

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

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ABSTRACT

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

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25 **Keywords:** bumblebee, flight control, optic flow, position, flight speed, viewing angle

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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; Gibson, 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; Srinivasan et al., 1996) or by maintaining the magnitude of unilateral optic flow about a set-point (Serres et al., 2008a, 2008b). Translational optic flow cues are also used by honeybees (Portelli et al., 2011, 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

62 its visual field) (Gibson, 1950). This means that the relative difference in the magnitude of
63 translational optic flow experienced by an insect approaching a change in the proximity of the
64 environment would be larger, and presumably easier to detect, in more lateral regions of the
65 visual field (assuming that the eyes are aligned with the direction of flight). However, the
66 larger the angle at which these changes are detected, the less time an insect will have to adjust
67 its flight before encountering the change. Thus, the viewing angle at which optic flow is
68 measured has important consequences for flight in densely cluttered environments, where
69 timely control of position and speed are necessary for effective collision avoidance.

70 In one of the first attempts to identify where in the visual field optic flow is measured for
71 flight control, Srinivasan et al., (1991) investigated how honeybees adjust their position in
72 response to a black bar presented in an otherwise featureless flight tunnel. The bees deflected
73 away from the bar only once they had flown past it, suggesting that they were measuring optic
74 flow for position control in the lateral region of the visual field. This was consistent with the
75 findings of an earlier study which showed that, to locate a frontally positioned target,
76 honeybees use image motion generated by landmarks in the lateral visual field (Lehrer, 1990).
77 In contrast to these earlier findings, however, more recent studies on honeybees (Portelli et al.,
78 2010), blowflies (Kern et al., 2012) and bumblebees (Baird et al., 2010) suggest that these
79 insects also respond to changes in optic flow that occur in the more frontal region of the
80 visual field. In the latter study, aimed at defining the minimum viewing angle at which
81 bumblebees measure translational optic flow for ground speed control, the changes in
82 translational optic flow were laterally symmetric, meaning that the same change occurred in
83 both the left and the right visual fields at the same time. What remains unclear, however, is
84 how position and speed control is affected when bumblebees experience a sudden change in
85 optic flow that occurs on only one side of the visual field, that is, when it becomes
86 asymmetric. Are bumblebees also able to detect and respond to these unilateral changes in the
87 proximity of the environment at low viewing angles? If so, how and when do they react to
88 these changes? Here, we aim to answer these questions by presenting bumblebees with abrupt
89 unilateral changes in translational optic flow – generated by flight past stationary patterns
90 whose visual properties change abruptly – and record the effect on position and flight speed.

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RESULTS

The effect of asymmetric translational optic flow cues on flight control

The centring response

When both walls of the 3 m long flight tunnel (Fig. 1) 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 the 30 cm and 15 cm wide tunnels 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. 2). 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 cm and 4.19 ± 0.38 cm from the midline in the 30 cm and 15 cm wide tunnels, respectively (Fig. 2). 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 cm and -0.56 ± 0.57 cm from the midline in the 30 cm 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 – checks on both walls (S_{checks}) – flight speed was significantly slower (30 cm: 68.3 ± 13.0 cm/s; 15 cm: 49.6 ± 11.3 cm/s) than when these cues were asymmetric – checks on one wall and stripes on the other (A) (30 cm: 82.4 ± 15.4 cm/s, $P < 0.001$; 15 cm: 60.6 ± 15.4 cm/s, $P = 0.004$) (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: 122.4 ± 27.0 cm/s, $P < 0.001$; 15 cm: 150.4 ± 40.2 cm/s, $P < 0.001$) (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

129 this relationship was inverted when both walls were lined with the stripe pattern ($S_{stripes}$)
130 ($P=0.03$) (Fig. 3).

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132 **The effect of abrupt unilateral changes in translational optic flow**

133 In this experiment, one wall of the tunnel displayed a checkerboard pattern while the pattern
134 on the other wall changed from checks to stripes (creating an abrupt change from symmetric
135 to asymmetric optic flow cues, $S_{checks} \rightarrow A$), or vice versa (creating an abrupt change from
136 asymmetric to symmetric optic flow cues, $A \rightarrow S_{checks}$) halfway along the tunnel (see Fig 1B).
137 For clarity, the experimental conditions have been abbreviated according to the combination
138 of patterns, with S_{checks} representing checkerboard pattern on both walls, $S_{stripes}$ representing
139 axial stripes on both walls and A representing an asymmetric pattern combination with checks
140 on one wall and axial stripes on the other. The first character in the abbreviation represents
141 the pattern combination in the first half of the tunnel (with respect to a bee flying towards the
142 feeder) and the second character represent the pattern combination in the second half. Thus,
143 the experimental condition $S_{checks} \rightarrow A$ indicates that the first half of the tunnel is lined with
144 checks on both walls and the second half of the tunnel is lined with checks on one wall and
145 axial stripes on the other wall. The experimental condition $A \rightarrow S_{checks}$ indicates that the first
146 half of the tunnel is lined with checks on one wall and axial stripes on the other and the
147 second half of the tunnel is lined with checks both walls.

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

152

153 *Lateral position*

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

162 adjusted their lateral position at a longitudinal distance of 16 cm and a lateral distance of 4 cm
163 from the pattern change ($P=0.04$; Fig. 4C), when it was located at a viewing angle of
164 approximately 14 deg.

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

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

181

182 *Flight speed*

183 When the translational optic flow cues changed from asymmetric to symmetric ($A \rightarrow S_{checks}$)
184 the bees decreased their flight speed with respect to the control condition (A) at a longitudinal
185 distance of 18 cm and a lateral distance of 5 cm before the pattern change in the 30 cm wide
186 tunnel ($P=0.04$; Fig. 5A). At this position, the pattern change was located at a viewing angle
187 of approximately 16 deg. In the 15 cm wide tunnel, flight speed decreased significantly from
188 the control condition at a longitudinal distance of 20 cm and a lateral distance of 3 cm before
189 the pattern change ($P=0.02$; Fig. 5B). At this position, the pattern change was located at a
190 viewing angle of approximately 9 deg.

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192 When the translational optic flow cues changed from symmetric to asymmetric ($S_{checks} \rightarrow A$),
193 the bees increased their flight speed significantly in response to the sudden decrease in optic
194 flow cues. In the 30 cm wide tunnel, this increase did not occur until the bees reached a

195 longitudinal distance of 22 cm *after* the pattern change, at a lateral distance of 9 cm from the
196 wall ($P=0.03$; Fig. 5C). The pattern change at this position occurs at a viewing angle of 157
197 deg. In the 15 cm wide tunnel, the acceleration did not occur until a longitudinal distance of
198 14 cm *after* the pattern change, and at a lateral distance of 5 cm from the wall ($P=0.01$; Fig.
199 5D), with the pattern change located at a viewing angle of 159 deg.

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

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DISCUSSION

208

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

210 When presented with a unilateral change from axial stripes to checks ($A \rightarrow S_{checks}$), the
211 bumblebees adjusted both their position and speed when the optic flow cues subtended only a
212 very small region of the frontal visual field. In the most extreme case, bumblebees decreased
213 their flight speed from 53 cm/s when the pattern change occupied a viewing angle of only 9
214 deg. (in the 15 cm tunnel). The maximum magnitude of translational optic flow that the bees
215 would have experienced at the location of the pattern change in this case would have been
216 approximately 24 deg./s. Considering that there is inevitably a delay between when the visual
217 system detects a change in optic flow and when a behavioural response is initiated – this delay
218 has been estimated at 100 ms in *Drosophila* (Fry et al., 2009) – it is likely that the bumblebees
219 were reacting to the pattern when it generated even lower magnitudes of optic flow. Can
220 bumblebees really detect and react to such low magnitudes of optic flow at such low viewing
221 angles?

222

223 Behavioural evidence that bumblebees can detect and respond to low magnitudes of optic
224 flow was first provided by Baird et al. (2010), which showed that bumblebees changed their
225 flight speed in response to a bilateral increase in tunnel width from 15 to 30 cm when the
226 view of the wider tunnel subtended a viewing angle of approximately 30 deg. While this is
227 larger than the viewing angle observed in the present study, the magnitude of translational

228 optic flow experienced by bees in this case was approximately 30 deg./s, in other words, not
229 far from the value of 24 deg./s measured in the present study. These findings are also
230 supported by physiological evidence that motion-sensitive neurons in the bumblebee visual
231 system respond to magnitudes of optic flow ranging from 5 deg./s to above 2000 deg./s
232 (O'Carroll et al. 1996). Thus, the response threshold recorded in the present study lies well
233 within the range of magnitudes that the visual system of bumblebees can detect, and provides
234 strong behavioural evidence that the motion-sensitive mechanism underlying position and
235 speed control is capable of detecting and responding to very low magnitudes of optic flow at
236 low viewing angles.

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

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

252

253 To investigate if the recorded increase in flight speed represents an attempt to hold the
254 magnitude of translational optic flow constant in one visual field, we can estimate the
255 maximum magnitude of optic flow experienced in the symmetric condition and compare it
256 with that experienced when the translational optic flow cues are asymmetric. When both walls
257 were lined with checkerboard pattern, the bees flew along the midline of the 30 cm wide
258 tunnel at an average forward speed of 68 cm/s. The maximum magnitude of optic flow
259 experienced by the bees in this case would be approximately 260 deg./s at a viewing angle of
260 90 deg. When the translational optic flow was asymmetric, the bees flew at an average lateral
261 distance of 25 cm from the checkerboard wall and increased their flight speed to 82 cm/s. In

262 this case, the maximum magnitude of translational optic flow (which occurs at a 90 deg.
263 viewing angle) would be approximately 188 deg./s. Given that the difference in the magnitude
264 of optic flow between these two conditions would only decrease with viewing angle – that is,
265 the lower the viewing angle at which translational optic flow is measured, the closer these
266 values become (at a viewing angle of 30 deg. for example, the values would be 130 and 94
267 deg.) – and that the bees are more likely to measure optic flow for speed control at frontal
268 rather than lateral viewing angles (discussed below), it is plausible that the increase in flight
269 speed that we observe in the asymmetric condition is due to the increased distance that the
270 bees are flying from the wall with the checkerboard pattern. This suggests that the bees are
271 able to regulate their flight speed even when optic flow cues are absent from one side of the
272 visual field.

273

274 Is this result consistent with the optic flow regulator model proposed by Serres et al. (2008a),
275 which is currently the only comprehensive model for how translational optic flow cues may
276 be used for speed control in flying insects? In this model, flight speed is controlled by
277 maintaining the sum of the optic flow in the dorsal/ventral *or* lateral visual fields at a set-point
278 – because the bees in our study changed their flight speed in response to changes in *lateral*
279 cues, we will assume that these were the dominant cues in this experiment. According to the
280 optic flow regulator model, the sum of the maximum magnitude of the translational optic flow
281 experienced by the bees when flying in the tunnel with checks on both sides should be equal
282 to that experienced in the tunnel with horizontal stripes on one wall. The sum of the
283 maximum magnitude of optic flow in the symmetrical condition is 520 deg./s (that is, 260×2
284 deg./s), while it is only 188 deg./s in the asymmetrical condition (in this case, only one wall is
285 generating translational optic flow). Thus, speed control in bumblebees does not seem to be
286 regulated by the summation of the magnitude of translational optic flow in the lateral visual
287 fields and is thus not fully explained by the optic flow regulator model. Instead, our results
288 suggest that, when translational optic flow cues are absent from one side of the visual field,
289 bumblebees control flight speed using unilateral translational optic flow cues – this is similar
290 to honeybees, which can use unilateral optic flow cues for calculating the distance flown to a
291 food source (Srinivasan et al., 1998). However, as soon as translational optic flow cues are
292 detected in the other eye, information from both sides of the visual field is used for speed
293 control.

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295

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

297 Unilateral changes from axial stripes to checks ($A \rightarrow S_{checks}$) caused a reduction in flight speed
298 when they occupied low visual angles (16 deg. and 9 deg. in the 30 cm and 15 cm wide
299 tunnels, respectively). When the unilateral translational optic flow cues changed from checks
300 to stripes ($S_{checks} \rightarrow A$), however, flight speed was adjusted only once the bees had already
301 moved closer to the axial stripes. At this point, the checkerboard pattern on the wall with the
302 pattern change is present only at very large viewing angles (157 deg. in the 30 cm tunnel and
303 159 deg. in the 15 cm tunnel). Thus, in the $A \rightarrow S_{checks}$ condition, the bees were changing their
304 flight speed when the translational optic flow cues occupied a very narrow region of the
305 frontal visual field, whereas in the $S_{checks} \rightarrow A$ condition, flight speed was reduced only once
306 the checkerboard pattern had passed 90 deg. Given the large difference in the visual angle of
307 the pattern at the time that the bees initiated a significant change in flight speed, our results
308 demonstrate that bumblebees are not measuring optic flow for speed control at one set
309 viewing angle. Instead, this angle appears to be modified depending on where in the visual
310 field translational optic flow is experienced (this hypothesis is discussed in more detail below).

311

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

313 One model that has been proposed to explain how optic flow may be used to control lateral
314 position in insects is the optic flow regulator model (Serres et al., 2008a). This model
315 proposes that position is controlled by a unilateral optic flow regulator that strives to maintain
316 the maximum magnitude of optic flow experienced in a lateral visual field about a set-point.
317 We can explore whether this model explains our data by comparing the maximum magnitude
318 of optic flow experienced in each lateral visual fields as the bees fly through the tunnel. This
319 can be done by calculating the maximum magnitude of translational optic flow at 1 deg.
320 intervals over each 180 deg. visual field and then calculating the difference between these
321 values (Fig. 6). If this relative difference in the maximum magnitude of translational optic
322 flow experienced in each visual field is zero then the maximum magnitude of translational
323 optic flow is equal in both eyes, negative values indicate that it is lower in the visual field
324 viewing the pattern change and vice versa. A prediction of the optic flow regulator model is
325 that, when the bees adjust their position in response to the pattern change, the maximum
326 magnitude of translational optic flow in the lateral visual field viewing the pattern change will
327 be larger than in the lateral visual field viewing the opposite wall – in other words, if the
328 magnitude of the optic flow on the wall with the constant check pattern was always larger

329 than on the wall with the pattern change, the bees would not need to initiate a change in
330 position. In the case where the pattern on one wall changes from stripes to checks ($A \rightarrow S_{checks}$),
331 the bees adjust their position when the maximum optic flow experienced in the lateral visual
332 field viewing the pattern change is 130 deg./s *higher* than the maximum optic flow generated
333 by the opposite wall in the 30 cm wide tunnel but 88 deg./s *lower* than the maximum optic
334 flow generated by the opposite wall in the 15 cm wide tunnel (red dotted lines, Fig. 6). In the
335 $S_{checks} \rightarrow A$ condition, position is adjusted when the maximum optic flow on the wall with the
336 change is 14 and 161 deg./s higher (in the 30 and 15 cm wide tunnels, respectively) than the
337 opposite wall (blue dotted lines, Fig. 6). Thus, in two of four conditions, the maximum optic
338 flow in the lateral visual field viewing the wall with the pattern change is less than, or very
339 close to the optic flow experienced in the visual field viewing the opposite wall (it is
340 important also to bear in mind that the bees would have already detected the change in optic
341 flow when we observe a behavioural response, so that the magnitude of the optic flow when
342 the bees detect the change would be lower). Overall, our results suggest that bumblebees do
343 not regulate their position using only one measure of the highest magnitude of optic flow
344 experienced in the visual field, indicating that the optic flow regulator model does not fully
345 explain position control in bumblebees.

346

347 A second model that describes how position might be regulated using translational optic flow
348 cues is the optic flow balancing model (Srinivasan et al., 1991). This model suggests that
349 position is controlled by balancing the magnitude of translational optic flow in the lateral part
350 of each visual field. The first prediction of this model, namely that position is controlled by
351 balancing the magnitude of optic flow experienced in each eye, can be explored by comparing
352 the maximum magnitude of optic flow experienced in each lateral visual field as the bees fly
353 through the tunnel (Fig. 6). Our analysis shows that, when one side of the visual field
354 experienced a large increase in the magnitude of translational optic flow, bumblebees adjusted
355 their speed and position so as to equalise the maximum magnitude of optic flow experienced
356 in each eye (red lines, Fig. 6). At the point when a behavioural response was observed, the
357 maximum magnitude of translational optic flow in the visual field viewing the pattern change
358 was located at the viewing angle occupied by the pattern change itself (speed: 9 deg. and 16
359 deg.; position: 14 deg. and 27 deg., 15 and 30 cm wide tunnels, respectively). When there was
360 a large decrease in the magnitude of translational optic flow, bumblebees again adjusted their
361 speed and position so as to equalise the maximum magnitude of optic flow experienced in
362 each eye (blue lines, Fig. 6). Once again, at the point when the behavioural responses

363 occurred, the maximum magnitude of translational optic flow in the visual field viewing the
364 pattern change occurred at the viewing angle occupied by the pattern change (speed: 159 deg.
365 and 157 deg.; position: 106 deg. and 75 deg., 15 and 30 cm wide tunnels, respectively) in all
366 but one case. In this case, position in the 15 cm wide tunnel was adjusted when the pattern
367 change subtended a viewing angle of 75 deg. (the maximum magnitude of optic flow in this
368 case would have been experienced at 90 deg.). Overall, our results indicate that, when
369 bumblebees experience a sudden increase or decrease in the magnitude of translational optic
370 flow, they change both their position and speed so as to equalise this value in each eye.

371

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

386

387 The second prediction of the optic flow balancing model is that optic flow is being balanced
388 in the lateral visual field of each eye. We can explore whether this is the case for bumblebees
389 by investigating when they reacted to the pattern changes. When bumblebees experienced a
390 sudden loss of translational optic flow on one side ($S_{checks} \rightarrow A$), they did not modify their
391 lateral position until the axial stripes subtended a relatively large viewing angle (75 deg. in the
392 30 cm wide tunnel and 106 deg. in the 15 cm wide tunnel), which is consistent with the
393 prediction of the optic flow balancing model. However, when the bumblebees experienced a
394 change from asymmetric to symmetric optic flow cues ($A \rightarrow S_{checks}$), they modified their lateral
395 position when the translational optic flow cues subtended very small viewing angles (27 deg.
396 in the 30 cm tunnel; 14 deg. in the 15 cm tunnel). Thus, they do not appear to be measuring

397 translational optic flow cues for position control at lateral viewing angles or even at one set
398 angular position in each visual field. Instead of balancing the magnitude of optic flow at a
399 large fixed viewing angle in each visual field to control position (as the optic flow balancing
400 model suggests), bumblebees appear to be balancing the maximum magnitude of optic flow
401 experienced in the frontal part of each visual field flexibly, that is, irrespective of whether this
402 value occurs at the same or different visual angles in each visual field.

403

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

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

431 this would ensure that the bees regulate their position and speed according to the nearest
432 obstacles, allowing them to maximise flight efficiency and to minimise the risk of collision.

433

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

440

441

METHODS

442

443

General

444 The experiments were conducted indoors in an aluminium net cage (2.1 m high, 3.2 m wide
445 and 2.7 m long) with constant light intensity (500 lux) and temperature (20°C). A bumblebee
446 hive, (*Bombus terrestris* L., Koppert UK) was placed in the cage at one end of a flight tunnel
447 (3 m long horizontal floor with two parallel 30 cm high vertical walls, Fig. 1A). Individual
448 bees (identified by small plastic number tags glued to their thorax) were trained to fly along
449 the tunnel towards a feeder hidden in a recess at the far end. The feeder was constructed of
450 two channels (1 cm deep and 1 cm wide) – one for sugar water and one for pollen – that ran
451 across the width of the tunnel. The construction and recessed position of the feeder was
452 designed to minimise the effect of the view of the feeder or its lateral position on the flight
453 trajectories of bees flying towards it.

454

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

461 For all experimental conditions, the floor of the flight tunnel was white and the top was
462 covered with plastic insect netting. The walls of the tunnel displayed a randomised black and
463 white 1 cm x 1 cm checkerboard pattern or 3 cm wide axial stripes (black and white). Both
464 patterns had a Michelson contrast of 0.76 and the randomised checkerboard pattern contained

465 a broad spectrum of frequencies (for an analysis of the spatial frequencies present in the
466 pattern, see Baird and Dacke 2012). The checkerboard pattern provided strong translational
467 optic flow cues for bees flying along the tunnel, while the axial stripes serve to minimize
468 translational optic flow cues. In two experimental conditions, we presented the bees with
469 symmetric (S) translational optic flow cues, that is, both tunnel walls displayed the same
470 pattern that was either checks (S_{checks}) or axial stripes ($S_{stripes}$). In a third experimental
471 condition, the bees were presented with asymmetric (A) translational optic flow cues by
472 placing the stripe pattern on one wall and the check pattern on the other. We also investigated
473 the effect of abrupt changes from symmetric to asymmetric ($S_{checks} \rightarrow A$) optic flow cues and
474 vice versa ($A \rightarrow S_{checks}$) on flight control by presenting the bees with different combinations of
475 the symmetric (S_{checks}) and asymmetric (A) conditions in the first or second half of the tunnel.
476 For each of the conditions in which asymmetric optic flow cues were presented, we varied the
477 side on which the stripe pattern was presented. This allowed us to test for and subsequently
478 exclude any side bias from these conditions. For an illustration of all experimental conditions,
479 see Fig. 1B.

480

481

Recording of flight trajectories

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

489

490

Data analysis and calculations of visual field

491 In each video frame, the centre of mass of the bumblebee was determined (in x- and y- pixel
492 coordinates) using an automated tracking program (Lindemann, 2005). The flight trajectories
493 were analysed over a distance of 100 cm (50 cm before and 50 cm after the midsection of the
494 tunnel). Flights in which the bees turned back towards the hive or where they crashed into the
495 walls, the floor or the net covering the tunnel were excluded from the analysis (these flights
496 only represented a small proportion of the total data set in any experimental condition).
497 Lateral position data was converted from pixels to millimetres using a reference pattern
498 placed 15 cm above the tunnel floor (the approximate height of the flight trajectories). Flight

499 speed was calculated by dividing the two-dimensional distance travelled between successive
500 frames by the time step between frames (8.3 ms). Lateral position and flight speed data was
501 averaged in 2 cm bins along the longitudinal axis of the tunnel (x-axis). Data from repeated
502 flights from the same individual were averaged so that each individual was considered only
503 once per experimental condition. To control for possible side biases, we inverted the lateral
504 (y-axis) values in conditions 3a, 4a and 5a and compared the lateral position data with the
505 values from the corresponding condition 3b, 4b and 5b (see Fig. 1B). In all cases, the data
506 were indistinguishable, indicating that there was no side bias in the data set. To simplify the
507 analysis, we therefore pooled the data together. The results from the pooled condition 4
508 ($S_{checks} \rightarrow A$) were then compared with control condition 1 (S_{checks}) and the results from the
509 pooled condition 5 ($A \rightarrow S_{checks}$) were compared with the pooled control condition 3 (A). A
510 response to a unilateral pattern change was considered to occur when the lateral position or
511 flight speed in the test condition differed significantly from the data in the control condition at
512 the corresponding longitudinal distance and continued to differ for the remainder of the
513 analysis region. To estimate the viewing angle occupied by the pattern change when a
514 response is observed, we took the inverse tangent of the ratio between the mean lateral
515 distance to the wall displaying the pattern change (dy) and the mean longitudinal distance
516 from the pattern change (dx) when the response occurred (Fig. 1C). This data was calculated
517 using data from all individuals in the respective condition and assumed that the visual field of
518 the bees was aligned parallel with the long axis of the tunnel. This assumption was based on
519 the observation that the bees flew along relatively straight trajectories with their body axis
520 aligned with the long axis of the tunnel and that the flight direction and the midline of the
521 bee's visual field are approximately equivalent. To further support this assumption, we
522 analysed the mean body angle for each flight over a distance of 20 cm before the first change
523 in behaviour was observed (for the conditions with a pattern change) or in the centre of the
524 tunnel when both walls were lined with checks (as a control). We found that the mean body
525 angle was very low in all conditions (ranging from -0.4 ± 6.8 deg. in S_{checks} to a maximum
526 9.2 ± 13.8 deg. towards the wall with the pattern change in the $S_{checks} \rightarrow A$). As a result,
527 references to viewing angle in the text are always given with respect to the midline of the
528 field of view (which has a viewing angle of 0 deg.). To estimate the magnitude of
529 translational optic flow generated by the unilateral pattern change at this viewing angle
530 (retinal angular velocity), we multiplied the sine of the viewing angle by the ratio between the
531 velocity and the distance to the change (the hypotenuse derived from dx and dy). This

532 calculation for the maximum magnitude of optic flow performed for each degree across the
533 region of the eye that viewed the check pattern (for the eye viewing the constant check pattern,
534 this region was 180 deg.) at each distance step. To calculate the relative difference between
535 the maximum magnitude of optic flow experienced in each eye (Fig. 6), we subtracted the
536 maximum value in the eye viewing the constant pattern from the maximum value in the eye
537 viewing the pattern that changed. Wilcoxon rank sum tests at the 5% significance level were
538 used for all statistical analyses.

539

540 **Competing interests**

541 The authors declare no competing financial interests.

542

543 **Author contributions**

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

546

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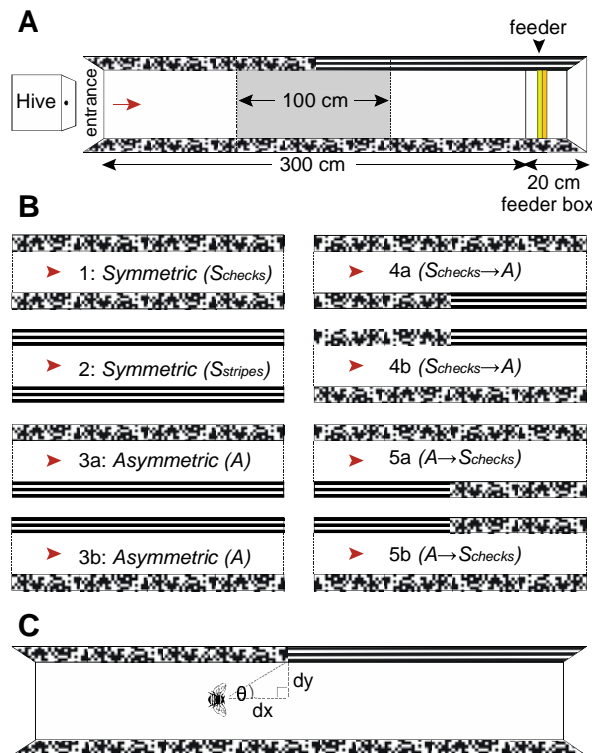
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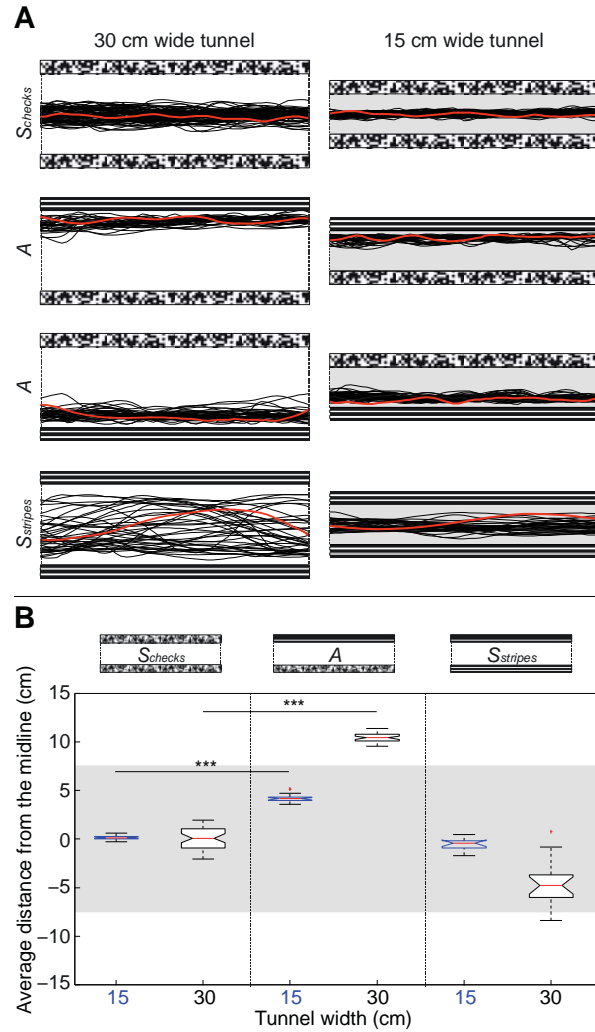
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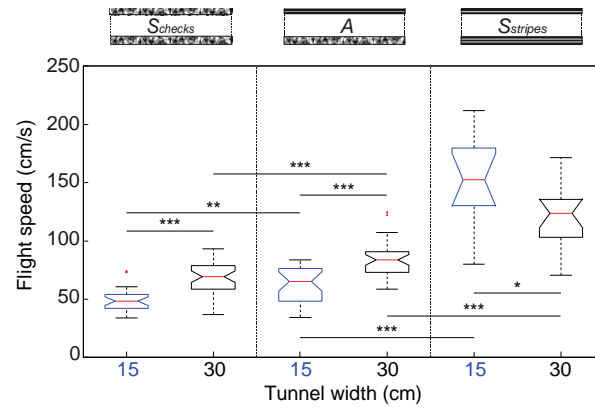
606 **Fig. 1. Experimental set-up.** (A) Schematic diagram of a flight tunnel (300 cm long x 30 cm
 607 wide x 30 cm high). The bumblebee hive was placed at one end of the flight tunnel (bees
 608 could enter anywhere along the opening of the tunnel) and a two-compartment feeder (marked
 609 in yellow), covering the whole width of the tunnel, was placed in a recess at the far end of the
 610 tunnel. A high-speed camera recorded trajectories of bees flying over the central (100 cm)
 611 section of the tunnel, indicated by the grey area. (B) Pattern combinations used in each
 612 experimental condition. In two of the experimental conditions, the same type of pattern was
 613 displayed on each wall, generating *symmetric* translational optic flow cues in the lateral visual
 614 field of bees flying along the midline of the tunnel: 1) randomised checkerboard on both walls
 615 (*Symmetric*, ' S_{checks} ') or 2) horizontal stripes on both walls (*Symmetric stripes*, ' $S_{stripes}$ '). In the
 616 third condition 3) one wall displayed the checkerboard pattern, while the other wall displayed
 617 the horizontal stripe pattern: 3a) left wall: checks, right wall: stripes; or 3b) vice versa. This
 618 presented the bees with *asymmetric* lateral optic flow cues (*Asymmetric*, ' A '). In the next four
 619 conditions, one wall displayed the checkerboard pattern along the length of the tunnel, while
 620 the pattern on the other wall changed abruptly half way along the tunnel: 4a) left wall: checks,
 621 right wall: checks then stripes; or 4b) vice versa; 5a) left wall: checks, right wall: stripes then
 622 checks; or 5b) vice versa. The red arrows indicate the direction of flight. (C) Illustration of the



623 calculation of the viewing angle (θ) occupied by the unilateral pattern change when the bees
 624 adjusted their flight speed and position in response to the abrupt change in the magnitude of
 625 optic flow. Condition $A \rightarrow S_{checks}$ is shown in the example.
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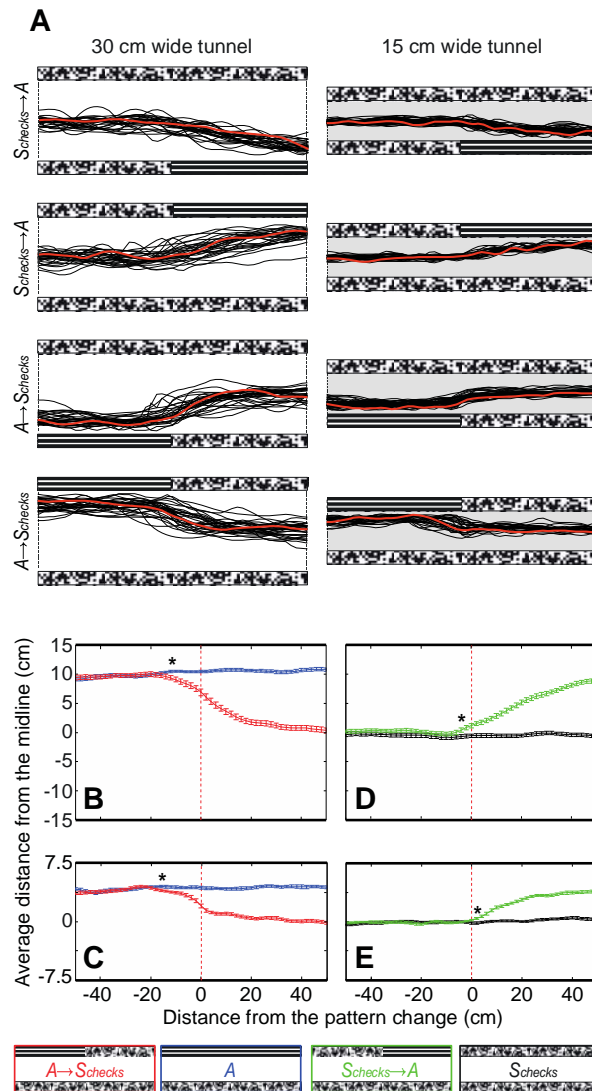
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 628 **Fig. 2. Effect of asymmetric translational optic flow cues on the centring response.** (A)
 629 Raw flight trajectories for the conditions S_{checks} , A , and $S_{stripes}$. A typical flight trajectory for
 630 each condition is highlighted in red. (B) The average lateral position of bees flying in a 15 cm
 631 (boxes outlined in blue) or 30 cm (boxes outlined in black) wide tunnel, lined with either
 632 checks on both walls (S_{checks}), stripes on one wall and checks on the other (A), or stripes on
 633 both walls ($S_{stripes}$). Grey shading indicates the width of the 15 cm wide tunnel. Boxes indicate
 634 the distance between the lower and upper quartile values, red lines indicate the median values
 635 and whiskers indicate the entire spread of the data. Stars indicate the significance level:
 636 $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



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640 **Fig. 3. Effect of asymmetric translational optic flow cues on speed control.** Average flight
 641 speed of bees flying in a 15 cm (boxes outlined in blue) or 30 cm (boxes outlined in black)
 642 wide tunnel, lined with either checks on both walls (S_{checks}), stripes on one wall and checks on
 643 the other (A), or stripes on both walls ($S_{stripes}$). Boxes indicate the distance between the lower
 644 and upper quartile values, red lines indicate the median values and whiskers indicate the
 645 entire spread of the data. Stars indicate the level of significance: $*P < 0.05$, $**P < 0.01$,
 646 $***P < 0.001$.

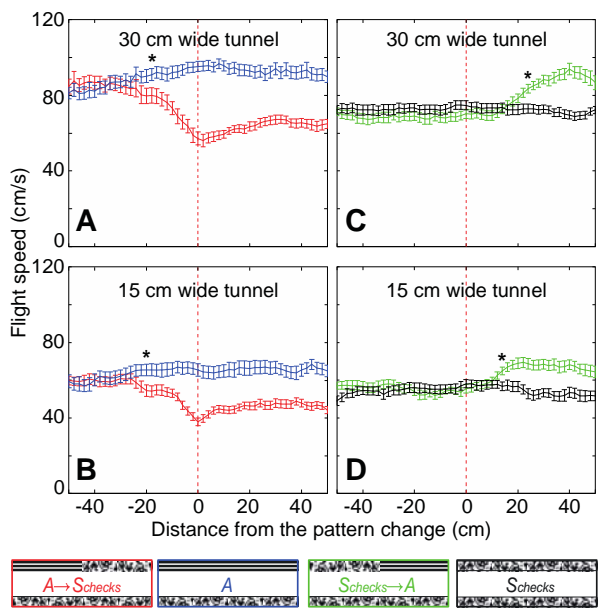


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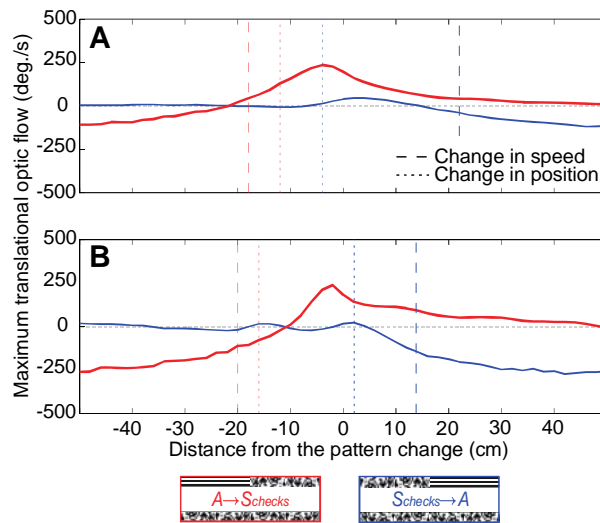
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649 **Fig. 4. Effect of a unilateral change in optic flow on lateral position.** (A) Raw flight
 650 trajectories for the conditions $S_{checks} \rightarrow A$ and $A \rightarrow S_{checks}$. A typical flight trajectory for each
 651 condition is highlighted in red. Grey shading indicates the width of the 15 cm wide tunnel.
 652 Average lateral position of bees flying along a 30 cm wide tunnel (B, D) or a 15 cm wide
 653 tunnel (C, E). Red lines represent condition $A \rightarrow S_{checks}$, blue lines represent control condition A,
 654 green lines represents condition $S_{checks} \rightarrow A$ and black lines represent control condition S_{checks} .
 655 The red dotted line illustrates the point in the tunnel where the unilateral pattern change
 656 occurred, generating an asymmetric change in the magnitude of translational optic flow. Means
 657 are calculated over 2 cm bins, error bars represent the standard error of the mean. Asterisks
 658 indicate where the lateral position of the bees in condition $A \rightarrow S_{checks}$ or $S_{checks} \rightarrow A$ deviates

659 significantly from the lateral position in the corresponding control condition (A or S_{checks} ,
 660 respectively).
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 664 **Fig. 5. Effect of a unilateral change in optic flow on flight speed.** Average flight speed of
 665 bees flying along a 30 cm wide tunnel (A, C) or a 15 cm wide tunnel (B, D). Red lines
 666 represent condition $A \rightarrow S_{checks}$, blue lines represent control condition A , green lines represents
 667 condition $S_{checks} \rightarrow A$ and black lines represent control condition S_{checks} . The red dotted line
 668 illustrates the point in the tunnel where the unilateral pattern change occurred, generating a
 669 asymmetric change in the magnitude of translational optic flow. Means are calculated over 2
 670 cm bins, error bars represent the standard error of the mean. Asterisks indicate where the
 671 flight speed in condition $A \rightarrow S_{checks}$ or $S_{checks} \rightarrow A$ deviates significantly from the flight speed in
 672 the corresponding control condition (A or S_{checks} , respectively).
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679 **Fig. 6. Comparison of the maximum magnitude of translational optic flow in each visual**
 680 **field.** The maximum magnitude of optic flow over 180 deg. was calculated for the visual field
 681 viewing the pattern change and compared with the visual field viewing the constant pattern
 682 for the two conditions, $A \rightarrow S_{checks}$ (solid red lines) or $S_{checks} \rightarrow A$ (solid blue lines) in both the 30
 683 cm (A) and 15 cm (B) wide tunnels. A value of zero indicates that the maximum magnitude of
 684 translational optic flow is equal in each visual field, a negative value indicates that it is lower
 685 in the visual field viewing the pattern change and vice versa. Note that the calculation for the
 686 visual field viewing the pattern change was made only for the region occupied by the check
 687 pattern as the stripe pattern generated no translational optic flow cues. Vertical lines indicate
 688 the longitudinal distance from the pattern change at which a change in speed (dashed lines) or
 689 position (dotted lines) was recorded for each condition (red lines indicate the data for the
 690 $A \rightarrow S_{checks}$ condition and blue lines for the $S_{checks} \rightarrow A$ condition).

691 **Table 1. Details of statistical analysis.** Wilcoxon rank sum tests at a 5% significance level
 692 were used for all statistical analyses. N is the total number of flights included in the analysis.
 693 For illustrations of the different experimental conditions (S_{checks} , A, $S_{stripes}$, $S_{checks} \rightarrow A$,
 694 $A \rightarrow S_{checks}$) see Fig. 1B.
 695

$S_{checks} \rightarrow A$ vs. S_{checks}		$A \rightarrow S_{checks}$ vs. A		S_{checks} vs. A		A vs. $S_{stripes}$	15 cm vs. 30 cm		
	position	speed	position	speed	position	speed	speed	speed	
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	S_{checks} Z=-5.0 N=67 P<0.001	$S_{stripes}$ Z=2.15 N=29 P=0.032
	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	A Z=-4.81 N=67 P<0.001	

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Table 2. Sample size for each condition. See Fig. 1B for illustration and description of the pattern combinations used for all conditions.

Condition	Number of individuals (15 cm / 30 cm tunnel)	Number of flights (15 cm / 30 cm tunnel)
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 ($S_{checks} \rightarrow A$)	12/21; 11/20	42/27; 25/30
5a;b ($A \rightarrow S_{checks}$)	21/13; 15/18	41/29; 27/32

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