

SHORT COMMUNICATION

Honeybees can learn the relationship between the solar ephemeris and a newly experienced landscape: a confirmation

Jordan R. Kemfort and William F. Towne*

Department of Biology, Kutztown University of Pennsylvania, Kutztown, PA 19530, USA

*Author for correspondence (towne@kutztown.edu)

SUMMARY

Honeybees learn the spatial relationship between the sun's pattern of movement and the landscape immediately surrounding their nest, which allows bees to locate the sun under overcast skies by reference to the landscape alone. Surprisingly, when bees have been transplanted from their natal landscape to a rotated twin landscape – such as from one treeline to a similar but differently oriented treeline – they fail to learn the relationship between the sun and the second landscape. This raises the question of whether bees can ever learn the relationship between the sun's pattern of movement and a landscape other than their natal one. Here we confirm, with new and necessary controls, that bees can indeed learn the relationship between the sun's pattern of movement and a second (that is, non-natal) landscape, if the second landscape is panoramically different from the bees' natal site. We transplanted bees from their natal site to a panoramically different second site and, 3 days later, tested the bees' knowledge of the relationship between the sun and the second landscape. The test involved observing the bees' communicative dances under overcast skies at a third site that was a rotated twin of the second. These bees oriented their dances using a memory of the sun's course in relation to the second landscape, indicating that they had learned this relationship. Meanwhile, control bees transplanted directly from the natal site to the third site, skipping the second, danced differently, confirming the importance of the experimental bees' experience at the second site.

Key words: orientation, sun compass, learning, panorama.

Received 29 January 2013; Accepted 1 July 2013

INTRODUCTION

The waggle dances of honeybees, *Apis mellifera* Linnaeus, performed on the vertical combs inside the nest, indicate the directions toward food sources in the field, such that directly upward on the comb represents the sun's azimuth in the field. Thus, one can infer a bee's estimate of the sun's azimuth by observing the bee's dances for a known food source. Dyer used this method to show that honeybees learn the relationship between the sun's pattern of azimuthal movement (the solar ephemeris function) and the landscapes near their nests (Dyer and Gould, 1981; Dyer, 1987; Towne and Moscrip, 2008). In a typical experiment, Dyer transplanted a hive from its home site beside a large treeline to a panoramically similar but differently oriented twin treeline. When these transplanted bees visited a feeder at the new site under overcast skies, they oriented their dances as if they were still at the first site; unable to detect the sun directly, the bees located the sun (erroneously) using a memory of the solar ephemeris in relation to their home landscape. This memory of the connection between the sun and landscape is strictly a backup system for cloudy days, as bees orient by direct views of the sun and sky whenever the latter are available (Dyer, 1987), and the memory encompasses the entire landscape panorama near the nest, not only (if at all) specific familiar flight routes (Towne and Moscrip, 2008). Further, this learned connection between the solar ephemeris and the bees' natal landscape can sometimes strongly resist revision: if bees are transplanted from their natal site to a rotated twin site and then left at the new site for several sunny days, the bees dance on subsequent overcast days as if they were still at their natal site, using their (now

outdated) memories of the relationship between the sun and landscape (Towne and Kirchner, 1998; Towne et al., 2005).

Towne (Towne, 2008) therefore asked whether bees can ever learn the relationship between the solar ephemeris and a new landscape (that is, other than the bees' natal one). He transplanted bees from their natal site in a thinly wooded valley (Fig. 1Ai) to a visually dissimilar recipient site along a sloping treeline (Fig. 1Aii). He then allowed the bees to visit a novel feeder placed uphill and to the south along the treeline (dashed white line in Fig. 1Aii) for at least 3 days. Next, the bees were transplanted under overcast skies to a third site that was a rotated twin of the second (Fig. 1Aiii; sequential transplantations are indicated by red arrowheads between 1Ai, 1Aii and 1Aiii). This was just like Dyer's (Dyer and Gould, 1981; Dyer, 1987) treeline-to-treeline transplantation experiment, except that the bees' natal site was the wooded valley, not the first treeline. Towne's twice-transplanted bees danced under overcast skies as if they were at the first treeline (Fig. 1B red, which shows the results of Towne's first trial of this experiment; $N=7$ bees), suggesting that they had learned the relationship between the solar ephemeris and the first treeline during the sunny days they had spent there. Thus, Towne concluded that bees can learn the relationship between the solar ephemeris and a second (i.e. non-natal) landscape, if the landscape surrounding the nest at the second site is panoramically different from the bees' natal landscape. The broader theoretical context of this experiment is reviewed in Towne's study (Towne, 2008).

Recently, however, Dovey and colleagues (Dovey et al., 2013) found that bees transplanted from Towne's valley (natal) site directly

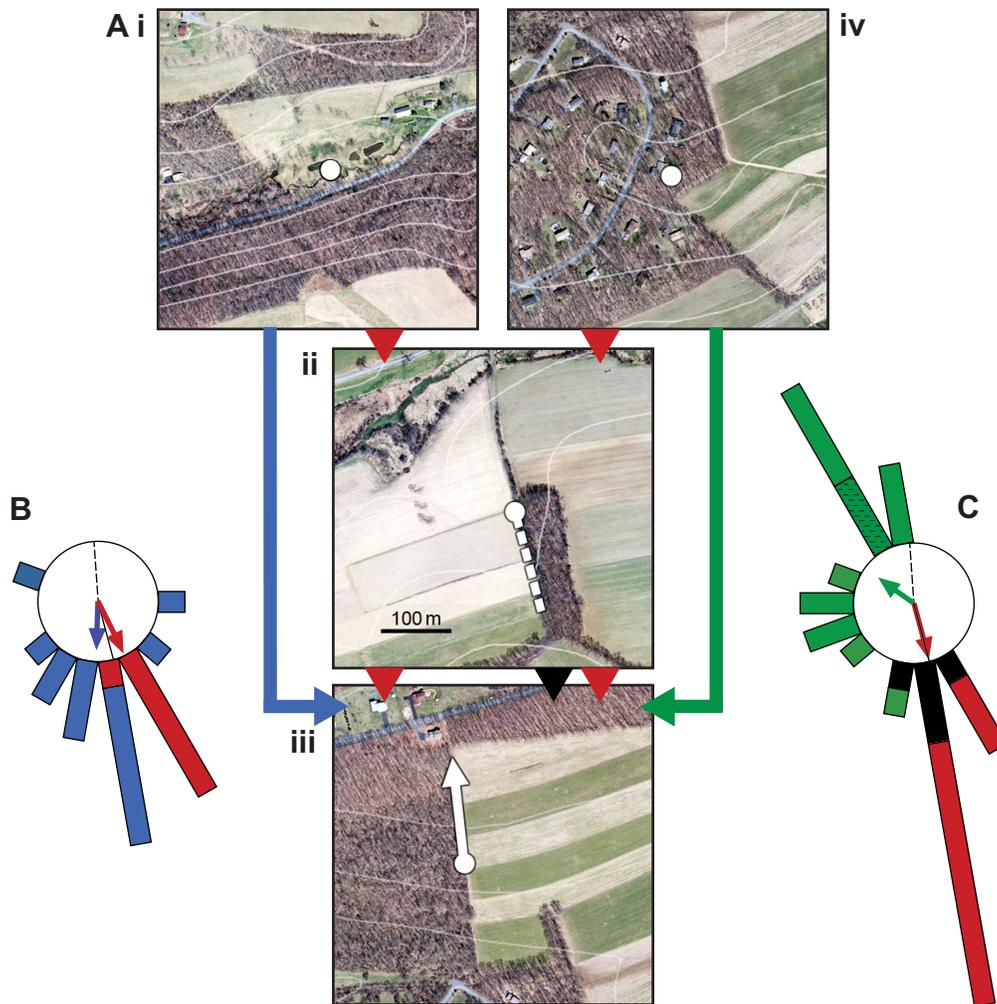


Fig. 1. Aerial photographs of sites (A) and results of the previous (B) and current (C) experiments. North is directly upward in all panels. (A) Aerial photographs: white dots show hive locations; adjacent contour lines are separated by 12.2 m of elevation. Colored arrows and arrowheads between panels show transplants, and the colors match those in which the corresponding dances are displayed in the histograms. (B,C) Circular histograms: solid black lines show the predicted direction for dances oriented by memory of the solar ephemeris in relation to the training site (broken white line in Aii), and dashed black lines show the direction of the feeder at the test site (white arrow in Aiii). Small colored arrows in the histograms show mean vectors. Site locations: the wooded site (Aiv) and the test site (Aiii) were only 450 m apart but not visible from each other; their relationship can be seen by noting that the upper left corner of panel Aiv shows a 90 deg turn in a roadway that can also be seen at the bottom center of panel Aiii. The other sites were all at least 1.6 km apart. The valley natal site (Ai) is at $75^{\circ}47'43.4''\text{W}$, $40^{\circ}36'41.4''\text{N}$; the training site (Aii) is at $75^{\circ}46'10.5''\text{W}$, $40^{\circ}36'28''\text{N}$; the test site (Aiii) is at $75^{\circ}47'9.5''\text{W}$, $40^{\circ}37'14''\text{N}$; and the wooded natal site (Aiv) is at $75^{\circ}47'4''\text{W}$, $40^{\circ}37'52''\text{N}$. Photographs courtesy of the US Geological Survey.

to his test site (blue arrow connecting Fig. 1Ai and 1Aiii) danced under overcast skies much like Towne's twice-transplanted bees (Fig. 1B, blue, $N=15$ bees), even with no opportunity to learn the sun's course in relation to the first treeline. Dovey and colleagues argue that their bees danced according to the best match between the somewhat dissimilar skyline panoramas at the natal and test sites, a possibility Towne did not foresee. The question arises, then, whether Towne's bees had learned the relationship between the solar ephemeris and the first treeline (Fig. 1Aii) or whether, instead, his bees danced according to the best match between the skylines at the natal and test sites (Fig. 1Aiii). We have therefore repeated Towne's experiment with two modifications: first, we used a different natal site that had no obvious similarities to the test site; and second, because we clearly perceive landscapes differently from insects (Wystrach et al., 2011a; Wystrach and Graham, 2012), we transplanted control bees directly from their natal site to the test site to reveal any unexpected matches the bees might make between them.

MATERIALS AND METHODS

All basic methods were as detailed previously (Towne et al., 2005; Towne, 2008). One hive was located at a wooded site near the highest point in the landscape (Fig. 1Aiv), and a feeder there was placed near the hive. A second, 'training' hive was located along the same sloping treeline used by Towne (Towne, 2008) (Fig. 1Aii), which was different from the wooded site in every obvious way. In order to determine whether bees native to the wooded site (Fig. 1Aiv) could learn the sun's course in relation to the landscape at the training site (Fig. 1Aii), groups of individually marked bees with at least 3 days of foraging experience were transplanted in small cages from the wooded site into the hive at the training site (transplantation is indicated by the red arrowhead between Fig. 1Aiv and 1Aii). When these bees were released into the recipient hive the next day, many of them were ejected by the hive's residents, so the cages were thereafter left in the recipient hive for two nights, after which the success rate increased (such that 34 out of 122 transplants became

foragers at the training site). These transplanted bees then visited a feeder placed ~2 m from the hive at the training site under sunny skies for at least 3 days, giving them the opportunity to learn the relationship between the solar ephemeris and the landscape panorama near the hive there. The feeder was placed near the hive so that the bees would not learn (if they were able to do so) the relationship between the sun's course and a single, specific flight route at the training site. Also, the feeder at the training site was different from that at the natal site in color, shape and scent, so that familiarity with the feeder would not block (if it can) the bees' learning of the relationship between the sun and landscape at the second site (Towne, 2008).

After the transplanted bees had had at least 3 days of experience at the training site, the hive at the training site was closed before dawn on an overcast morning (10 August 2012) and moved to the test site (Fig. 1Aiii), thereby transplanting the experimental bees a second time (red arrowhead between Fig. 1Aii and 1Aiii). Meanwhile, a second group of bees from the wooded site had been transplanted in cages into the same hive, but these bees were not released until the hive was moved to the test site (Fig. 1Aiii), so these bees had no experience at the training site (net transplantation indicated by the green arrow in Fig. 1A).

The hive was opened at the test site at 06:45 h, the caged control bees native to the wooded site were released, and a feeder was put out and moved uphill to the north–northwest along the treeline (white arrow in Fig. 1Aiii), eventually reaching 190 m from the hive. The bees visiting the feeder, all individually marked, included both groups of bees from the wooded site (with and without experience at the training site) as well as natives of the training site. The dances of these bees were recorded as previously (Towne et al., 2005), starting at 08:45 h as the feeder passed through 50 m and continuing under a full cloud cover with light to heavy rain for the next 2.5 h. Each bee's overall mean dance direction for the day was used in the statistical analysis, so that each bee was represented by a single data value regardless of how often she danced. Statistical calculations were made using Oriana circular statistics software (Kovach Computing Services, Anglesey, Wales, UK; www.kovcomp.co.uk).

RESULTS

On the day of the experiment at the test site (Fig. 1Aiii), the hive contained three groups of bees: an 'experimental group' native to the wooded site but with 3 days of experience at the training site (red arrowheads between Fig. 1Aiv, 1Aii and 1Aiii, and red histogram in 1C); a 'first control group' native to the training site (black arrowhead between Fig. 1Aii and 1Aiii, and black histogram in 1C); and a 'second control group' native to the wooded site with no experience at the training site (green arrow between Fig. 1Aiv and 1Aiii, and green histogram in 1C). Because the sky was overcast, the bees had to orient their dances by memory of the sun's course in relation to the landscape (Dovey et al., 2013). And because the landscape at the test site (Fig. 1Aiii) was a rotated twin of that at the training site (Fig. 1Aii), the control bees native to the training site danced as if they were still at the training site (Fig. 1C, black bars; angle of the mean vector, $\Theta=172$ deg; length of the mean vector, $r=0.98$; $N=5$ bees that danced 1–14 times each, 34 dances total; $P<0.001$, V -test with a predicted direction of 165 deg), which was roughly opposite to the feeder's actual direction. This confirms that these bees matched their visual representations of the landscapes at the twin sites as expected and that there were no celestial cues available to the bees during the test.

Meanwhile, the experimental bees native to the wooded site but with experience at the training site also danced as if they were at

the training site (Fig. 1C, red bars; $\Theta=165$ deg; $r=0.99$; $N=13$ bees that danced 1–10 times each, 77 dances total; $P<0.001$, V -test with a predicted direction of 165 deg). This matches Towne's (Towne, 2008) result (Fig. 1B, red) and suggests that these bees had learned the relationship between the sun's course and the landscape at the training site during their time there. Further, because the feeder was moved away from the hive (white arrow in Fig. 1Aiii) under cloudy skies only after the hive was transplanted to the test site, these bees must have used a memory of the solar ephemeris in relation to the entire panorama at the training site, not only the corresponding flight route there (indicated by the dashed white line in Fig. 1Aii), as the bees had never visited a feeder along that route.

The results of Dovey and colleagues (Dovey et al., 2013) (Fig. 1B, blue) suggest the possibility, however, that the experimental bees might have noticed some correspondence not obvious to us between the skyline panoramas at the wooded site (Fig. 1Aiv) and the test site (Fig. 1Aiii), causing the bees to dance in directions that coincided (by chance) with the prediction based on the bees' having learned the relationship between the solar ephemeris and the landscape at the training site. This is why we included a second control group of bees native to the wooded site but with no experience at the training site (Fig. 1A, green arrow from 1Aiv to 1Aiii).

The dances of this second control group were quite different from those of the other groups (Fig. 1C, hatched green bar; $N=3$ bees that danced 2–8 times each, 17 dances altogether). However, because only three such bees danced in the first trial, we increased the sample by transplanting the entire hive from the wooded site directly to the test site (Fig. 1A, green arrow) on the next overcast day, 14 August 2012. The hive was opened at the test site at 09:10 h, and the feeder was again moved to the north–northwest along the treeline. The bees' dances were recorded in a light rain from 10:38 h–11:35 h (ending when blue sky became visible). The bees danced in various directions (Fig. 1C, plain green bars; $N=15$ bees that danced 1–4 times each, 30 dances altogether), although the modal direction was the same as for the control bees from the first trial (Fig. 1C, hatched green bar), and most dances were again quite different from the dances of the experimental bees in the first trial (Fig. 1C, red bars). The mean direction for this group from both trials combined is 308 deg (green mean vector in Fig. 1C; $r=0.73$; $N=18$ bees).

While the dances of these control bees were widely scattered, they were not random ($P<0.001$, Rayleigh test, $N=18$). Both Dyer (Dyer, 1984) and Dovey et al. (Dovey et al., 2013) observed similar scattered but non-random dances when they transplanted bees between visually dissimilar landscapes under clouds, and these authors attributed the dance directions to the bees' attempts to match their internal representations of the dissimilar landscapes. We discuss this orientation further below. Regardless, the behavior of these control bees indicates that the dances of our experimental bees, which were native to the wooded site (Fig. 1Aiv) but had experience at the training site (Fig. 1Aii), depended crucially on the bees' experience at the training site. That is, the bees had learned the relationship between the solar ephemeris and the landscape panorama around the nest at the training site during the few sunny days they had spent here.

DISCUSSION

Honeybees can fail strikingly to learn the relationship between the solar ephemeris and a second (that is, non-natal) landscape when they are transplanted from their natal site to a panoramically similar site and visit a (seemingly) familiar food source there (Towne and Kirchner, 1998; Towne et al., 2005). Here, however, we confirm

Towne's (Towne, 2008) finding that when bees are transplanted from their natal site to a panoramically dissimilar site and visit a novel food source there, they can learn the relationship between the sun and the second landscape. Further, we can rule out the possibility that the bees in our test danced according to some spurious match between their natal site and the test site, as control bees transplanted directly between these sites (skipping the second site) danced differently.

Towne (Towne, 2008) gives a full discussion of the implications of these findings in the context of the bees' adaptively specialized sun-compass learning (Dyer and Dickinson, 1994; Dyer, 1996; Gallistel, 2003; Towne et al., 2005). Here, we briefly summarize just three main points from that discussion. First, we know that bees acquire their solar ephemerides starting with an innate expectation that the sun's azimuth in the morning is 180 deg from its azimuth in the afternoon (Dyer and Dickinson, 1994). The bees then learn the details of the local ephemeris with experience (Lindauer, 1971; Dyer, 1996). In the current experiment, we do not know whether our bees merely brought their mature ephemerides into the proper relationship with the new landscape or whether, instead, they re-learned the sun's pattern of movement entirely anew at the training site. Second, we have seen repeatedly (Towne et al., 2005) that bees transplanted between panoramically similar sites fail to learn a new relationship between the sun and landscape. We do not know, however, whether this failure is best considered a case of irreversible imprinting, in which the sun's pattern of movement is permanently linked to the landscape around the nest where it was first learned (thereby preventing the connection from being re-learned in a twin landscape), or a case of blocking, in which the apparent familiarity of a food source or flight route at a second (non-natal) site blocks the bees' re-learning of the sun's course in relation to the landscape there. These two questions could now be explored using variations on the methods we have used here. Third, in the current experiment, we moved the feeder away from the hive only at the test site under cloudy skies, and our bees nonetheless oriented correctly according to the sun's location in relation to a route at the training site that they had never flown, which shows that the bees had learned the relationship between the solar ephemeris and the entire landscape panorama around the hive at the training site, not only familiar flight routes (Towne and Moscrip, 2008). Towne (Towne, 2008) discusses these and other issues more fully, so here we limit ourselves to a few additional points not raised by Towne.

The results of Dovey et al. (Dovey et al., 2013) that led to the current study emphasize the need for controls when studying animals with visual worlds so different from our own (Wystrach and Graham, 2012). The dances of the twice-transplanted bees in Towne's (Towne, 2008) original study matched an otherwise unlikely prediction based on the hypothesis that the bees had learned the relationship between the sun's course and a novel non-natal landscape. But Dovey and colleagues later showed that the dances Towne observed might be explained by the bees confusing the skylines at the natal and test sites. The differences between our own visual perceptions, with our narrow views that emphasize individual landmarks, and the perceptions of insects, with their low-resolution, panoramic views that probably emphasize the entire panorama over individual landmarks (Wystrach et al., 2011a; Wystrach et al., 2011b; Wystrach and Graham, 2012; Wystrach et al., 2012; Baddeley et al., 2011; Baddeley et al., 2012; Graham and Cheng, 2009a; Graham and Cheng, 2009b; Legge et al., 2010; Philippides et al., 2011; Cheng, 2012; Zeil, 2012; Zeil et al., 2003), led Towne to overlook a cue (the skyline panorama around the nest) that is probably important to the bees.

We cannot fully explain the dances of our control bees transplanted directly between the wooded and test sites (Fig. 1A, green arrow; Fig. 1C, green bars). These bees might have matched their internal representations of the two sites' skylines (Towne and Moscrip, 2008; Dovey et al., 2013), a mechanism that was first clearly demonstrated in desert ants (Graham and Cheng, 2009a) and that has recently been elucidated to some degree in wood ants (Lent et al., 2013). However, because free-flying bees may view skylines from above the canopy (Dovey et al., 2013), and because our wooded site (Fig. 1Aiv) is surrounded by tall trees, we were unable to compare the skylines at our wooded and test sites meaningfully. The issue clearly deserves further study. It is also possible that our control bees estimated the sun's course at the test site based on direct experience with the site, which was only 450 m from the bees' natal site, and the bees' modal dance direction was nearly correct (Fig. 1C, green bars and dashed line). However, it seems likely – although untested – that bees connect the solar ephemeris only to views of the landscape near the nest (Towne et al., 2005), possibly within the peripheral correction area described by Palikij and colleagues (Palikij et al., 2012). Regardless, the dances of our control bees make it clear that our experimental bees had learned the relationship between the solar ephemeris and the training site, which is the first properly controlled demonstration that bees can indeed learn the relationship between the solar ephemeris and a landscape other than their natal one.

ACKNOWLEDGEMENTS

We thank Paul M. Bauscher and Walter and Doris Fink for permission to work on their properties, and Rudolf Jander and an anonymous reviewer for many useful suggestions on the manuscript.

AUTHOR CONTRIBUTIONS

W.F.T. designed the experiments, J.R.K. and W.F.T. carried out the experiments, J.R.K. analyzed the data, J.R.K. and W.F.T. wrote the paper, and W.F.T. prepared the figure.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This work was supported by a grant from the Kutztown University Research Committee [2012 grant to W.F.T.].

REFERENCES

- Baddeley, B., Graham, P., Philippides, A. and Husbands, P. (2011). Holistic visual encoding of ant-like routes: navigation without waypoints. *Adapt. Behav.* **19**, 3-15.
- Baddeley, B., Graham, P., Husbands, P. and Philippides, A. (2012). A model of ant route navigation driven by scene familiarity. *PLoS Comput. Biol.* **8**, e1002336.
- Cheng, K. (2012). How to navigate without maps: the power of taxon-like navigation in ants. *Comp. Behav. Cogn. Rev.* **7**, 1-22.
- Dovey, K. M., Kemfort, J. R. and Towne, W. F. (2013). The depth of the honeybee's backup sun-compass systems. *J. Exp. Biol.* **216**, 2129-2139.
- Dyer, F. C. (1984). Comparative Studies of the Dance Language and Orientation of Four Species of Honey Bees. PhD thesis, Princeton University, Princeton, NJ, USA.
- Dyer, F. C. (1987). Memory and sun compensation in honey bees. *J. Comp. Physiol. A* **160**, 621-633.
- Dyer, F. C. (1996). Spatial memory and navigation by honeybees on the scale of the foraging range. *J. Exp. Biol.* **199**, 147-154.
- Dyer, F. C. and Dickinson, J. A. (1994). Development of sun compensation by honeybees: how partially experienced bees estimate the sun's course. *Proc. Natl. Acad. Sci. USA* **91**, 4471-4474.
- Dyer, F. C. and Gould, J. L. (1981). Honey bee orientation: a backup system for cloudy days. *Science* **214**, 1041-1042.
- Gallistel, C. R. (2003). The principle of adaptive specialization as it applies to learning and memory. In *Principles of Learning and Memory* (ed. R. H. Kluwe, G. Lüer and F. Rösler), pp. 259-280, Basel, Switzerland: Birkhäuser Verlag.
- Graham, P. and Cheng, K. (2009a). Ants use the panoramic skyline as a visual cue during navigation. *Curr. Biol.* **19**, R935-R937.
- Graham, P. and Cheng, K. (2009b). Which portion of the natural panorama is used for view-based navigation in the Australian desert ant? *J. Comp. Physiol. A* **195**, 681-689.

- Legge, E. L., Spetch, M. L. and Cheng, K.** (2010). Not using the obvious: desert ants, *Melophorus bagoti*, learn local vectors but not beacons in an arena. *Anim. Cogn.* **13**, 849-860.
- Lent, D. D., Graham, P. and Collett, T. S.** (2013). Visual scene perception in navigating wood ants. *Curr. Biol.* **23**, 684-690.
- Lindauer, M.** (1971). *Communication Among Social Bees*, 2nd edn. Cambridge, MA: Harvard University Press.
- Palikij, J., Ebert, E., Preston, M., McBride, A. and Jander, R.** (2012). Evidence for the honeybee's place knowledge in the vicinity of the hive. *J. Insect Physiol.* **58**, 1289-1298.
- Philippides, A., Baddeley, B., Cheng, K. and Graham, P.** (2011). How might ants use panoramic views for route navigation? *J. Exp. Biol.* **214**, 445-451.
- Towne, W. F.** (2008). Honeybees can learn the relationship between the solar ephemeris and a newly-experienced landscape. *J. Exp. Biol.* **211**, 3737-3743.
- Towne, W. F. and Kirchner, W. H.** (1998). Honey bees fail to update their solar ephemerides after a displacement. *Naturwissenschaften* **85**, 459-463.
- Towne, W. F. and Moscrip, H.** (2008). The connection between landscapes and the solar ephemeris in honeybees. *J. Exp. Biol.* **211**, 3729-3736.
- Towne, W. F., Baer, C. M., Fabiny, S. J. and Shinn, L. M.** (2005). Does swarming cause honey bees to update their solar ephemerides? *J. Exp. Biol.* **208**, 4049-4061.
- Wystrach, A. and Graham, P.** (2012). What can we learn from studies of insect navigation? *Anim. Behav.* **84**, 13-20.
- Wystrach, A., Beugnon, G. and Cheng, K.** (2011a). Landmarks or panoramas: what do navigating ants attend to for guidance? *Front. Zool.* **8**, 21.
- Wystrach, A., Schwarz, S., Schultheiss, P., Beugnon, G. and Cheng, K.** (2011b). Views, landmarks, and routes: how do desert ants negotiate an obstacle course? *J. Comp. Physiol. A* **197**, 167-179.
- Wystrach, A., Beugnon, G. and Cheng, K.** (2012). Ants might use different view-matching strategies on and off the route. *J. Exp. Biol.* **215**, 44-55.
- Zeil, J.** (2012). Visual homing: an insect perspective. *Curr. Opin. Neurobiol.* **22**, 285-293.
- Zeil, J., Hofmann, M. I. and Chahl, J. S.** (2003). Catchment areas of panoramic snapshots in outdoor scenes. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **20**, 450-469.