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## RESEARCH ARTICLE

# Bumblebee calligraphy: the design and control of flight motifs in the learning and return flights of *Bombus terrestris*

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## SUMMARY

**Many wasps and bees learn the position of their nest relative to nearby visual features during elaborate 'learning' flights that they perform on leaving the nest. Return flights to the nest are thought to be patterned so that insects can reach their nest by matching their current view to views of their surroundings stored during learning flights. To understand how ground-nesting bumblebees might implement such a matching process, we have video-recorded the bees' learning and return flights and analysed the similarities and differences between the principal motifs of their flights. Loops that take bees away from and bring them back towards the nest are common during learning flights and less so in return flights. Zigzags are more prominent on return flights. Both motifs tend to be nest based. Bees often both fly towards and face the nest in the middle of loops and at the turns of zigzags. Before and after flight direction and body orientation are aligned, the two diverge from each other so that the nest is held within the bees' fronto-lateral visual field while flight direction relative to the nest can fluctuate more widely. These and other parallels between loops and zigzags suggest that they are stable variations of an underlying pattern, which enable bees to store and reacquire similar nest-focused views during learning and return flights.**

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## INTRODUCTION

Effective learning in natural surroundings is difficult for animals to implement. Insects often simplify learning by restricting it to pre-specified cues (Ômura and Honda, 2005; Balkenius et al., 2006) that may be learnt during specific phases of a behavioural sequence, as in the case considered here. Many bees and wasps perform elaborate learning flights on their first few departures from a significant place, such as a nest or a feeding site (Wagner, 1907; Opfinger, 1931; Vollbehr, 1975; Lehrer, 1993; Zeil, 1993a). The major function of these flights is to gather visual information about the surroundings of an insect's departure point, so that the insect can find its way back there (Tinbergen, 1932; Becker, 1958; Zeil, 1993b; Capaldi and Dyer, 1999). We have examined the learning flights of the bumblebee, *Bombus terrestris* Linnaeus, to understand how these flights are generated and controlled. The flights are quite variable, but embedded within this variability are stereotyped motifs that we analyse on the hypothesis that they are likely to be involved in information acquisition and storage.

Learning flights resemble human handwriting in that the spatial patterns of both are scale invariant, similar across individuals and can be hard for an observer to decode. We examine the notion that bumblebees read the information acquired on learning flights by replicating components of their learning manoeuvres on their return and so obtain similar visual input for guiding their approach to the nest (Zeil, 1993b; Collett, 1995; Zeil et al., 1996). The most prominent motifs of learning flights (loops) differ from those of returns (zigzags) in part because the flights have different functions. The former involves information acquisition as part of a controlled departure, in which bees scan their surroundings; in the latter, bees return to the nest guided by that information. We explore whether segments of these different motifs have features in common.

The study of learning flights is made more interesting by species differences. Bees and wasps construct their nests in a variety of locations. To some degree, learning flights seem to vary with the location of the nest (Jander, 1997) and incorporate different stereotyped manoeuvres. Thus the orchid bee, *Euglossa cyanipes*, which nests in cavities or hollows of vertical plant stems (D. W. Roubik, personal communication), alternates phases of hovering while facing the nest with rapid horizontal or vertical displacements (Jander, 1997). The solitary sweat bee (*Lasioglossum figueresi*) digs nests in the vertical face of banks. During learning flights it backs away from its nest in a serpentine pattern and then returns directly to the nest to hover 5 cm in front of it, repeating this loop-like pattern two to five times before departing (Wcislo, 1992). Similarly, honeybees (*Apis mellifera*), which in their native state nest in holes in trees, hover in front of the nest entrance during learning flights (Vollbehr, 1975). Much evidence indicates that insects guide their returns to a familiar place using remembered views of the immediate surroundings of the place (reviewed in Collett and Collett, 2002; Zeil, 2012). The hovering of these bees while facing the nest suggests that they may acquire nest-directed views during these phases of their learning flights. Intriguingly, desert ants performing learning walks when leaving their nest frequently turn back and face it (Müller and Wehner, 2010). Bumblebee learning flights have prominent

loops, already sketched by Wagner (Wagner, 1907), which we will show often bring bees close to the nest several times during a flight.

Analysis of video-recordings of the learning flights of some solitary and social wasps also suggest the importance of facing the nest or goal for acquiring information. The first flights to be analysed were those of the solitary wasp *Cerceris* sp. (Zeil, 1993b; Zeil et al., 2007) leaving its nest. Somewhat similar data came from the social wasp *Vespula vulgaris* (Collett and Lehrer, 1993; Collett, 1995) leaving a feeder. Flights were recorded at high spatial resolution within a small area around the nest-hole or feeder. After leaving this departure point, a wasp pivots about it in a series of arc-like movements of increasing radius that gradually take it out of the view of the camera. The wasp tends to look briefly at the goal at the ends of the arcs (Zeil, 1993a; Collett and Lehrer, 1993). In *Vespula* leaving a newly discovered feeder, but not obviously in *Cerceris* leaving the nest, the ends of the arcs are aligned so that the wasp views the feeder along specific compass bearings that can be maintained over a sequence of learning flights (Collett, 1995).

Correlations found between learning and return flights have led to several suggestions about when learning occurs. When *Vespula* is close to its goal on return flights, it tends to adopt body orientations that are similar to its body orientation at the end of arcs on learning flights (Collett, 1995), reinforcing the notion that views are stored when insects face the nest at the end of arcs. There are different resemblances between the learning and return flights of *Cerceris* (Zeil, 1993b; Zeil et al., 1996; Zeil et al., 2007). Here a measure of the similarity of different segments of the flights leads to a more extensive region of similarity that exists around the beginnings (Zeil et al., 2007) and ends (Zeil et al., 1996) of arcs. For most of the period of maximum similarity, wasps view the nest with lateral retina, suggesting that *Cerceris* may learn views at the boundaries of a flight corridor to the nest and keep within the corridor on its returns (Zeil et al., 1996). A third possibility is that learning occurs during short segments of learning flights (Dittmar et al., 2010), or continuously except when flying away from the nest (Baddeley et al., 2012).

In what follows, we show first the importance of the nest in structuring bumblebee flights. Bees change the angle between their flight direction and body orientation to keep the nest within their fronto-lateral visual field, despite large fluctuations in flight direction. We then identify which phases of learning and return flights contain points in which bees fly towards and face the nest. These coincident points occur frequently during the loop motifs of learning flights and the zigzag motifs of return flights. A comparison of these two stereotyped motifs shows that the nest-facing segments of loops and zigzags closely resemble each other, suggesting that bumblebees store visual information during flight segments in which they are broadly facing the nest.

## MATERIALS AND METHODS

### Data

This paper presents a further analysis of the learning and return flights of the bumblebee *B. terrestris* recorded out of doors (Hempel de Ibarra et al., 2009). Bees emerged from and returned to a nest-hole ~1 cm in diameter in the centre of a tabletop that was carpeted with a white bath mat to give the bees stabilizing visual texture. The location of the hole was marked by one or more vertical cylinders (2.3 cm wide and 20 cm high) placed either 8 or 20 cm from the nest-hole.

Flights were recorded at 25 frames $s^{-1}$ (50 half-frames $s^{-1}$) at high spatial resolution with a single Sony HD camera (Sony HDR HC7E, Tokyo, Japan) suspended 2 m above the nest-hole. The area surveyed by the camera was about 80 cm by 60 cm around the nest. Within this area we could capture the initial phases of learning flights and the final phases of return flights. Bees, in both cases, fly close to the surface of the tabletop.

The relevant portions of the video tapes were extracted using Adobe Premiere Pro (San Jose, CA, USA). The yaw orientation of the bee's longitudinal body axis was obtained from each frame using custom software written in MATLAB (MathWorks, Natick, MA, USA). The algorithm fits an ellipse to the image of the bee. Horizontal position ($x$–$y$ coordinate) is taken as the centre of the bee's image. Orientation is given by the angle of the major axis of the ellipse. Positions and orientations were checked by eye and corrected if necessary.

Rates of change of flight parameters were estimated using the MATLAB function 'gradient'. The bees' speed and direction of flight were estimated from the time derivate of the bees' position (a vector) by transforming it into polar coordinates. The retinal position of the nest or of a cylinder is equivalent to the positions of these objects relative to the bees' longitudinal axis. Retinal position here is an approximation that ignores the bumblebees' small head movements. The absolute mean difference between head and body orientation is 5.6±4.3 deg (±s.d.) [see fig. 3 in Hempel de Ibarra et al. (Hempel de Ibarra et al., 2009)].

### Analysis

In order to analyse individual loops and sequences of zigzags, we extracted short segments exhibiting these features from learning and return flights. Loops were extracted semi-automatically. The first step was to locate all points where a bee's path crosses itself within 4 s and to select segments joining these crossing points. Very short segments (<3 cm) were discarded. From the remaining pool, segments were considered loops, provided that they contained just a single crossing point and were not both large and multi-lobed. The crossing point was taken as the beginning and end of the loop.

Zigzags are harder to extract automatically. They are characterized by alternating phases of changing flight direction, but we have not found a principled way to define the length of each clockwise (CW) and counter-clockwise (CCW) phase. Thus for most of our analysis, zigzags were selected manually and limited to very clear sequences, as shown in example figures. The selected zigzags had several phases of zigs or zags in which flight direction oscillated about the axis of the zigzag, as given by the mean direction of flight over the zigzag sequence. The axis was relatively constant along the selected zigzags so that the sequence could be subdivided into zigs and zags at points where the absolute angles between the direction of flight and the axis of the zigzag are at a maximum. These selected zigzags were relatively common in return and learning flights. They occurred in 51% of return flights and comprised 22% of all return flights. Selected zigzags occurred in 54% of learning flights and occupied 10% of all learning flights.

Coincident points were defined as moments during flights in which flying towards the nest, within ±10 deg, coincided with facing the nest (i.e. the bee's body axis pointed at the nest within ±10 deg). These moments were usually brief. Most (81%) lasted only a single frame. Moments of coincidence were considered to be distinct from each other when separated by more than two half-frames of non-coincidence. When deciding which coincident points were in loops or zigags, we were less conservative in the selection of zigzags and included both shorter zigzags and those in which the direction of its axis changed along the motif. The frequency of zigzags in return flights estimated this way increased from 51% in the selected zigzags to at least 67%. Thus 89% of return flights had at least one coincident point, and 75% of these return flights had coincident points associated with zigzags.

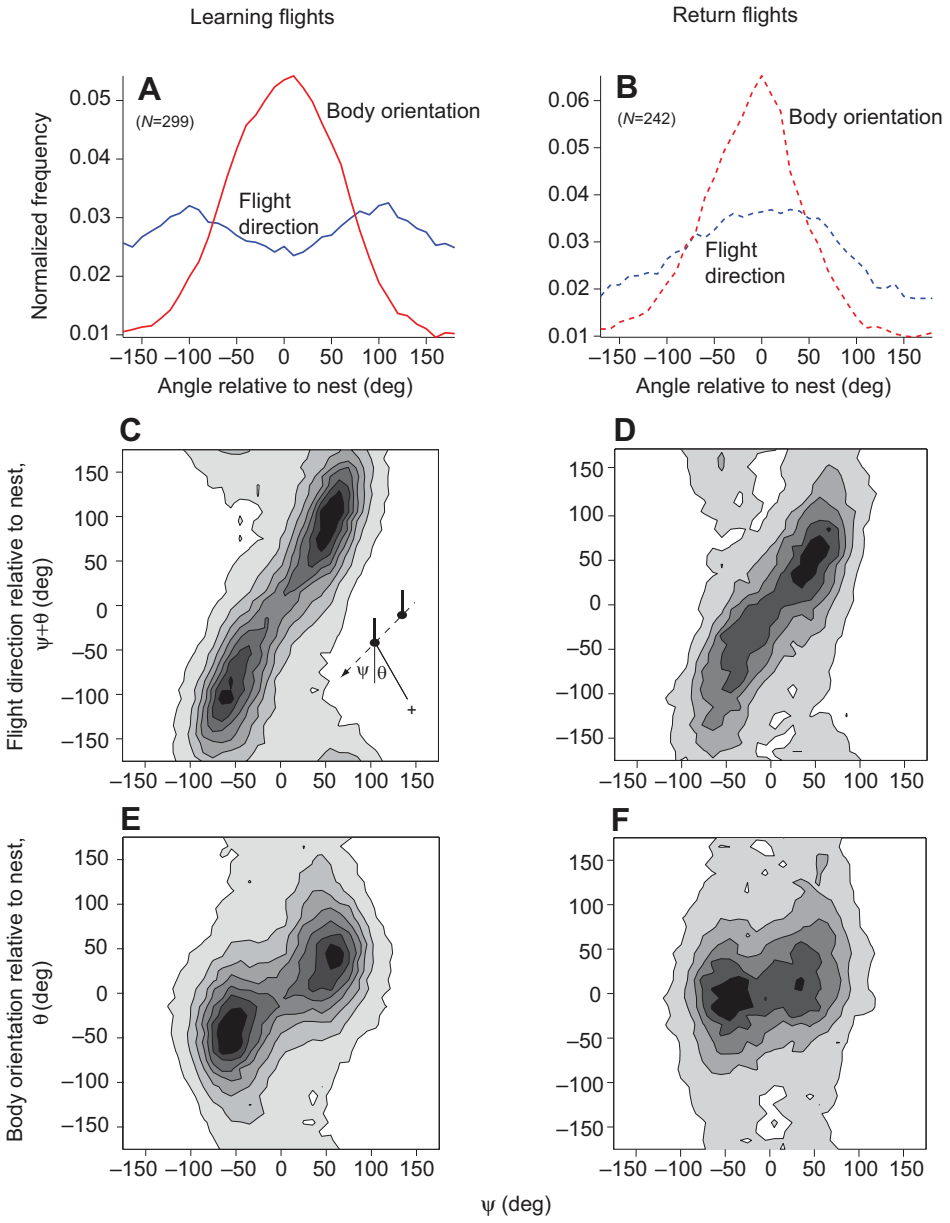


Fig. 1. The nest-directed nature of learning and return flights. (A,B) Frequency distributions of body orientations (red lines, θ, see inset in C) and flight directions (blue lines, ψ+θ) relative to the nest are pooled over 299 learning (left-hand column) and 242 return (right-hand column) flights. The flights come from different groups of bees each with one or two cylinders near the nest. (C,D) Contour plot of flight direction relative to nest (ψ+θ) *versus* ψ; inset illustrates ψ, θ and the nest (+). (E,F) Contour plot of body orientation (θ) *versus* ψ. Contour plots in (C,E) comprise 169,798 data points and both plots step in multiples of 100 from white (<100) to black (≥700). The plots of (D,F) comprise 64,015 data points and both plots step in multiples of 50 from white (<50) to black (≥250). Here and in other figures angular values increase clockwise (see inset to E), frequency distributions are normalized so that their area is 1, and, unless specified otherwise, bin widths are 10 deg.

When bees are close to the nest at the beginning of learning flights or at the end of return flights, their flight manoeuvres are very small in extent and not well-resolved in our recordings. To exclude these portions of the flights (on average 22% of learning and 15% of return flights), we began our analysis of loops, zigzags and coincident points when bees first left a 5 cm radius circle around the nest on learning flights, or last entered it on return flights.

Statistical tests of differences between data distributions use each flight or incidents within flights as independent data points. The significance of the differences was assessed with non-parametric Wilcoxon paired or unpaired rank tests or the Kolmogorov–Smirnov test using the MATLAB statistics toolbox. Calculations and statistics on circular data were performed using another MATLAB toolbox (Berens, 2009).

## RESULTS

### Flight and facing directions are controlled relative to the nest

The nest-directed nature of learning and return flights emerges clearly from the relationship between the bees' flight direction and their body orientation. Many insects fly sideways and backwards as well as forwards (Collett and Land, 1975; Taylor, 2001; Ristroph et al., 2009) and so vary the angle (ψ, Fig. 1C) between their direction of flight and the orientation of their body. In bumblebees, the value of ψ changes during learning flights in such a way that the nest-hole remains within the fronto-lateral region of the visual field, while flight direction fluctuates more widely as bees perform manoeuvres close to the nest (see example in Fig. 2A).

Details of the changing pattern of ψ over whole learning flights are documented in the plots of Figs 1 and 2. When bees fly towards the nest, they tend to fly forwards (ψ=0 deg) facing the nest, with flight direction and body orientation aligned (Fig. 1C). As flight direction rotates away from the nest, the value of ψ diverges increasingly from 0 deg, but by half as much (Fig. 1C). Correspondingly, body orientation changes at about half the rate of flight direction (supplementary material Fig. S1) and stays closer to the direction of the nest. The bee's flight direction relative to the nest (ψ+θ in Fig. 1) is distributed broadly with symmetrical peaks

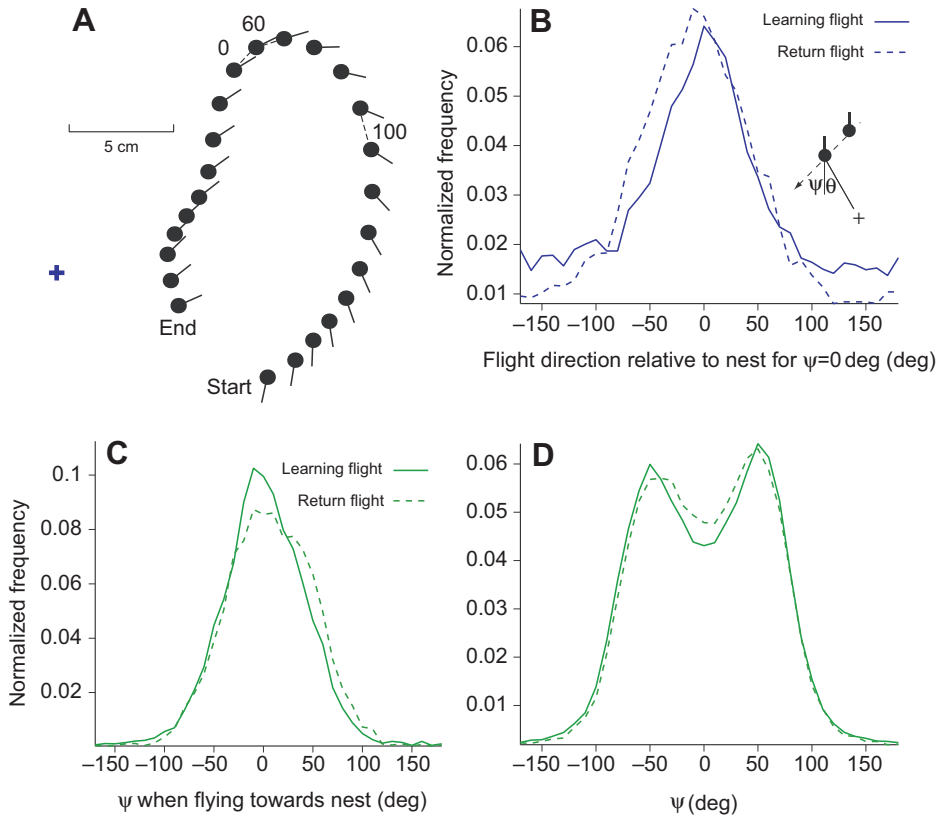


Fig. 2. Relationship between body orientation, ψ and flight direction during learning and return flights. (A) Part of a loop to illustrate that body orientation points closer to the nest (blue +) than flight direction. Bee's position (●) and orientation (/) are plotted every 20 ms. Dashed lines and associated numbers show direction of flight relative to the nest for illustrative moments. (B) Frequency distributions of flight directions relative to the nest during learning and return flights when ψ is within ±10 deg of 0 deg. Flight directions cluster more tightly around the position of the nest than distributions covering the whole range of ψ ($P$<0.001). (C) Frequency distribution of ψ when the bees' flight direction was within ±10 deg of the nest. (D) Frequency distributions of the values of ψ during complete learning and return flights. Data come from the 299 learning and 242 return flights used for Fig. 1.

at 100 deg either side of the nest (Fig. 1A), comparable to the wasp *Cerceris* (Zeil, 1993a). Body orientations relative to the nest (θ) and equivalently the retinal position of the nest have a narrower distribution with a single prominent peak in the direction of the nest (Fig. 1A), unlike *Cerceris* (Zeil, 1993a).

When ψ lies within the range ±10 deg, the distributions of flight directions (Fig. 2B) are clustered more closely on the nest than are distributions encompassing the whole range of ψ (Fig. 1A). The statistical significance of this difference was tested by obtaining the mean vectors for each flight, either with all frames included, or when the range of ψ is limited to ±10 deg. The mean directions of the vectors do not differ, but the lengths of the vectors are significantly longer for the limited range of ψ than they are for the whole flight ($P$<$10^{-9}$, paired rank-sum test).

On return flights (Fig. 1B,D,F), with no need to explore around the nest, the range of flight directions relative to the nest is narrower than in learning flights and the distribution peaks towards the nest (Fig. 1B). Despite differences in the distributions of flight directions, learning and return flights have similar distributions of ψ values, both when flight directions are within ±10 deg of the nest (Fig. 2C) and over complete flights (Fig. 2D). As in learning flights, flight directions are focused significantly more closely on the nest, when ψ lies within the range ±10 deg (Fig. 2B) than it is when ψ is unconstrained (Fig. 1B). The distribution of body orientations in return flights is correspondingly tighter and almost independent of the value of ψ (Fig. 1F). It is unclear why bees avoid ψ values that are greater than about 90 deg. It may be for aerodynamic reasons, or because visual control is impeded if the direction of flight lies far outside the fronto-lateral visual field. The coincidence of facing and flying towards the nest emphasizes the centrality of the nest in the patterning of learning and return flights.

### Coincidences between flying towards and facing the nest during the motifs of learning and return flights

Coincidences of flying towards and facing the nest (±10 deg) seem to be key points within learning and return flights. To determine how these coincident points are distributed within the flights analysed in Figs 1 and 2, we extracted the segments of the flights containing them. Eighty-eight percent of learning flights and 89% of return flights had coincident points. When possible, we identified the motif to which each coincident point belonged. Of the 1872 coincident points of learning flights, 13% occurred in zigzags, 37% in loops, 8% in segments that belonged to both zigzags and loops, and 42% fell outside these categories. The distribution between these categories differed for return flights. Of 773 points, 54% occurred in zigzags, 7% in loops, 4% in both zigzags and loops, and 35% could not be categorized. Because coincident points occur mostly in loops during learning flights and in zigzags during return flights, in what follows we focus on these two combinations. However, it is important not to forget the large minority of uncategorized coincident points, many of which occurred at sharp bends.

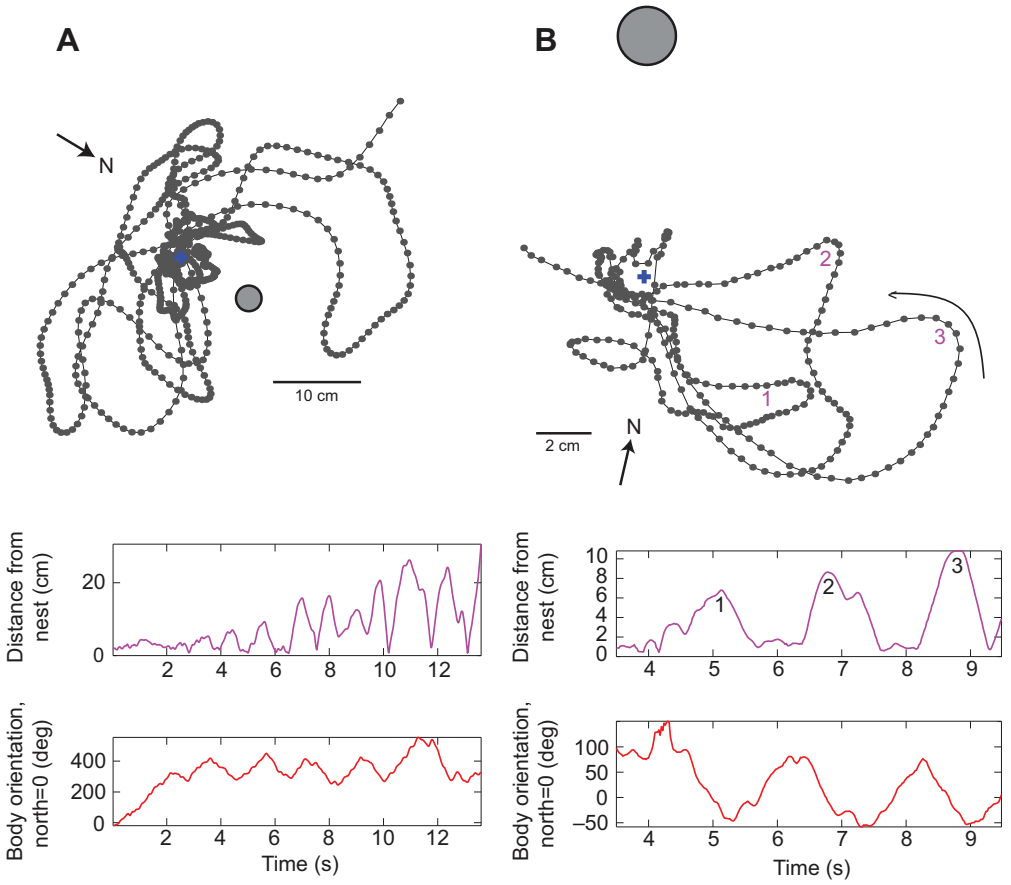


Fig. 3. Loops that return bees to the vicinity of the nest. (A) Complete flight with sequence of returns to the nest (blue +). Each excursion tends to take the bee further from the nest. Time plots of distance and body orientation indicate that the bee turns in one direction during an excursion, alternating turn direction on each excursion. (B) A different pattern of returns. The large loops numbered 1 to 3 are all CCW. Between these loops, small CW manoeuvres keep the bee close to the nest. Bees' position is shown every 20 ms. Cylinder is shown by the grey disc. Time plots below show distance from the nest (magenta) and body orientation (red) in compass coordinates; north=0.

### The loops of bumblebee learning flights

Bumblebee learning flights have a loop-like structure that differs somewhat from the arcing pattern described in *Cerceris* and *Vespula*. Loops occur on most flights. Ninety-eight percent of flights on the bees' first three departures from the nest contained loops. The percentage fell to 89% in flights 4–6, to 85% in flights 7–9, and to 71% in flights 10–12. Loops occupied at least 39% of the durations of all learning flights. Loops are also seen on return flights (e.g. Fig. 4B). They occur in 61% of return flights and take up 14% of those flights. The prevalence of loops can be seen in the partial sequence of learning and return flights displayed in supplementary material Fig. S2.

A possible reason for the prominence of loops in learning flights is the bee's tendency to fly away and return close to their nest several times during a single flight (e.g. Fig. 3A,B). Unlike wasps or honeybees which 'turn back and look' at their goal (Lehrer, 1993), bumblebees often fly back and look at it. Loops that return bees close to the nest are common in flights with a single cylinder 8 cm from the nest (supplementary material Fig. S3A,C). Loops in flights with two cylinders 20 cm from the nest are more likely to bring the bees part way rather than all the way to the nest (e.g. Fig. 4A, supplementary material Fig. S2, Fig. S3B,D). Bumblebees may return towards the nest because of the complexity of the nest surroundings and in order to supplement visual cues with odour information (Hempel de Ibarra et al., 2009).

### The predominantly nest-centred structure of loops

A common feature of loops is that bees tend to face and fly towards the nest at a particular phase of the motif. We take the start and end points of a loop to be where the bee's flight path crosses. At the start (* in Fig. 5A) of a prototypical tear-shaped loop, the bee's direction of flight relative to the nest is oblique to its body orientation and the angle between body orientation and flight direction, ψ, tends to be relatively large. The lateral region of one eye is directed towards the nest, when the bee enters the loop, and the fronto-lateral region of the other eye, when the bee leaves, in a way that bears some similarity to the behaviour of *Cerceris* at the transition between two arcs (Zeil, 1993a). The value of ψ and the bee's flight direction relative to the nest changes smoothly, but asymmetrically, over the course of the loop, with the result that the body is more closely oriented towards the nest than its flight direction. The value of ψ reaches 0 deg just beyond the middle of the loop, the point at which the bee faces the nest. The absolute value of ψ then grows again, but to a smaller extent, during the second half of the loop.

The example of Fig. 5A generalizes over many loops of varying sizes and shapes and, in some respects, across different landmark arrangements (but see supplementary material Fig. S4). We combined loops of different sizes, shapes and durations by normalizing the duration of the loop between 0 at the start and 1 at the end. The value of ψ is initially large and drops to 0 deg at about 0.6 through the loop. It grows again during the second half (Fig. 5B). The ridges of the contour plot that curve to the left correspond to CCW loops and those curving to the right to CW loops. The median values of ψ calculated for each loop differ significantly between 0 and 0.6 through the loop (test for circular medians, $P<10^{-9}$) and the absolute difference between the medians of ψ at these points is 60 deg. Flight direction relative to the nest follows a similar pattern (Fig. 5C), reflecting the coincidence between facing the nest and flying towards it. The median values of flight direction relative to

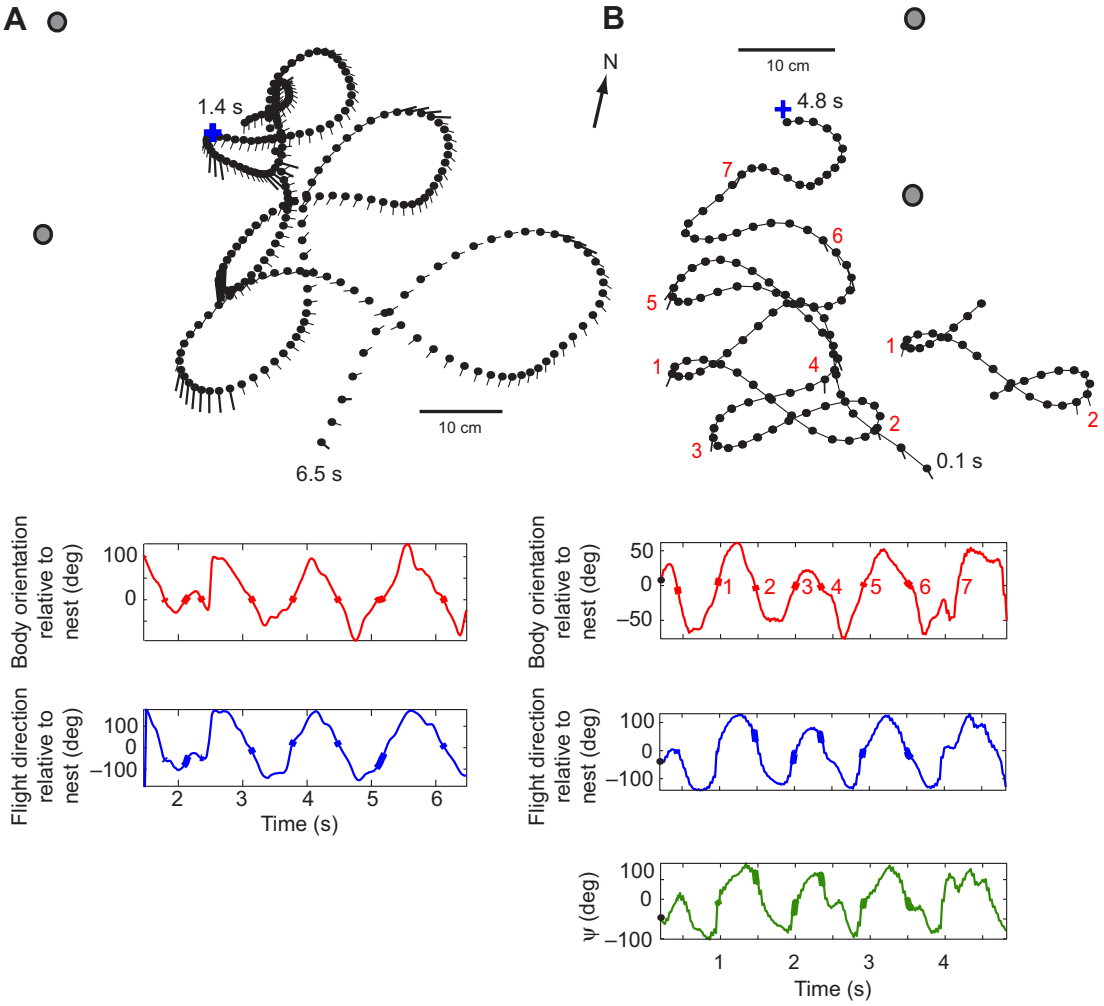


Fig. 4. Loops and zigzags. (A) Sequence of loops from a learning flight with two cylinders west of the nest. (B) Excerpt from return flight with two cylinders east of the nest showing an uncommon transition between loops and zigzags. Body orientations are lengthened and thickened (A) or added (B) when bees face within 10 deg of the nest. Time plots of body orientation (red) and flight direction (blue) relative to the nest (A,B) and the value of ψ (B, green) every 20 ms indicate the alternating handedness of loops and of zigs and zags. Inset in B shows loops extracted from the excerpt. The data line is thickened when bees face within 10 deg of the nest. Conventions as in Figs 2 and 3.

the nest (ψ+θ) at 0 and at 0.6 also differ significantly ($P<10^{-9}$), with an absolute difference of 128 deg. As bees probably cannot see the nest-hole, the coincidence of flying towards the nest and a value of ψ close to 0 deg suggests that they may 'know' its position through path integration (Müller and Wehner, 2010).

The relation between facing the nest and the value of ψ during loops depends to some degree on the arrangement of cylinders around the nest (supplementary material Fig. S4). With two cylinders 20 cm to the east or west of the nest-hole, the bees' facing direction was centred on the nest when ψ was 0±10 deg. In contrast, with a single cylinder either 8 cm north or east of the nest-hole, bees tended to face it, and not the nest, when ψ was 0±10 deg. It may be that, if a landmark is close to the nest-hole, it provides a reliable and easily locatable substitute for the nest-hole.

### Zigzags

Zigzags on return flights (Fig. 4B, supplementary material Figs S2, S5) share several properties with the loops of learning flights. The changing values of ψ (Fig. 5D) and of flight direction relative to the nest (ψ+θ) during normalized half-zigzags follow a somewhat similar course to those of loops (Fig. 5E). In both loops and zigzags, ψ is close to zero when bees fly towards the nest (Fig. 5). As with loops, the median values of ψ and ψ+θ are significantly smaller at 0.6 through a zig or zag than at the start of the motif (test for circular medians, $P<10^{-9}$). Thus the absolute difference in ψ between the two positions is 34 deg, and in flight direction (ψ+θ) it is 66 deg, roughly half the values found in loops. These differences (see supplementary material Fig. S6) arise because the loops of learning flights tend to take bees away from and towards the nest and so cover a wider range of flight directions relative to the nest than do zigzags with their axis towards the nest.

### The similar patterning of loops and zigzags

During both loops and zigzags, the bees' body orientation and flight direction relative to the nest oscillate approximately in synchrony (see Fig. 4 for example and Fig. 5 for general trends). A period of rotation in one direction corresponds to a single loop or to half a zigzag centred on a turn. The repeated oscillations reflect a pattern of alternating handedness between neighbouring loops during learning flights (Fig. 4A) and a sequence of zigzags on return flights (Fig. 4B). The pattern of alternating handedness of loops seen in Fig. 4A is not always as clear, but alternation occurs frequently. Of

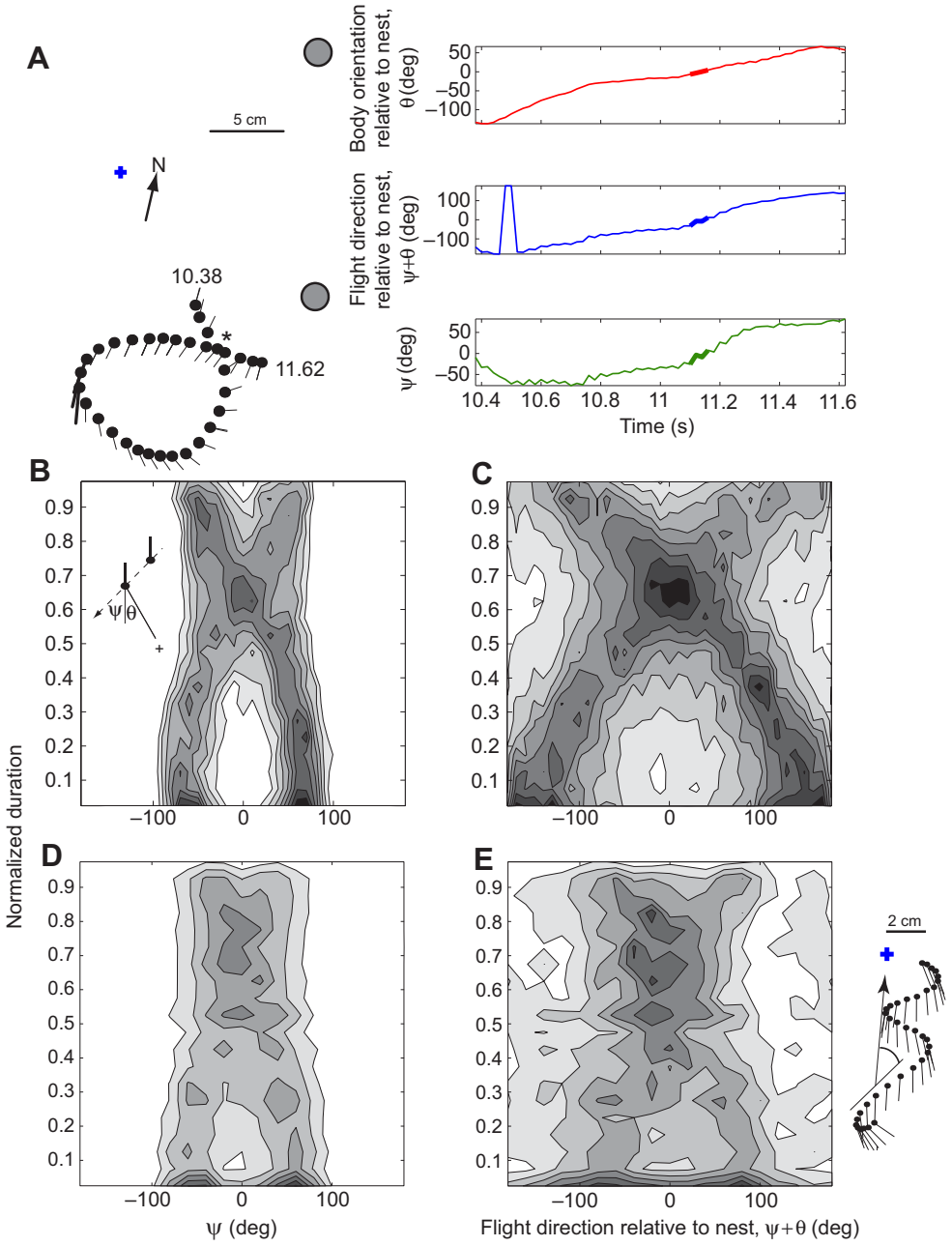


Fig. 5. Some common properties of loops and zigzags. (A) Example loop, ∗ indicates start and end points. On the right are time plots of body orientation (θ, red), flight direction relative to the nest (θ+ψ, blue), and ψ (green). Traces are thickened when bee faces within ±10 deg of nest. (B,C) Contour plots of ψ (B) and flight direction relative to the nest (C) during the course of 1306 normalized loops from 299 learning flights. '0' on ordinate denotes start of loop, and '1' the end. Width of normalized time bins is 0.05. Plots contain 47,578 data points. In B, contours step by 25 data points from <25 (white) to ≥200 (black). In C, steps are 20 data points from <20 (white) to ≥160 (black). (D,E) Similar contour plots of flight direction relative to the nest (D) and ψ (E) during the course of 488 half-zigzags from 242 return flights. Both plots contain 9407 data points. Contours step by 10 data points from 0 (white) to ≥70 (black). Inset: example of zigzag with arrow to show mean flight direction over the course of a zigzag.

824 pairs of neighbouring loops extracted from all learning flights, 572 (69%) are of opposite handedness.

Alternating loops (Fig. 4A) have in common with the parallel 'rungs' of alternate zigzags (Fig. 4B, supplementary material Fig. S2) that the entrance to one loop or zig tends to be parallel to the exit from the neighbouring loop or zag. The similarities and differences between a full zigzag cycle and a pair of loops of opposite handedness are particularly obvious when loops and zigzags are mingled. In the example of Fig. 4B, the excerpt starts with a zigzag towards the nest. It is followed by a pair of loops carrying the bee away from the nest, and it ends with a run of zigzags to the nest. Time plots of body orientation, flight direction relative to the nest and their difference (ψ) show no discontinuity, when one motif turns into the other. However, the ranges of body orientations, flight directions and ψ during the two loops are larger during the loops than in the adjacent zigzags (cf. supplementary material Fig. S6). The continuity between loops and zigzags seen in Fig. 4B suggests that their control mechanisms are closely related.

Broad similarities also exist in the way that rotational and translational speeds change over the course of individual loops and zigzags (Fig. 6). Bees tend to begin the motif at a relatively fast speed, slow down towards the middle and speed up at the end. These trends are superimposed on a general increase in a bee's translational speed with its distance from the nest (Fig. 6A) that occurs across learning and return flights, and is also found in *Cerceris* (Zeil, 1993a). Rotational speed, as in *Cerceris*, barely changes (Fig. 6B). To accommodate the systematic change in translational speeds with distance, translational speed during each loop and half-zigzag is normalized between 0 and 1 (Fig. 6G,H). The contour plots show absolute rotational speed (Fig. 6E,F).

Translational speeds during loops are lowest about 0.6 through the motif (Fig. 6G), just after ψ reaches 0 deg and bees face the nest.

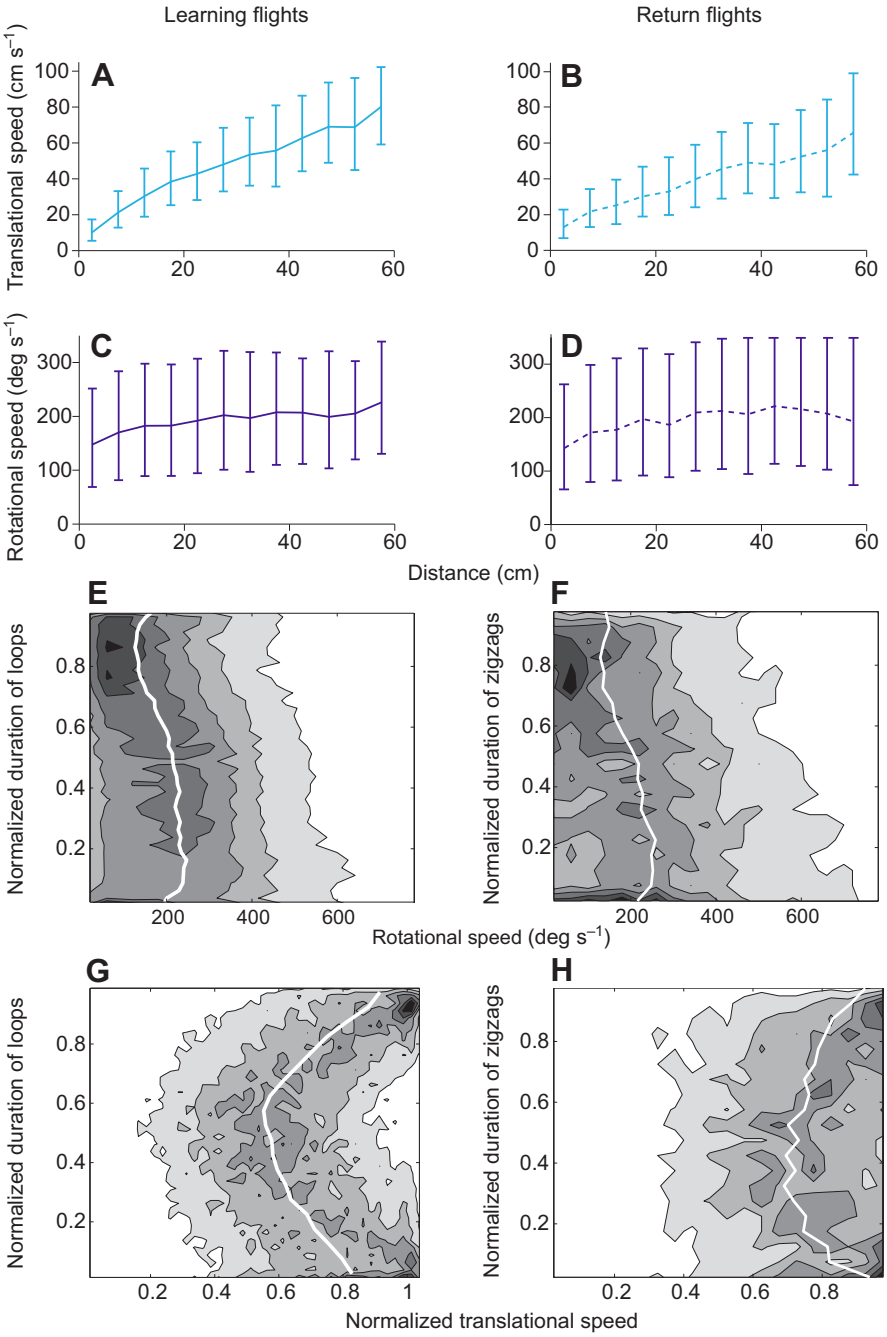


Fig. 6. Translational and rotational speeds during learning and return flights. (A–D) Median translational (A,B, light blue) and angular speeds (C,D, purple) plotted against the bees' distance from the nest during 299 learning (A,C, continuous lines) and 242 return (B,D, dashed lines) flights. Bin size is 5 cm and error bars show interquartile range (IQR). Data points in successive bins of increasing distance decrease from 84,000 in first bin to <1000 in last bin for learning flights and from 17,000 to <1000 for return flights. (E–H) Contour plots of normalized translational speed (E,F bin width 0.05) and absolute rotational speed (G,H, bin width 60 deg $s^{-1}$) during the course of 1306 normalized loops from 299 learning flights (E,G) and of 488 half-zigzags from 242 return flights (F,H). The white line shows median speed. Speeds within each loop or zigzag are normalized by the 90th percentile of the speed within that loop. Contour plots of loops consist of 47,578 data points and step by 50 data points from <50 (white) to ≥300 (black); zigzags contain 6483 data points and step by 10 data points from <10 (white) to ≥60 (black).

Zigzags exhibit a similar slowing with a broader minimum (Fig. 6E,F). Rotational speeds of both loops and zigzags tend to be lowest just before bees fly out of the loop (Fig. 6E,F). The statistics of these minima are considered in supplementary material Fig. S7 and its legend.

### Bees obtain similar views during loops and zigzags

If loops and zigzags have the role of acquiring and using visual information for view-based navigation, we would expect them to generate similar visual input. To compare the views gained during loops and zigzags, we selected motifs from flights with two cylinders to the east or west of the nest in which flying towards the nest (within ±10 deg) coincided with facing the nest (within ±10 deg). For these selected loops and zigzags, the duration of the overlap between facing and flying towards the nest was only one 20 ms frame in ~90% of cases. The selection procedure means that loops and zigzags are aligned on the coincident points, which makes it easier to spot similarities and differences away from this point.

The values of ψ, the retinal positions of the nest and of the more northerly cylinder are plotted against the bees' flight direction relative to the nest in Fig. 7. Each row displays a different set of loops and zigzags with cylinders to the east or west of the nest during CW or CCW turns. The changing values of ψ examines the similarity of egocentric flight parameters across loops and zigzags. The retinal position of the nest tests the similarity of the motifs in terms of nest-based co-ordinates. The retinal position of the cylinder adds information about the similarity of the directions of the motifs in compass co-ordinates and tests whether bees obtain similar views of their surroundings during loops and zigzags.

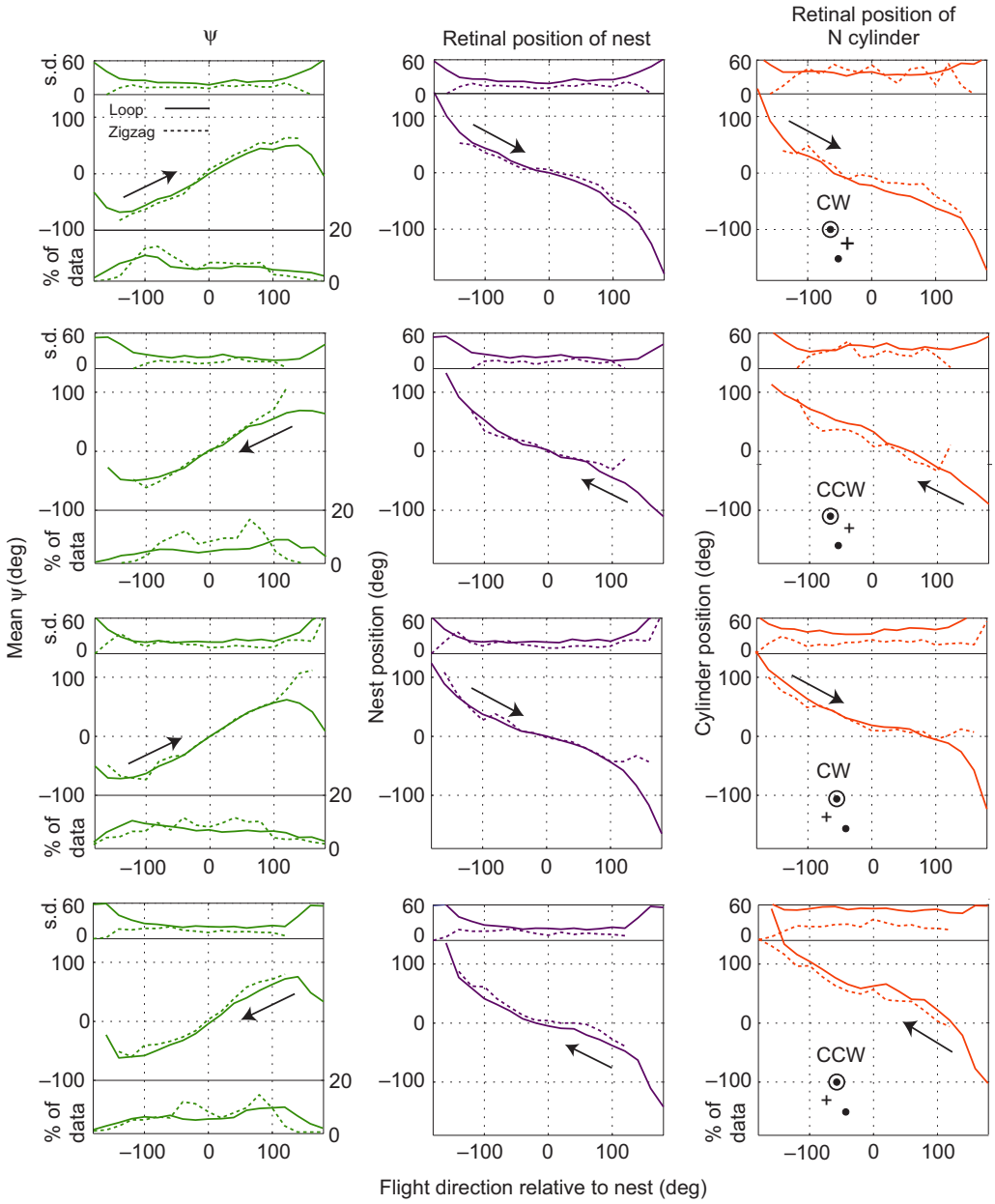


Fig. 7. Values of ψ (green), retinal positions of nest (purple) and of northerly cylinder (orange) plotted against flight direction relative to nest during loops and zigzags. Each row shows CW or CCW turns from flights with two cylinders (•) west or east of the nest (+); see insets of third column. North is up and the N cylinder is encircled. Error plot at the top of each panel shows the circular standard deviation. Bottom of left-hand panel gives the relative percentage of data points in each 20 deg bin with ordinate between 0 and 20% on the right. Motifs progress in time from negative to positive values of flight direction for CW turns and from positive to negative for CCW turns, as indicated by the arrow; 20 deg bins are centred on 0, 20, etc. Data for each row are: (1) 3608 data points in 113 loops from 50 flights from 9 bees; 234 data points in 23 zigzags from 14 flights from 8 bees; (2) 2565 data points in 89 loops from 40 flights from 9 bees; 188 data points in 18 zigzags from 12 flights from 7 bees; (3) 4112 data points in 112 loops from 53 flights from 9 bees; 427 data points in 27 zigzags from 15 flights from 8 bees; (4) 4443 data points in 118 loops from 47 flights from 8 bees; 264 data points in 21 zigzags from 13 flights from 5 bees.

Over a substantial range of flight directions relative to the nest (approximately ±50 deg), the profiles of ψ and the retinal positions of the nest are similar across loops and zigzags. Because changes in ψ act to keep body orientation pointing close to the nest, the frontal retinal position of the nest changes relatively slowly as flight direction diverges from the nest over a range of about of ±50 deg.

Because loops explore the surroundings in a variety of compass directions and zigzags are more concentrated on a particular return direction, plots of the retinal position of the more northerly cylinder are less similar between loops and zigzags, particularly for flights with two cylinders to the west (Fig. 7, right-hand column, rows 1 and 2). For CW and CCW zigzags during these flights bees face the cylinder when flying towards the nest, indicating that bees may have learnt to take up a compass orientation along a bearing that runs from the nest to the cylinder. By flying along this bearing while facing the cylinder, the bee can potentially reach the nest (cf. Zeil, 1993a; Hempel de Ibarra, 2009). With two cylinders east of the nest (Fig. 7, right-hand column, rows 3 and 4), the same pattern occurs during CW turns. During CCW turns, the cylinder is in the right visual field when the bee flies towards the nest.

The spatial similarity of loops and zigzags over a limited range of flight directions around coincident points is paralleled by similar temporal rates of changes of flight direction, but marked temporal differences exist across the complete motifs. Fig. 8A shows how flight direction relative to the nest changes over the course of a set of loops and half-zigzags that were selected to have similar durations. Flight direction in loops changes relatively slowly at the start and end of the motif and more rapidly in the middle. Changes in flight direction are more uniform over the course of a zigzag. These differences are shown for the complete set of loops and zigzags in Fig. 8B. The abscissa is flight direction relative to the nest with CCW motifs reflected so that all loops and half-zigzags start on the left. The ordinate shows the median time that it takes the bees to change flight direction by 20 deg. At the start of the motifs, but less clearly at the end, flight direction changes more slowly in loops than in zigzags.

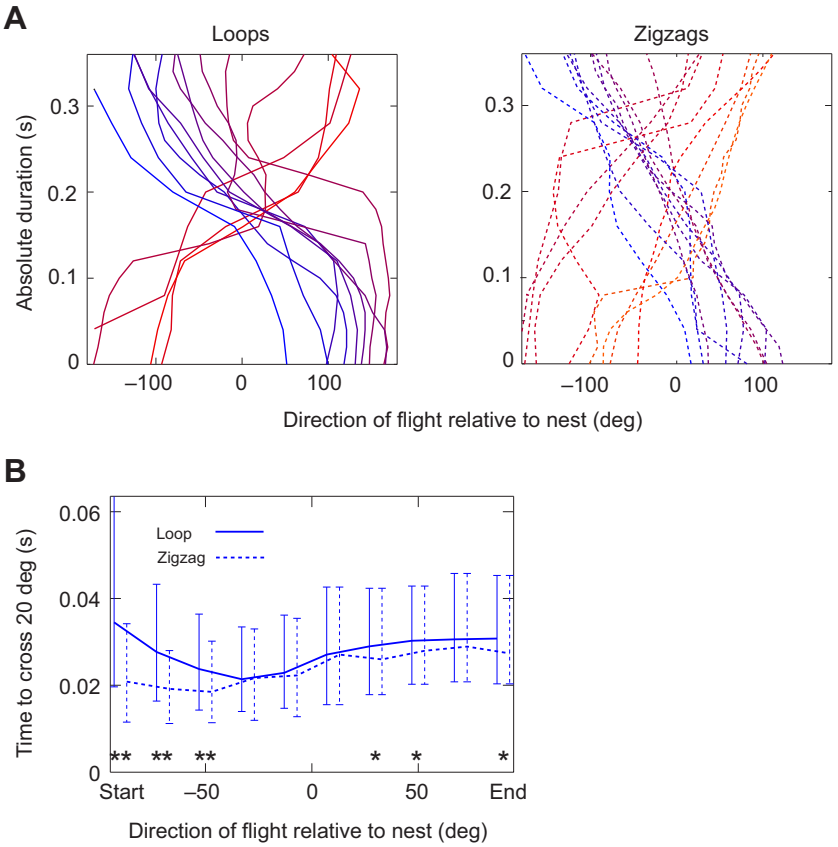


Fig. 8. Temporal patterning of loops and zigzags. (A) Time during loop plotted against flight direction relative to the nest for 13 loops and 18 zigzags with similar durations (340–360 ms). (B) Median duration and 25% and 75% IQR over successive segments of loops (continuous line) and half-zigzags (dashed line). In each segment bees change their flight direction relative to the nest by 20 deg. Data comprise 556 loops and 253 half-zigzags from flights with two cylinders east and west of the nest. CCW motifs are reflected so that all start on the left. Sample size drops towards the edges of the plot, reaching a minimum of 412 for loops and 78 for half-zigzags. Loops and zigzags are staggered to avoid overlap. Probability levels are two-tailed (*0.01<$P$<0.05; **$P$<0.001).

For flight directions close to the nest, the directional changes of loops and zigzags follow a similar pattern and give bees similar visual input on outward and return flights.

### The similarity of views across less constrained loops and zigzags

The selection process to provide the data for Fig. 7 discarded about 50% of loops and 80% of zigzags. An analogous plot for loops and zigzags without coincident points for flights with two cylinders to the west is shown in supplementary material Fig. S8A. The relationships between flight direction relative to the nest and ψ, the retinal positions of the nest and the northerly landmark are similar to those of Fig. 7, but with somewhat greater scatter. The same plot for segments of learning and return flights with two cylinders to the west that lie outside identified loops and zigzags (supplementary material Fig. S8B) differs qualitatively. The value of ψ does not increase in an orderly fashion with increasing flight direction from the nest and remains relatively flat. In consequence, the retinal position of the nest diverges more quickly from the bee's frontal visual field. The same plots for flights with two cylinders to the east (not shown) are less tidy, but the patterns generated by flight segments that lie outside loops and zigzags are more similar to those of loops and zigzags. Taken together, the data of Fig. 7 and supplementary material Fig. S8 reinforce the notion that loops and zigzags are specialized motifs for the uptake of nest-related information.

## DISCUSSION

### Storing and using views for guidance

The close similarity in the visual input generated during segments of bumblebee loops and zigzags is consistent with the suggestion from several species of bees and wasps (see Introduction) that brief segments of flight in which insects face the nest are good candidate points for information acquisition. One possibility, then, is that bumblebees store visual information during the loops and possibly zigzags of their learning flights, while facing and flying towards the nest, and access the same visual information during these coincident points in the zigzags and possibly loops of return flights. Modelling shows that images stored when facing a goal can guide returns to it (Graham et al., 2010). However, as suggested by the use of parallax information during homing (see 'Visual feedback during loops and zigzags' section), learning may be less restricted and occur over longer segments of loops and zigzags surrounding coincident points (cf. Dittmar et al., 2010; Dittmar et al., 2011).

As we have seen, bumblebee learning and return flights resemble those of *Cerceris* in several respects. Most notably, in both insects, visual input is similar across relatively long segments of learning and return flights, but periods of direct fixation on the nest are brief. Major differences are the prevalence of loops in the learning flights of bumblebees and the retinal position of the nest. In bumblebees, the nest tends to be located mostly in the frontal visual field of the insect, whereas in *Cerceris* it is positioned significantly more laterally (Zeil, 1993a; Zeil, 1993b; Zeil et al., 1996). It is not yet known whether these differences reflect slightly different homing strategies in the two insects.

### Visual feedback during loops and zigzags

The central segments of loops and zigzags are similar and relatively stereotyped. In addition to providing static views, the visual feedback generated during these segments can inform bees about the 3-D structure of the surroundings near the nest. Wasps and bees are known to obtain parallax-derived distance information from their learning and return flights for two somewhat different functions. It can allow returning wasps (Zeil, 1993a), honeybees (Lehrer and

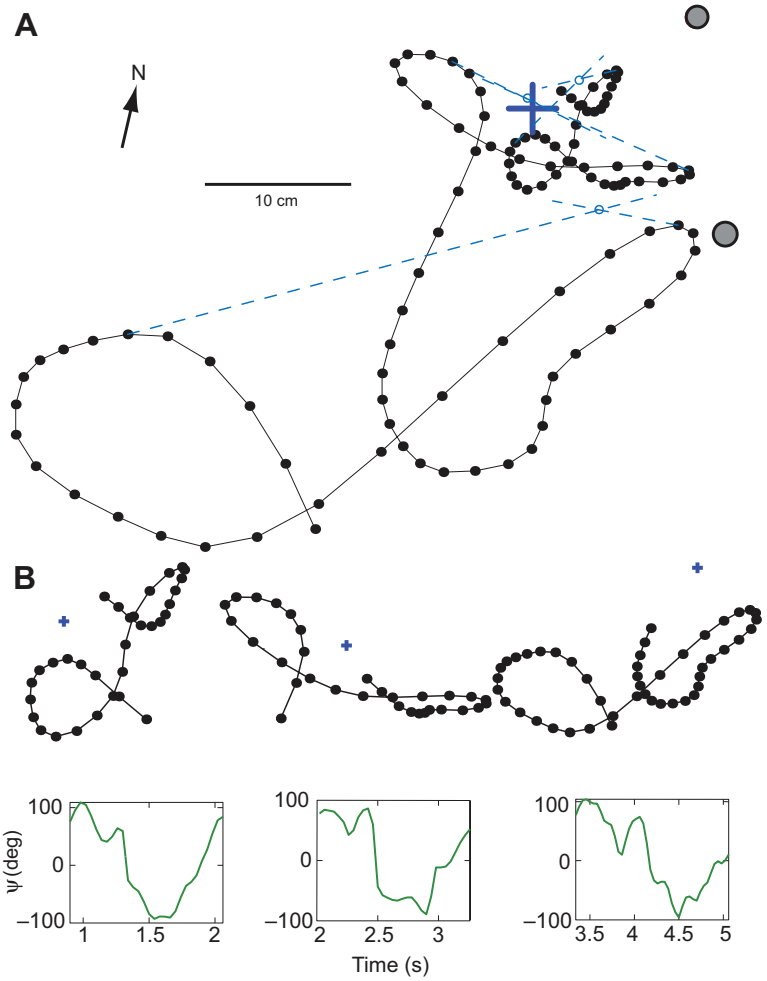


Fig. 9. Invariance of loop shape to compass direction and size. (A) Excerpt from the ninth learning flight of a bee with its position shown every 20 ms. Dashed blue lines show flight direction from points in loops where ψ is zero. Cylinders are marked by grey discs, and the nest by the blue cross. (B) Similarity of resized pairs with time plot of ψ over the course of each pair.

Collett, 1994) and ground nesting bees (Brünnert et al., 1994) to control their distance from a familiar landmark near to a goal. It also enables wasps and bees to check their surroundings for nearby visual features that can then be emphasized in stored views (Cheng et al., 1987; Dittmar et al., 2011). We will consider in more detail elsewhere the likelihood that nearby features are highlighted during the first part of a loop, as bees scan their surroundings while turning to face the nest. Similar information is available during the analogous segments of zigzags to guide returns.

Bumblebee loops, like the arcs of wasps (Zeil, 1993a), tend to grow in size during a learning flight, largely independently of their shape (Figs 4, 9, supplementary material Fig. S2). Larger loops tend to be flown at higher speeds and often start further from the nest. This increase in size and speed will lead to a progressive scaling of distance measurements. At the start of a flight, the scan will only differentiate between features close to the nest. The increase in speed with loop size allows the range of discriminable distances to enlarge as the bee moves further from the nest.

### Generating loops and zigzags

Loops and zigzags are organized both relative to a nest-based reference frame (Fig. 5) and to one that is intrinsic to the motifs. Thus sequences of zigzags on return flights occasionally point in directions that are 90 deg or more away from the nest (e.g. supplementary material Fig. S5). The compass direction of the axis of a sequence of loops may be maintained over several pairs of loops (Fig. 4A). It can also rotate after each pair of loops. The rare example of Fig. 9 shows a pair of loops repeated three times in different orientations at increasing size. The first loop of each pair is distinctly elongated with a slight protuberance near the crossing point, and the second loop is rounder. Despite large changes in orientation, speed and size, the individual loops retain their shape across each repetition, indicating that shape is controlled independently of overall travel speed and loop size.

This example is helpful in suggesting that pairs of loops are distinct entities and, by analogy, that complete zigzag cycles are as well. We thus conjecture that loops and zigzags are generated by a fixed pattern of changing flight direction relative to an intrinsically chosen axis, which is often aligned with the nest. Much of the basic pattern is similar across loops and zigzags and is, in some ways, comparable to the arcs of wasps (Collett and Lehrer, 1993; Zeil, 1993a). We do not discount the possibility that zigzags and loops are assembled hierarchically from arc-like components. The bee switches between loops and zigzags by enlarging or restricting the range of flight directions relative to the axis of the motifs (Fig. 4B, supplementary material Fig. S6) and by adjusting its speed.

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