Visual control of flight speed in honeybees

Emily Baird[1],*, Mandyam V. Srinivasan[1], Shaowu Zhang[1] and Ann Cowling[2]

[1]*Centre for Visual Science, Research School of Biological Sciences* and [2]*The Statistical Consulting Unit, Australian National University, PO Box 475, Canberra, ACT 2601, Australia*

*Author for correspondence (e-mail: emily.baird@anu.edu.au)

Summary

Visual control of flight speed in honeybees (*Apis mellifera* L.) was investigated by training them to fly through a specially constructed tunnel in which the motion, contrast and texture of the patterns lining the walls could be varied. Manipulation of pattern motion revealed that the speed of flight is controlled by regulating the image motion that is experienced by the eyes. Flight speed is surprisingly robust to changes in the contrast and/or spatial texture of the visual environment, suggesting that the underlying movement-detecting mechanisms estimate the speed of image motion in the eye largely independently of these parameters. This ensures that flight speed depends primarily on the distances to nearby surfaces and not upon their particular visual properties, such as contrast or visual texture. The removal of image motion cues drastically compromises the regulation of flight speed, underscoring their role in this function.

Key words: insect flight, honeybee, *Apis mellifera*, image motion, pattern motion, pattern texture, pattern contrast.

Introduction

Honeybees rely heavily on information from the visual system to navigate. Despite the perceptual limitations imposed by their immobile eyes, fixed-focus optics and a lack of stereo vision, bees are able to acquire a three-dimensional percept of their world from the pattern of image motion that their visual system experiences as they fly through the environment. This so-called 'optic flow' pattern is rich in cues that bees exploit to achieve a number of navigational goals such as flight stabilisation, landing, estimation of object range, safe negotiation of narrow gaps and estimation of the distance flown to a food source (Kirchner and Srinivasan, 1989; Srinivasan et al., 1991, 1996, 2000).

Here, we investigate whether honeybees use visual cues to control flight speed and attempt to uncover the properties of the underlying mechanisms. A few earlier studies have suggested that flying insects use visual cues to regulate flight speed. Heran (1955) observed that tethered flying bees altered their wing-stroke amplitudes when exposed to moving visual patterns in the ventral visual field. While this suggests that flight thrust is influenced by the image motion that is experienced by the eye, it does not reveal how effective the motion-induced response is in regulating flight speed. David (1982) found that *Drosophila*, flying in a wind tunnel with movable patterns on the walls, adjusted their flight speed to maintain a constant velocity of image motion in the eye. Srinivasan et al. (1996) found that bees flying through a tapered tunnel slowed down as the tunnel narrowed, and

sped up as it widened. This suggests that the bees were adjusting their flight speed in such a way as to hold the image velocity in the eye constant, despite the changes in the width of the tunnel. Here, we test this hypothesized mechanism for speed control in bees, directly and rigorously, by investigating the effects of pattern motion on flight speed and examining the influences of pattern texture and contrast on this behaviour.

Investigation of the influence of pattern texture and contrast on flight speed is an important component of this study, given the current controversy over the nature of the mechanisms that underlie movement detection. The classical studies of optomotor behaviour (Hassenstein and Reichardt, 1956; Reichardt, 1969) and the electrophysiological investigations of the 1980s and 1990s on movement-detecting neurons in the fly have suggested that the perception of movement depends strongly on the spatial texture and the contrast of the moving image (Egelhaaf et al., 1988; Hausen, 1993). In other words, movement detection does not involve an accurate perception of image velocity. On the other hand, David's study of flight speed regulation in fruit flies (David, 1982), and the investigations of centring behaviour (Srinivasan et al., 1991) and of visually mediated odometry (Si et al., 2003) in freely flying honeybees suggest that image velocity is indeed sensed veridically by the visual system. These studies also show that this velocity measurement is quite robust to variations in pattern texture and contrast.

Materials and methods

General

The experiments were carried out in an All Weather Bee Flight Facility at the Australian National University's Research School of Biological Sciences. The temperature inside the facility was maintained at $24\pm5°C$ during the day and $17\pm3°C$ at night. A beehive mounted on the wall of the facility supplied the bees (*Apis mellifera* L.) used in the experiments.

Experimental apparatus

All of the experiments were conducted in a rectangular tunnel that had clear Perspex walls, which allowed bees flying through the tunnel to view a variety of stationary or moving visual patterns (see below). The tunnel was 320 cm long, 20 cm high and 22 cm wide. A clear Perspex ceiling permitted observation and filming of the bees as they flew in the tunnel (Fig. 1A). The floor of the tunnel was white and provided no visual texture. For each experiment, up to 20 bees were individually marked and trained to fly to a feeder containing sugar solution placed at the far end of the tunnel. Flights to the feeder were filmed in the central 1.45 m segment of the tunnel by a digital video camera (Sony DCR-TRV410E; Sony Corporation, Toyko, Japan) positioned 2.5 m above the tunnel floor (Fig. 1A). The recorded flights were analysed by an automated tracking program developed in-house, using Matlab software (v.6.5.0; The Mathworks Inc., Natick, MA, USA).

In Experiments 1–3 (described below), a motorized conveyor belt was placed along the length of the tunnel on each side (Fig. 1A). Each belt was white in colour and carried a pattern of randomly positioned black dots on its surface. The dot diameter was 2 cm, and the spacing between dots varied randomly between 2 cm and 10 cm. The conveyor belt system allowed the pattern to be moved towards or away from the closed end of the tunnel, at a range of speeds (see below).

For Experiment 3 (described below), the tunnel walls were lined with a pattern of randomly placed black dots over one half of the length of the tunnel, such that the external, moveable pattern was occluded when a bee was in this section. Thus, half of the tunnel presented a stationary pattern on either side, while the other half presented moving patterns.

For Experiments 4–6 (described below), the side walls of the tunnel were lined with various black-and-white patterns. These patterns were created on a computer using a graphics program and were printed on a laser printer using sheets of A4 paper. Adjacent sheets were joined and attached to the walls using transparent adhesive tape. Care was taken 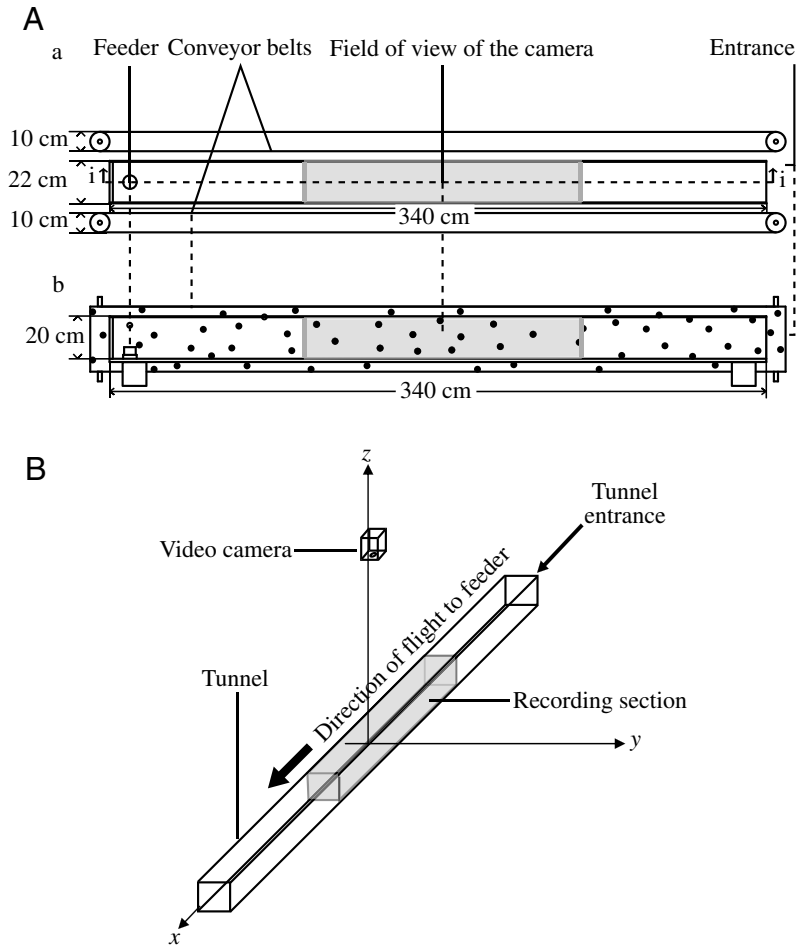to minimise visual artefacts and flaws at the junctions. The contrasts of the patterns were calibrated using a photodiode that had a linear intensity-response function. Percentage contrast was defined as:

$$\% \text{ Contrast} = 100 \times (I_{max} - I_{min}) / (I_{max} + I_{min}) . \quad (1)$$

Experiment 1. Measurement of the effect of pattern motion on flight speed

The flight speed of trained bees flying to the feeder was recorded for six pattern velocities in each direction and for one condition in which the pattern was static. When the pattern was moved in the direction of flight to the feeder, the highest speed was limited by the maximum speed of the motor. The speeds used for pattern motion in this direction were 15, 22, 30, 37, 45 and 52 cm s^{-1} (these velocities were regarded as positive). When the pattern was moved against the direction of flight to the feeder, at high pattern speeds, the bees were unable to enter the tunnel. The maximum speed used in this condition was therefore limited to the highest speed at which the bees could enter the tunnel and fly to the feeder. The speeds tested in this

Fig. 1. (A) Experimental apparatus showing (a) plan and (b) vertical section through i–i in a. The field of view of the camera is shown in grey. It spans a central 1.45 m segment of the tunnel. (B) Illustration of tunnel coordinates. Flight speed was calculated as the projection of the flight vector along the x axis.

condition were 6, 12, 18, 24, 30 and 36 cm s^{-1} (these velocities were regarded as negative).

Experiment 2. Measurement of the effect of temporal changes of pattern velocity on flight speed

This experiment was designed to investigate how bees responded to temporal changes in pattern speed. The flight speed profile of bees flying to the feeder was recorded under two different conditions, stationary-to-moving and moving-to-stationary. This was carried out for six different pattern velocities. For the stationary-to-moving condition, the pattern motor was initially stationary and was turned on as the bee approached the mid-point of the tunnel. The pattern velocity profiles for this condition are shown in Fig. 2A. For the moving-to-stationary condition, the pattern was initially in motion at a prescribed speed as the bees entered the tunnel, and the pattern motor was turned off as they reached the mid-point of the tunnel. The pattern velocity profiles for this condition are shown in Fig. 2B. In each condition, flights to the feeder were recorded for pattern speeds of 23, 30 and 37 cm s^{-1} with the pattern moving towards the feeder and for speeds of 8, 23 and 30 cm s^{-1} with the pattern moving away from the feeder. In addition, there was one control condition in which the pattern was stationary.

Experiment 3. Measurement of the effect of spatial changes of pattern velocity on flight speed

This experiment was designed to investigate how bees responded to spatial changes in pattern speed. In one half of the tunnel, the bees were exposed to a moving pattern; in the other half of the tunnel, the walls were lined with a static pattern such that the moving pattern was occluded. The flight speed profile of bees flying to the feeder was recorded for six pattern velocities under two different conditions: static-to-moving and moving-to-static. For the static-to-moving condition, the stationary pattern lining the walls was placed in the first half of the tunnel. Thus, the bees encountered a

stationary pattern in the first half of their flight towards the feeder, and a moving pattern in the second half. In the moving-to-static condition, the stationary pattern was placed in the second half of the tunnel. Here, the bees encountered a moving pattern in the first half, followed by a stationary pattern in the second half. In each condition, flights to the feeder were recorded for pattern speeds of 23, 30 and 37 cm s^{-1} with the pattern moving towards the feeder, and 8, 23 and 30 cm s^{-1} with the pattern moving away from the feeder. In addition, there was one control condition in which the pattern was static.

Experiment 4. Measurement of the effect of pattern texture on flight speed

This experiment was designed to investigate how bees responded to changes in the visual texture of the patterns lining the tunnel. The influence of spatial texture was examined by measuring flight speeds with the tunnel lined with vertical sinusoidal gratings of spatial periods 7.2 cm, 3.6 cm and 1.8 cm, in turn. For a bee flying along the midline of the tunnel, the spatial frequency of these gratings as seen by the lateral field of the eye would be 0.03, 0.05 and 0.10 cycles deg.$^{-1}$, respectively. To enable comparisons between the flight speeds with the various gratings, the 3.6 cm grating (0.05 cycles deg.$^{-1}$) was arbitrarily designated as the control pattern. This pattern was used throughout the course of the training (24 h before testing began) and between the testing periods when the other gratings were used.

Experiment 5. Measurement of the effect of pattern contrast on flight speed

This experiment was designed to investigate how bees responded to changes in the visual contrast of the patterns lining the tunnel. The influence of pattern contrast was examined by measuring flight speeds with the tunnel lined with vertical square-wave gratings of various contrasts, in turn. Six contrasts were tested: 100, 70, 50, 30, 10 and 0% (0% contrast being a uniformly grey surface of luminance equal to the mean
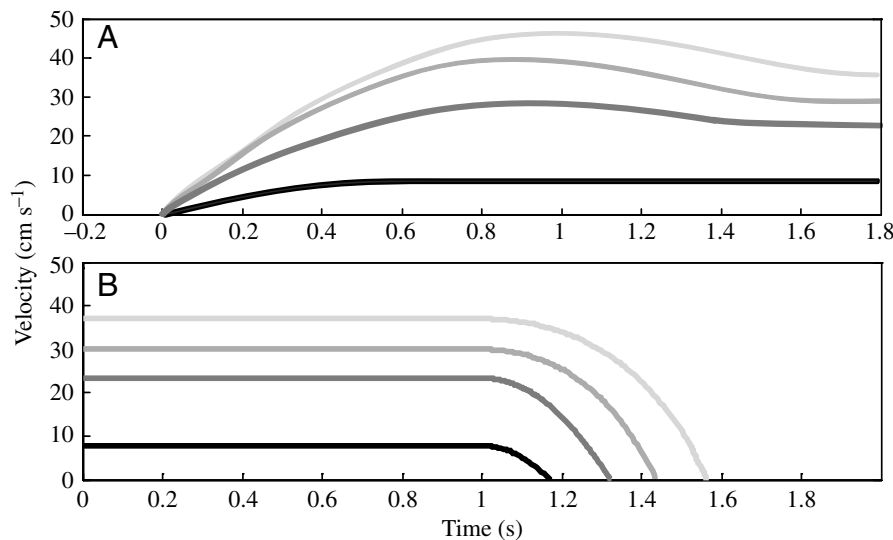

Fig. 2. Measured velocity profiles of pattern motion. (A) Velocity profiles for the stationary-to-moving condition for the four tested pattern velocities: 8 cm s^{-1} (black), 23 cm s^{-1} (dark grey), 27 cm s^{-1} (mid grey) and 37 cm s^{-1} (light grey). The motor was switched on at 0 s. There was no difference between the pattern velocity profiles for positive and negative pattern velocities. (B) Velocity profiles for the moving-to-stationary condition for the four tested pattern velocities: 8 cm s^{-1} (black), 23 cm s^{-1} (dark grey), 27 cm s^{-1} (mid grey) and 37 cm s^{-1} (light grey). The motor was switched off at 1 s. There was no difference between the pattern velocity profiles for positive and negative pattern velocities.

luminance of the other gratings). The gratings had a spatial period of 1.8 cm, which, for a bee flying along the midline of the tunnel, would present a spatial frequency of 0.10 cycles deg.$^{-1}$ as seen by the lateral field of the eye. To enable comparisons between the flight speeds with the various gratings, the 50% contrast grating was arbitrarily designated as the control pattern. This pattern was used throughout the course of the training (24 h before testing began) and between the testing periods when the other gratings were used.

Experiment 6. Measurement of the effect of removing optic flow on flight speed

This experiment was designed to examine the contribution of optic flow cues to the control of flight speed. The influence of optic flow was examined by recording flight speeds when the tunnel was lined with two different types of stationary pattern: vertical stripes and axial stripes. The vertical pattern consisted of alternating black and white, vertically oriented stripes, with a spatial period of 1.8 cm. The axial pattern consisted of alternating black and white, horizontally oriented stripes, each of width 4 cm. The vertical pattern was used as a control because the alternating black and white vertical stripes would provide strong image motion cues to a bee flying along the tunnel. The axial pattern, on the other hand, was used to create a condition in which the optic flow cues were very weak. This is because flight in the direction of the stripes would produce very little apparent motion of the images of the walls on the retina. Because of the rather dramatic difference in texture between the two patterns, it was necessary to place each pattern in the tunnel for about 1 h before commencing flight measurements. This ensured that the bees were accustomed to the change in the appearance of the tunnel environment. Four testing periods were used for each pattern.

Analysis of flight trajectories

The number and frequency of trained bees that visited the apparatus were usually such that, most of the time, only one bee was flying in the tunnel, either towards the feeder or away from it. Only flights towards the feeder were analysed. To avoid possible effects of interaction between bees, flights in which more than one bee was flying simultaneously in the tunnel were not analysed.

An automated tracking program was developed, using Matlab (v.6.5.0), to track individual bees and analyse the recordings of flights obtained in each experiment. For each flight, the program identified the position of the bee in consecutive frames (every 40 ms). The position of the bee was defined in relation to the tunnel co-ordinates x, y and z, where x denotes axial direction, y the transverse direction and z the vertical direction (Fig. 1B). Only x and y could be measured, as the system used a single camera (rather than a stereo pair). z was assumed to be constant. Visual observation indicated that, although bees decreased their height as they approached the feeder towards the end of the flight, this assumption was a good approximation within the segment of the flight that was filmed by the camera (Fig. 1A). The resolution of the tracking

system, based on the pixel density of the camera is estimated to be 2 mm in x and 2 mm in y.

The data were analysed to calculate the component of the flight velocity in the axial (x) direction (V_x). The program generated plots of bee position (x,y) as a function of time, as well as plots of V_x as a function of time or position. It was also possible to calculate the mean values of V_x over any desired section of the tunnel or during any specified time window. Preliminary analysis revealed that the lateral component of flight velocity (V_y) was much smaller in magnitude compared with the axial component (V_x). In other words, the flight trajectories were essentially straight, with only small deviations from side to side. Given this, and the observation that flight height was reasonably constant within the filmed section of the tunnel (see above), it follows that V_x provides a good approximation of the actual magnitude of the flight speed.

Estimation of image velocities

It was of interest to estimate the angular velocity of the image (the speed of the image on the retina) under various stimulus conditions. We estimated the image angular velocity (ω; in deg. s^{-1}) in the lateral field of view of the eye by using the relationship:

$$\omega = (180/\pi) \, [(V_x - V_p)/d] \,, \qquad (2)$$

where V_x and V_p are the linear velocities of the bee and the pattern, respectively (in cm s^{-1}), and d is the distance between the bee and either wall (in cm). [The bee was assumed to fly close to the midline of the tunnel, so that d is equal to half the width of the tunnel. Examination of flight trajectories showed that this was indeed the case, as first reported in Kirchner and Srinivasan (1989)].

Statistical analysis

Statistical models accounting for multiple levels of variation were developed to assess whether covariates such as treatment, time, temperature, light intensity or humidity affected bee flight speed and to eliminate their effects. To account for the two principal levels of variation in the study – variation between bees and variation within bees – linear mixed models (McCulloch and Searle, 2001) were used, with bee identity as a random effect. The response variables of flight speed, treatment, time, temperature, light intensity and humidity were included in the models as fixed effects. The significance of each explanatory variable was assessed using Wald tests, and non-significant terms (at the 5% level) were deleted from models. Analyses were performed using Genstat (release 6.1; VSN International Ltd, Oxford, UK).

Results
Experiment 1. Effect of pattern motion on flight speed

In this experiment, the mean flight speed was measured for six positive pattern velocities, six negative pattern velocities and one condition when the pattern was static (see Materials
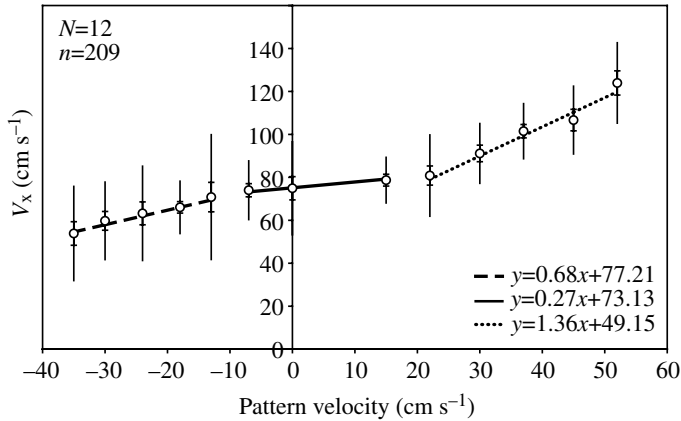
Fig. 3. Experiment 1. Effect of pattern motion on flight speed. The graph shows mean axial flight speed (V_x) when the pattern on the walls was static (0 pattern velocity), moved in the direction of flight (positive pattern velocity values) or against the direction of flight (negative pattern velocity values). The open circles represent V_x values for various pattern speeds. N denotes the number of bees, n denotes the number of flights. The horizontal lines on the error bars denote standard error of the mean; the uncapped bars denote the standard deviation. The dashed line represents a model of the flight speed data for large negative pattern velocities; the slope of this line is slightly smaller than 1. The solid line represents a model of the flight speed data for the positive and negative pattern velocities near zero; the slope of this line is not significantly different from zero. The dotted line represents a model of the flight speed data for large positive pattern velocities; the slope of the regression line was slightly greater than 1. The equations for each regression are shown. Note: only five bees participated in the data shown for negative pattern velocities, due to difficulties in getting them to enter the tunnel under these conditions.

and methods). A minimum of 10 flights were analysed for each condition.

The dependence of flight speed on pattern velocity is shown in Fig. 3. Flight speed increases when the pattern moves in the direction of flight, as shown by the data points in the right-hand part of the graph. Higher pattern velocities elicit higher flight speeds. In this domain, flight speed depends significantly on the velocity of pattern motion ($t_{189}=7.07$, $P<0.001$). By contrast, flight speed decreases with higher pattern velocities when the pattern is moving against the direction of flight. In this domain, there is some evidence to suggest that flight speed is also influenced by pattern movement against the direction of flight ($t_{189}=3.70$, $P=0.0003$). Interestingly, for low pattern velocities about zero (–0.07, 0 and 0.15 cm s^{-1}) flight speed is not significantly affected by pattern movement ($t_{189}=0.63$, $P=0.53$).

If bees regulate their flight speed by maintaining a constant rate of optic flow in the eye, flight speed should vary linearly with pattern velocity and the change of flight speed should be equal to the change of pattern velocity. Thus, the equation for the hypothesised flight speed adjustment (solid line, Fig. 3) takes the form: $y=mx+c$, where y is flight speed, x is pattern velocity and c is the flight speed that bees maintain when the

pattern is stationary. If the bees maintain a constant optic flow in the eye, regardless of pattern velocity (i.e. if they compensate perfectly for changes in pattern velocity) then $m=1$. This assumes that at zero pattern velocity, flight speed is set to achieve the desired optic flow.

An analysis of the data indicates that a model that includes three lines of different slopes provides a good approximation of the effect of large positive pattern velocities, large negative pattern velocities and small positive and negative pattern velocities (including zero pattern velocity) on flight speed. To fit this model, the pattern velocities were classified into three categories: high positive, near zero and high negative. A separate line was fitted within each class. The allocation of data points to each category was determined by comparing four possible groupings. At each of the two category boundaries, there was one point that could be included in one of two possible categories. Models were therefore created for each of the four possible combinations with the two boundary points. The best model was chosen as the one with the lowest Akaike's information criterion (AIC) (Akaike, 1973). A comparison of the AIC was also used to determine whether the three-line model provided a better approximation of the data than a one- or two-line model.

For large positive pattern velocities, the model revealed a slope of $m=1.36$ (dotted line, Fig. 3). There is some evidence that this slope is significantly greater than 1 (two-sided t-test, $t_{189}=1.86$, $P=0.06$). This result suggests that, when the pattern is moved in the direction of flight, the bees respond by increasing their flight speed by a greater amount, thus overcompensating for the changes in pattern speed.

For large negative pattern velocities, the slope of the model was $m=0.68$ (dashed line, Fig. 3). There is some evidence that this slope is significantly different from 1 (two-sided t-test, $t_{189}=-1.78$, $P=0.077$). Thus, when the pattern moved against the direction of flight, the bees were not making a complete adjustment of flight speed to counter the changes in pattern speed: they were experiencing increased optic flow.

For small pattern velocities about zero, the slope of the model was 0.27 (solid line, Fig. 3). This slope is not significantly different from zero (two-sided t-test, $t_{189}=0.63$, $P=0.53$). Thus, at low image velocities, the bees were not adjusting their flight speed to compensate for the small changes in image velocity.

Experiment 2. Effect of temporal changes of pattern velocity on flight speed

This experiment was designed to investigate how bees responded to temporal changes in pattern speed. Flights of bees flying to the feeder were recorded under two stimulus conditions: stationary-to-moving and moving-to-stationary, as detailed in Materials and methods. For each flight, the mean flight speed was measured over two time windows: one window corresponding to the period when the pattern was stationary and the other corresponding to the period when the pattern was in motion. A minimum of 27 flights were analysed for each experimental condition.

The results are shown in Fig. 4. In the stationary-to-moving condition (Fig. 4A), bees clearly change their flight speed in response to the onset of pattern motion. This is true regardless of the direction of pattern motion: in either case, the change of flight speed is such as to counter the effect of pattern motion. The effect of pattern velocity on flight speed is significant (Wald/d.f.=346.14, $P<0.001$) and the relationship is approximately linear for positive as well as negative pattern velocities. However, the compensation is not complete: the slope of the regression line is 0.63, a value significantly lower than 1 (two-sided t-test, $t_{222}=10.32$, $P<0.001$). Thus, bees respond to temporal increases or decreases in pattern velocity by adjusting their flight speed in such a way as to partially compensate for such changes.

The same is true for the moving-to-stationary condition (Fig. 4B). Here, too, bees respond to changes in pattern velocity by adjusting their flight speed in such a way as to partially compensate for such changes. Linear regression analysis reveals that the change in flight speed is again proportional to the change in pattern velocity. The slope of the regression line (0.65) is very similar to that for the stationary-to-moving condition and is not significantly different from it (two-sided t-test, $t_{202}=0.18$, $P<0.86$). This slope is also significantly lower than 1 (two-sided t-test, $t_{199}=6.94$, $P<0.001$).

Taken together, the results of Fig. 4 show that flight speed is sensitive to temporal changes of pattern motion and is equally responsive to changes in either direction.

Experiment 3. Effect of spatial changes of pattern velocity on flight speed

This experiment was designed to investigate how bees respond to spatial changes of pattern motion. Flights of bees flying to the feeder were recorded for two experimental conditions: one with the static pattern in the first half of the tunnel and another with the static pattern in the second half. In each condition, flights were recorded for a range of pattern speeds and directions, as detailed in Materials and methods. For each flight, the mean flight speed was measured in two sections of the tunnel: the static section and the moving section. A minimum of 24 flights were analysed for each experimental condition.

The results are shown in Fig. 5. In the static-to-moving condition (Fig. 5A), bees clearly change their flight speed in response to the existence of pattern motion in the second half of the tunnel. This is true regardless of the direction of pattern motion: in either case, the change of flight speed is such as to counter the effect of pattern motion. The effect of pattern velocity on flight speed is significant (Wald/d.f.=191.08, $P<0.001$) and the relationship is again approximately linear, for positive as well as negative pattern velocities. Again, the compensation is not complete: the slope of the regression line is 0.63, a value significantly lower than 1 (two-sided t-test, $t_{208}=8.29$, $P<0.001$). Thus, bees respond to spatial increases or decreases in pattern velocity by adjusting their flight speed in such a way as to partially compensate for such changes.

The same is true for the moving-to-static condition (Fig. 5B). Here, too, bees respond to the changes in pattern velocity by adjusting their flight speed in such a way as to partially compensate for such changes. Linear regression analysis reveals that the change in flight speed is again proportional to the change in pattern velocity. The slope of the regression line (0.56) is somewhat lower that for the static-to-moving condition but is not significantly different from it (two-sided t-test, $t_{177}=0.99$, $P=0.32$). This slope is also significantly lower than 1 (two-sided t-test, $t_{174}=6.24$, $P<0.001$).

In summary, the results of Fig. 5 show that flight speed is sensitive to spatial changes in pattern motion and is equally responsive to changes in either direction.
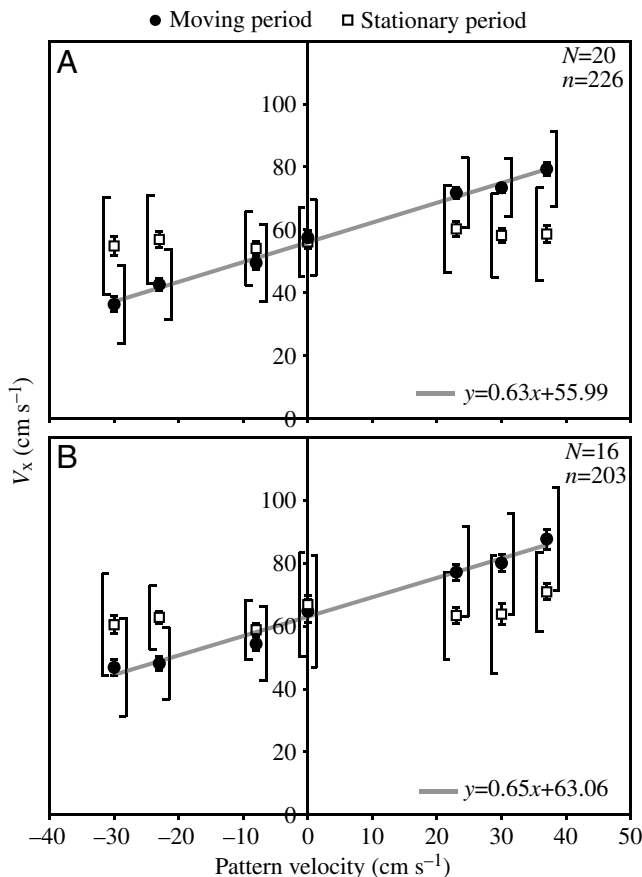

Fig. 4. Experiment 2. Effect of temporal changes of pattern velocity on flight speed. (A) Mean flight speeds when the pattern is stationary in the first phase and moving in the second phase. (B) Mean flight speeds when the pattern is moving in the first phase and stationary in the second. In each case, the open squares and filled circles indicate mean flight speeds during the stationary and moving phases, respectively. The solid line represents a regression model of the data; the equation for this line is shown on each graph. The error bars through the centre of each point indicate the standard error of the mean. The error bars to the left of each data point indicate the standard deviation for the stationary period (open squares); the bars to the right of each data point indicate the standard deviation for the moving period (filled circles). Other details are as in Fig. 3.

Experiment 4. Effect of pattern texture on flight speed

This experiment was designed to investigate whether flight speed is influenced by the spatial texture of the visual environment. Bees were trained to fly to the end of a tunnel in which the walls were lined with patterns displaying stationary sinusoidal gratings of various spatial periods, as detailed in Materials and methods. A minimum of 20 flights were analysed for each condition.

The results are shown in Fig. 6A. The data indicate that flight speed is largely insensitive to changes in spatial period. The mean flight speed varied by only 5 cm s^{-1} over the three conditions and there was no strong evidence to suggest that the differences were statistically significant (Wald/d.f.=2.09, P=0.10). Thus, flight speed is relatively robust to variations in the spatial texture of the visual environment.

Experiment 5. Effect of pattern contrast on flight speed

This experiment was designed to investigate whether flight speed is influenced by changes in the contrast of the visual environment. Bees were trained to fly to the end of a tunnel in which the walls were lined with vertical gratings of different contrasts, as detailed in Materials and methods. A minimum of 14 flights were analysed for each condition.
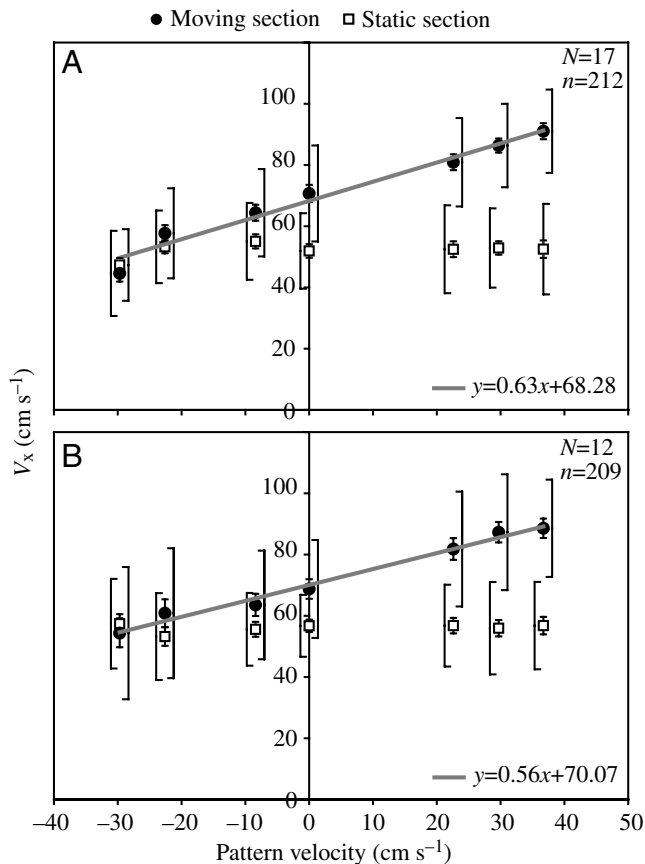

Fig. 5. Experiment 3. Effect of spatial changes of pattern velocity on flight speed. (A) Mean flight speeds when the pattern is static in the first half of the tunnel and moving in the second half. (B) Mean flight speeds when the pattern is moving in the first half of the tunnel and static in the second half. In each case, the open squares and filled circles indicate mean flight speeds in the static and moving sections, respectively. The solid line represents a regression model of the data; the equation for this line is shown on each graph. The error bars to the left of each data point indicate the standard deviation for the stationary section (open squares); the bars to the right of each data point indicate the standard deviation for the moving section (filled circles). Other details are as in Fig. 3.
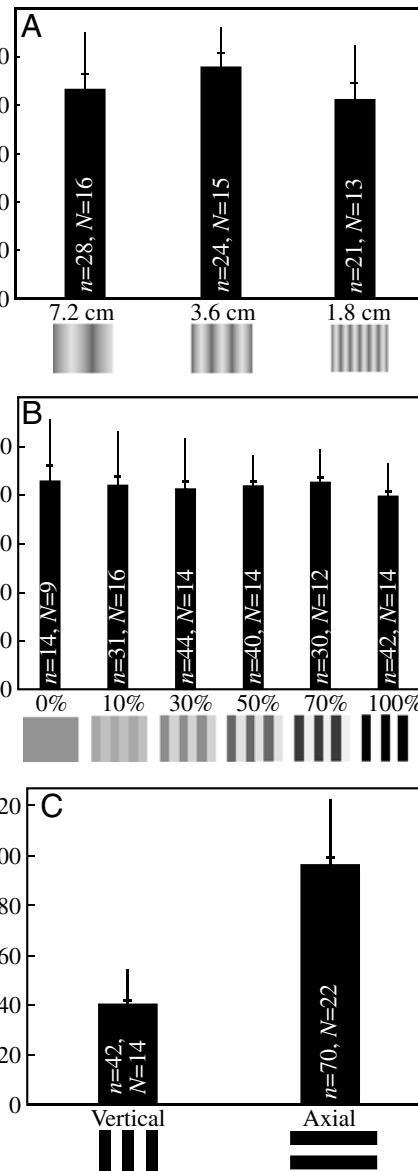

Fig. 6. (A) Experiment 4 – effect of pattern texture on flight speed. Comparison of mean flight speeds when the walls are lined with vertical sinusoidal gratings of spatial period 1.8 cm, 3.6 cm or 7.2 cm. (B) Experiment 5 – effect of pattern contrast on flight speed. Comparison of mean flight speeds when the walls are lined with vertical square-wave gratings of contrast 0%, 10%, 30%, 50%, 70% or 100%. (C) Experiment 6 – comparison of mean flight speeds when the walls are lined with vertical stripes (producing strong optic flow cues) or axial stripes (producing very weak optic flow cues). Other details are as in Fig. 3.

The results are shown in Fig. 6B. The data indicate that flight speed is largely insensitive to changes in the contrast of the visual environment. The effect of contrast on flight speed is not significant (Wald/d.f.=0.25, P<0.94). Flight speed is highest in the zero-contrast condition, although this value is not significantly different from that at any other contrast. The possible reasons for this outcome will be discussed below. Overall, this experiment reveals that flight speed is rather robust to variations in the contrast of the visual environment.

Experiment 6. Effect of removing optic flow on flight speed

This experiment was designed to examine the contribution of optic flow cues to the control of flight speed. Flight speeds were measured when the tunnel was lined with (1) vertical stripes and (2) axial stripes, as detailed in Materials and methods. Forty-two flights were analysed for the vertical stripe condition and 70 flights for the axial stripe condition.

The results are shown in Fig. 6C. The data indicate that bees fly substantially faster in the presence of axial stripes (when optic flow cues are weak) than in the presence of the vertical stripes (when optic flow cues are strong). The mean flight speed is 96±23 cm s^{-1} (± S.D.) with axial stripes, compared with 42±10 cm s^{-1} with the vertical stripes. The difference in mean flight speeds between the two conditions is significant at the P<0.001 level (Wald/d.f.=107.13). Furthermore, flight speeds are much more variable in the axial stripe condition: the standard deviation is approximately twice that in the vertical-stripe condition. We used a restricted maximum likelihood (REML) test (Patterson and Thompson, 1971) to test the hypothesis that the variances for the two conditions are the same. This test showed that there was strong evidence against the hypothesis (P=0.0032), indicating that flight speeds are significantly more variable in the absence of optic flow. It is possible that absence of optic flow leads to greater variation in flight speed even within individual trajectories, but we did not perform this analysis.

Discussion

The findings of this study demonstrate quite clearly that honeybees use optic flow information to regulate flight speed. In varying environments, flight speed tends to be adjusted so as to hold the angular velocity of the image – that is, the speed of the image on the retina – constant. This finding is in general agreement with the conclusions drawn by David (1982) in relation to the control of flight speed in the fly *Drosophila* and confirms the conjecture put forward by Srinivasan et al. (1996) for the honeybee.

The phenomenon is clearest in Experiment 1, for pattern motion in the direction of flight. There, bees encountering the moving pattern increase their flight speed by an amount that is slightly greater than the speed of the pattern. When the pattern is moved against the direction of flight, bees decrease their flight speed by an amount that is slightly lower than the speed of the pattern. The changes in flight speed that are observed in response to the movement of the pattern suggest that the bees are holding the optic flow in the eye within a certain, preferred range. Interestingly, when the pattern is moved at slow speeds, either with or against the direction of flight, there is no associated change in flight speed. This suggests that the honeybee's flight speed control system responds only when the image velocity deviates from its 'set point' by an amount that exceeds a certain threshold. From Fig. 3, we see that this threshold is between 10 and 15 deg. s^{-1} (as calculated from the flight speed values at pattern velocities that are close to zero). However, once the deviation exceeds this threshold – in either direction – the flight speed is adjusted to reduce the deviation back to a level below threshold. As we see below, this 'dead zone' in the speed control does not lead to statistically significant changes in the angular velocity of the image, except in two conditions.

In order to test whether the bees were maintaining a constant rate of retinal image flow, we estimated angular velocity as perceived by a bee flying along the tunnel when the patterns

Table 1. *Estimates of image angular velocity for different pattern velocities*

Pattern velocity (cm s^{-1})	Mean flight speed (cm s^{-1})	Estimated image angular velocity (deg. s^{-1})	t-value*	P-value*
0	74.3	265		
−7	73.0	287	0.813	0.417
−13	70.2	283	0.567	0.572
−18	65.5	299	1.291	0.198
−24	62.7	310	1.622	0.107
−30	59.2	320	1.984	0.049
−35	53.3	316	1.847	0.066
15	78.1	224	−1.698	0.091
22	80.3	207	−2.379	0.018
30	90.6	215	−1.834	0.068
37	100.9	227	−1.585	0.115
45	106.1	217	−1.674	0.096
52	123.4	254	−0.419	0.676

*These values represent the probability that the estimated image angular velocity for the various pattern velocities is different from the estimated value when the pattern is stationary.

were in motion (Experiment 1). To calculate retinal image flow, we either added the pattern velocity to the bees' flight speed (when the pattern was moving against the direction of flight) or subtracted the pattern velocity from the bees' flight speed (when the pattern was moving in the direction of flight). Interestingly, statistical analysis using linear mixed models (as shown in Table 1) reveals that the estimated angular velocity for each pattern velocity does not differ significantly from that obtained when the pattern is stationary, except in two instances, namely when the pattern moved at 22 cm s^{-1} in the direction of flight and at 30 cm s^{-1} against the direction of flight. This suggests that, in all but a few cases, the bees were adjusting their flight speed to maintain a constant rate of image flow across their retina.

We have seen that, in Experiments 2 and 3 (which examined the effects of temporal and spatial changes of pattern motion), the bees seemed to compensate only partially for the changes in motion. There could be a number of reasons for this. In Experiment 2, the bees would have required a finite time to respond to the change in pattern speed. Furthermore, the pattern did not change speed instantaneously from the stationary to the moving phase, and *vice versa*: the full transition required anywhere between 0.25 and 1.0 s, depending upon the magnitude of the final speed (see Fig. 4). A bee flying at a speed of, say, 56 cm s^{-1} in the tunnel would be visible in the camera's field of view for only 1.25 s after the onset of pattern motion. Because of these delays and observation constraints, a simple comparison of the mean flight speeds during the stationary and movement periods (as we have done here) would tend to underestimate the magnitude of the full change in flight speed. A more accurate estimate of this total change could have been obtained by filming a larger section of the tunnel, using a longer time window and comparing the mean flight speed during the stationary phase with the flight speed well after the onset of pattern motion. However, such a measurement was not feasible owing to the experimental constraints described above.

A similar underestimation of the change in flight speed could have occurred in Experiment 3. Here, it is possible that the bees' response to the spatial change in pattern motion was not complete within the relatively short distance (70 cm) over which the mean flight speed in the moving section was measured. A better estimate of the change in flight speed could have been obtained by comparing the flight speed in the static section with that observed towards the end of the moving section. However this, again, was not practicable owing to the experimental constraints indicated above.

Experiment 3 reveals another phenomenon that is worthy of mention. When the pattern in the 'moving' section was stationary, there was a small, but significant, increase of flight speed in that section compared with the 'static' section (see Fig. 5). This is because the separation between the stationary patterns in the 'moving' section (32 cm) was larger than in the 'static' section (22 cm). Consequently, the bees had to fly faster in the wider section to maintain the same optic flow. This observation adds a further dimension to the results because it means that, when the external pattern was moving, the bees were responding to changes in optic flow that were caused not only by the changes in pattern velocity but also by the changes in pattern distance. In each case, the bees were responding by countering the resulting changes in optic flow.

The results of Experiment 4 reveal that flight speed is relatively insensitive to changes in the spatial texture of the patterns lining the tunnel walls (Fig. 6A). Specifically, when the patterns are sinusoidal gratings, the flight speed does not vary substantially when the spatial frequency of these gratings is changed by a factor of two or four. Because the flight speed is more or less constant at all of these spatial frequencies, it follows that each spatial frequency must induce a proportionally different temporal frequency of intensity fluctuations in the visual system. Therefore, we may conclude that flight speed is largely independent of the spatial as well as the temporal frequency of the gratings.

The results of Experiment 5 reveal that flight speed is relatively insensitive to changes in the contrast of the patterns lining the tunnel walls over a broad range of contrasts (100% through to 10%; see Fig. 6B). When the contrast is reduced to a nominal value of 0% (by lining the walls with uniformly grey sheets of paper), the mean flight speed is somewhat greater than at other contrasts, although the difference is not statistically significant. This suggests that the visual system is capable of extracting motion cues even in this impoverished condition. It should be noted, however, that the contrast of the grey walls is not truly zero: rather, it is very low. Residual contrast features arising from imperfections in the printing process, from light and shade effects, and from the junctions between adjacent sheets must contribute to the detection of image movement. Indeed, it is known that the insect visual system is sensitive to motion at contrasts as low as 7% (Dvorak et al., 1980). It is possible that contrast adaptation – a phenomenon whereby the contrast sensitivity of the visual system is enhanced in the presence of low ambient contrast and is suppressed in the presence of high ambient contrast (Harris et al., 2000) – plays a role in amplifying the sensitivity to low contrasts. This possibility is supported by the finding that regulation of flight speed is better at zero contrast (Fig. 6B) than when the walls are lined with high-contrast axial stripes (Fig. 6C). In the former case, the absence of high environmental contrast would have made the visual system highly sensitive to the low-contrast flaws in the stimulus, whereas in the latter case the high contrast of the axial stripes would have made the system insensitive to the flaws. Since the axial stripes, by themselves, carry no horizontal image motion cues, it is reasonable to expect that, in the presence of contrast adaptation, the motion signals will be weaker with the axial stripes, causing the flight speed to be generally higher and more variable. This is indeed what occurs (Fig. 6C).

Our findings suggest that the visual pathways that control flight speed are capable of measuring and regulating the angular velocity of the images of the walls in the eye, largely independently of the spatial texture and contrast of the environment. A similar phenomenon has been observed in

Table 2. *Estimates of image angular velocity under different stimulus conditions*

Experimental condition	Tunnel width (cm)	Pattern velocity (cm s⁻¹)	Mean flight speed (cm s⁻¹)	Estimated image angular velocity (deg. s⁻¹)
Stationary patterns (data averaged over all spatial frequencies and contrasts except 0% contrast)	22	0	45.8	239
Stationary patterns (random dots, data from the static region in Experiment 3)	22	0	54.5	284
Stationary pattern (random dots, data from Experiment 1)	32	0	74.3	265
Stationary pattern (random dots, data averaged from both conditions in Experiment 2)	32	0	60.1	215
Stationary pattern (random dots, data averaged from both conditions in Experiment 3)	32	0	70.0	251
Moving pattern (random dots, data from Experiment 1)	32	7 (against flight direction)	74.0	287
Moving pattern (random dots, data from Experiment 1)	32	30 (against flight direction)	59.7	320
Moving pattern (random dots, data from Experiment 1)	32	37 (in flight direction)	101.9	227
Moving pattern (random dots, data from Experiment 1)	32	52 (in flight direction)	123.4	254

experiments investigating odometry in honeybees. The perception of distance flown depends upon the extent of image motion that is experienced by the eye but is largely independent of the contrast or the spatial texture of the image (Esch and Burns, 1995; Hrncir et al., 2003; Si et al., 2003; Srinivasan et al., 1996; Srinivasan and Zhang, 1997, 2000). Robust perception of image motion has also been observed in the context of another visually mediated response – the centring behaviour. Bees flying through a narrow gap or tunnel maintain equidistance between the walls by adjusting their lateral position so as to balance the image velocities in the two eyes (Srinivasan et al., 1991). Bees continue to fly down the middle of the tunnel even when the spatial frequencies or contrasts of the gratings on the two walls are substantially different. This indicates that the visual pathway that mediates this behaviour is again measuring image speed largely independently of the contrast or spatial texture of the environment.

The movement-detecting mechanisms underlying the three behaviours discussed above seem to have properties that are rather different from those of the well-studied optomotor behaviour in insects. The optomotor response is a behaviour in which a flying insect compensates for unwanted body rotations by detecting the resulting rotations of the image in the eye and generates motions to compensate for these deviations (Reichardt, 1969). The optomotor response appears to be characterised by a movement-detecting system that is sensitive to changes in the contrast, spatial frequency and temporal frequency of the moving image. This system appears not to encode image angular velocity in a manner that is robust to variations in these parameters. Therefore, it is very likely that the visual pathways that control flight speed, mediate the centring response and generate the odometric signal have properties that are different from the pathway that drives the optomotor response. There is an extensive literature on the anatomy and physiology of movement-detecting neurons in the insect brain, whose response properties mirror the characteristics of the optomotor response (reviewed by Egelhaaf et al., 1988; Hausen, 1993). On the other hand, there is relatively little evidence, so far, for the existence of motion-sensitive neurons with response properties that meet the requirements for centring behaviour, odometry and flight speed control. These latter behaviours require movement-detecting mechanisms that measure image angular velocity relatively independently of contrast and spatial texture. Future work should identify the neural pathways that subserve flight speed control, odometry and centring behaviour and investigate the response properties of neurons in these pathways. Some reports of velocity-tuned neurons are now beginning to appear (Dror et al., 2001; Ibbotson, 2001), although it remains to be ascertained whether these neurons indeed participate in the behaviours discussed above.

The present study has demonstrated that flight speed is regulated by holding constant the image angular velocity in the eye. What is the level of image angular velocity that is maintained? Table 2 shows estimates of the image angular velocities that the bees were maintaining under four different stimulus conditions. These angular velocities were calculated as described in Materials and methods. It is evident from the table that, despite the widely varying stimulus conditions, the bees were adjusting their flight speeds to hold the image angular velocity in the range of 215–320 deg. s⁻¹ (the spread of angular velocities shown in this table is somewhat larger than in Table 1 because the data in Table 2 span a wider range of stimulus patterns and experimental conditions). Another way of evaluating the efficacy of this regulatory process would be to examine the extent to which the image angular velocities would vary between different stimulus conditions if the bees did not react to the various conditions and instead maintained a constant flight speed. For example, with a stationary random dot pattern, the average flight speed in the 32 cm tunnel is 68.3 cm s⁻¹ (average of flight speeds in rows 3–5 of Table 2), generating an average image angular velocity of 245 deg. s⁻¹ (average of image angular velocities in rows 3–5 of Table 2).

If the bees were to maintain the same flight speed when the pattern was moving at 52 cm s^{-1} in the same direction, they would experience an image angular velocity of only 58 deg. s^{-1}, a value substantially lower than that actually experienced (254 deg. s^{-1}; row 8, Table 2). Thus, the bees are compensating fully for the motion of the pattern by adjusting their flight speed by an amount that is appropriate to restore the image velocity to a level very close to its original value.

What are the consequences of maintaining a constant image angular velocity during flight? One outcome would be that flight speed would tend to be high when travelling in open fields, for example, and low during flight through densely cluttered vegetation. Thus, maintaining a constant image angular velocity in the eye would ensure that the speed of flight is automatically adjusted to a level that is safe and appropriate to the environment. The relative insensitivity of the underlying movement-detecting mechanism to the contrast or the spatial texture of the environment would enhance the robustness of this control system, ensuring that flight speed depends primarily on the distances to nearby surfaces and not on their visual properties such as contrast or visual texture.

A recent study found that bumblebees flying outdoors in natural environments cruise at an average speed of 7.1 m s^{-1}, at a height of ~2 m above the ground (Riley et al., 1999), thus maintaining an image angular velocity of ~200 deg. s^{-1}. This figure is very similar to that maintained by bees during flight in our experimental tunnels. Thus, although flight is fast in open environments, the image velocities experienced are very similar to those experienced in the confined environment of the tunnel, because flight in the tunnel is correspondingly slower.

Maintaining a constant image angular velocity would also simplify certain difficult manoeuvres, such as making a grazing landing on a flat surface. If a bee holds the image velocity of a surface constant whilst approaching it, flight speed would automatically be reduced as the surface is neared, thus ensuring a smooth touchdown. Indeed, bees performing grazing landings do hold the image velocity of the surface constant. This image angular velocity is in the range of 250–750 deg. s^{-1}, with the value varying depending upon the particular bee or the particular landing trajectory (Srinivasan et al., 2000). Interestingly, these image velocities are comparable with those maintained by bees whilst cruising through tunnels (see Table 2). Thus, the visual mechanisms that regulate cruising flight speed may be similar, or identical, to those used to control grazing landings.

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