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RESEARCH ARTICLE

Innate turning preference of leaf-cutting ants in the absence of external orientation cues

Thomas Endlein* and Metin Sitti

ABSTRACT

Most ants use a combination of cues for orientation but how do ants find their way when all external cues are suppressed? Do they walk in a random way or are their movements spatially oriented? Here, we show for the first time that leaf-cutting ants (*Acromyrmex lundii*) have an innate preference for turning counter-clockwise (left) when external cues are precluded. We demonstrated this by allowing individual ants to run freely on the water surface of a newly developed treadmill. The surface tension supported medium-sized workers but effectively prevented ants from reaching the wall of the vessel, which was important to avoid wall-following behaviour (thigmotaxis). Most ants ran for minutes on the spot but also slowly turned counter-clockwise in the absence of visual cues. Reconstructing the effective path walked revealed a looping pattern which could be interpreted as a search strategy. A similar turning bias was shown for groups of ants in a symmetrical Y-maze where twice as many ants chose the left branch in the absence of optical cues. Wall-following behaviour was tested by inserting a coiled tube before the Y-fork. When ants traversed a left-coiled tube, more ants chose the left box and vice versa. Adding visual cues in the form of vertical black strips either outside the treadmill or on one branch of the Y-maze led to oriented walks towards the strips. It is suggested that both turning bias and wall following are employed as search strategies for an unknown environment which can be overridden by visual cues.

KEY WORDS: Lateralisation, Thigmotaxis, Visual cues, Treadmill, Y-maze, *Acromyrmex lundii*

INTRODUCTION

Ants are known to use a variety of signals and cues for orientation (Jaffé et al., 1990). In most ant species, trail pheromones are the predominant signals for recruiting and homing, but they can only be detected in close proximity and thus can be easily lost, especially in complex environments. In very hot or wet environments, pheromone trails are also difficult to sustain. Alone or combined with pheromones, homing ants are known to rely on visual information (landmarks, optic flow or polarised light), tactile information (substrate features or wall contact) or geomagnetic information gained from proprioceptive sensors (inertia, stride length and azimuth) for orientation. *Acromyrmex octospinosus* leaf-cutting ants, for instance, use gravitational and visual cues for orientation although they have relatively small eyes with probably poor vision, and thereby rely more on pheromones (Vilela et al., 1987).

Many animals also use egocentric information for homing. Idiothetic memory is one form of spatial memory where information on the relative position of the body in space is recalled from egocentric cues such as the number of steps taken, position of limbs, etc. For example, desert ants (*Cataglyphis fortis*) memorise stride length and azimuth while leaving the nest and orient back home by integrating these values to form a straight line (Wittlinger et al., 2006). Idiothetic memory has been shown for several other ant species, for example when workers move along a maze to find their way back to the nest (Cosens and Toussaint, 1985; Jaffé et al., 1990; Aksoy and Camlitepe, 2005; Gerbier et al., 2008; Diez et al., 2011) or when ant queens determine the length of their excavated nest tunnel so as to initiate the digging of the first nest chamber (Fröhle and Roces, 2012). This shows that idiothetic memory is an important fall-back option for directed orientation when visual and other external cues are absent (Mittelstaedt and Mittelstaedt, 1973). However, several other studies on ants showed no such effect (Fourcassie and Beugnon, 1988; Goss et al., 1989; Salo and Rosengren, 2001), indicating that this may not be a general feature of all ant species and that some ants may use other fall-back options.

Egocentric cues can be error prone (Huber and Knaden, 2015) and need constant adjustment through external information, e.g. from the magnetic field (for leaf-cutter ants: Banks and Srygley, 2003), polarised light (for spiders and desert ants, respectively: Görner, 1958; Wehner, 1992) or other visual landmarks (for some mammals; Etienne et al., 1996). Furthermore, egocentric and external cues may become unreliable in some cases: for example, ants might find themselves in a new environment when suddenly displaced from an established pheromone trail or nest site by a strong gust of wind or a flash flood, or through the action of a larger animal. In such situations, the recall of landmarks or other idiothetic information might not be sufficient and a more robust search strategy has to be applied. Is there a 'default' search pattern that animals use in order to find their way back to a lost trail? Many animals apply random-walk strategies to explore unknown areas (Bartumeus et al., 2005; Schultheiss et al., 2015) but in order to find their way back to a previously known patch or trail, moving in loops or spirals is known to be an effective strategy (Langetepe, 2010), and this was shown, for example, in bugs and beetles (Wilson and Hoy, 1968), isopods (Hoffman, 1983), wood ants (Fourcassié and Traniello, 1994), cockroaches (Bell and Kramer, 1979), dung beetles (Dacke et al., 2013) and desert ants (Müller and Wehner, 1994; Wehner and Srinivasan, 1981). When moving in spirals, an animal turns at a decreasing rate in either the clockwise or counter-clockwise direction.

Interestingly, some animals have a preference for one direction over the other. For example, bumblebees circle around a flower in a preferred direction (Kells and Goulson, 2001) and some cockroaches show a bias for turning right in a Y-maze (Cooper et al., 2011). In ants, it is not clear whether such a turning bias is

Department for Physical Intelligence, Max Planck Institute for Intelligent Systems, Heisenbergstr. 3, 70569 Stuttgart, Germany.

*Author for correspondence (endlein@gmail.com)

(iD) T.E., 0000-0002-6358-5523; M.S., 0000-0001-8249-3854

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innate or linked to idiothetic memory and whether it has an adaptive value and/or is a result of morphological or neuronal asymmetries. Jander (1990) showed that ants explore leaves and branches by following the outside edge, which is similar to wall-following behaviour in a maze. He suggested that in ants, an intrinsic turning bias together with the suppression of random turns could have evolved as a search strategy. Hunt et al. (2014) provided evidence that the rock ant *Temnothorax albipennis* exhibits such an innate turning bias, but that this can be masked from the observer by wall-following behaviour (thigmotaxis). For the same ant species, Hunt et al. (2018) showed furthermore that the turning bias can be associated with a left–right asymmetry in the ommatidia count.

In this study, we investigated whether leaf-cutting ants (*Acromyrmex lundii*) would exhibit a default search behaviour when all external orientation cues are precluded and whether tactile and optical cues would act as fall-back options. First, to overcome the possible interference of wall-following behaviour (thigmotaxis), our study used a newly developed treadmill where ants are able to run freely on a slippery water surface without any wall contact. This set-up allowed us to investigate the control of directional movements in single ants. Second, we allowed ants to walk in a social context using simple Y-mazes, in which we tested the involvement of wall-following and optical cues for orientation.

MATERIALS AND METHODS
Study animals
Two mature colonies of leaf-cutting ants, *Acromyrmex lundii* (Guérin-Méneville 1838), from Argentina were kept in the laboratory under controlled conditions (25°C, >45% relative humidity and 12 h:12 h light:dark cycle) and fed *ad libitum* with freshly cut leaves of bramble (*Rubus fruticosus*) and a mix of honey and water. Prior to the experiments, a randomly chosen subset of ants (<300 of mostly medium-sized worker ants; body mass 3–5 mg) from either colony were kept in a separate box and were given only water for 12 h in order to stimulate their foraging behaviour.

Water surface treadmill
A small Petri dish (5 cm diameter) was filled with regular tap water and placed on a level surface. The surface tension of the water was sufficient to support medium-sized worker ants (3–5 mg body mass), whereas the low friction of the surface together with the slight concave meniscus effectively trapped the ants in the centre of the dish. Thus, ants were not able to reach the edge of the Petri dish but could run on the spot like on a treadmill (Fig. 1; Movie 1). The advantage of this set-up, in addition to its simplicity, is that it excludes deposited pheromones and contact with the wall of the container.

In many cases, ants ran freely for several minutes and only occasionally a front leg would penetrate the water surface. Ants often managed to pull out the leg after a few attempts and continued to run. If the ant could not free itself, the trial was stopped and only used when there was at least 30 s of uninterrupted running exhibited. In order to prevent the ants from using any visual cues, a dome-shaped, semi-transparent white box was placed over the Petri dish which diffused the light evenly and reduced shadows.

In order to test whether ants would follow a moving visual cue like a vertical black stripe, i.e. whether ants show an optomotor response (Hassenstein, 1951), we introduced a small strip of printed paper (15×50 mm) at the edge of the Petri dish. The strip could be moved slowly (4–6 deg s^{-1}) around the perimeter of the dish by manually pulling on a string wound around a cylinder underneath the Petri dish. By covering everything uniformly in white paper (including the turning mechanism), we attempted to exclude any other visual cues from the ants.

An overhead video camera (Basler acA1300-200um, 1280×1024 pixels) filmed the ant at a frame rate of either 10 or 20 frames s^{-1} for longer recordings of the ant's turning behaviour; a few sequences were also shot at 169 frames s^{-1} for a more detailed observation (by eye) of the running gait. To extract the ant's angular position, we converted the image into a binary image. By carefully choosing the threshold, we managed to exclude the legs of the ant, which left the body as a triangular shape with a broad head and narrow thorax and gaster. Using custom-written Matlab routines
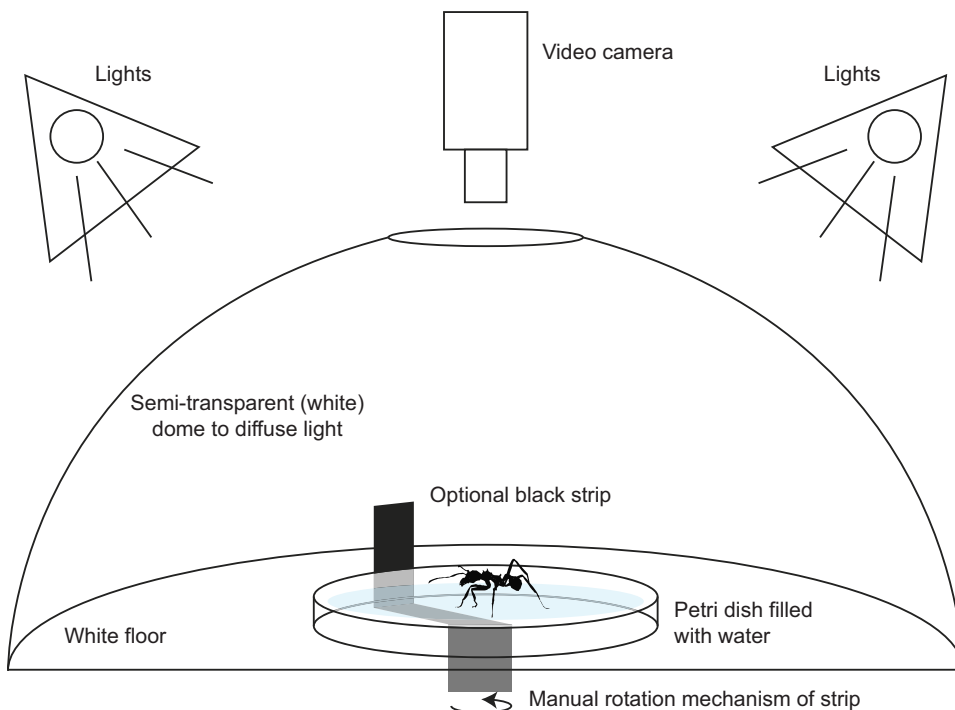

Fig. 1. Surface tension 'treadmill'. The surface tension of water was enough to support medium-sized leaf-cutting ant workers. The low friction and slight concave meniscus of the water surface effectively prevented the ants from reaching the edge. Thus, ants could run on the spot like on a treadmill. In order to preclude any optical cues, the Petri dish was placed on white paper under a semi-transparent white plastic dome which diffused the light evenly. To test whether ants would follow a black stripe, a printed paper strip (15×50 mm) was slowly moved manually around the dish by pulling on a string wound around a cylinder beneath the Petri dish. Please see also Movie 2.

(MathWorks, Natick, MA, USA), we fitted a narrow, pointed triangle around the body where the short side of the triangle was placed in front of the head and the two long sides were along the left and right side of the body of the ant. This way, the bisecting line of the smallest angle provided us with a directional axis of the ant's body for an automated readout of the angular position of the ant. In order to remove high-frequency noise resulting from slight variations of the 'shape' of the ant (coming from different leg positions), we filtered the raw data using a 2nd-order, low-pass Butterworth filter with a cut-off frequency of 1 Hz.

Y-maze

A small subset of ants (<300) were placed in a rectangular plastic container ('home' box in Fig. 2; L×W×H: 20×10×10 cm) with mineral oil applied to the walls to prevent them from climbing the walls and escaping. The container was connected to two identical boxes ('foraging' boxes) via a glass tube (25 mm diameter, 50 cm long) which branched half-way through into a Y-piece at an angle of approximately 22 deg (Fig. 2). The entire set-up was surrounded by white paper with diffused lighting in order to suppress any optical landmarks the ants would use otherwise when foraging. The two boxes were provided with small Petri dishes (5 cm diameter) containing regular tap water. The visiting ants in the two boxes were filmed at the same time using an overhead digital video camera. An image was taken every 2 s and ants were counted in real time using custom-written Matlab routines. Each trial ran for a minimum of

about 10 min and a maximum of 2 h. After a trial was completed, ants were killed in order to avoid a training effect where ants would possibly memorise one branch over the other (idiothetic memory); all boxes and tubes were rinsed thoroughly with 95% ethanol and blow-dried using compressed air in order to remove any residues of pheromones used by the ants.

In order to avoid the effect of any other possible orientation cues like the magnetic field of the earth (Banks and Srygley, 2003; Camlitepe and Stradling, 1995) or a possible temperature gradient of the room (Kleineidam et al., 2007), we turned the entire set-up by 180 deg between each experiment.

To investigate whether ants are able to use idiothetic memory during orientation, we inserted a coiled glass tube (Fig. 2 inset; 5 full turns of 360 deg; approximately 15 cm diameter) before the Y-branch. The coil forced the ants to make a few left or right turns before entering the fork.

To test whether ants would use a black stripe against a white background as a visual cue for orientation, a repeating pattern of vertical black strips (1×5 cm; 5 cm white gap) was printed on white paper and taped to one side of the Y-maze branch (Fig. 2). A repeating pattern (instead of a single stripe) was used in order to ensure that ants traversing the tube would perhaps perceive a stronger signal before reaching the forking point. Black stripes have been used in orientation studies on other animals, such as moths (Preiss and Kramer, 1984) and ants (Handlon, 2015; Yanoviak and Frederick, 2014). Movements towards a dark object (skototaxis) are
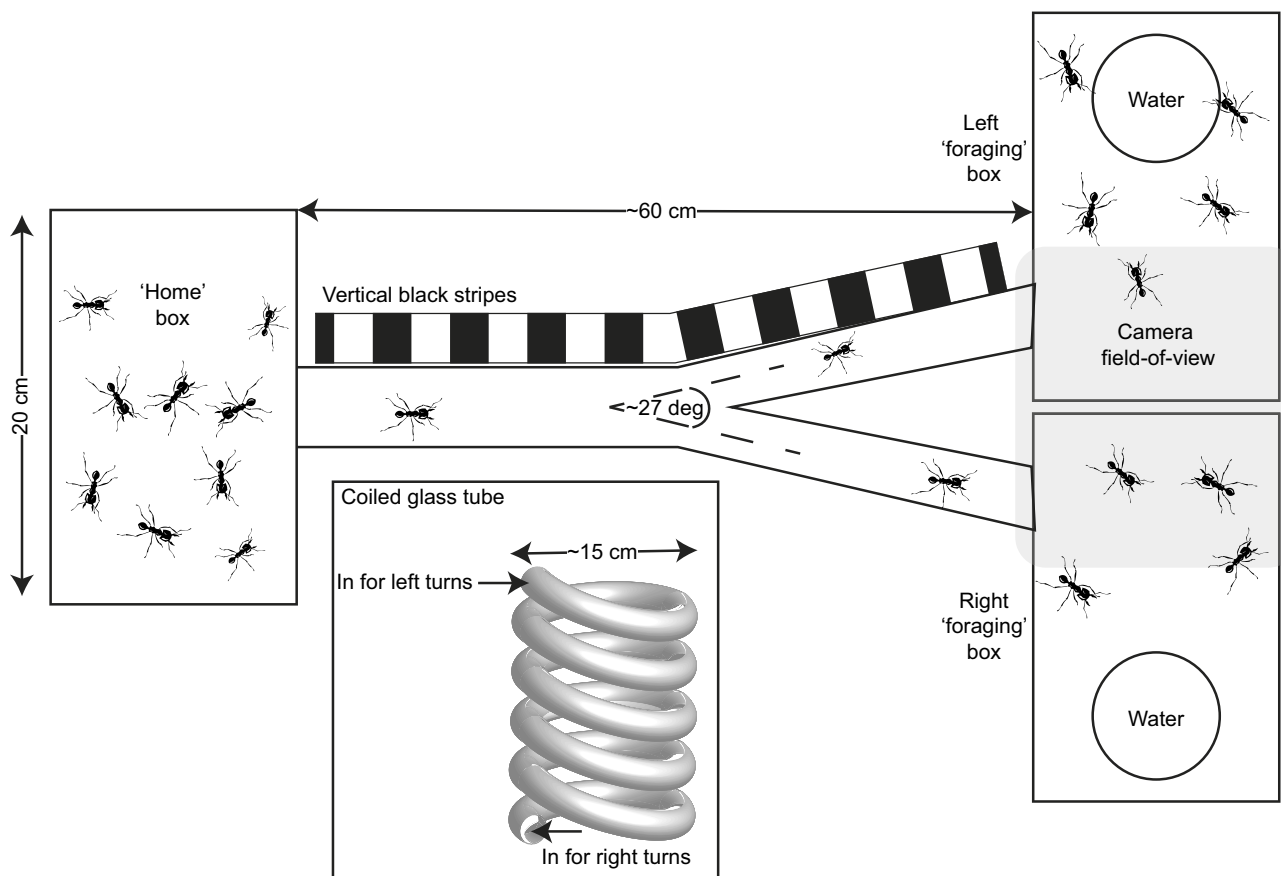

Fig. 2. Set-up to test the turning preference of ants. A small group of ants (<300) were given the choice of visiting either a left or right box when leaving their 'home' box. The set-up was surrounded by white paper and diffused light was used to minimise any optical landmarks for visual orientation other than the vertical black stripes along one branch (for more details, see Materials and Methods). Ants were counted in both 'foraging' boxes by filming with an overhead camera (shaded area). Inset: a coiled glass tube (5 turns of 360 deg) was inserted before the Y-branch to mechanically force multiple left or right turns on the ants when traversing the tube.

also known for other ground-dwelling arthropods, including meal worm beetles (Varjú, 1987), flat bugs (Taylor, 1988) and crickets (Atkins et al., 1987).

RESULTS

Turning preference of individual ants on the treadmill

Individual ants were placed on the water surface of a small Petri dish which allowed them to run on the spot, similar to a treadmill. Despite the ants slipping on the surface, their gait was not altered much from a regular tripod gait. However, noticeably, the hindlegs never left the water surface but were instead dragged forward on the surface during the swing phase. This is presumably to ensure greater stability on the slippery surface and possibly for steering. The midlegs were splayed out further from the body than when running on a firm surface and the front legs seemed to be mainly responsible for the propulsion. A very similar behaviour was described for other ants able to run or swim on the water surface in a coordinated way (Bohn et al., 2012; Yanoviak and Frederick, 2014).

In many cases, the ants would run for minutes (5.0±4.8 min, N=18; mean±s.d. here and throughout unless mentioned otherwise) and only occasionally stop or break the water surface with a leg (mostly the front legs). While running, the ants also turned (2.0±1.1 deg s^{-1}, or 0.33±0.18 full turns per minute, N=18). The turn was often in one direction with only intermittent turns in the other direction (e.g. the left-turning ant in Fig. 3A,B). We thus summed the turning angles to the left and the right (Fig. 3C) and formed a ratio of the two (Fig. 3D) for an overall comparison of the turning preference. The resulting average proportion of 1.33 was significantly higher than an equal proportion of 1 (Student's t-test to test against the mean of 1: t=2.34, P=0.032; Fig. 3C) and thus shows the ants' preference for turning left.

Reconstruction of the walked path

The slippery water surface and the concave meniscus restricted the ants' movements to the centre of the petri dish (Fig. 4, left column). Despite slipping, the front legs moved in a regular and stereotypical way and contributed most to the forward propulsion and turning of the animal. In order to reconstruct the effective path an ant would have taken on a firm substrate, we digitised the footfall positions of the front legs, with the 'stance' phase being the retraction of the leg from front to back. This fore–aft component of each stride length was added to the position of the ant.

Fig. 4 shows three examples of the position of the ant in the Petri dish (left column) and the reconstructed tracks (right column). In each case, the ant exhibited a looping pattern which could be interpreted as a search strategy to find its way back when external cues are absent or have become unreliable. However, it is possible that the circular pattern is a result of the geometry and/or size of the vessel used here and further tests using a much larger (rectangular) vessel or a trackball system are needed to verify the search behaviour of the ants in the absence of external cues.

Orientation towards visual cues

When we presented ants with a single vertical black strip which was fixed to one side of the Petri dish, the ants would align themselves towards the strip, indicated by a very restricted angular movement (Fig. 5A). In the example given, the average angle of the ant was 180.2 deg with a deviation of 10.1 deg, which coincided very well with the position and angular width of the strip.

When we moved the strip slowly around the outer perimeter of the Petri dish (Fig. 5), the ants followed the strip closely in both directions (clockwise and counter-clockwise), clearly showing an optomotor response, as known for several insect species (Hassenstein, 1951). Occasionally (4 times within the 70 s in this instance), the ants would stray away for a short time (deviation of >50 deg and back within a few seconds), visible in the deviations of the ant's path with respect to the pattern position (see Fig. 5B); however, in most cases the ant reoriented itself back towards the strips within a few seconds. The strong response of the ants towards the visual pattern indicated the ability to use visual signals in the absence of other orientation cues like pheromones.

Turning preference of groups of ants in the Y-maze

Fig. 6 shows the turning preference of workers in the Y-maze under different scenarios. When no visual cues were provided, about twice the number of ants preferred the left-hand box, showing a similar left-turn preference to that in our treadmill set-up (Fig. 6, left plot; exact test of goodness-of-fit: average number of ants left/right-46/ 22, P=0.005).

To investigate the involvement of tactile cues by wall following, a left-hand coil was introduced before the Y-piece and any visual signals precluded. This enhanced the ants' preference for the left, whereas none of the trials had a bias to the right (Fig. 6, second plot from the left; left/right 22/10, P=0.05). When the coil was reversed
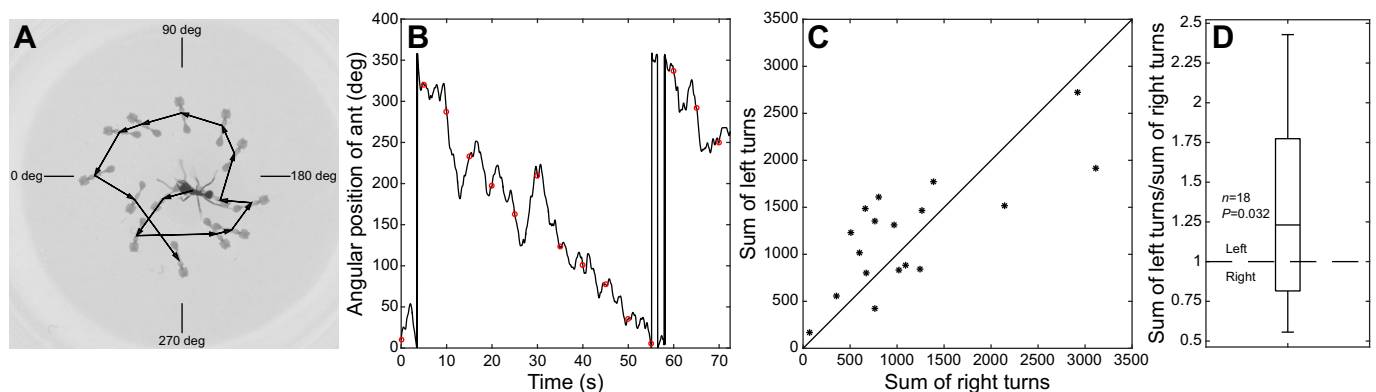

Fig. 3. Turning preference of single ants running freely on a water surface. (A) Example of a left-turning ant. Different positions of the same ant are superimposed and connected with a line to highlight the direction of the ant's path. (B) Angular position of the ant over the course of 70 s. Decreasing angles are left turns; increasing angles are right turns. Circled data points refer to the positions of the ant in A at 5 s intervals. (C) Proportions of left and right turns in n=18 ants. The horizontal bar inside the box is the median, and the box itself denotes the 25th and 75th percentiles. The whiskers cover approximately 99% of the data when normally distributed. Please also see Movie 1.
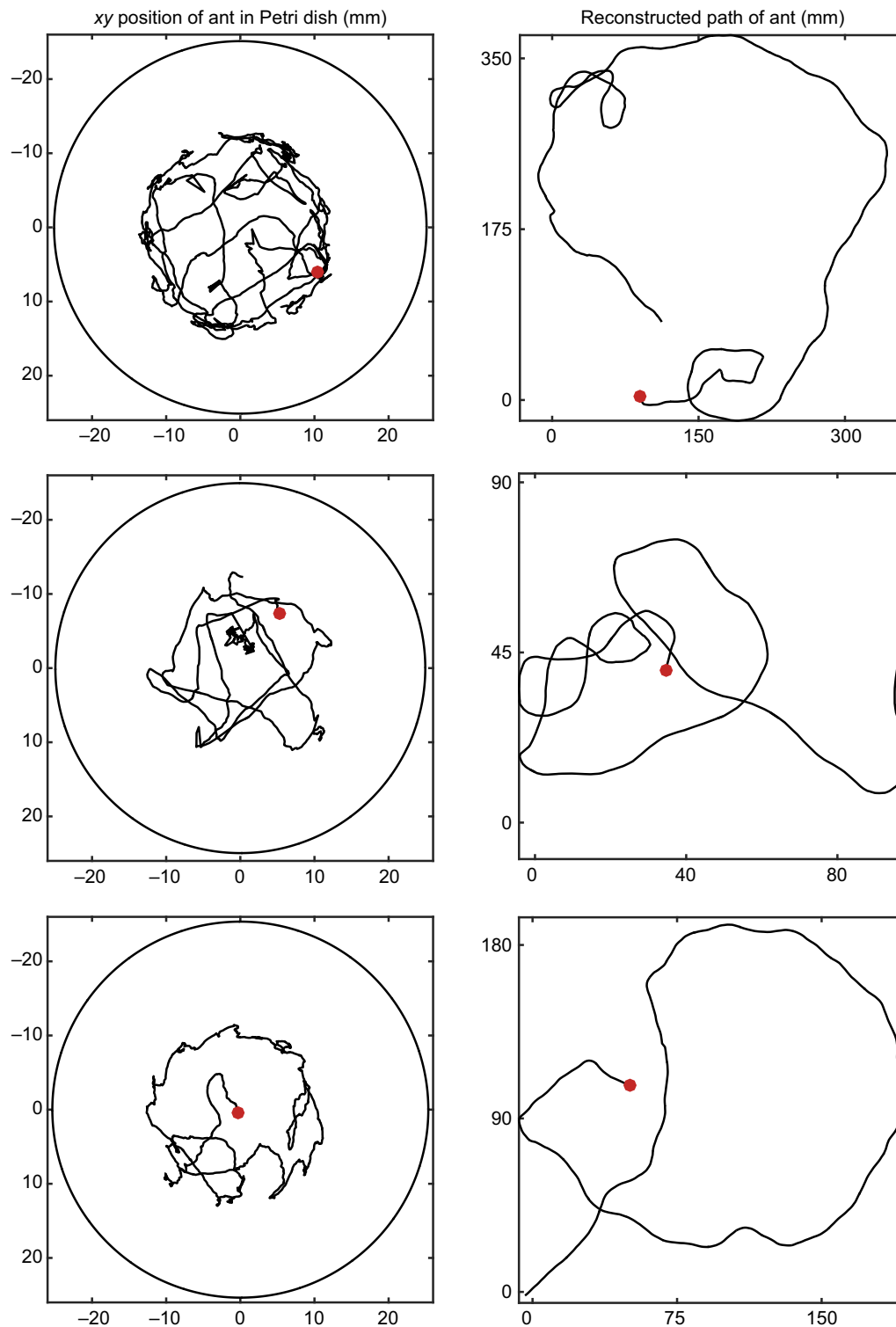
Fig. 4. Three examples of different tracks using the water treadmill. Left column: x–y positions of the ant in the Petri dish (circle). Right column: reconstructed tracks by adding the stride length of the (slipped) front legs to the ant's position. Red dots indicate the starting position in all graphs. Please also see Movie 1.

(right turns), more ants chose the right-hand box, resulting in a ratio closer to 1:1, similar to the scenario where stripes were attached to the right side of the maze (Fig. 6, middle plot; left/right=37/50, P=0.6).

When ants were presented with a pattern of vertical stripes along the left-hand side of the branch, their preference to the left side was not much altered compared with the bare set-up (Fig. 6, second plot from the right; left/right=87/57, P=0.015). When the stripes were placed on the right-hand side of the branch, more ants were guided

towards the right, resulting in a nearly 1:1 ratio of visiting ants (Fig. 6, right plot; left/right=131/132, P=0.6).

DISCUSSION
Turning preference in the absence of visual cues
Our treadmill set-up allowed us to observe ants running in an untethered way over several minutes without the possible interference of a wall contact. Thus, our study is the first to address the turning behaviour of ants in the absence of external cues.
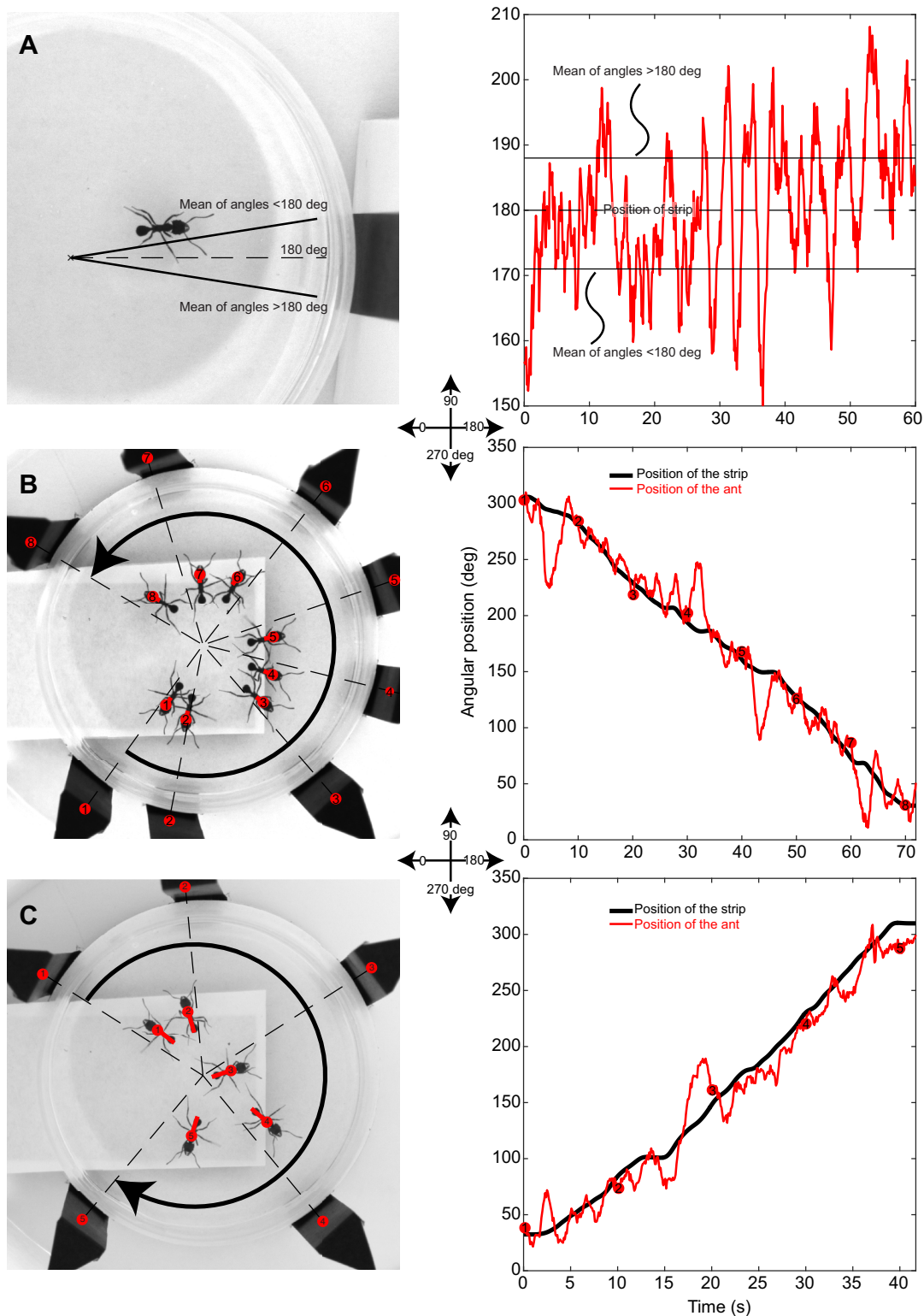
Fig. 5. Ants use a visual cue in the absence of other orientation cues. Individual ants followed a vertical black strip that was either fixed or moved slowly around the outside perimeter of the Petri dish. (A) An ant tried to move forward with a heading angle of about ±10 deg around the fixed strip. (B) Counter-clockwise and (C) clockwise turns of the black strip. In B and C, several images of the same single ant were superimposed at time intervals of 10 s, which correspond to the red, numbered labels of the right-hand data plots. Please also see Movie 2.

Most other methods either tether the animal and let them turn a Y-maze globe or ball underneath them (Hassenstein, 1951; Buchner, 1976) or use a computerised motor system to automatically compensate for the animal's movements (Gras and Hörner, 1992).

Given an insect is able to 'walk' on the water surface, this set-up offers an extremely simple alternative and opens up a wide range of possibilities to investigate orientation mechanisms in freely walking insects.
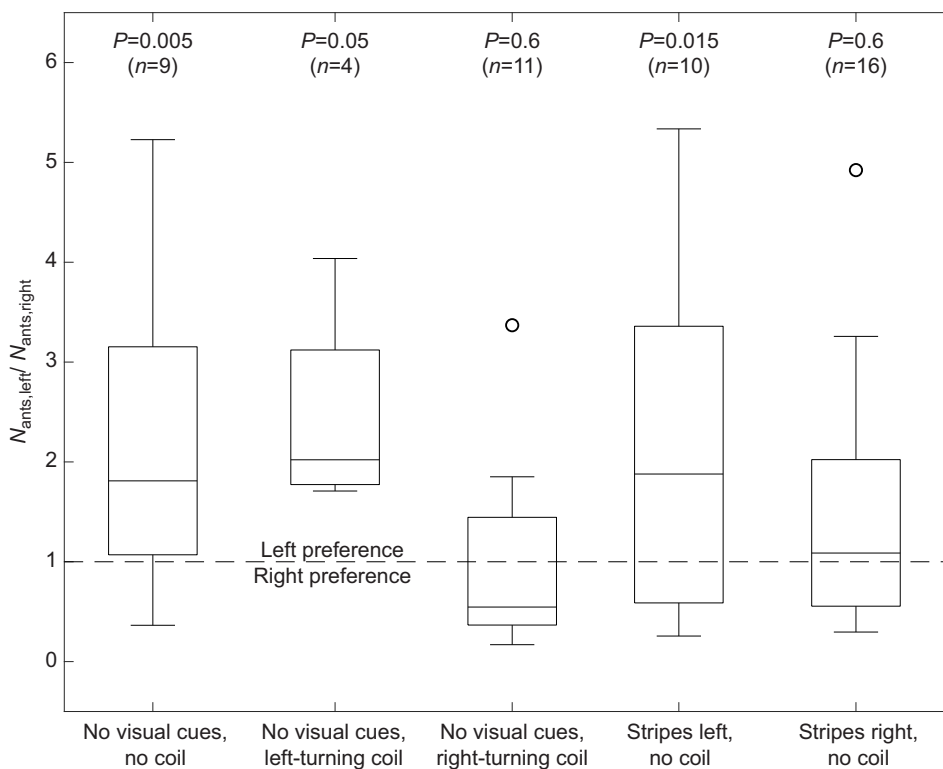
Fig. 6. Turning preference of ants in a Y-maze. Data are presented as the proportion of ants that visited the left box ($N_{ants,left}$) divided by the number of ants in the right box ($N_{ants,right}$) for different scenarios; a value of 1 (dashed horizontal line) thus indicates no preference. The P-values indicate whether there is a significant deviation from a 1:1 ratio; n-values give the number of repeated trials for each scenario. The horizontal bar inside the boxes is the median, and the box denotes the 25th and 75th percentiles. The whiskers extend to cover approximately 99% of the data when normally distributed. Any values outside this range are outliers (shown here as circles).

Our present experiments showed that *A. lundii* has a left-turning preference in the absence of external (visual) orientation cues. When ants ran on the treadmill, the turning happened spontaneously, suggesting that the turning bias is innate and not the result of idiothetic memory formed before the ants were tested. Also, a bias from inertia can be ruled out as ants turned rather slowly and did show some intermittent turns in the opposite direction (visible in Fig. 3B). The experiments also showed that not every ant had a preference for turning left – some preferred to turn right. It thus appears that individual ants may have individual preferences with an overall bias, evident with a larger number of tested ants. More detailed investigations into the innate preference of individuals would require repeated testing of the same individual, which was not carried out in this study.

Despite the possible use of pheromones and potential aggregation effects, mediated by contact with nestmates in the maze, ants showed a similar left-hand preference in the Y-maze. It is interesting to note that *Temnothorax albipennis* and *Formica pratensis* exhibit the same turning bias towards the left, as was shown by Hunt et al. (2018) and Aksoy and Camlitepe (2005), using similar maze set-ups. Hunt et al. (2014) also showed recently that this turning behaviour is associated with an asymmetry in the ommatidia count of the left and right eye. It would be interesting to investigate this aspect in the leaf-cutting ant species here and in other (ant) species to determine whether this is indeed the cause of the turning bias. The turning bias might be adaptive and used as a search and foraging strategy as shown for ladybird beetles (Girling et al., 2007) and ants (Jander, 1990), where the turns lead to a looping pattern. Even we humans tend to turn in surprisingly small circles (diameter <20 m) when blindfolded (Souman et al., 2009). However, Souman and colleagues (2009) suggested that neither biomechanical nor other asymmetries are responsible for this behaviour; instead, accumulated noise in the sensorimotor system would, without correction from an external reference, cause such a

bias. Further studies are required to ascertain the underlying mechanism for behavioural lateralisation as this might be different for individual behaviours and organisms.

Turning preference after traversing a coiled tube

Our experiments also showed that ants continued to turn in the same direction when exiting the coiled tube. Most likely, this is the result of wall-following behaviour (thigmotaxis), where ants may have experienced more wall contact on one side of the coil and continue to seek this at the Y-fork. Wall-following behaviour has been shown in other ants (Jander, 1990; Dussutour et al., 2005; Hunt et al., 2014) and cockroaches (Chapman and Webb, 2006) and is considered to be an effective strategy for exploring unknown spaces with boundaries; it is also a good strategy for solving a simply connected maze (Walker, 1986) and is thus employed as a search algorithm in robots (Weber et al., 1996; Mishra and Bande, 2008). When woodlice traverse a bent tube, they continue in the same direction after exiting (Beale and Webster, 1971), similar to what we have observed in the present study. The authors interpreted this behaviour as being due to the fact that the legs facing the inside of the curved path are more active during the turn and remain more active after exit. In our experiment, the tube diameter was about twice the body length of the ants and had a relatively large radius of curvature (approximately 150 times an ant's body length). It is thus difficult to tell whether the ants experienced an asymmetry in leg use or increased tactile stimuli from one side of the wall, or perhaps both.

In our experiment, ants experienced an unknown environment due to the lack of visual and chemical cues and thus their behaviour was probably more of a search-and-explore rather than a directed homing behaviour. For a directed search, animals instead try to maintain a straight line by initiating compensatory turns after a deviation from a straight line. For example, when crawling through a narrow bent tube, millipedes and isopods experience a mechanical

bending of their body and show a compensatory turn after exiting the tube (Burger, 1971; Hughes, 1989). Similar correcting turns are also shown by animals which use path integration with the help of idiothetic memory. To establish a spatial map and maintain a certain path, idiothetic memory is an important component and back-up strategy and further experiments need to be done to test this in *A. lundii*. Such memory feats have already been shown in various ant species, including *Cataglyphis fortis* (Ronacher and Wehner, 1995), *Pseudomyrmex termitarius* (Jaffé et al., 1990) and *Temnothorax albipennis* (Hunt et al., 2014) and are probably prevalent in other ant species too. Leaf-cutting ants of the genus *Atta* even use idiothetic memory to size up brood chambers (Fröhle and Roces, 2012). The use of idiothetic information for orientation also occurs in many other animals including honey bees (Collett and Collett, 2000), fiddler crabs (Layne et al., 2003) and some mammals (Etienne et al., 1996; Kimchi and Terkel, 2001), including humans (Mittelstaedt and Mittelstaedt, 2001).

Turning preference when visual cues are provided

From our experiments, we can conclude that *A. lundii* do use visual cues as a fall-back option when other cues are absent. Leaf-cutting ants probably have poor vision and it is unclear the extent to which they rely on visual cues for orientation. However, as they are night or day active depending on the season and forage in a complex environment, it appears to be important to not rely on pheromone trails alone. Vilela et al. (1987) showed that leaf-cutting ants (*Atta cephalotes*, *Atta laevigata* and *Acromyrmex octospinosus*) predominantly rely on pheromones but also use visual and gravitational cues and the spatial layout of the trail. The authors noted that *Acromyrmex* gives more importance to odour cues on the trail. Likewise, many other ants use a combination of cues for orientation, including visual (Wehner and Müller, 2006; Evison et al., 2008; Ronacher and Wehner, 1995; Aksoy and Camlitepe, 2012), magnetic (Banks and Srygley, 2003) and kinesthetic cues (Jaffé et al., 1990), which helps them to navigate in challenging environments and ensures fail-safe homing. However, despite the strong optomotor reaction towards the single black stripe visible in our treadmill set-up, ants in the Y-maze were only shifted in their bias to the extent that the left/right ratio evened out. Therefore, in this set-up, ants may have used more tactile cues and pheromones (with the left-turning preference quickly established) and thus relied less on visual cues.

Similar optomotor responses towards black stripes have also been found in many other walking insects, including beetles (Frantsevich and Mokrushov, 1980), stick insects (Gruhn et al., 2006) and ants (Ronacher and Wehner, 1995; Lent et al., 2013), and thus they seem to be a widely used attribute in orientation behaviour. A single vertical black strip against a white/bright background might resemble a tree or some larger object against the bright sky. Handlon (2015) has recently shown that, when trying to escape from a water surface, ants immediately oriented themselves towards a black stripe which might resemble a tree trunk near the water edge. However, many insects are able to use much less obvious features for navigation. For example, dung beetles use celestial objects at night (like the Milky Way; el Jundi et al., 2016) and even a seemingly featureless desert ground provides enough information for desert ants to utilise self-induced optic flow using a constant running speed (Ronacher and Wehner, 1995). The visual and cognitive capabilities of some ants seem to go beyond the recognition of a dark object. For example, wood ants (*Formica rufa*) are able to differentiate between vertical and horizontal stripes and can use those as cues to successfully traverse a maze (Vowles, 1965).

Conclusions

Our experiments showed that *A. lundii* has an innate preference to turn counter-clockwise in the absence of visual cues. Using our newly developed water treadmill, we were able to first exclude thigmotaxis, which showed the innate nature of the turning behaviour. This behaviour may lead to a circular searching pattern to enable ants to find their way back to a lost trail or nest site but might also be used to explore new sites when foraging. The wall-following behaviour is possibly another search strategy for environments with boundaries (like leaves or tunnels) whereas the use of visual cues supports the ants in a more directed homing. Our results give evidence that a lateral bias in the behaviour of insects can be innate and therefore might have an adaptive value. However, it remains unclear what exactly causes such a bias and to what extent this would affect other aspects of their behaviour (e.g. nest site preference, building of nest tunnels, traffic rules on trails, foraging, etc.).

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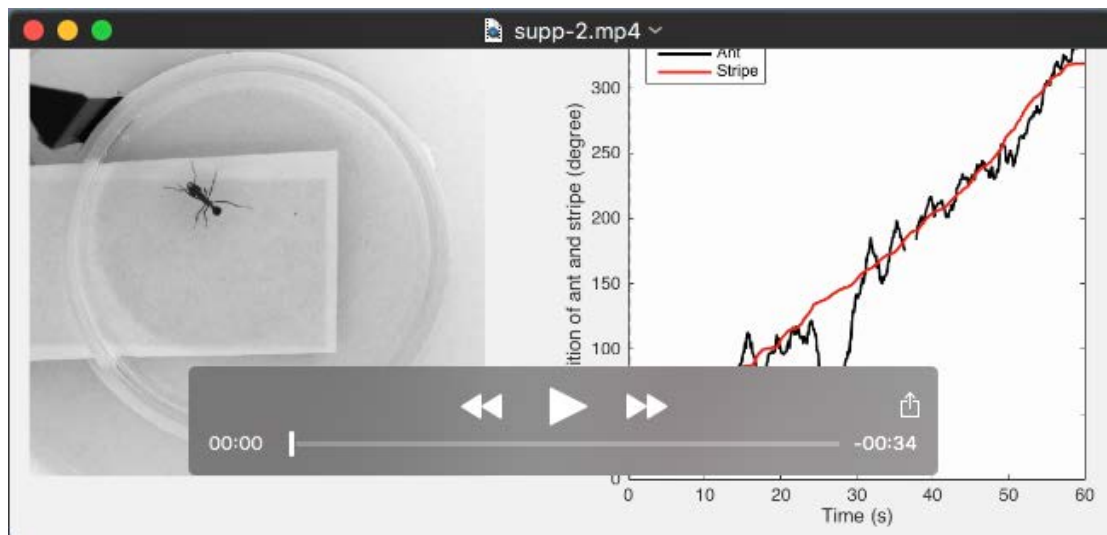
Movie S1

The water surface of a petri dish was used as a treadmill for Acromyrmex lundii worker ants. The surface tension supported medium-sized workers but the slippery surface together with the concave meniscus prevented the ants from reaching the wall of the vessel (first and second graphs showing the video image and the XY-position of the tracked position in the petri dish). In the absence of any visual cues, ants showed an innate bias to turn counter-clockwise (angular position of ant, third graph from left). By adding the slipped steps of the front limbs to the ant's position, the effectively walked path was reconstructed which revealed a looping pattern (right-most graph).
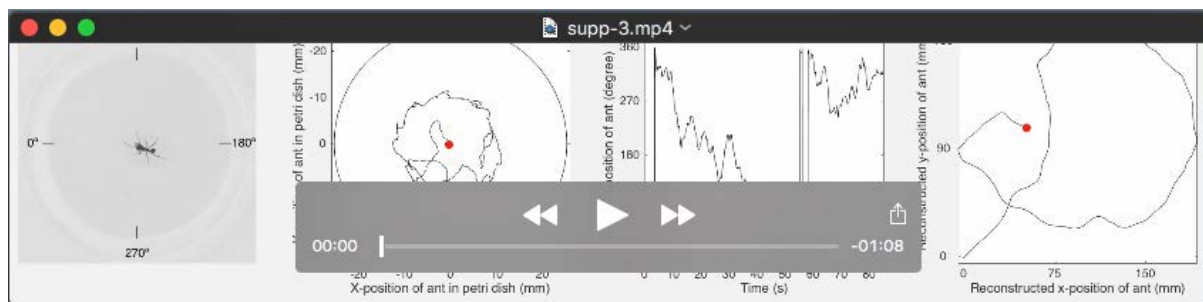
Movie S2

The water surface of a petri dish was used as a treadmill for Acromyrmex lundii worker ants. A single vertical black strip was provided as a visual cue which was slowly moved around the petri dish. The angular position of the ant and stripe are shown in the right-hand graph. The ant exhibited directed movement towards the strip which indicates that visual cues are recognised by this species. Visual cues are probably an important fallback option when chemical cues are absent.