

RESEARCH ARTICLE

Bumblebees measure optic flow for position and speed control flexibly within the frontal visual field

Nellie Linander*, Marie Dacke and Emily Baird

ABSTRACT

When flying through narrow spaces, insects control their position by balancing the magnitude of apparent image motion (optic flow) experienced in each eye and their speed by holding this value about a desired set point. Previously, it has been shown that when bumblebees encounter sudden changes in the proximity to nearby surfaces – as indicated by a change in the magnitude of optic flow on each side of the visual field – they adjust their flight speed well before the change, suggesting that they measure optic flow for speed control at low visual angles in the frontal visual field. Here, we investigated the effect that sudden changes in the magnitude of translational optic flow have on both position and speed control in bumblebees if these changes are asymmetrical; that is, if they occur only on one side of the visual field. Our results reveal that the visual region over which bumblebees respond to optic flow cues for flight control is not dictated by a set viewing angle. Instead, bumblebees appear to use the maximum magnitude of translational optic flow experienced in the frontal visual field. This strategy ensures that bumblebees use the translational optic flow generated by the nearest obstacles – that is, those with which they have the highest risk of colliding – to control flight.

KEY WORDS: *Bombus terrestris*, Flight control, Image motion, Centring, Flight speed, Viewing angle

INTRODUCTION

When an animal moves through the environment, the image of the world moves across its retina, creating a pattern of apparent image motion known as optic flow (Gibson, 1950, 1979). During forward motion, translational optic flow (the term ‘translational optic flow’ in this context refers to image motion along the animal’s longitudinal axis) varies inversely with the distance to nearby surfaces so that closer objects appear to move faster than those that are further away. Thus, translational optic flow provides important information about an animal’s self-motion and the spatial layout of the environment (Collett, 2002; Koenderink, 1986; Lappe, 2000). Flying insects use this information to control various aspects of their flight. When flying through narrow spaces, honeybees and bumblebees use translational optic flow to control their position so as to maintain an equal distance to the nearby surfaces – a behaviour known as centring. It has been proposed that this centring behaviour is achieved by balancing the magnitude of the lateral optic flow experienced in each eye (Dyhr and Higgins, 2010; Kirchner and Srinivasan, 1989; Srinivasan et al., 1991, 1996) or by maintaining the magnitude of unilateral optic flow about a set point (Serres et al., 2008a,b). Translational optic flow cues are also used by honeybees (Portelli et al., 2010; Baird et al., 2005; Barron and

Srinivasan, 2006; Srinivasan et al., 1996), bumblebees (Baird et al., 2010) and *Drosophila* (David, 1982; Fry et al., 2009) to control their ground speed. By holding the magnitude of translational optic flow about a set point, these insects ensure that their speed automatically decreases as the distance to nearby obstacles (and therefore the risk of collision) decreases. Thus, by utilising information contained in the translational optic flow field, insects have developed computationally simple strategies for solving the rather complex problem of controlling flight and avoiding collisions with nearby obstacles.

Although we now understand quite a lot about how insects use translational optic flow cues for position and speed control, one thing that remains unclear is how they use this information to detect and respond to changes in the proximity of the environment, such as those which might occur when flying from a cluttered forest into an open field. The key lies in understanding where in the visual field translational optic flow for flight control is being measured. For an insect that is flying at a constant forward speed, the magnitude of translational optic flow is not constant over the entire visual field. Instead, it is greatest at an angle of 90 deg from the direction of motion and decreases to a value of zero in the direction of flight (in pure forward translation, this would be aligned with the midline of the insect and its visual field) (Gibson, 1950). This means that the relative difference in the magnitude of translational optic flow experienced by an insect approaching a change in the proximity of the environment would be larger, and presumably easier to detect, in more lateral regions of the visual field (assuming that the eyes are aligned with the direction of flight). However, the larger the angle at which these changes are detected, the less time an insect will have to adjust its flight before encountering the change. Thus, the viewing angle at which optic flow is measured has important consequences for flight in densely cluttered environments, where timely control of position and speed are necessary for effective collision avoidance.

In one of the first attempts to identify where in the visual field optic flow is measured for flight control, Srinivasan et al. (1991) investigated how honeybees adjust their position in response to a black bar presented in an otherwise featureless flight tunnel. The bees deflected away from the bar only once they had flown past it, suggesting that they were measuring optic flow for position control in the lateral region of the visual field. This was consistent with the findings of an earlier study which showed that, to locate a frontally positioned target, honeybees use image motion generated by landmarks in the lateral visual field (Lehrer, 1990). In contrast to these earlier findings, however, more recent studies on honeybees (Portelli et al., 2010), blowflies (Kern et al., 2012) and bumblebees (Baird et al., 2010) suggest that these insects also respond to changes in optic flow that occur in the more frontal region of the visual field. In the study by Baird et al. (2010), aimed at defining the minimum viewing angle at which bumblebees measure translational optic flow for ground speed control, the

Lund Vision Group, Department of Biology, Lund University, Lund SE-22362, Sweden.

*Author for correspondence (nellie.linander@biol.lu.se)

changes in translational optic flow were laterally symmetric, meaning that the same change occurred in both the left and the right visual fields at the same time. What remains unclear, however, is how position and speed control are affected when bumblebees experience a sudden change in optic flow that occurs on only one side of the visual field; that is, when it becomes asymmetric. Are bumblebees also able to detect and respond to these unilateral changes in the proximity of the environment at low viewing angles? If so, how and when do they react to these changes? Here, we aimed to answer these questions by presenting bumblebees (*Bombus terrestris* L.) with abrupt unilateral changes in translational optic flow – generated by flight past stationary patterns whose visual properties change abruptly – and record the effect on position and flight speed.

RESULTS

The effect of asymmetric translational optic flow cues on flight control

The centring response

When both walls of a 3 m long flight tunnel (Fig. 1; see Materials and methods) were lined with checkerboard patterns (S_{checks}), providing laterally symmetric translational optic flow cues (indicated by the letter S in the abbreviation S_{checks}), the flight paths in both a 30 cm wide and a 15 cm wide tunnel were relatively straight (Fig. 2A). The low values for the mean lateral distance from the midline in both the 30 cm wide tunnel, 0.04 ± 1.18 cm (mean \pm s.d.), and the 15 cm wide tunnel, 0.14 ± 0.21 cm, indicate that the bumblebees centred accurately between the two walls (Fig. 2B). When the lateral translational optic flow in the tunnel was asymmetric (indicated by the letter A in abbreviations below), with horizontal stripes (providing only weak translational optic flow cues) on one wall and checks (providing strong translational optic flow cues) on the other, the trajectories were still relatively straight but they were shifted towards the striped wall ($P < 0.001$; for details of this and all statistical analyses shown below, see Table 1), with mean lateral positions of 10.42 ± 0.47 and 4.19 ± 0.38 cm from the midline in the 30 and 15 cm wide tunnels, respectively (Fig. 2B). When both walls were lined with stripes (S_{stripes}), providing laterally symmetric translational optic flow cues (indicated by the letter S in the abbreviation S_{stripes}), the flight trajectories were more widely distributed across the tunnel (-4.49 ± 2.38 and -0.56 ± 0.57 cm from the midline in the 30 and 15 cm wide tunnels, respectively). Some bees even flew from wall to wall, suggesting that they were no longer able to control their position (Fig. 2A).

Flight speed

When the translational optic flow cues were laterally symmetric, i.e. with checks on both walls (S_{checks}), flight speed was significantly slower (30 cm wide tunnel: 68.3 ± 13.0 cm s^{-1} ; 15 cm wide tunnel: 49.6 ± 11.3 cm s^{-1}) than when these cues were asymmetric, with checks on one wall and stripes on the other (A) (30 cm wide tunnel: 82.4 ± 15.4 cm s^{-1} , $P < 0.001$; 15 cm wide tunnel: 60.6 ± 15.4 cm s^{-1} , $P = 0.004$) (Fig. 3). However, flight speed in the asymmetric condition was still significantly slower than when both walls of the tunnel were lined with horizontal stripes (S_{stripes}) (30 cm wide tunnel: 122.4 ± 27.0 cm s^{-1} , $P < 0.001$; 15 cm wide tunnel: 150.4 ± 40.2 cm s^{-1} , $P < 0.001$) (Fig. 3). In addition, the bees flew significantly faster in the 30 cm wide tunnel than in the 15 cm wide tunnel in both the asymmetric (A) and the symmetric condition with checkerboard pattern on both walls (S_{checks}) ($P < 0.001$) (Fig. 3), but the opposite relationship was found when both walls were lined with the stripe pattern (S_{stripes}) ($P = 0.03$) (Fig. 3).

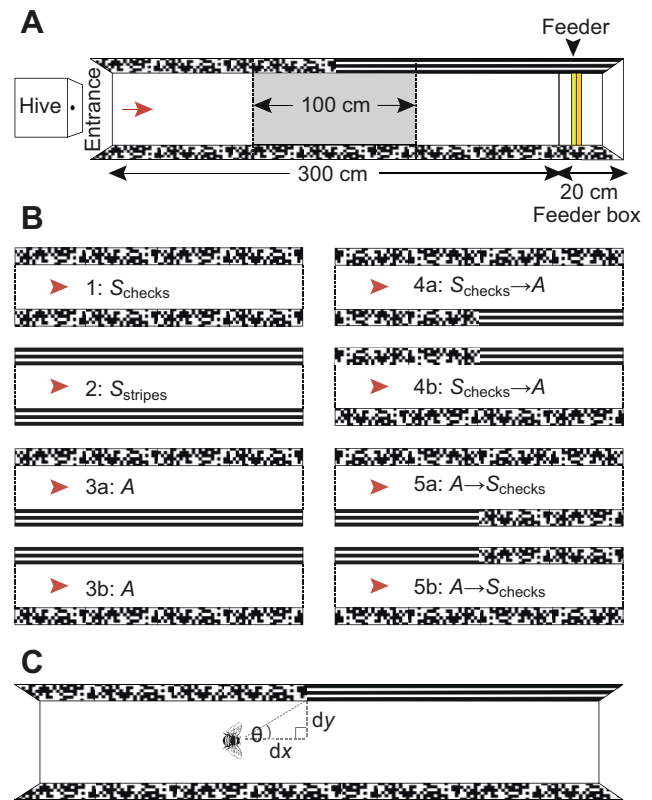


Fig. 1. Experimental set-up. (A) Schematic diagram of a flight tunnel (300 cm long \times 30 cm wide \times 30 cm high). The bumblebee hive was placed at one end of the flight tunnel (bees could enter anywhere along the opening of the tunnel) and a two-compartment feeder (marked in yellow), covering the whole width of the tunnel, was placed in a recess at the far end of the tunnel. A high-speed camera recorded trajectories of bees flying over the central (100 cm) section of the tunnel, indicated by the grey area. (B) Pattern combinations used in each experimental condition. In two of the experimental conditions, the same type of pattern was displayed on each wall, generating symmetric translational optic flow cues in the lateral visual field of bees flying along the midline of the tunnel: (1) randomised checkerboard on both walls (S_{checks}) or (2) horizontal stripes on both walls (S_{stripes}). In the next two conditions, one wall displayed the checkerboard pattern, while the other wall displayed the horizontal stripe pattern: (3a) left wall: checks, right wall: stripes; (3b) vice versa. This presented the bees with asymmetric lateral optic flow cues (A). In the next four conditions, one wall displayed the checkerboard pattern along the length of the tunnel, while the pattern on the other wall changed abruptly half-way along the tunnel: (4a) left wall: checks, right wall: checks then stripes; (4b) vice versa; (5a) left wall: checks, right wall: stripes then checks; (5b) vice versa. The red arrows indicate the direction of flight. (C) Illustration of the calculation of the viewing angle (θ) occupied by the unilateral pattern change when the bees adjusted their flight speed and position in response to the abrupt change in the magnitude of optic flow. Condition $A \rightarrow S_{\text{checks}}$ is shown in the example.

The effect of abrupt unilateral changes in translational optic flow

In this experiment, one wall of the tunnel displayed a checkerboard pattern while the pattern on the other wall changed from checks to stripes (creating an abrupt change from symmetric to asymmetric optic flow cues, $S_{\text{checks}} \rightarrow A$), or vice versa (creating an abrupt change from asymmetric to symmetric optic flow cues, $A \rightarrow S_{\text{checks}}$) halfway along the tunnel (see Fig. 1B). For clarity, the experimental conditions have been abbreviated according to the combination of patterns, with S_{checks} representing checkerboard pattern on both walls, S_{stripes} representing axial stripes on both walls and A representing an asymmetric pattern combination with checks on one wall and axial stripes on the other. The first character in the abbreviation represents the pattern

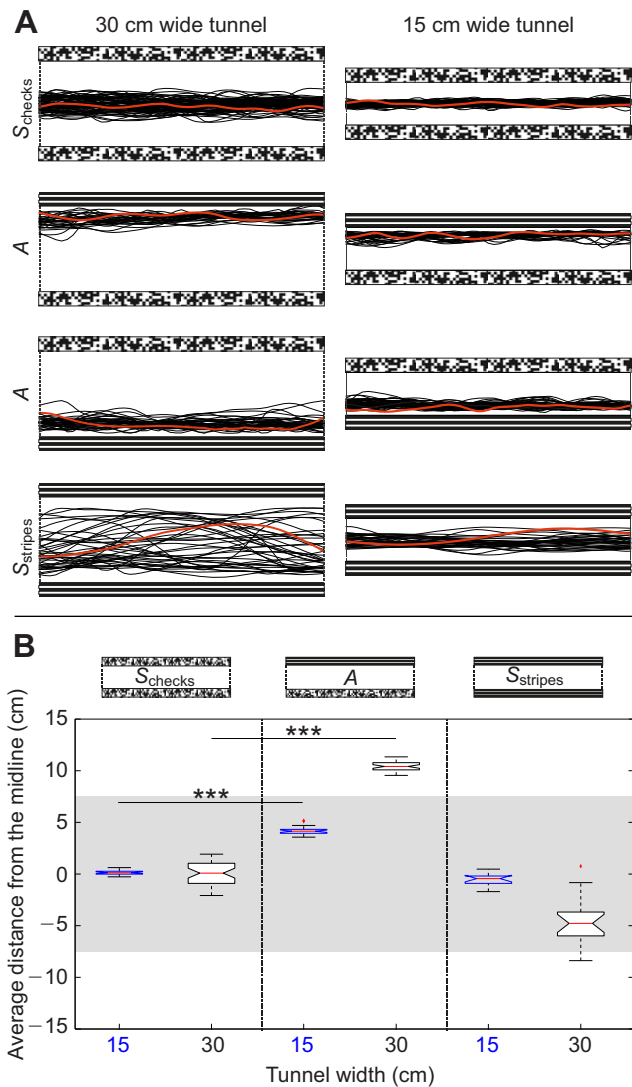


Fig. 2. Effect of asymmetric translational optic flow cues on the centring response. (A) Raw flight trajectories for the conditions S_{checks} , A and $S_{stripes}$. A typical flight trajectory for each condition is highlighted in red. (B) The average lateral position of bees flying in a 15 cm (boxes outlined in blue) or 30 cm (boxes outlined in black) wide tunnel, lined with checks on both walls (S_{checks}), stripes on one wall and checks on the other (A), or stripes on both walls ($S_{stripes}$). Grey shading indicates the width of the 15 cm wide tunnel. Boxes indicate the distance between the lower and upper quartile values, red lines indicate the median values and whiskers indicate the entire spread of the data. Asterisks indicate the significance level: *** $P < 0.001$.

combination in the first half of the tunnel (with respect to a bee flying towards the feeder) and the second character represents the pattern combination in the second half. Thus, the experimental condition $S_{checks} \rightarrow A$ indicates that the first half of the tunnel is lined with checks on both walls and the second half of the tunnel is lined with checks on one wall and axial stripes on the other wall. The experimental condition $A \rightarrow S_{checks}$ indicates that the first half of the tunnel is lined with checks on one wall and axial stripes on the other and the second half of the tunnel is lined with checks on both walls.

Condition A refers to the control condition that generates an asymmetric optic flow field (checks on one wall and axial stripes on the other) along the full length of the tunnel. Condition S_{checks} refers to the control condition S generating a symmetric optic flow field (checks on both walls) along the full length of the tunnel.

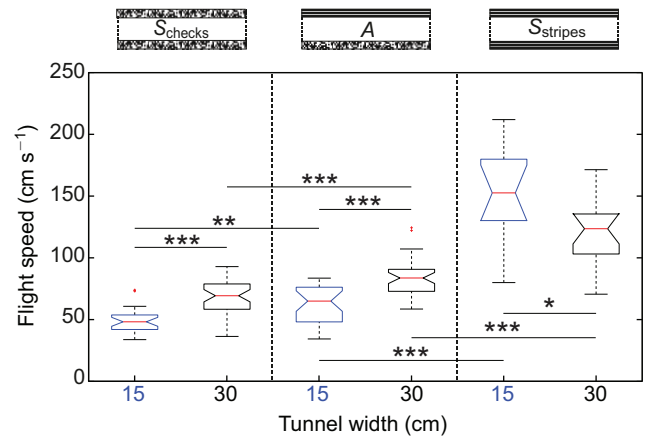


Fig. 3. Effect of asymmetric translational optic flow cues on speed control. Average flight speed of bees flying in a 15 cm (boxes outlined in blue) or 30 cm (boxes outlined in black) wide tunnel, lined with checks on both walls (S_{checks}), stripes on one wall and checks on the other (A), or stripes on both walls ($S_{stripes}$). Boxes indicate the distance between the lower and upper quartile values, red lines indicate the median values and whiskers indicate the entire spread of the data. Asterisks indicate the level of significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Lateral position

When the bees experienced an abrupt change from asymmetric to symmetric optic flow cues ($A \rightarrow S_{checks}$), they responded by shifting their flight trajectories from a position that was close to the striped wall (and not significantly different from the average lateral position in the control condition, A) to a position that was close to the tunnel's midline (Fig. 4A). In the 30 cm wide tunnel, this response occurred at an average lateral distance of 6 cm from the striped wall and at a longitudinal distance of 12 cm before the pattern change ($P = 0.02$; Fig. 4B). At this position, the pattern change was located at a viewing angle of 27 deg lateral to the midline of the bee (see Fig. 1C for details of this calculation). In the 15 cm wide tunnel, the bees adjusted their lateral position at a longitudinal distance of 16 cm and a lateral distance of 4 cm from the pattern change ($P = 0.04$; Fig. 4C), when it was located at a viewing angle of approximately 14 deg.

When the pattern on one wall instead changed from symmetric to asymmetric optic flow cues ($S_{checks} \rightarrow A$), the position of the bees in the 30 cm wide tunnel did not differ significantly from the control condition (S_{checks}) until they reached a longitudinal distance of 4 cm before the pattern change and an average lateral distance of 15 cm from the wall ($P = 0.02$; Fig. 4D). At this position, the pattern change was located at a viewing angle of approximately 75 deg. In the 15 cm wide tunnel, the change in lateral position did not occur until the bees reached a longitudinal distance of 2 cm after the pattern change at a lateral distance of 7 cm from the wall ($P = 0.002$; Fig. 4E). This corresponds to a viewing angle of 106 deg.

Together, these results suggest that, when bumblebees are presented with an abrupt unilateral change from axial stripes to checks, they adjust their lateral position well before they reach the change itself or, more specifically, when the change occurs at low frontal viewing angles (approximately 14–27 deg). In contrast, when the unilateral change is from checks to axial stripes, bumblebees do not adjust their lateral position until the change occupies much larger, more lateral viewing angles (approximately 75–106 deg).

Flight speed

When the translational optic flow cues changed from asymmetric to symmetric ($A \rightarrow S_{checks}$), the bees decreased their flight speed with

Table 1. Details of statistical analysis

Tunnel width	$S_{\text{checks}} \rightarrow A$ vs S_{checks}		$A \rightarrow S_{\text{checks}}$ vs A		S_{checks} vs A		A vs S_{stripes}	15 cm vs 30 cm speed	
	Position	Speed	Position	Speed	Position	Speed	Speed	S_{checks}	S_{stripes}
15 cm	Z=3.04 N=49 P=0.002	Z=2.53 N=49 P=0.011	Z=2.05 N=64 P=0.040	Z=2.27 N=64 P=0.023	Z=-6.29 N=54 P<0.001	Z=-2.85 N=54 P=0.004	Z=4.86 N=40 P<0.001	Z=-5.0 N=67 P<0.001	Z=2.15 N=29 P=0.032
30 cm	Z=-2.32 N=82 P=0.020	Z=-2.17 N=82 P=0.030	Z=-2.39 N=70 P=0.017	Z=-2.03 N=70 P=0.042	Z=7.69 N=80 P<0.001	Z=4.35 N=80 P<0.001	Z=4.44 N=56 P<0.001	Z=-4.81 N=67 P<0.001	A

Wilcoxon rank sum tests at a 5% significance level were used for all statistical analyses. N is the total number of flights included in the analysis. For illustrations of the different experimental conditions (S_{checks} , A , S_{stripes} , $S_{\text{checks}} \rightarrow A$, $A \rightarrow S_{\text{checks}}$) see Fig. 1B.

respect to the control condition (A) at a longitudinal distance of 18 cm and a lateral distance of 5 cm before the pattern change in the 30 cm wide tunnel ($P=0.04$; Fig. 5A). At this position, the pattern change was located at a viewing angle of approximately 16 deg. In the 15 cm wide tunnel, flight speed decreased significantly from the control condition at a longitudinal distance of 20 cm and a lateral distance of 3 cm before the pattern change ($P=0.02$; Fig. 5B). At this position, the pattern change was located at a viewing angle of approximately 9 deg.

When the translational optic flow cues changed from symmetric to asymmetric ($S_{\text{checks}} \rightarrow A$), the bees increased their flight speed significantly in response to the sudden decrease in optic flow cues. In the 30 cm wide tunnel, this increase did not occur until the bees reached a longitudinal distance of 22 cm after the pattern change, at a lateral distance of 9 cm from the wall ($P=0.03$; Fig. 5C). The pattern change at this position occurred at a viewing angle of 157 deg. In the 15 cm wide tunnel, the acceleration did not occur until a longitudinal distance of 14 cm after the pattern change, and at a lateral distance of 5 cm from the wall ($P=0.01$; Fig. 5D), with the pattern change located at a viewing angle of 159 deg.

These results suggest that, when the unilateral optic flow cues change from weak (axial stripes) to strong (checks), condition $A \rightarrow S_{\text{checks}}$, bumblebees decelerate well before passing the change in optic flow. When the pattern instead changes from checks to axial stripes, condition $S_{\text{checks}} \rightarrow A$, they respond by accelerating but not until after they have passed the pattern change.

DISCUSSION

Bumblebees respond to low magnitudes of translational optic flow at low viewing angles

When presented with a unilateral change from axial stripes to checks ($A \rightarrow S_{\text{checks}}$), the bumblebees adjusted both their position and speed when the optic flow cues subtended only a very small region of the frontal visual field. In the most extreme case, bumblebees decreased their flight speed when the pattern change occupied a viewing angle of only 9 deg (in the 15 cm tunnel). The maximum magnitude of translational optic flow that the bees would have experienced at the location of the pattern change in this case would have been approximately 24 deg s^{-1} . Considering that there is inevitably a delay between when the visual system detects a change in optic flow and when a behavioural response is initiated – this delay has been estimated at 100 ms in *Drosophila* (Fry et al., 2009) – it is likely that the bumblebees were reacting to the pattern when it generated even lower magnitudes of optic flow. Can bumblebees really detect and react to such low magnitudes of optic flow at such low viewing angles?

Behavioural evidence that bumblebees can detect and respond to low magnitudes of optic flow was first provided by Baird et al. (2010), who showed that bumblebees changed their flight speed in

response to a bilateral increase in tunnel width from 15 to 30 cm when the view of the wider tunnel subtended a viewing angle of approximately 30 deg. While this is larger than the viewing angle observed in the present study, the magnitude of translational optic flow experienced by bees in this case was approximately 30 deg s^{-1} ; in other words, not far from the value of 24 deg s^{-1} measured in the present study. These findings are also supported by physiological evidence that motion-sensitive neurons in the bumblebee visual system respond to magnitudes of optic flow ranging from 5 deg s^{-1} to above 2000 deg s^{-1} (O'Carroll et al., 1996). Thus, the response threshold recorded in the present study lies well within the range of magnitudes that the visual system of bumblebees can detect, and provides strong behavioural evidence that the motion-sensitive mechanism underlying position and speed control is capable of detecting and responding to very low magnitudes of optic flow at low viewing angles.

Bumblebees can use both unilateral and bilateral optic flow cues for speed control

Bumblebees adjust their trajectories so that they fly further away from the wall that generates higher translational optic flow when presented with asymmetric optic flow cues (checks on one wall and stripes on the other, A). This is consistent with the findings of similar experiments performed on bumblebees (Baird et al., 2011; Dyhr and Higgins, 2010) and honeybees (Kirchner and Srinivasan, 1989; Srinivasan et al., 1996, 1991). In addition, we found that bumblebees fly significantly faster when the translational optic flow is asymmetric than when the optic flow cues are strong in both eyes (checks on both walls, S_{checks}). A possible explanation for this result is that, as the bees increase the distance to the wall that provides strong optic flow cues (the checks), they experience a decrease in the apparent magnitude of optic flow generated by that wall. As bumblebees regulate their ground speed by holding the magnitude of optic flow around a set point (Baird et al., 2010), this change would induce a compensatory increase in flight speed provided optic flow from one visual field offers sufficient information for speed control.

To investigate whether the recorded increase in flight speed represents an attempt to hold the magnitude of translational optic flow constant in one visual field, we can estimate the maximum magnitude of optic flow experienced in the symmetric condition and compare it with that experienced when the translational optic flow cues are asymmetric. When both walls were lined with checkerboard pattern, the bees flew along the midline of the 30 cm wide tunnel at an average forward speed of 68 cm s^{-1} . The maximum magnitude of optic flow experienced by the bees in this case would be approximately 260 deg s^{-1} at a viewing angle of 90 deg. When the translational optic flow was asymmetric, the bees

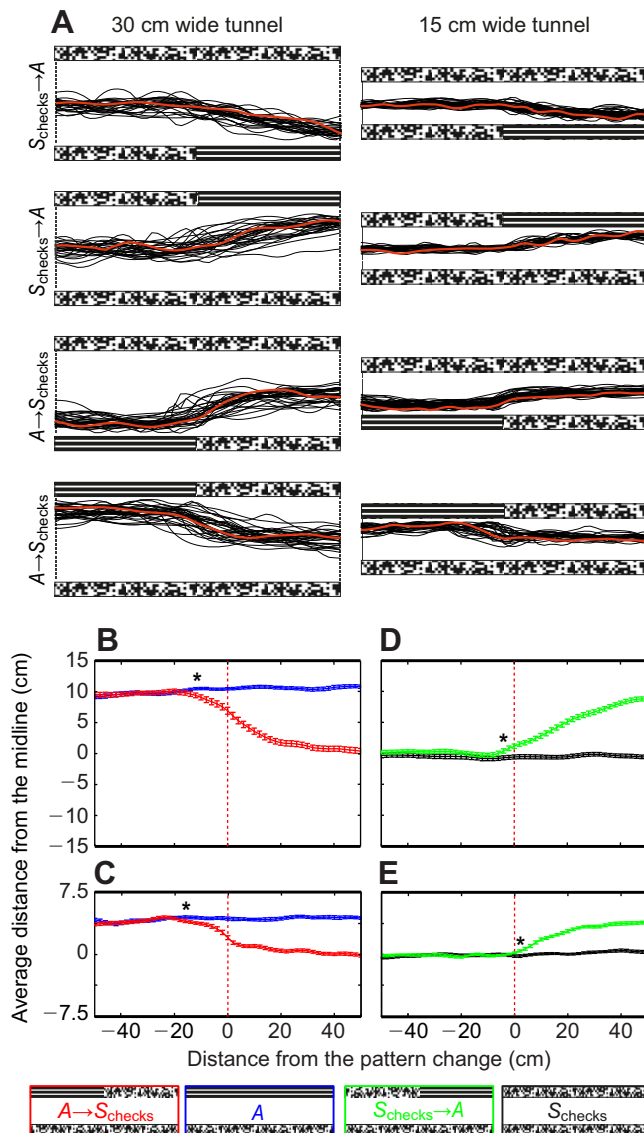


Fig. 4. Effect of a unilateral change in optic flow on lateral position.

(A) Raw flight trajectories for the conditions $S_{\text{checks}} \rightarrow A$ and $A \rightarrow S_{\text{checks}}$. A typical flight trajectory for each condition is highlighted in red. Grey shading indicates the width of the 15 cm wide tunnel. (B–E) Average lateral position of bees flying along a 30 cm wide tunnel (B, D) or a 15 cm wide tunnel (C, E). Red lines represent condition $A \rightarrow S_{\text{checks}}$, blue lines represent control condition A, green lines represents condition $S_{\text{checks}} \rightarrow A$ and black lines represent control condition S_{checks} . The red dotted line illustrates the point in the tunnel where the unilateral pattern change occurred, generating an asymmetric change in the magnitude of translational optic flow. Means are calculated over 2 cm bins, error bars represent s.e.m. Asterisks indicate where the lateral position of the bees in condition $A \rightarrow S_{\text{checks}}$ or $S_{\text{checks}} \rightarrow A$ deviates significantly from the lateral position in the corresponding control condition (A or S_{checks} , respectively).

flew at an average lateral distance of 25 cm from the checkerboard wall and increased their flight speed to 82 cm s^{-1} . In this case, the maximum magnitude of translational optic flow (which occurs at a 90° viewing angle) would be approximately 188 deg s^{-1} . Given that the difference in the magnitude of optic flow between these two conditions would only decrease with viewing angle – that is, the lower the viewing angle at which translational optic flow is measured, the closer these values become (at a viewing angle of 30° , for example, the values would be 130 and 94 deg s^{-1}) – and that

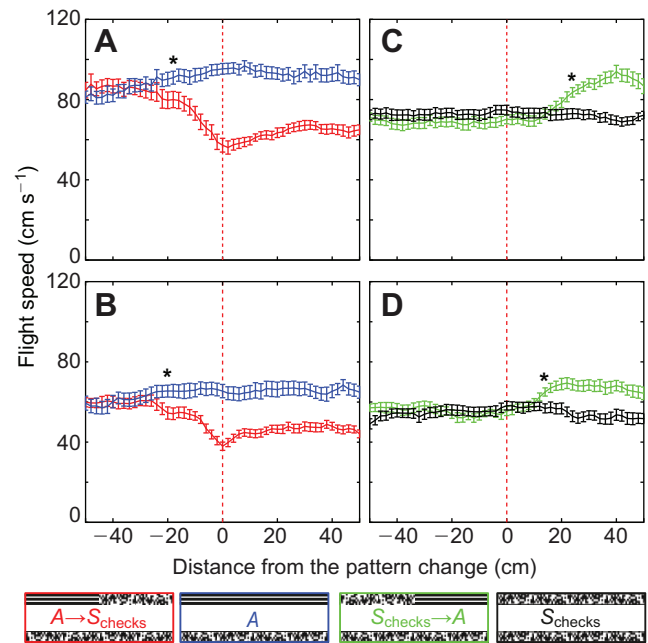


Fig. 5. Effect of a unilateral change in optic flow on flight speed. Average flight speed of bees flying along a 30 cm wide tunnel (A, C) or a 15 cm wide tunnel (B, D). Red lines represent condition $A \rightarrow S_{\text{checks}}$, blue lines represent control condition A, green lines represents condition $S_{\text{checks}} \rightarrow A$ and black lines represent control condition S_{checks} . The red dotted line illustrates the point in the tunnel where the unilateral pattern change occurred, generating an asymmetric change in the magnitude of translational optic flow. Means are calculated over 2 cm bins, error bars represent s.e.m. Asterisks indicate where the flight speed in condition $A \rightarrow S_{\text{checks}}$ or $S_{\text{checks}} \rightarrow A$ deviates significantly from the flight speed in the corresponding control condition (A or S_{checks} , respectively).

the bees are more likely to measure optic flow for speed control at frontal rather than lateral viewing angles (discussed below), it is plausible that the increase in flight speed that we observed in the asymmetric condition is due to the increased distance that the bees are flying from the wall with the checkerboard pattern. This suggests that the bees are able to regulate their flight speed even when optic flow cues are absent from one side of the visual field.

Is this result consistent with the optic flow regulator model proposed by Serres et al. (2008a), which is currently the only comprehensive model for how translational optic flow cues may be used for speed control in flying insects? In this model, flight speed is controlled by maintaining the sum of the optic flow in the dorsal/ventral or lateral visual fields at a set point – because the bees in our study changed their flight speed in response to changes in lateral cues, we will assume that these were the dominant cues in this experiment. According to the optic flow regulator model, the sum of the maximum magnitude of the translational optic flow experienced by the bees when flying in the tunnel with checks on both sides should be equal to that experienced in the tunnel with horizontal stripes on one wall. The sum of the maximum magnitude of optic flow in the symmetrical condition is 520 deg s^{-1} (that is, $2 \times 260 \text{ deg s}^{-1}$), while it is only 188 deg s^{-1} in the asymmetrical condition (in this case, only one wall is generating translational optic flow). Thus, speed control in bumblebees does not seem to be regulated by the summation of the magnitude of translational optic flow in the lateral visual fields and is thus not fully explained by the optic flow regulator model. Instead, our results suggest that, when translational optic flow cues are absent from one side of the visual field, bumblebees control flight speed using unilateral translational

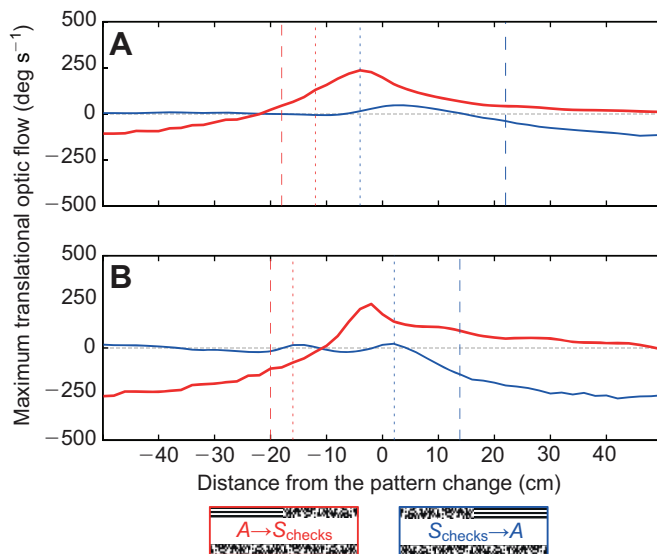


Fig. 6. Comparison of the maximum magnitude of translational optic flow in each visual field. The maximum magnitude of optic flow over 180 deg was calculated for the visual field viewing the pattern change and compared with the visual field viewing the constant pattern for the two conditions $A \rightarrow S_{\text{checks}}$ (solid red lines) or $S_{\text{checks}} \rightarrow A$ (solid blue lines) in both the 30 cm (A) and 15 cm (B) wide tunnels. A value of zero indicates that the maximum magnitude of translational optic flow is equal in the two visual fields, a negative value indicates that it is lower in the visual field viewing the pattern change and vice versa. Note that the calculation for the visual field viewing the pattern change was made only for the region occupied by the checked pattern as the stripe pattern generated no translational optic flow cues. Vertical lines indicate the longitudinal distance from the pattern change at which a change in speed (dashed lines) or position (dotted lines) was recorded for each condition (red lines indicate the data for the $A \rightarrow S_{\text{checks}}$ condition and blue lines for the $S_{\text{checks}} \rightarrow A$ condition).

optic flow cues – this is similar to honeybees, which can use unilateral optic flow cues for calculating the distance flown to a food source (Srinivasan et al., 1998). However, as soon as translational optic flow cues are detected in the other eye, information from both sides of the visual field is used for speed control.

Translational optic flow for speed control is measured flexibly in the visual field

Unilateral changes from axial stripes to checks ($A \rightarrow S_{\text{checks}}$) caused a reduction in flight speed when they occupied low visual angles (16 and 9 deg in the 30 and 15 cm wide tunnels, respectively). When the unilateral translational optic flow cues changed from checks to stripes ($S_{\text{checks}} \rightarrow A$), however, flight speed was adjusted only once the bees had already moved closer to the axial stripes. At this point, the checkerboard pattern on the wall with the pattern change is present only at very large viewing angles (157 deg in the 30 cm tunnel and 159 deg in the 15 cm tunnel). Thus, in the $A \rightarrow S_{\text{checks}}$ condition, the bees were changing their flight speed when the translational optic flow cues occupied a very narrow region of the frontal visual field, whereas in the $S_{\text{checks}} \rightarrow A$ condition, flight speed was reduced only once the checkerboard pattern had passed 90 deg. Given the large difference in the visual angle of the pattern at the time that the bees initiated a significant change in flight speed, our results demonstrate that bumblebees are not measuring optic flow for speed control at one set viewing angle. Instead, this angle appears to be modified depending on where in the visual field translational optic flow is experienced (this hypothesis is discussed in more detail below).

Translational optic flow for position control is measured flexibly in the visual field

One model that has been proposed to explain how optic flow may be used to control lateral position in insects is the optic flow regulator model (Serres et al., 2008a). This model proposes that position is controlled by a unilateral optic flow regulator that strives to maintain the maximum magnitude of optic flow experienced in a lateral visual field about a set point. We can explore whether this model explains our data by comparing the maximum magnitude of optic flow experienced in each lateral visual field as the bees fly through the tunnel. This can be done by calculating the maximum magnitude of translational optic flow at 1 deg intervals over each 180 deg visual field and then calculating the difference between these values (Fig. 6). If this relative difference in the maximum magnitude of translational optic flow experienced in each visual field is zero, then the maximum magnitude of translational optic flow is equal in the two eyes; negative values indicate that it is lower in the visual field viewing the pattern change and vice versa. A prediction of the optic flow regulator model is that, when the bees adjust their position in response to the pattern change, the maximum magnitude of translational optic flow in the lateral visual field viewing the pattern change will be larger than in the lateral visual field viewing the opposite wall – in other words, if the magnitude of the optic flow on the wall with the constant check pattern was always larger than on the wall with the pattern change, the bees would not need to initiate a change in position. In the case where the pattern on one wall changes from stripes to checks ($A \rightarrow S_{\text{checks}}$), the bees adjust their position when the maximum optic flow experienced in the lateral visual field viewing the pattern change is 130 deg s^{-1} higher than the maximum optic flow generated by the opposite wall in the 30 cm wide tunnel but 88 deg s^{-1} lower than the maximum optic flow generated by the opposite wall in the 15 cm wide tunnel (red dotted lines, Fig. 6). In the $S_{\text{checks}} \rightarrow A$ condition, position is adjusted when the maximum optic flow on the wall with the change is 14 and 161 deg s^{-1} higher (in the 30 and 15 cm wide tunnels, respectively) than on the opposite wall (blue dotted lines, Fig. 6). Thus, in two of the four conditions, the maximum optic flow in the lateral visual field viewing the wall with the pattern change is less than, or very close to the optic flow experienced in the visual field viewing the opposite wall (it is also important to bear in mind that the bees would have already detected the change in optic flow when we observe a behavioural response, so that the magnitude of the optic flow when the bees detect the change would be lower). Overall, our results suggest that bumblebees do not regulate their position using only one measure of the highest magnitude of optic flow experienced in the visual field, indicating that the optic flow regulator model does not fully explain position control in bumblebees.

A second model that describes how position might be regulated using translational optic flow cues is the optic flow balancing model (Srinivasan et al., 1991). This model suggests that position is controlled by balancing the magnitude of translational optic flow in the lateral part of each visual field. The first prediction of this model, namely that position is controlled by balancing the magnitude of optic flow experienced in each eye, can be explored by comparing the maximum magnitude of optic flow experienced in each lateral visual field as the bees fly through the tunnel (Fig. 6). Our analysis shows that, when one side of the visual field experienced a large increase in the magnitude of translational optic flow, bumblebees adjusted their speed and position so as to equalise the maximum magnitude of optic flow experienced in each eye (red lines, Fig. 6). At the point when a behavioural response was observed, the

maximum magnitude of translational optic flow in the visual field viewing the pattern change was located at the viewing angle occupied by the pattern change itself (speed: 9 and 16 deg; position: 14 and 27 deg; 15 and 30 cm wide tunnels, respectively). When there was a large decrease in the magnitude of translational optic flow, bumblebees again adjusted their speed and position so as to equalise the maximum magnitude of optic flow experienced in each eye (blue lines, Fig. 6). Once again, at the point when the behavioural responses occurred, the maximum magnitude of translational optic flow in the visual field viewing the pattern change occurred at the viewing angle occupied by the pattern change (speed: 159 and 157 deg; position: 106 and 75 deg; 15 and 30 cm wide tunnels, respectively) in all but one case. In this case, position in the 15 cm wide tunnel was adjusted when the pattern change subtended a viewing angle of 75 deg (the maximum magnitude of optic flow in this case would have been experienced at 90 deg). Overall, our results indicate that, when bumblebees experience a sudden increase or decrease in the magnitude of translational optic flow, they change both their position and speed so as to equalise this value in each eye.

It is interesting to note that we observed a difference in the timing of the speed and position changes depending on whether the magnitude of translational optic flow increased or decreased. When the magnitude of translational optic flow increased, speed and position were adjusted when the pattern change subtended similar viewing angles (speed: 9 and 16 deg; position: 14 and 27 deg; 15 and 30 cm wide tunnels, respectively). However, when the magnitude of optic flow decreased abruptly, bumblebees adjusted their position before they adjusted their speed. One major difference between the speed response to the abrupt increase and decrease in translational optic flow is that in the former case, the bees responded by slowing down, whereas in the latter case, they responded by speeding up. It is likely that the bees take longer to speed up than to slow down, which would mean that a significant difference between the test and control condition would not be observed until some time after the bees initiated a response. It is therefore possible that speed and position are indeed being adjusted at the same time in this condition (as they are when there is an abrupt increase in the translational optic flow cues).

The second prediction of the optic flow balancing model is that optic flow is being balanced in the lateral visual field of each eye. We can explore whether this is the case for bumblebees by investigating when they reacted to the pattern changes. When bumblebees experienced a sudden loss of translational optic flow on one side ($S_{\text{checks}} \rightarrow A$), they did not modify their lateral position until the axial stripes subtended a relatively large viewing angle (75 deg in the 30 cm wide tunnel and 106 deg in the 15 cm wide tunnel), which is consistent with the prediction of the optic flow balancing model. However, when the bumblebees experienced a change from asymmetric to symmetric optic flow cues ($A \rightarrow S_{\text{checks}}$), they modified their lateral position when the translational optic flow cues subtended very small viewing angles (27 deg in the 30 cm tunnel and 14 deg in the 15 cm tunnel). Thus, they do not appear to be measuring translational optic flow cues for position control at lateral viewing angles or even at one set angular position in each visual field. Instead of balancing the magnitude of optic flow at a large fixed viewing angle in each visual field to control position (as the optic flow balancing model suggests), bumblebees appear to be balancing the maximum magnitude of optic flow experienced in the frontal part of each visual field flexibly; that is, irrespective of whether this value occurs at the same or different visual angles in each visual field.

Table 2. Sample size for each condition

Condition		No. of individuals (15 cm/30 cm tunnel)	No. of flights (15 cm/30 cm tunnel)
No.	Description		
1	S_{checks}	26/41	66/75
2	S_{stripes}	12/17	34/41
3a;b	A	14/23; 14/16	48/38; 30/30
4a;b	$S_{\text{checks}} \rightarrow A$	12/21; 11/20	42/27; 25/30
5a;b	$A \rightarrow S_{\text{checks}}$	21/13; 15/18	41/29; 27/32

See Fig. 1B for illustration and description of the pattern combinations used for all conditions.

Bumblebees respond primarily to translational optic flow information generated by the nearest obstacles

Overall, the findings of this study indicate that the visual angle at which optic flow cues are being used for position and speed control varies depending on where in the visual field the highest magnitudes of translational optic flow are experienced. One potential explanation for these results is that bumblebees are selecting the translational optic flow used for flight control from one or more non-overlapping regions of the visual field. The position and size of these ‘measurement’ regions would be determined by the areas of the visual field in which the magnitude of optic flow is maximal. Such a strategy would explain how bumblebees are able to control their speed and position when translational optic flow cues are available on only one side of the visual field, as in the case of the asymmetrical tunnel. The idea that bumblebees may be extracting information about the maximum magnitude of optic flow in the visual field is consistent with the findings of a recent study showing that tethered *Drosophila* make corrective adjustments in response to the highest magnitude of optic flow encountered in the visual field (Cabrera and Theobald, 2013). Because the magnitude of optic flow increases when the distance to surfaces decreases, such a system would ensure that speed and position are being controlled relative to the nearby obstacles. This suggestion also agrees with the finding that, when presented with a three-dimensional visual environment, bumblebees control their flight according to the translational optic flow generated primarily by the nearby obstacles – that is, those that generated the highest magnitudes of translational optic flow in the visual field – rather than the optic flow generated by the background (Baird and Dacke, 2012). The fine sensitivity of the bumblebee visual system to even very low increases in the magnitude of translational optic flow at low viewing angles also suggests that optic flow information in these areas may be enhanced, resulting in a tendency to preferentially use measurements in this area for flight control. The apparent flexibility and sensitivity of the bumblebee’s optic flow measuring system would readily allow them to detect and respond to changes in the proximity of nearby obstacles. In a cluttered environment, this would ensure that the bees regulate their position and speed according to the nearest obstacles, allowing them to maximise flight efficiency and to minimise the risk of collision.

It would be of interest to know the limit of the flexibility in this system; for example, how much of a visual angle would the region of translational optic flow need to subtend to have an effect on position control? It would also be of interest to know how the translational optic flow that is used for position (and speed) control is calculated in the visual system of bumblebees. Answering these questions requires more detailed investigations that will be the focus of future work.

MATERIALS AND METHODS

General

The experiments were conducted indoors in an aluminium net cage (2.1 m high, 3.2 m wide and 2.7 m long) with constant light intensity (500 lx) and temperature (20°C). A bumblebee hive (Koppert UK) was placed in the cage at one end of a flight tunnel (3 m long horizontal floor with two parallel 30 cm high vertical walls, Fig. 1A). Individual bees (identified by small plastic number tags glued to their thorax) were trained to fly along the tunnel towards a feeder hidden in a recess at the far end. The feeder was constructed of two channels (1 cm deep and 1 cm wide) – one for sugar water and one for pollen – that ran across the width of the tunnel. The construction and recessed position of the feeder were designed to minimise the effect of the view of the feeder or its lateral position on the flight trajectories of bees flying towards it.

Two different tunnel widths (30 and 15 cm) were used in the experiments investigating the effect of sudden changes in translational optic flow cues on the flight control behaviour of bumblebees. By using two different tunnel widths, we could perform a more rigorous assessment of where in the visual field bees measure optic flow for position and speed control because their trajectories are differently restricted and they naturally fly faster in the wider tunnel (Baird et al., 2010).

For all experimental conditions, the floor of the flight tunnel was white and the top was covered with plastic insect netting. The walls of the tunnel displayed a randomised black and white 1 cm×1 cm checkerboard pattern or 3 cm wide axial stripes (black and white). Both patterns had a Michelson contrast of 0.76 and the randomised checkerboard pattern contained a broad spectrum of frequencies (for an analysis of the spatial frequencies present in the pattern, see Baird and Dacke, 2012). The checkerboard pattern provided strong translational optic flow cues for bees flying along the tunnel, while the axial stripes served to minimize translational optic flow cues. In two experimental conditions, we presented the bees with symmetric (S) translational optic flow cues, i.e. both tunnel walls displayed the same pattern that was either checks (S_{checks}) or axial stripes (S_{stripes}). In a third experimental condition, the bees were presented with asymmetric (A) translational optic flow cues by placing the striped pattern on one wall and the checked pattern on the other. We also investigated the effect of abrupt changes from symmetric to asymmetric ($S_{\text{checks}} \rightarrow A$) optic flow cues and vice versa ($A \rightarrow S_{\text{checks}}$) on flight control by presenting the bees with different combinations of the symmetric (S_{checks}) and asymmetric (A) conditions in the first or second half of the tunnel. For each of the conditions in which asymmetric optic flow cues were presented, we varied the side on which the stripe pattern was presented. This allowed us to test for and subsequently exclude any side bias from these conditions. For an illustration of all experimental conditions, see Fig. 1B.

Recording of flight trajectories

Bumblebees were allowed to visit the feeder at the end of the flight tunnel for at least 1 day before recording commenced. A camera (Mikrotron MotionBLITZ EoSens, Unterschleisheim, Germany), mounted above the centre of the tunnel, recorded flights to the feeder at 120 Hz. The experimental conditions were presented in a randomised order and the bees were given at least 30 min to adapt to the new experimental condition before recording commenced. A maximum of five flights were recorded for each individual in each condition. The number of flights and individuals analysed in each condition is listed in Table 2.

Data analysis and calculations of visual field

In each video frame, the centre of mass of the bumblebee was determined (in x - and y -pixel coordinates) using an automated tracking program (Lindemann, 2005). The flight trajectories were analysed over a distance of 100 cm (50 cm before and 50 cm after the mid-section of the tunnel). Flights in which the bees turned back towards the hive or where they crashed into the walls, the floor or the net covering the tunnel were excluded from the analysis (these flights only represented a small proportion of the total data set in any experimental condition). Lateral position data were converted from pixels to millimetres using a reference pattern placed 15 cm above the tunnel floor (the approximate height of the flight trajectories). Flight speed was calculated by dividing the two-dimensional distance travelled between successive frames by the time step

between frames (8.3 ms). Lateral position and flight speed data were averaged in 2 cm bins along the longitudinal axis of the tunnel (x -axis). Data from repeated flights from the same individual were averaged so that each individual was considered only once per experimental condition. To control for possible side bias, we inverted the lateral (y -axis) values in conditions 3a, 4a and 5a and compared the lateral position data with the values from the corresponding condition 3b, 4b and 5b (see Fig. 1B). In all cases, the data were indistinguishable, indicating that there was no side bias in the data set. To simplify the analysis, we therefore pooled the data. The results from the pooled condition 4 ($S_{\text{checks}} \rightarrow A$) were then compared with control condition 1 (S_{checks}) and the results from the pooled condition 5 ($A \rightarrow S_{\text{checks}}$) were compared with the pooled control condition 3 (A). A response to a unilateral pattern change was considered to occur when the lateral position or flight speed in the test condition differed significantly from the data in the control condition at the corresponding longitudinal distance and continued to differ for the remainder of the analysis region. To estimate the viewing angle occupied by the pattern change when a response is observed, we took the inverse tangent of the ratio between the mean lateral distance to the wall displaying the pattern change (dy) and the mean longitudinal distance from the pattern change (dx) when the response occurred (Fig. 1C). These data were calculated using data from all individuals in the respective condition and assuming that the visual field of the bees was aligned parallel with the long axis of the tunnel. This assumption was based on the observation that the bees flew along relatively straight trajectories with their body axis aligned with the long axis of the tunnel and that the flight direction and the midline of the bee's visual field are approximately equivalent. To further support this assumption, we analysed the mean body angle for each flight over a distance of 20 cm before the first change in behaviour was observed (for the conditions with a pattern change) or in the centre of the tunnel when both walls were lined with checks (as a control). We found that the mean body angle was very low in all conditions (ranging from -0.4 ± 6.8 deg in S_{checks} to a maximum 9.2 ± 13.8 deg towards the wall with the pattern change in the $S_{\text{checks}} \rightarrow A$). As a result, references to viewing angle in the text are always given with respect to the midline of the field of view (which has a viewing angle of 0 deg). To estimate the magnitude of translational optic flow generated by the unilateral pattern change at this viewing angle (retinal angular velocity), we multiplied the sine of the viewing angle by the ratio between the velocity and the distance to the change (the hypotenuse derived from dx and dy). This calculation for the maximum magnitude of optic flow was performed for each degree across the region of the eye that viewed the check pattern (for the eye viewing the constant check pattern, this region was 180 deg) at each distance step. To calculate the relative difference between the maximum magnitude of optic flow experienced in each eye (Fig. 6), we subtracted the maximum value in the eye viewing the constant pattern from the maximum value in the eye viewing the pattern that changed. Wilcoxon rank sum tests at the 5% significance level were used for all statistical analyses.

Acknowledgements

We would like to thank Julien LeCoer for useful discussions about the data and the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

N.L., M.D., and E.B. designed the experiments. N.L. collected the data. N.L. and E.B. analysed the data. N.L., M.D. and E.B. wrote the manuscript.

Funding

This study was funded by the Swedish Foundation for Strategic Research (FFL09-056), Swedish Research Council (2011-4701) and Swedish Research Council Formas (229-1010-829).

References

- Baird, E. and Dacke, M. (2012). Visual flight control in naturalistic and artificial environments. *J. Comp. Physiol. A* **198**, 869-876.
- Baird, E., Srinivasan, M. V., Zhang, S. and Cowling, A. (2005). Visual control of flight speed in honeybees. *J. Exp. Biol.* **208**, 3895-3905.
- Baird, E., Kornfeldt, T. and Dacke, M. (2010). Minimum viewing angle for visually guided ground speed control in bumblebees. *J. Exp. Biol.* **213**, 1625-1632.
- Baird, E., Kreiss, E., Wcislo, W., Warrant, E. and Dacke, M. (2011). Nocturnal insects use optic flow for flight control. *Biol. Lett.* **7**, 499-501.

- Barron, A. and Srinivasan, M. V.** (2006). Visual regulation of ground speed and headwind compensation in freely flying honey bees (*Apis mellifera* L.). *J. Exp. Biol.* **209**, 978-984.
- Cabrera, S. and Theobald, J. C.** (2013). Flying fruit flies correct for visual sideslip depending on relative speed of forward optic flow. *Front. Behav. Neurosci.* **7**, 76.
- Collett, T. S.** (2002). Insect vision: controlling actions through optic flow. *Curr. Biol.* **12**, R615-R617.
- David, C. T.** (1982). Compensation for height in the control of groundspeed by *Drosophila* in a new, 'barber's pole' wind tunnel. *J. Comp. Physiol. A* **147**, 485-493.
- Dyhr, J. P. and Higgins, C. M.** (2010). The spatial frequency tuning of optic-flow-dependent behaviors in the bumblebee *Bombus impatiens*. *J. Exp. Biol.* **213**, 1643-1650.
- Fry, S. N., Rohrseitz, N., Straw, A. D. and Dickinson, M. H.** (2009). Visual control of flight speed in *Drosophila melanogaster*. *J. Exp. Biol.* **212**, 1120-1130.
- Gibson, J. J.** (1950). *The Perception of the Visual World*. Boston, MA: Houghton Mifflin.
- Gibson, J. J.** (1979). *The Ecological Approach to Visual Perception*. Boston, MA: Houghton Mifflin.
- Kern, R., Boeddeker, N., Dittmar, L. and Egelhaaf, M.** (2012). Blowfly flight characteristics are shaped by environmental features and controlled by optic flow information. *J. Exp. Biol.* **215**, 2501-2514.
- Kirchner, W. H. and Srinivasan, M. V.** (1989). Freely flying honeybees use image motion to estimate object distance. *Naturwissenschaften* **76**, 281-282.
- Koenderink, J. J.** (1986). Optic flow. *Vision Res.* **26**, 161-179.
- Lappe, M.** (2000). *Neuronal Processing of Optic Flow*. San Diego, CA: Academic Press.
- Lehrer, M.** (1990). How bees use peripheral eye regions to localize a frontally positioned target. *J. Comp. Physiol. A* **167**, 173-185.
- Lindemann, J.** (2005). Visual Navigation of a Virtual Blowfly. PhD Thesis. Universität Bielefeld, Germany.
- O'Carroll, D. C., Bidweil, N. J., Laughlin, S. B. and Warrant, E. J.** (1996). Insect motion detectors matched to visual ecology. *Nature* **382**, 63-66.
- Portelli, G., Ruffier, F. and Franceschini, N.** (2010). Honeybees change their height to restore their optic flow. *J. Comp. Physiol. A* **196**, 307-313.
- Serres, J., Dray, D., Ruffier, F. and Franceschini, N.** (2008a). A vision-based autopilot for a miniature air vehicle: joint speed control and lateral obstacle avoidance. *Auton. Robot.* **25**, 103-122.
- Serres, J. R., Masson, G. P., Ruffier, F. and Franceschini, N.** (2008b). A bee in the corridor: centering and wall-following. *Naturwissenschaften* **95**, 1181-1187.
- Srinivasan, M. V., Lehrer, M., Kirchner, W. H. and Zhang, S. W.** (1991). Range perception through apparent image speed in freely flying honeybees. *Vis. Neurosci.* **6**, 519-535.
- Srinivasan, M. V., Zhang, S. W., Lehrer, M. and Collett, T.** (1996). Honeybee navigation en route to the goal: visual flight control and odometry. *J. Exp. Biol.* **199**, 237-244.
- Srinivasan, M. V., Zhang, S. W. and Lehrer, M.** (1998). Honeybee navigation: odometry with monocular input. *Anim. Behav.* **56**, 1245-1259.