (see Fig. 2). These two observations indicate that a bee makes many 'trips' across the swarm as the swarm travels to the new nest site, and that a bee's detection of the swarm 'edge' and center, in addition to its attraction to fast-flying bees, could be key to the swarm flight dynamics. The bimodality present in Fig. 3E,F could be indicative of this boundary behavior. For instance, a bee that is at the rear of the swarm and away from the swarm's central axis (in the rear 'corner') presumably would avoid flight angles that would take it away from the edge of the swarm whereas a bee that is in the rear of the swarm and near the central axis presumably would not face this restriction. Another observation that is consistent across the three swarm fly-bys is that the mean angle for the bottom-middle is on the opposite side of 1.5π rad compared with the top-rear. This could be a result of corrective steering by the leader bees.

Table 3. Summary of ANOVA results and correlation-based analysis of velocity peaking

	Probability>F	ρ	$P(\rho \geq 0)$	ρ_{med}	$P(\rho_{med} \geq 0)$
2 July – front–top	8.36e–06	–0.52	6.07e–16	–0.62	0.0020
2 July – front–bottom	0	–0.39	1.95e–68	–0.79	2.09e–05
2 July – middle–top	0	–0.39	7.14e–68	–0.50	0.013
2 July – middle–bottom	0	–0.35	0	–0.85	0
2 July – rear–top	0	–0.26	3.44e–77	–0.904	4.82e–07
2 July – rear–bottom	0	–0.29	0	–0.98	3.25e–06
3 July – front–top	0.24	–0.41	1.45e–05	–0.71	0.00033
3 July – front–bottom	8.80e–06	–0.013	0.42	0.044	0.57
3 July – middle–top	2.22e–16	–0.39	3.25e–36	–0.73	0.00015
3 July – middle–bottom	0	–0.42	8.45e–105	–0.89	4.66e–08
3 July – rear–top	0	–0.55	2.08e–154	–0.95	3.37e–11
3 July – rear–bottom	3.24e–05	–0.39	1.29e–71	–0.86	6.41e–07
29 June – front–top	0.07	–0.17	0.033	–0.37	0.056
29 June – front–bottom	1.97e–11	–0.20	6.18e–06	–0.70	0.00042
29 June – middle–top	1.15e–13	–0.19	9.18e–11	–0.471	0.018
29 June – middle–bottom	0	–0.025	0.036	–0.14	0.27
29 June – rear–top	0	–0.12	6.85e–06	–0.32	0.082
29 June – rear–bottom	0	–0.0045	0.42	–0.033	0.45

ρ , Spearman’s rank correlation coefficient calculated for flight speed vs angle; ρ_{med} , Spearman’s rank correlation coefficient of median flight speed vs flight angle.

The results of this study complement several of the findings reported by Beekman and colleagues (Beekman et al., 2006). Let x denote the direction of swarm travel, y the direction parallel to the ground and perpendicular to x , and z the height above ground. We report data gathered with a video camera oriented with a bottom view of a swarm, hence our data indicate the velocity vectors of the bees projected in the x - y plane, with approximate information about bee position on the z -axis (a bee is either in the top or the bottom portion of the swarm). Beekman and colleagues report data gathered with a photo camera oriented with a side view of a swarm (Beekman et al., 2006), hence their data indicate the velocity vectors of bees projected in the x - z plane, with no information about bee position on the y -axis. Viewing swarms from the side, they showed conclusively that bees in the top of the swarm travel faster and with greater directionality (in the x - z plane) than the bees in the bottom, but their photos did not allow them to determine the direction of the bees’ movements in the x - y plane (whether toward the new nest

site, away from it, or some angle in between). Viewing swarms from the bottom, we have confirmed their report that bees in the top of the swarm have greater directionality than the bees in the bottom – especially in the middle portion of the swarm – and we have shown that this greater directionality is oriented toward the new nest site.

Another feature of individual bee motion that is revealed by our video data, but not by the photo data of Beekman and colleagues (Beekman et al., 2006), is that many bees in a swarm are flying in directions (in the x - y plane) other than that of the new nest site. This was particularly true for the bottom–front and bottom–middle portions of the swarms we studied (see Fig. 3), for which the flight angle distributions are nearly uniform. This suggests that the uninformed bees are mostly flying in random directions when in the center of the swarm cloud, though given that a swarm maintains cohesiveness, these uninformed bees must be orienting themselves towards the swarm center when they approach its edge.

If the stalker bee hypothesis is indeed correct, then the higher flight speed of bees flying toward the nest box that was observed throughout the swarm (top and bottom, front to rear) suggests that the informed bees induced the uninformed bees not only to fly toward the nest box but also to fly faster. Probably, the uninformed, follower bees ‘latched onto’ the informed, stalker bees by some sort of velocity attraction to the fast-flying bees. Both the informed and uninformed bees flying rapidly forward must, however, eventually slow down and change direction lest they shoot ahead, leaving the rest of the swarm behind. We did not see a concentrated band of flight angles and flight speeds at the front of the swarm, which is consistent with the idea that the high-speed, forward-flying bees did indeed slow down and veer from the swarm’s flight axis upon reaching the front of the swarm.

We conclude by noting that although this study shows that the informed bees are evidently providing flight guidance by streaking, this study has not provided detailed information about the streaking behavior of the informed bees. Presumably, each informed bee makes repeated streak flights through the swarm, which raises the question of how the repetition is achieved. There seem to be two logical possibilities: (1) stop at the front of the swarm and permit the swarm to fly past, or (2) fly inconspicuously (slowly? along the

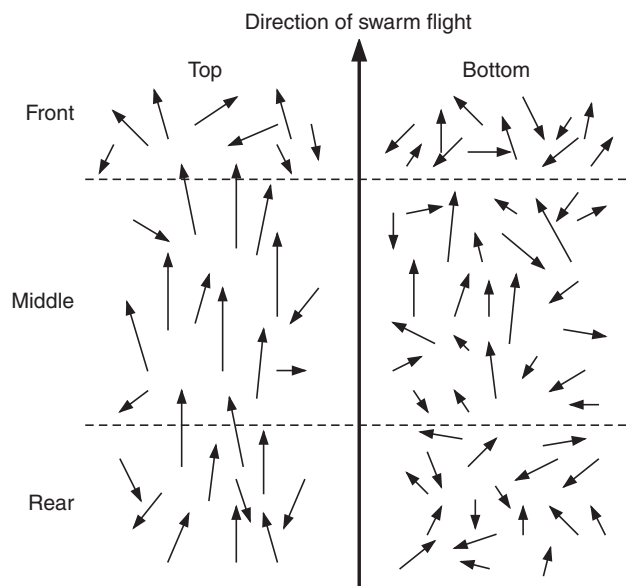


Fig. 5. Schematic summary of velocity vectors.

bottom?) to the rear of the swarm. Do the informed bees perform slow returns along the lateral edges of the swarm, thus flying circuits in the x - y plane? This might promote swarm cohesiveness by helping to define its boundary. It is also possible that the informed bees could return slowly along the bottom of the swarm, so that they are flying circuits in the x - z plane. This might make them inconspicuous against a dark background below. The streaking behavior of the informed bees is a mystery that merits close investigation.

LIST OF ABBREVIATIONS

N	sample size
r	length of the mean vector
s^2	angular variance
s_{bottom}^2	angular variance of flight angles of bottom of the swarm
s_{top}^2	angular variance of flight angles of top of the swarm
$\bar{\Delta}_{\text{bottom}}$	angular concentration around direction of the nest for the bottom of the swarm
$\bar{\Delta}_{\text{top}}$	angular concentration around direction of the nest for the top of the swarm
μ	mean vector
ρ	Spearman's rank correlation coefficient (calculated for flight speed vs angle)
ρ_{med}	Spearman's rank correlation coefficient of median flight speed vs flight angle
$\bar{\phi}$	angle of the mean vector

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