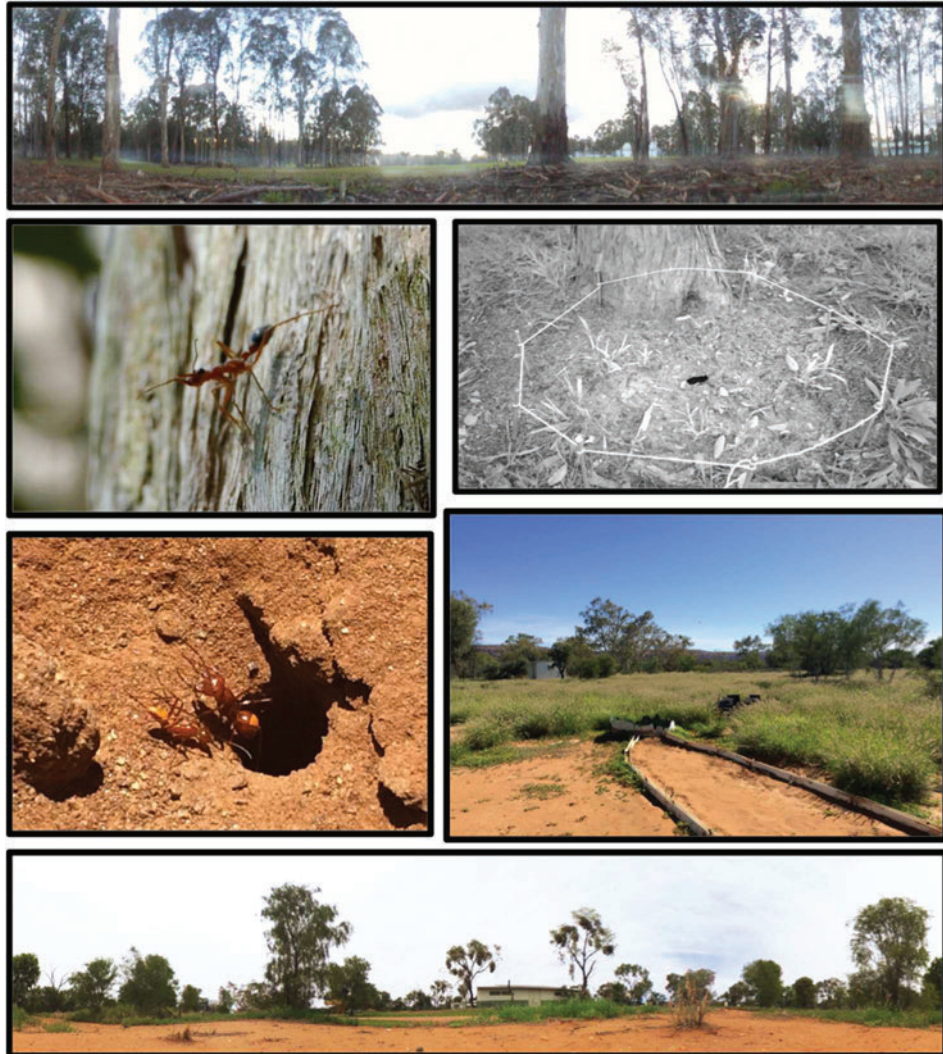


Learning, Memory and Cue Choice in Navigating Ants



Cody A Freas, M.S., M.Res.

Department of Biological Sciences

Macquarie University, Sydney Australia

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Summary

A critical reoccurring challenge facing mobile animals is the need to reliably find goal locations (e.g. food or nest). To solve this problem, animal navigators acquire and use multiple cue sets within their environment, which designate direction and distance estimates of these locations. Navigational cues can conflict, which adds a further challenge, requiring the navigator to integrate these cues before moving. Foraging ants are expert visual navigators known to use both learned panorama cues and path integration to navigate. This thesis examines navigational memory, learning and cue choice in two Australian ant species, the diurnal ant *Melophorus bagoti* and the nocturnal bull ant *Myrmecia midas*. *M. bagoti* is a solitary foraging desert ant endemic to Central Australia. Foragers are shown to retain robust memories of both the nest skyline and multiple non-nest site skylines. Forager orientation performance shows evidence of retroactive interference after changes are made to the skyline at the same site. When presented with cue conflicts between the terrestrial and celestial cue sets, foragers appear to choose dynamically based on experience and cue reliability in accordance with the Temporal Weighting Rule. Foragers with directional conflicts in their inbound and outbound routes show evidence of rapid vector calibration to the inbound route. This calibration appears to have a directional limit of around 45°. When foragers are restricted to the nest site, they are unable to extrapolate panorama cues from the nest to local sites but only require one experience of the homeward route to learn the correct nest direction. Furthermore, exposure to the outbound foraging path appears critical for efficient homeward route formation in this species. *M. midas* is a previously unstudied solitary foraging bull ant that forages nocturnally. Foragers show a strong reliance on terrestrial panorama cues in cue choice tests and only weak use of accumulated vectors. Foragers that are subjected to polarised light manipulations during route navigation dynamically weight the terrestrial and celestial cues during the foraging trip. *M. midas* foragers also navigate using the panorama while on the tree face, exhibiting similar solutions to navigational challenges on the ground and when navigating in three dimensions. Both species show heavy use of panorama learning and memory along with flexible use of navigation strategies.

Candidate Declaration

I hereby certify that all work contained in this thesis, entitled ‘Learning and Cue Integration in Navigating Ants’ has not been previously submitted in full or in part for a degree to any other university or institution other than Macquarie University. I also certify that the work within this thesis represents an original piece of research and it has been written by me.

All assistance received in this research and the preparation of this thesis has been appropriately acknowledged. Additionally, I certify that all sources and literature used in the creation of this work has been cited in the thesis.

All work was conducted on invertebrates and as such it was not subject to approval by the Macquarie University Ethics Review Committee.

Cody A Freas

ID: 43352227

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Chapter 1. Introduction

Background

All mobile animals need steering mechanisms to find goal locations within their environment. These locations can include a potential mate, known food source or the route back home. Leaving the safety of home can be dangerous, and an animal's survival can be dependent on its ability to accurately navigate. To accomplish this task, the navigator needs to acquire and then use information that can specify both the distance and direction of a goal location. Often, multiple environmental cues can be used in tandem to direct movement. The use of multiple streams of information poses an additional cognitive challenge: these signals need to be integrated before the navigator makes a course-plotting decision.

Researchers have explored the navigational abilities of insects for over a century. Early displacement experiments in wasps and bees found that these foragers could home successfully over great distances (Fabre 1882, Romanes 1885). These findings suggested that bees and wasps retain some information around the nest in order to navigate back. Through further research, naturalists have begun to grasp the underlying mechanisms behind the collection and use of these spatial strategies across a wide range of insect groups (Collett and Land 1975; Wehner and Raber 1979; Dyer 1996; Dacke et al. 2003; Wessnitzer et al. 2008; Ofstad et al. 2011; el Jundi et al. 2016). Solitary foraging ants solve navigational problems using two navigational strategies: path integration (Collett & Collett 2000a; Wehner 2003, 2008) and learned panorama cues (Cheng et al. 2009; Wehner 2003) with systematic search as a backup mechanism (Müller & Wehner 1994; Schultheiss et al. 2015).

Path Integration

Path integration is a mechanism that allows the forager to keep track of the nest location by creating a homeward vector that combines the nest's current distance and

direction (Collett and Collett 2000; Wehner 2003). As the forager travels away from the nest entrance searching for food, the individual will continually update its homeward vector. Once a piece of food is found, the forager can then return to the nest entrance by following the homeward vector in a straight line to the nest. To successfully maintain the vector, the path integrator combines two mechanisms, a celestial compass for directional cues and an odometer for distance estimates. Celestial compass information is derived from multiple sources including the sun or moon's position in the sky and the sky's distinct pattern of polarised light. Polarised light is comprised of light waves that oscillate along a single plain. In the sky, polarised light is the result of light scattering as it enters the atmosphere. The pattern of this light is arranged in concentric circles around the sun's (or moon's) position in the sky, called an e-vector (Fent 1986; Wehner 1994). The e-vector pattern of the sky remains stable even when the sun/moon is behind cloud cover and is even present during twilight when the sun's position is below the horizon, making it a useful directional cue (Rozenberg 1966). Ants are able to use either the sun's position or the polarised light pattern for directional cues when they are presented alone yet can also integrate them when they conflict, where either the polarised light pattern dominates (Wehner and Müller 2006) or ants choose a compromise direction between the cues (Lebhart and Ronacher 2014).

Insects estimate distance through a number of mechanisms. In flying honeybees, foragers record distances through optic flow, a visual cue that integrates the motion of objects as they move across the navigator's optic field (Srinivasan and Gregory 1992; Esch and Burns 1995, 1996; Srinivasan et al. 1997; Esch et al. 2001). Optic flow appears to play only a minor role in walking ants and its effects on distance estimation have only been shown with cues below the ant on the ground and not in the lateral visual fields (Ronacher and Wehner 1995; Ronacher et al. 2000). Ants largely rely on internal mechanisms for distance estimates using a stride integrator or pedometer (Wittlinger et al. 2006, 2007; Seidl and Wehner 2008).

Together, the pedometer and celestial compass permit the forager to accumulate the

distance of each path segment along with the forager's current orientation, allowing a quick return to the nest at the end of a foraging trip (Müller and Wehner 1988). Path integration is a navigational system crucial to ant species living in environments with few terrestrial cues, such as the saltpan dwelling *Cataglyphis fortis* (Wehner 2003; Wehner and Srinivasan 2003; Bühlmann et al. 2011). Path integration is also employed by ant species inhabiting cluttered, landmark-rich environments, though it is typically overshadowed by the available panorama cues (Narendra 2007; Cheng et al. 2009; Mangan and Webb 2012; Freas and Cheng 2017; Freas et al. 2017a).

Path integration continually updates both during the outbound and inbound portions of the foraging trip. As the animal reaches and enters its nest, the path integrator is reset to zero (Knaden and Wehner 2005, 2006). The use of this cue set is generally studied in the short term during the current foraging trip (Collett and Collett 2000a; Wehner 2003, 2008). Yet, both ants and honeybees can also retain long-term vector memories of path integration information from previous foraging trips. Foragers can use these memories from previous trips to return to profitable food locations or return to the nest after delays (Ziegler and Wehner 1997; Collett and Collett 2000b; Beugnon et al. 2005; Bolek et al. 2012).

Panoramas

As previously noted, path integration is only one of the strategies employed by ant navigators. Foragers can also retain long-term memories of terrestrial cues in order to navigate successfully. Recent research has focused on the use of the full panorama rather than specific elements of the navigator's visual scene (Graham and Cheng, 2009ab; Wystrach et al. 2012; Narendra et al. 2013ab; Zeil et al. 2014; Schultheiss et al. 2016). The prevailing theory of how ants use these panorama cues is by view-based matching. In the view-based matching strategy, panorama information is stored and then compared to the forager's current views while it is away from the nest (Collett 2010; Zeil et al. 2014). For successful nest-ward orientation, the forager will try to match the current view with the stored views from around

the nest or another goal location. The resulting best match between these views occurs when the forager is oriented toward this goal direction (Wehner et al. 1996; Collett et al. 2001; Collett et al. 2006; Cheng et al. 2009; Wystrach et al. 2011ab). Navigation through view-based matching requires foragers to first acquire and retain the views around the nest site. Acquisition of the nest panorama is accomplished through learning flights in insects such as wasps and bees and learning walks in ants (Nicholson et al. 1999; Baddeley et al. 2011; Zeil et al. 2014; Fleischmann et al. 2016; 2017). Before the onset of foraging, foragers will perform a series of short stereotypical arcing walks around the nest. During these arcs, foragers will face the nest location from a number of directions, storing views of the nest panorama for future use while away from the nest. As foragers travel away from the nest during the first few foraging trips, they will occasionally look back toward the nest direction. During this ‘turn back’ behaviour foragers are likely acquiring changes in the panorama along their current route (Nicholson et al. 1999; Zeil 2012; Zeil et al. 2014) and retaining these non-nest site views for future use (Graham and Cheng 2009ab; Schultheiss et al. 2016). These stored panorama memories are robust, allowing for successful navigation back to the nest location even after extended delays (Wehner et al. 1983; Ziegler and Wehner 1997; Narendra 2007).

Cue Conflict and Choice

Cue sets can be manipulated to signal two conflicting directions to the navigator. When foragers are displaced away from their current foraging route, the available panorama cues will update to their new position, yet their path integrator will not. In these experiments, navigating ants will choose a compromise heading between the two cue sets (Collett 2012; Legge et al. 2014; Narendra et al. 2007; Wehner 2016) or individuals will ignore their vector and orient to the panorama cues alone (Collett et al. 1998; Narendra et al. 2007).

Within path integration, the forager’s current accumulated vector and retained vector memories of previous foraging trips can also be experimentally put into directional conflict

(Collett et al. 1999; Collett and Collett 2000b; Wehner et al. 2002). When the outbound and inbound vectors of a foraging trip do not coincide, the result is a non-zero vector value when the forager reaches the nest entrance. The disagreement between these trip segments can result in foragers altering their future route directions toward the direction of previous inbound trips as a corrective mechanism, which is dubbed vector calibration (Collett and Collett 2000b; Wehner et al. 2002). Vector calibration testing in honeybees and the ant species *Cataglyphis fortis* resulted in marked shifts toward the inbound route direction, yet these shifts remained incomplete, with foragers choosing an intermediate path direction between the two routes (Collett et al. 1999; Collett and Collett 2000b; Wehner et al. 2002).

Desert Ant Species

Desert ants are specialized species that solitarily forage during the hottest portions of the day, collecting insects that have perished due to the intense heat. They can remain active at temperatures over 50° Celsius, close to their thermal maximum (Marsh 1985; Christian and Morton 1992). These species are highly tolerant to heat due to a specialized physiological response, the ability to synthesize heat shock proteins at extreme temperatures (Gehring and Wehner 1995). Additionally, the behavior and morphology of these species are specialized to survive in harsh desert climates. Long legs reduce periods foraging in the extreme temperatures by allowing for fast movement and also giving individuals the ability to lift their bodies away from the high surface temperatures on the ground (Sommer and Wehner 2012). Another behavioral strategy for blunting the high temperatures is the use of thermal refuges during the hottest periods of the day. Foragers will climb up into cooler air layers away from the ground using leaves of grass or other objects (Christian and Morton 1992). These mechanisms allow desert ants to occupy a temporal niche when other species are unable to survive, reducing both competition for resources and predation risks (Briese and Macauley 1980; Wehner et al. 1992).

Desiccated insects are scattered randomly throughout the desert surface, and this

spread of resources coupled with the intense heat makes solitary foraging advantageous to desert ants. Individual foragers are skilled navigators, efficiently moving through the environment to find food and returning accurately to their nest. Desert ants have the capacity to use both path integration and the panorama (Wehner et al. 1996; Wehner 2003; Cheng et al. 2009); nevertheless, cue set preferences are mediated by their habitat and cue availability. In barren environments with few terrestrial cues, such as the saltpans that *Cataglyphis fortis* inhabits, foragers rely heavily on path integration to judge the distance and direction to previously visited goal sites (Wehner and Srinivasan 2003; Bühlmann et al. 2011; Cheng et al. 2014). Though *C. fortis* is well known for its use of path integration, these foragers can also use learned panorama cues when they exist nearby (Wehner et al. 1996).

Melophorus bagoti, inhabits a very different environment in central Australia from the north African *C. fortis* and relies heavily on visual navigation to return to the nest. This cluttered semi-arid environment is filled with bushes, trees, and grass tussocks, and *M. bagoti* forms idiosyncratic routes between these objects using terrestrial cue memories (Cheng et al. 2009, 2014). While *M. bagoti* is most reliant on the panorama for navigation, foragers also concurrently accumulate a celestial based vector for returning to the nest and other goal locations. Yet these foragers are significantly worse at accurately estimating the distance to the nest and are quicker to switch to systematic search when compared to *C. fortis* (Cheng et al. 2006; Narendra et al. 2007; Bühlmann et al. 2011).

Melophorus bagoti has been widely studied in its use of both path integration and panorama cues (Cheng et al. 2009, 2014), yet less well studied is how these ants learn panorama cues and the mechanisms underlying cue choice. In this (Chapters 2, 3, 4, & 5) thesis, we explore panorama learning and memory in *M. bagoti* and explore how these ants resolve conflicts both between the terrestrial and celestial cues sets and within vector based navigation when the outbound and inbound routes differ.

Nocturnal bull ants

Visual navigation is widely studied in desert ants and other diurnal ants that navigate when ambient light levels are high and visual information is easy to detect (Wehner et al. 1996; Fukushi and Wehner 2004; Cheng et al. 2009; Jayatilaka et al. 2011; Narendra et al. 2013a). As light levels drop during twilight and overnight, these visual cues become increasingly hard to detect (Warrant 2008; Warrant and Dacke 2011). Yet, nocturnal members of the genus *Myrmecia* are able to use very similar vision based navigational strategies to that of diurnal ants while foraging (Narendra et al. 2010; Jayatilaka et al. 2011; Reid et al. 2011, 2013). *Myrmecia* foragers typically navigate as they travel between the nest entrance and nearby Eucalyptus trees where they forage in the canopy. The nocturnal species of this genus have specialized visual systems adapted for navigation at low light levels (Greiner et al. 2007; Narendra et al. 2010, 2016). Navigation in this genus depends heavily on the visual cues of the panorama. The day active *Myrmecia croslandi* navigate primarily using the surrounding panorama, with path integration employed only in unfamiliar locations (Narendra et al. 2013a). *Myrmecia pyriformis*, which navigates during twilight, uses both celestial cues such as the polarized light pattern and the panorama while navigating and will compromise their heading when these cue sets conflict (Reid et al. 2011).

In this (Chapters 6, 7, & 8) thesis, we explore the navigational abilities and preferences of the previously unstudied nocturnal bull ant species, *Myrmecia midas*. *M. midas* foragers leave the nest just after sunset and travel to one of 3-5 nest specific foraging trees where they spend the night foraging in the canopy. They descend these trees and return to the nest in the pre-dawn twilight. Unlike previously studied *Myrmecia* species, *M. croslandi* and *M. pyriformis*, this species nests within stands of trees and at the base of one of their foraging trees, with a subset of the nest foraging force travelling straight up this nest tree. *Myrmecia midas* is an interesting candidate for study due to its ability to find goal locations during the low light levels of twilight and its unique nesting ecology within this genus.

Chapter outline

M. bagoti foragers have been shown to retain the visual cues around the nest location for the lifetime of the forager (Narendra et al. 2007b). It remains unknown if these foragers retain similar long-term memories of the skyline cues of locations that are experienced while away from the nest, such as a known food source. In **Chapter 2**, we test foragers' memory retention of skyline cues of resource patches far from the nest site, characterized by skyline cues independent of the nest's skyline. We find that foragers retain robust memories of non-nest skylines and that exposure to the panorama around the nest does not interfere with this memory retention. In a second experiment we replaced the non-nest skyline after training. Our results suggest that new skyline memories at the same site cause retroactive interference in the retention of previously learned skylines. Skyline memories may compete during memory retrieval, or may be retrieved in association with context cues such as vector length.

This chapter has been published in the *Journal of Comparative Physiology A* (Freas et al. 2017b), and was co-authored by Ken Cheng and Christopher White. Ken Cheng provided suggestions on experimental methodology as well as revisions to the manuscript prior to publication. Christopher White assisted with fieldwork. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

M. bagoti is known to use both terrestrial landmark information and path integration to navigate using independent navigational systems including path integration and panorama learning. When cue sets are presented in conflict, the Australian desert ant species, *Melophorus bagoti*, will choose a compromise heading or when on well-known routes, panorama cues will override the celestial cues. In **Chapter 3**, we explore both foragers' learning of panorama cues and the effect of temporal delays on cue choice decisions by testing foragers with varying levels of panorama experience with a 180° cue conflict.

Foragers restricted to the nest area are unable to extrapolate landmark information from the nest to local sites 8m away but show one-trial learning of panorama cues given one homeward experience. This experience level is insufficient to override a conflicting 1m vector. After multiple experiences of the homeward route, terrestrial cue strength increases and overrides the vector cues. This change in cue choice is dynamic as foragers discount the reliability of the terrestrial cues over time and will revert back to orienting to the vector cues when the last exposure of the terrestrial cues was 24 hours in the past. We further find that foragers with outbound and inbound conflicts of 180° show no evidence of vector calibration during training. We discuss the decision-making that underlies these navigational choices, which can be explained by the temporal weighting rule.

This chapter has been published in the journal *Ethology* (Freas and Cheng 2017a) and was co-authored by Ken Cheng. Ken Cheng provided suggestions on experimental methodology as well as revisions to the manuscript prior to publication. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

Given the absence of vector calibration in the subjects of Chapter 3, in **Chapter 4** we explore the limits of vector calibration in *M. bagoti* by testing foragers with directional conflicts in the inbound and outbound foraging routes (45°, 90°, 135°, 180°). We find that homeward vectors calibrate rapidly after repeated training, with significant shifts toward the inbound route direction occurring after only three foraging trips. The limit of the vector's directional plasticity appears to be 45°, as under this conflict the forager's vector will recalibrate the full 45°. At larger directional conflicts, calibration decreases, showing intermediate shifts between the inbound memories and the current outbound vector, and at the maximum conflict (180°), foragers show no calibration.

This chapter is published in *Insectes Sociaux* (Freas and Cheng 2017b) and was co-authored by Ken Cheng. Ken Cheng provided suggestions on experimental methodology as

well as revisions to the manuscript prior to submission. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

In **Chapter 5**, we expand on the experiments in Chapter 3, testing both foragers' ability to extrapolate nest site views and landmark learning during the first foraging trips. In experiment 1, foragers were allowed variable amounts of exposure to the nest panorama and then tested at a local site where their return trips were recorded. In experiment 2, foragers' return trips from a local site were recorded with the panorama cues blocked during the outbound or inbound segment of the trip and with/without a homeward vector. We show that foragers are unable to orient or return home efficiently regardless of the exposure level to the nest panorama. During experiment 2, foragers were able to use their experience of either the outbound or inbound views to orient, but were unable to quickly return to the nest when only exposed to the inbound route. Our results suggest foragers cannot extrapolate views from learning walks and supports a recognition-based model for panorama navigation. Furthermore, exposure to the outbound foraging path appears critical for efficient homeward route formation.

This chapter is in review at *Journal of Experimental Psychology: Animal Learning and Cognition* and was co-authored by Ken Cheng. Ken Cheng provided suggestions on experimental methodology as well as revisions to the manuscript prior to submission. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

The study of vision based navigation and cue choice is widely studied in diurnal ants, when visual cues are easy to distinguish (Wehner et al. 1996; Fukushi 2001; Beugnon et al. 2005; Cheng et al. 2009; Bühlmann et al. 2011), yet less is known of these abilities and preferences in nocturnal ant species. These ants are presented with the additional challenge of navigating when visual cues are more difficult to detect (Warrant 2008; Warrant and Dacke

2011). In **Chapter 6**, we explored the compass cues used by the previously unstudied nocturnal bull ant *Myrmecia midas*. We found that *M. midas* foragers with accumulated vectors of under 5m do not orient to home vectors after displacement, yet when displaced with larger home vectors (≥ 10 m), foragers do orient to their vector. When presented a cue choice test with a conflict between the home vector and the panorama cues, foragers oriented using the panorama cues alone regardless of vector length. When the panorama cues were blocked, foragers were unable to orient, suggesting heavy reliance on visual panorama cues in this species. Our final testing condition focused on nest-tree foragers. *M. midas* nest at the base of a tree and a subset of the nest forage on that tree. These foragers were unable to orient to the nest after local displacements (5m), suggesting an inability to extrapolate panorama cues at the nest site to close displacement sites.

This chapter is published in *Journal of Experimental Biology* (Freas et al. 2017a) and was co-authored by Ajay Narendra and Ken Cheng. Ajay Narendra and Ken Cheng provided suggestions on experimental methodology as well as revisions to the manuscript prior to publication. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

The navigational tool kit of solitary foraging ants includes both the use of celestial cues and the panorama. One such celestial cue is the polarisation pattern in the sky. In **Chapter 7**, we tested polarised light use during foraging and with different home vectors in *M. midas* by rotating the overhead polarization pattern by $\pm 45^\circ$. Foragers responded to these manipulations during both the outbound and inbound foraging trips, yet the degree to which they responded to the rotation varied. On the outbound journey, foragers compensated by about half of the change in the overhead polarization pattern, and this intermediate direction was regardless of the forager's vector length. On the inbound journey, foragers compensated by about half the manipulation when their vector was short but by more than half when the vector was longer (> 4 m). Our results suggest that these ants dynamically weight the

terrestrial and celestial cues during the foraging trip.

This chapter is published in *Royal Society Open Science* (Freas et al. 2017c) and was co-authored by Ajay Narendra, Corentin Lemesle and Ken Cheng. Ajay Narendra and Ken Cheng provided suggestions on experimental methodology as well as revisions to the manuscript prior to publication. Corentin Lemesle assisted with fieldwork. All other work concerning the conception, data collection, analysis, and manuscript preparation was conducted by Cody Freas.

Solitary foraging ants are known to store the panorama to navigate while on the ground, yet several species also travel in three dimensions by climbing trees. How these foragers solve navigational challenges in three dimensions while their body is perpendicular to the ground is largely unknown. In **Chapter 8**, we explore if *Myrmecia midas* foragers use visual information while travelling down trees to return to the nest. During displacement tests, regardless of their location on the tree, foragers travelled to the side of the trunk facing the nest during their descent and oriented to the panorama cues when displaced on non-foraging trees. When the panorama around the tree was blocked, foragers could no longer orient while descending, suggesting the panorama is critical. We further explored the potential for panorama-based navigation through rotational image analysis, which showed that views acquired along the ground foraging route could provide information for successful orientation while on the tree.

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Limits of vector calibration in the Australian desert ant, *Melophorus bagoti*

Cody A Freas¹, & Ken Cheng¹

¹ Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Abstract

Desert ants that forage solitarily continually update their position relative to the nest through path integration. This is accomplished by combining information from their celestial compass and pedometer. The path integration system can adapt when memories of previous inbound routes do not coincide with the outbound route, through vector calibration. Here we test the speed and limit of vector calibration in the desert ant *Melophorus bagoti* by creating directional conflicts between the inbound and outbound routes (45°, 90°, 135°, 180°). The homeward vector appears to calibrate rapidly after training with shifts occurring after three foraging trips, yet the limit of the vector's plasticity appears to be a maximum of 45°. At 45° conflicts, the vector calibrates the full 45°, suggesting dominance of the previous inbound memories over the outbound cues of the current trip. Yet at larger directional conflicts, vector shifts after training diminish, with foragers in the 90° and 135° conditions showing smaller intermediate shifts between the inbound memories and the current outbound vector. When the conflict is at its maximum (180°), foragers show no calibration, suggesting the outbound vector is dominant. Panorama exposure during training appears to aid foragers orienting to the true nest, but this also appears limited to about a 45° shift and does not change with training.

Introduction

Solitary foraging ants continually update their current position relative to their nest, termed their vector, via path integration (Collett and Collett 2000b; Wehner 2003; Wehner 2008). Path integration combines two mechanisms, which together create the vector (Wehner 1994; Wittlinger et al. 2007). The first is a distance estimate, calculated primarily through a pedometer (Wittlinger et al. 2007). The second is a celestial compass and is dependent chiefly on the pattern of polarized light in the sky (Wehner, 1994; Wehner and Müller 2006; Cheng and Freas 2015). Ants sense these celestial cues through the dorsal rim area of their eyes, which is sensitive to UV light (Fent, 1986; Labhart and Meyer 1999; Zeil et al. 2014; Narendra et al. 2016). Using path integration, foragers can return to the nest along the shortest route despite a meandering outbound search for food. Foragers retain memories of these vectors for subsequent foraging trips where outbound foragers at the nest can use the 180° mirror of the inbound vector to return to profitable spatial locations (Collett et al. 1999; Collett and Collett 2000b).

Path integration is susceptible to the accumulation of error, meaning that the vector will only direct a returning forager to the general area of the nest (Wehner and Wehner 1986). This imprecision necessitates the use of corrective mechanisms to reach the exact nest location. These mechanisms can include learned panorama cues when they are present (Collett 1992; Wehner et al. 1996; Narendra et al. 2007b; Schultheiss et al. 2016; Freas et al. 2017b), systematic search (Wehner and Srinivasan 1981; Müller and Wehner 1994; Wehner 2003; Narendra 2007; Schultheiss and Cheng 2011), olfactory cues (Buehlmann et al. 2015), and vector calibration (Collett et al. 1999; Wehner et al. 2002).

Vector calibration occurs when the outbound and inbound vectors do not coincide, resulting in a non-zero value when the forager reaches the nest. The disagreement between the inbound and outbound vectors during previous foraging trips can cause foragers to alter their subsequent inbound and outbound route directions toward the vector memory of previous

trips' inbound routes (Collett et al. 1999; Collett and Collett 2000a; Wehner et al. 2002). In *Cataglyphis fortis*, a desert ant species navigating with few available visual panorama cues, foragers were shown to rapidly calibrate their vector directions in response to vector conflicts in the inbound and outbound route, shifting subsequent foraging directions to align with the previous trips' inbound routes. Yet, vector calibration in this species did not result in full shifts to the inbound direction but instead suggested an intermediate direction between the two routes. These individuals were tested with directional conflicts under 90°, so that the extent of the vector's directional calibration remains unknown (Collett et al. 1999; Wehner et al. 2002). Furthermore, the barren landscape *C. fortis* inhabits means it may only rely on systematic search to find the nest after displacement off-route (Wehner et al. 2002). Therefore, it is currently unknown what role the availability of the visual panorama may play in vector calibration when the panorama aligns with the inbound vector of previous trips and conflicts with their current outbound vector.

Here we study vector calibration in the Australian desert ant, *Melophorus bagoti*, which lives in a landmark rich environment, and relies both on the surrounding panorama and path integration to navigate (Narendra et al. 2007ab; Graham and Cheng 2009; Cheng et al. 2009; Legge et al. 2014). Only one study (Freas and Cheng 2017) has explored vector recalibration in *Melophorus bagoti* and these experiments only tested foragers with no accumulated outbound vector with a 180° conflict, resulting in no observable vector calibration. In the current study, foragers were trained and tested by displacing individuals off their outbound foraging route by 45°, 90°, 135°, or 180° on every successful foraging trip. Foragers were collected and tested on their first visit to a feeder before displacement training and then at regular training intervals, within an arena representing an unfamiliar, uniform skyline. This arena forced individuals to use celestial cues to orient, allowing us to examine the magnitude and speed of their vector calibration after displacement training. Additionally, we recorded forager headings at the displacement sites to explore how the added presence of

the panorama may alter inbound headings of foragers with conflicting outbound and inbound vectors.

Methods

Field Site & Subjects

Experiments were conducted at a field site (Fig. 1) located 10 km south of Alice Springs, Northern Territory, Australia ($23^{\circ}45'28.12''$ S, $133^{\circ}52'59.77''$ E). The study site consists of semi-arid desert populated primarily with buffel grass (*Cenchrus ciliaris*), and scattered Eucalyptus trees and *Acacia* bushes (Muser et al. 2005). Experiments were conducted in February and March 2017, during the Australian summer. The current study focused on foragers of *Melophorus bagoti*, a species of desert ant, which acts as a diurnal scavenger and therefore is active during the hottest parts of the day, collecting dead insects (Wehner 1987; Christian and Morton 1992).



Fig. 1: Overhead (Google Earth, 2016) image of the nest location and surrounding field site, located 10 km south of Alice Springs, Northern Territory, Australia. Reference: Google Earth 9.8.2017. Centre for Appropriate Technology Campus. $23^{\circ}45'29.68''$ S, $133^{\circ}53'00.31''$ E, elevation ~555m.

Experimental Set-up

4m from the nest entrance, a plastic feeder ($15 \times 15 \times 9$ cm) was dug into the ground (Fig. 2). The feeder was stocked with crushed cookie (Arnott™) pieces and cut up pieces of mealworms. Foragers that fell into the feeder were unable to escape without being removed manually. A training corridor was erected around the nest and feeder using 10cm tall plastic similar to the feeder walls. This corridor was 75cm wide and 4.5m in length. The lack of natural food within the corridor encouraged foragers to return to the feeder continuously for food. On the outer side of the corridor, a dirt ramp was erected, allowing one-way movement back to the nest entrance from the displacement sites (Fig. 2). All vegetation was cleared in a 4.3m radius semi-circle around the nest entrance and four displacement sites, each 4m from the nest entrance and 45°, 90°, 135° and 180° clockwise from the feeder were marked using metal pegs (Fig. 2). 20-30cm beyond the displacement sites, the ground remained uncleared and was populated with grass tussocks and brush. In the nest-feeder direction 15m from the nest, we constructed a 1m diameter, 60cm high uniform arena. This arena was made out of black plastic and blocked the entire surrounding natural panorama, forcing foragers to orient using vector cues. Initial headings in this arena were recorded through direct observation, randomising the observer's location with each release.

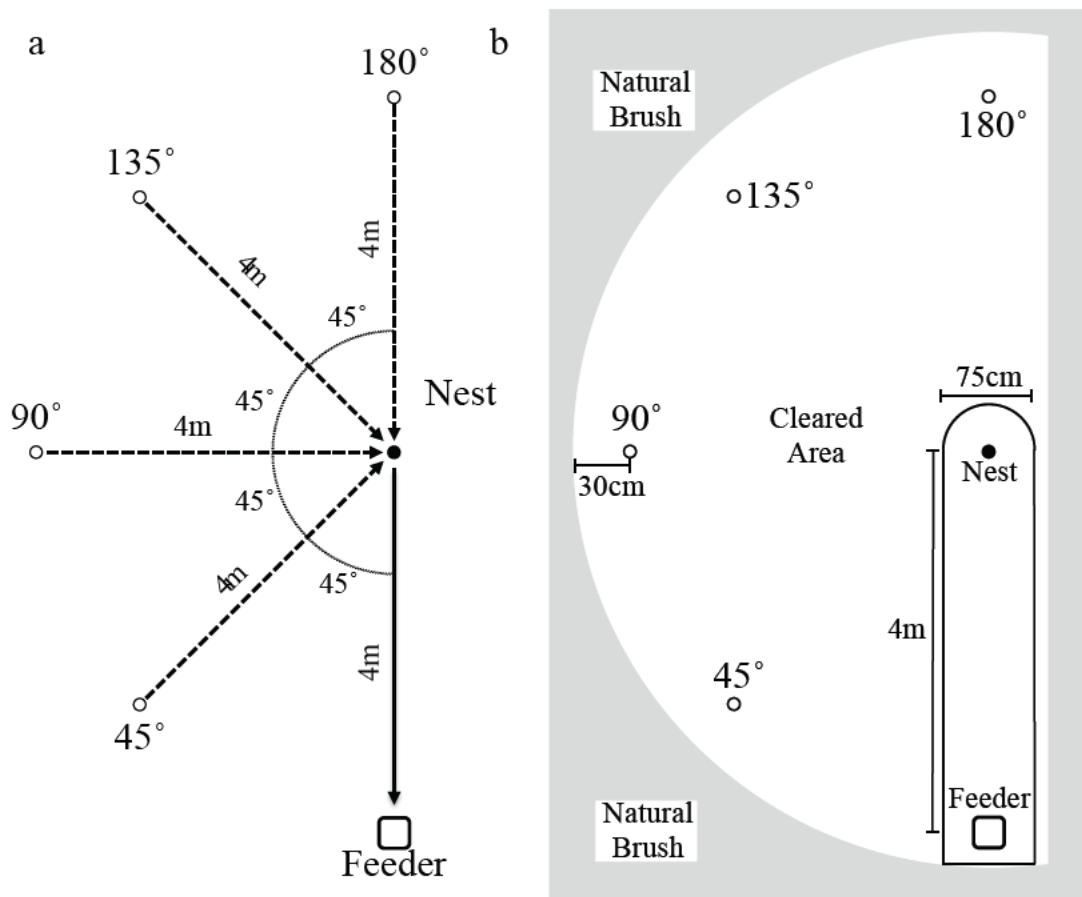


Fig. 2 Diagrams of the displacement training set-up. (a) Diagram showing the outbound and inbound route distances and angles. (b) Diagram of the training corridor and the surrounding area which was cleared of natural brush in a ~5m radius semi-circle around the nest.

45° Calibration Tests

The 45° condition tested foragers with similar vector discrepancies tested in *C. fortis* where paths differed by 55° (Wehner et al. 2002). On a forager's first trip to the feeder, it was collected and individually marked by placing a small amount of acrylic paint (Tamiya™) on the abdomen. It was then allowed to gather a piece of food. Foragers were collected from the feeder using a darkened plastic vial and transferred to the uniform arena. Within the testing arena foragers were released onto a wooden board with a goniometer drawn on the surface. The goniometer consisted of a 60cm diameter circle divided into 15° wedges, and initial headings of individual foragers were measured by recording the sector number of forager crossings at 30cm. After arena testing, foragers were re-collected in the darkened vial and transferred to the displacement site 45° off-route from the nest-feeder route (Fig. 2). At the displacement site, foragers were released and tested at the site on an identical goniometer, and

after running off this goniometer, foragers were allowed to return to the nest naturally. On each subsequent return trip to the feeder, foragers were displaced in a darkened vial to the 45° displacement site (Fig. 2) and allowed to return home, resulting in every successful foraging trip having distinct and conflicting outbound and inbound routes. After 3, 5, and 10 trips with this setup, foragers were again tested within the uniform arena before displacement, and after 10 trips foragers were also tested on the goniometer at the displacement site.

90°, 135°, & 180° Calibration Tests

Identical to the previous, 45° condition, on Trip 1 foragers were marked and tested on the goniometer within the uniform arena before training and then randomly assigned one of three displacement sites (90°, 135°, & 180°) off the homeward route (Fig. 2). After the 3rd, 5th, 10th, 15th, 20th, & 25th displacements, foragers were tested within the uniform arena before displacement and after the 10th & 25th trips foragers were tested at the displacement site. All individuals completed at least 10 training trips. As a forager may only make a few training trips a day and mean life span above ground is 4.9 days (Muser et al. 2005), we experienced forager attrition over the longer training regimens (25th trip survivorship 90°, 46.9%; 135°, 56.3%; 180°, 78.1%).

Statistical analysis

Data were analyzed with circular statistics (Batschelet 1981; Zar 1998) with Oriana Version 4 (Kovach Computing Services, UK). Rayleigh's Tests were used to determine if data met the conditions of a uniform distribution ($p > 0.05$). V tests, with alpha set at $p = 0.05$, and the mean vector's 95% confidence interval (95% CI) were used to determine if initial orientations were oriented to the outbound or inbound vector direction. Paired Watson & Wheeler F-tests were used to compare mean vectors between pre-training orientations and each testing condition. To test if the observed shifts were compromise directions or complete shifts to the inbound vector, we rotated the pre-training orientations by condition, +45° +90°

+135° or +180° and compared these orientations to the post training testing conditions using paired Watson & Wheeler F-tests. We used the Holm-Bonferroni sequential method (Holm 1979) to correct for multiple paired Watson & Wheeler F-tests. In the 180° condition, calibration could result in individual directional shifts in either direction. These changes may not be detectable when comparing mean vectors but could manifest as increases in variance. We compared these variance levels between the pre and post training conditions in the 180° condition using a Var test (Wystrach et al. 2014). In the Var test, absolute differences from the mean vector were calculated for both the pre and post training conditions and then compared using a two-tailed non-parametric Wilcoxon rank sum test.

Results

Pre-training Arena Tests

Initial orientations of pre-training foragers tested within the uniform arena were significantly non-uniform and directed to the accumulated outbound vector at 0° in all conditions (Table 1; Fig. 3a; Fig. 4a,h,o).

45° Arena Tests

In the 45° displacement testing in the uniform arena, foragers remained oriented across all conditions (Table 1). After three displacements, forager's orientations shifted significantly away from the outbound vector (mean \pm s.e.m = $21.97 \pm 14.06^\circ$; Table 1; Table 2; Fig. 3b) and represented a compromise direction between the current outbound trip and previous inbound trips (Table 2). After five trips, forager's orientations had shifted away from the pre-training vector (mean \pm s.e.m = $38.88 \pm 10.51^\circ$; Table 2; Fig. 3c). This shift was complete to the inbound vector direction (Table 2). This pattern continued with arena testing after 10 displacements (mean \pm s.e.m = $48.76 \pm 6.43^\circ$; Table 1; Fig. 3d) as orientations were shifted significantly away from the outbound vector and toward the inbound vector (Table 2).

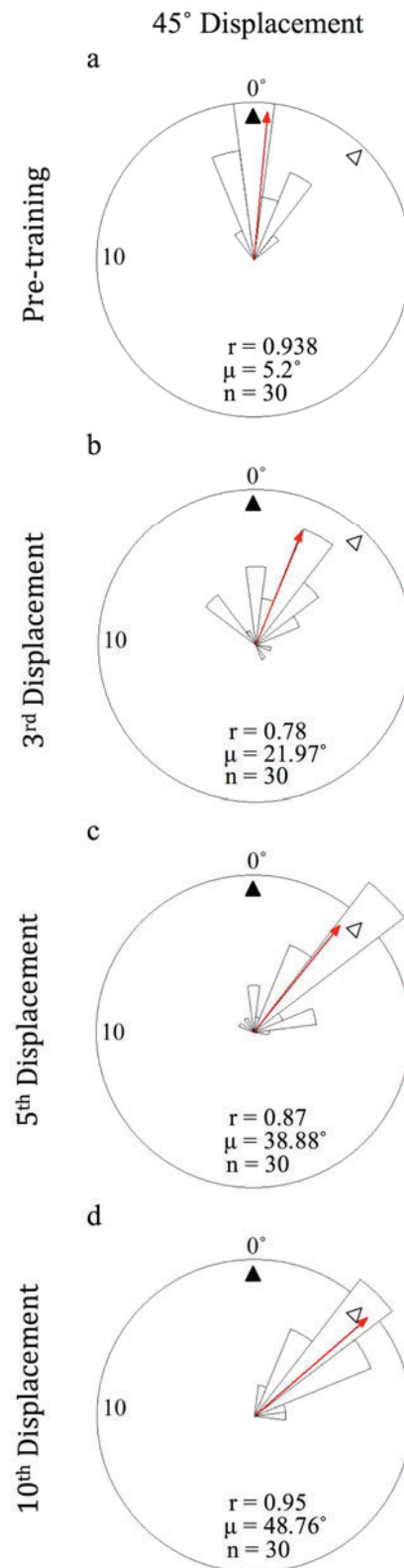


Fig. 3 Circular histograms of initial headings in the 45° arena tests. The current accumulated vector direction for all conditions is marked by a black triangle at 0°. The inbound route after displacement is marked by an open triangle at 45°. All initial orientations were taken at 30cm from release. The arrow in each histogram denotes the length of the mean vector and the direction of the average orientation of the condition. **a** On their first trip, foragers were collected from the feeder, transferred to the uniform arena and tested on a goniometer. After testing they were released at the 45° displacement site. After **(b)** 3, **(c)** 5, and **(d)** 10 displacements to the 45° site, foragers were collected from the feeder, transferred to the uniform arena and tested on a goniometer. n , number of individuals; μ , mean vector; r , length of the mean vector.

Table 1 Statistical results for the vector calibration tests. In all conditions degrees increased clockwise from 0° and the outbound vector direction for all conditions was set at 0°

	Mean Vector	95% Confidence interval		Rayleigh test		V test: Outbound Vector (0°)		V test: Inbound Vector (45°, 90°, 135°, or 180°)	
Arena Tests	μ (°)	Minus (°)	Plus (°)	Z	p	V	p	V	p
45° Pre-training	5 20	358 01	12 40	27 29	< 0 0001	0 93	< 0 0001	0 72	< 0 0001
45° 3 rd Trip	21 97	7 913	36 02	18 87	< 0 0001	0 72	< 0 0001	0 72	< 0 0001
45° 5 th Trip	38 88	28 37	49 40	23 59	< 0 0001	0 68	< 0 0001	0 87	< 0 0001
45° 10 th Trip	48 76	42 33	55 19	28 01	< 0 0001	0 63	< 0 0001	0 95	< 0 0001
90° Pre-training	355 13	348 10	2 16	28 22	< 0 0001	0 94	< 0 0001	-0 08	0 737
90° 3 rd Trip	15 10	2 78	27 42	21 63	< 0 0001	0 79	< 0 0001	0 21	0 043
90° 5 th Trip	23 41	9 16	37 66	18 9	< 0 0001	0 71	< 0 0001	0 31	0 007
90° 10 th Trip	37 29	25 82	48 76	22 84	< 0 0001	0 67	< 0 0001	0 51	< 0 0001
90° 15 th Trip	25 93	18 12	33 74	21 37	< 0 0001	0 85	< 0 0001	0 41	0 002
90° 20 th Trip	29 70	18 70	40 71	15 83	< 0 0001	0 79	< 0 0001	0 45	0 002
90° 25 th Trip	33 48	21 06	45 91	12 93	< 0 0001	0 73	< 0 0001	0 51	0 002
135° Pre-training	357 14	350 21	4 08	28 32	< 0 0001	0 94	< 0 0001	-0 70	1
135° 3 rd Trip	16 55	4 03	29 07	21 34	< 0 0001	0 78	< 0 0001	-0 39	0 99
135° 5 th Trip	18 29	359 32	37 27	13 25	< 0 0001	0 61	< 0 0001	-0 29	0 99
135° 10 th Trip	29 93	17 72	42 15	21 78	< 0 0001	0 72	< 0 0001	-0 21	0 96
135° 15 th Trip	25 26	16 03	34 49	21 80	< 0 0001	0 83	< 0 0001	-0 31	0 99
135° 20 th Trip	20 61	10 15	31 07	16 81	< 0 0001	0 86	< 0 0001	-0 38	0 99
135° 25 th Trip	25 87	16 90	34 83	16 05	< 0 0001	0 85	< 0 0001	-0 31	0 97
180° Pre-training	357 36	348 85	5 87	26 61	< 0 0001	0 91	< 0 0001	-0 91	1
180° 3 rd Trip	3 89	354 09	13 69	25 05	< 0 0001	0 88	< 0 0001	-0 88	1
180° 5 th Trip	4 22	351 84	16 61	21 53	< 0 0001	0 82	< 0 0001	-0 82	1
180° 10 th Trip	7 59	353 01	22 18	18 45	< 0 0001	0 75	< 0 0001	-0 75	1
180° 15 th Trip	4 89	349 83	19 96	16 73	< 0 0001	0 77	< 0 0001	-0 77	1
180° 20 th Trip	9 34	356 37	22 31	17 39	< 0 0001	0 84	< 0 0001	-0 84	1
180° 25 th Trip	355 77	344 03	7 51	16 68	< 0 0001	0 98	< 0 0001	-0 89	1
Displacement Site Tests									
45° 1 st Trip	33 70	26 16	41 25	26 20	< 0 0001	0 78	< 0 0001	0 92	< 0 0001
45° 10 th Trip	47 56	35 49	59 63	21 13	< 0 0001	0 57	< 0 0001	0 84	< 0 0001
90° 1 st Trip	30 97	24 86	37 08	29 11	< 0 0001	0 82	< 0 0001	0 49	< 0 0001
90° 10 th Trip	38 77	32 04	45 51	28 52	< 0 0001	0 74	< 0 0001	0 59	< 0 0001
90° 25 th Trip	31 07	9 68	52 46	9 57	< 0 0001	0 68	< 0 0001	0 41	0 011
135° 1 st Trip	33 85	21 14	46 56	21 07	< 0 0001	0 67	< 0 0001	-0 16	0 89
135° 10 th Trip	40 03	32 26	47 80	27 45	< 0 0001	0 71	< 0 0001	-0 08	0 739
135° 25 th Trip	42 95	25 85	60 04	11 76	< 0 0001	0 59	< 0 0001	-0 03	0 568
180° 1 st Trip	5 14	352 26	18 02	20 83	< 0 0001	0 80	< 0 0001	-0 80	1
180° 10 th Trip	18 29	3 78	32 80	18 55	< 0 0001	0 72	< 0 0001	-0 72	1
180° 25 th Trip	0 82	326 83	34 82	4 9	0 006	0 44	0 0007	-0 44	0 99

90° & 135° Arena Tests

During the 90° and 135° displacement testing in the uniform arena, after three displacements forager orientations were shifted away from the pre-training headings (Table 1; Table 2; Fig. 4b,i) in the direction of the inbound route (mean \pm s.e.m, 90° $\theta = 15.10 \pm 12.32^\circ$ & 135° $\theta = 16.55 \pm 12.52^\circ$ respectively). These shifts were incomplete, as each condition's mean vector significantly differed from both the outbound and inbound vector route (Table 2). This trend continued in subsequent tests. After 5, 10, 15, 20, & 25 trips in the 90° and 135° displacement conditions, forager orientation was shifted significantly way from the outbound route (Table 1; Table 2; Fig. 4c-g,j-n). These shifts remained incomplete, as mean vectors in both 90° and 135° conditions significantly differed from the inbound route across all tests (Table 2). These orientations remained compromise directions as the mean vector directions significantly differed from the inbound vector (Table 1).

Table 2 Mean vector comparisons between pre-training tests and after displacements. Probability values presented in this table are not corrected for multiple comparisons.

<u>Arena Tests</u>	<u>Watson-Williams F-Test</u>			<u>Watson-Williams F-Test</u>		
	<u>Pre-training</u>			<u>Pre-training + displacement angle</u>		
	F	p	df1, df2	F	p	df1, df2
45° Displacements						
3 rd Trip	4 256	0 043	1, 58	12 04	< 0 001	1, 58
5 th Trip	26 212	< 0 0001	1, 58	2 98	0 09	1, 58
10 th Trip	75 664	< 0 0001	1, 58	0 08	0 773	1, 58
90° Displacements						
3 rd Trip	7 476	0 008	1, 62	89 25	< 0 0001	1, 62
5 th Trip	11 957	< 0 001	1, 62	55 82	< 0 0001	1, 62
10 th Trip	36 776	< 0 0001	1, 62	47 18	< 0 0001	1, 62
15 th Trip	31 618	< 0 0001	1, 56	114 78	< 0 0001	1, 56
20 th Trip	28 305	< 0 0001	1, 49	71 66	< 0 0001	1, 49
25 th Trip	32 76	< 0 0001	1, 45	58 75	< 0 0001	1, 45
135° Displacements						
3 rd Trip	6 936	0 011	1, 62	230 36	< 0 0001	1, 62
5 th Trip	4 395	0 04	1, 62	117 59	< 0 0001	1, 62
10 th Trip	20 47	< 0 0001	1, 62	191 06	< 0 0001	1, 62
15 th Trip	22 942	< 0 0001	1, 56	317 73	< 0 0001	1, 56
20 th Trip	14 03	0 0005	1, 50	296 92	< 0 0001	1, 50
25 th Trip	23 41	< 0 0001	1, 48	304 21	< 0 0001	1, 48
180° Displacements						
3 rd Trip	0 953	0 333	1, 62			
5 th Trip	0 784	0 379	1, 62			
10 th Trip	1 384	0 244	1, 62			
15 th Trip	0 75	0 39	1, 58			
20 th Trip	2 396	0 128	1, 54			
25 th Trip	0 047	0 83	1, 51			
Displacement Site Tests						
45° 1 st Trip & 10 th Trip	3 573	0 064	1, 58			
90° 1 st Trip & 10 th Trip	2 768	0 101	1, 62			
90° 10 th Trip & 25 th Trip	0 0001	0 99	1, 45			
135° 1 st Trip & 10 th Trip	0 648	0 424	1, 62			
135° 10 th Trip & 25 th Trip	0 669	0 417	1, 48			
180° 1 st Trip & 10 th Trip	1 684	0 199	1, 62			
180° 10 th Trip & 25 th Trip	0 073	0 788	1, 51			

180° Arena Tests

In the 180° condition, initial orientations in the arena did not change after training. Forager remained oriented toward the outbound vector direction at 0° (Table 1; Fig. 4p-u). The mean vector of these orientations did not significantly differ from that of pre-training orientations (Table 2). Additionally, the variance of these orientations did not increase significantly after training (Var Test; 3rd trip, $Z = 0.6077$, $p > 0.05$; 5th trip, $Z = 1.318$, $p > 0.05$; 10th trip, $Z = 1.533$, $p > 0.05$; 15th trip, $Z = 0.178$, $p > 0.05$; 20th trip, $Z = 0.579$, $p > 0.05$; 25th trip, $Z = 0.461$, $p > 0.05$).

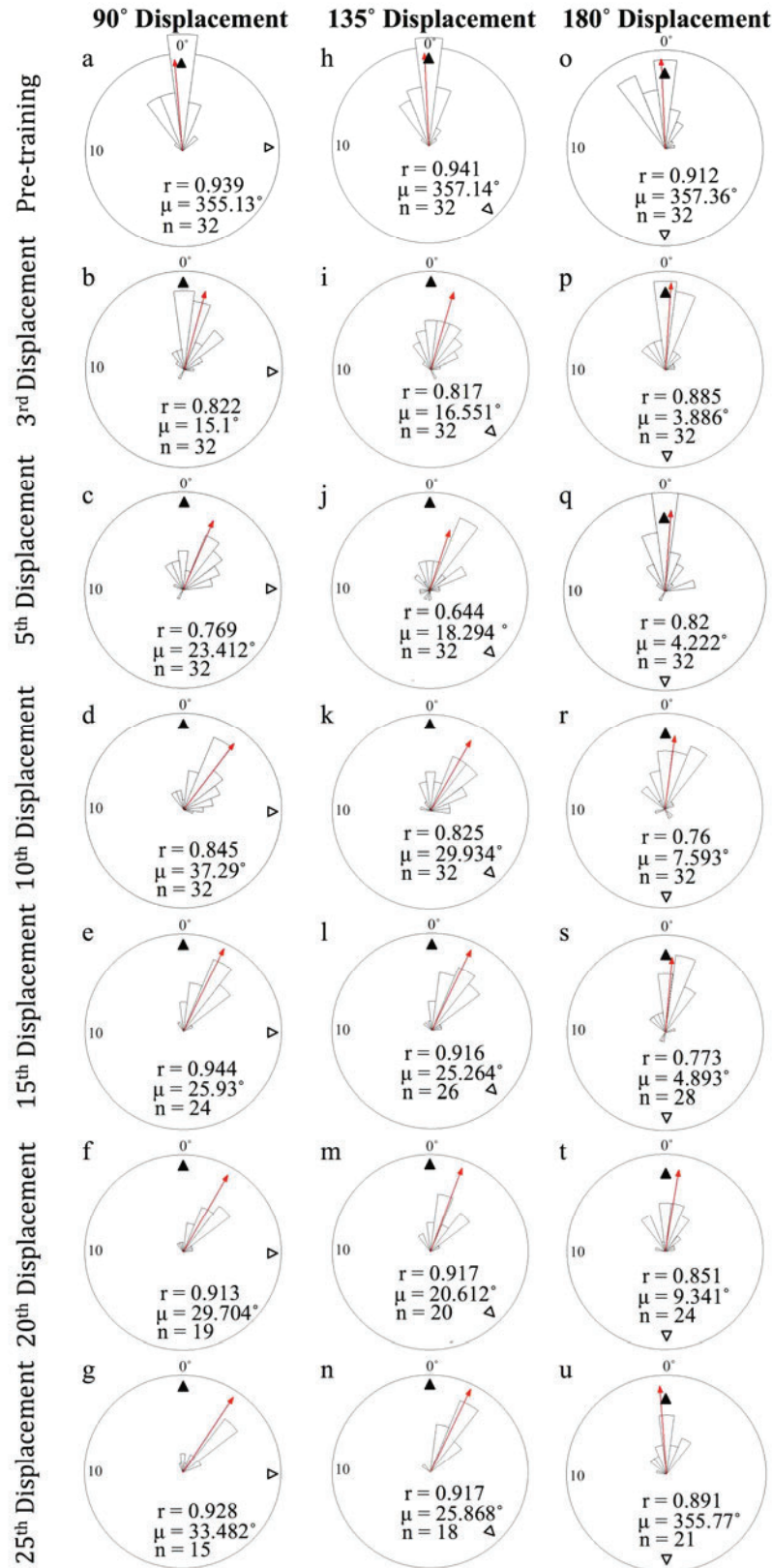


Fig. 4 Circular histograms of initial headings in the 90°, 135°, and 180° arena tests. The current accumulated vector direction for all conditions is marked by a black triangle at 0°. The inbound route after displacement is marked by an open triangle. All orientations were recorded at 30cm from release. The arrow in each histogram denotes the length of the mean vector and the direction of the average orientation of the condition. Foragers in the (a–g) 90°, (h–n) 135° and (o–u) 180° conditions were collected from the feeder and tested within the uniform arena on their first foraging trip, and after 3, 5, 10, 15, 20, or 25 training displacements. n, number of individuals; μ , mean vector; r, length of the mean vector.

Displacement Site Tests

In the 45° displacement site testing, when foragers were released (Fig. 2) they oriented in a direction (mean \pm s.e.m = $33.70 \pm 7.54^\circ$) between the true nest direction at 45° and the vector direction at 0° and both the true nest and vector directions fell outside the 95% CI (Table 1; Fig. 5a). After ten training trips, foragers were oriented (mean \pm s.e.m = $47.56 \pm 12.07^\circ$) toward the true nest site at 45°, (Table 1) but this shift to the true nest direction after training was not significant when the mean vectors of both the pre and post training conditions were compared (Table 2; Fig. 5b).

In the 90° & 135° displacement site testing, when foragers were released on the first displacement trip at the 90° or 135° site (Fig. 2) they oriented in a compromise direction between the true nest and the vector direction with both falling outside the 95% CI (30.972° and 33.847° respectively; Table 1; Fig. 5c,f). Observations suggest foragers on their first trip appeared unsure of how to navigate home, resulting in long inbound paths that resembled searching behavior. Orientations at the displacement site did not shift significantly on training trips 10 and 25 (Table 2; Fig. 5d,e,g,h), but observations of experienced foragers' inbound routes after the initial orientation suggest the formation of a homeward route that initially bowed out in the direction of the vector and then arched back toward the nest entrance.

In the 180° displacement site testing, when these foragers were released on the first displacement trip (Fig. 2) they oriented to the vector cues at 0° and not to the true nest at 180° (Table 1; Fig. 5i). Foragers traveled off the goniometer and into the brush beyond the training area. Foragers' observed homeward routes were indicative of search behavior. These orientations did not shift significantly after training on trips 10 and 25 (Table 2; Fig. 5j,k), with foragers still oriented to the vector direction and not to the true nest (Table 1). Observations of foragers suggested they did not develop efficient homeward routes, as most individuals still headed into the brush before returning past the release site and to the nest.

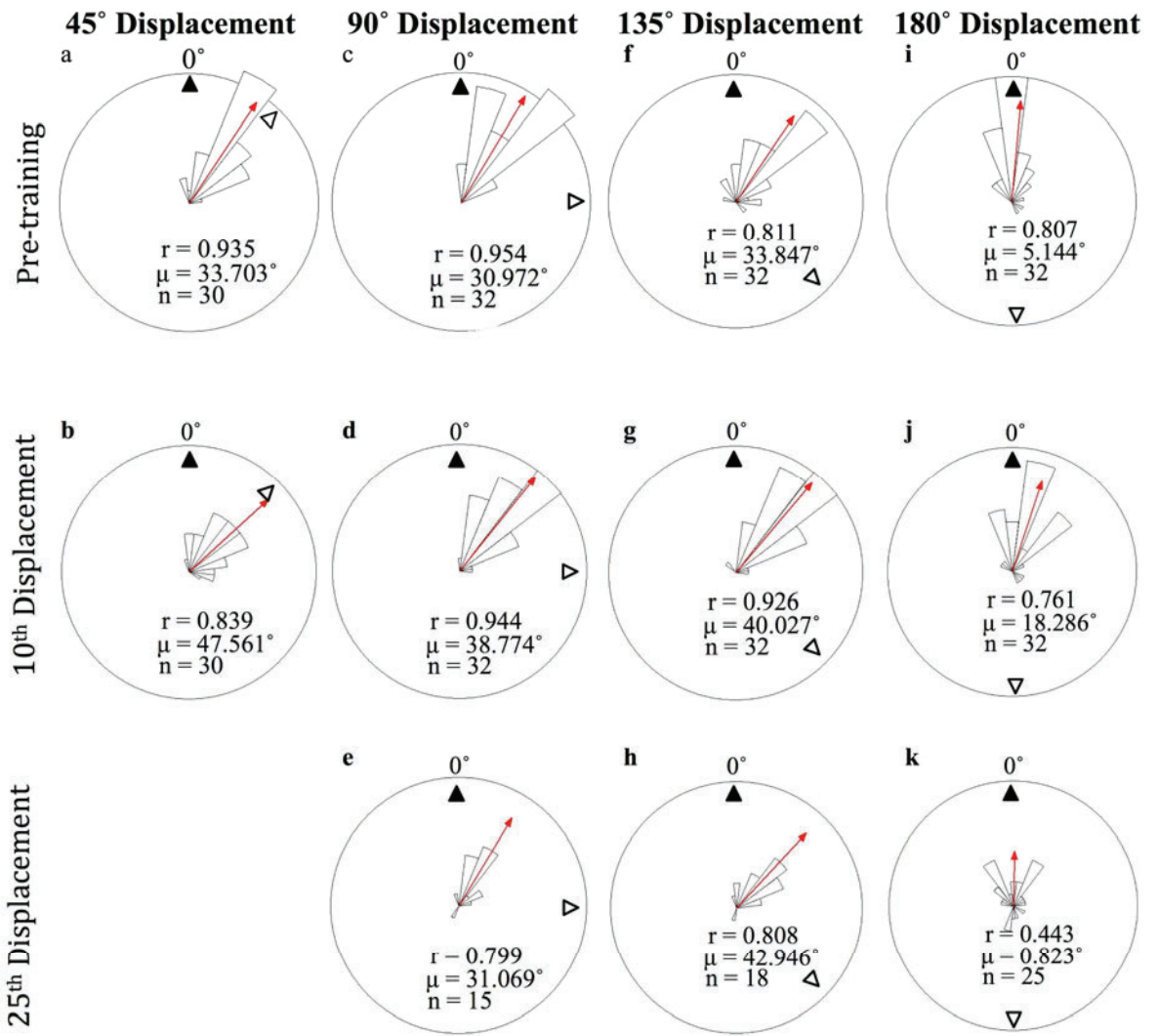


Fig. 5 Circular histograms of initial headings at the 45°, 90°, 135°, and 180° displacement site tests. The current accumulated vector direction for all conditions is marked by a black triangle at 0°. The true nest direction is marked by an open triangle and all orientations were recorded at 30cm from release. The arrow in each histogram denotes the length of the mean vector and the direction of the average orientation of the condition. After testing in the uniform arena, 45° condition foragers were collected and released on a goniometer at the 45° displacement site, on their (a) 1st displacement, and after their (b) 10th displacement. In the (c–e) 90°, (f–h) 135°, and (i–k) 180° condition, foragers were collected in the arena and tested at the respective displacement site on their 1st displacement and after their 10th and 25th displacement; μ , mean vector; r , length of the mean vector.

Arena Tests vs. Displacement Site Tests

In the 45°, 90° and 135° conditions on Trip 1, the mean vector of forager orientations within the uniform arena differed significantly from orientations when tested at the displacement site (Watson & Wheeler F-test; 45°, $F_{1,58} = 26.72$, $p < 0.001$; 90°, $F_{1,62} = 55.20$, $p < 0.001$; 135°, $F_{1,62} = 24.09$, $p < 0.001$). By Trip 10, the mean vector of orientations in the arena did not significantly differ from orientations at the displacement site (Watson & Wheeler F-test; 45°,

$F_{1,58} = 0.07, p > 0.05$; $90^\circ, F_{1,62} = 0.05, p > 0.05$; $135^\circ, F_{1,62} = 1.83, p > 0.05$). In the 180° condition the mean vector of forager orientations within the uniform arena did not differ significantly from orientations when tested at the displacement site on Trip 1 or Trip 10 (Watson & Wheeler F-test; Trip 1, $F_{1,62} = 0.96, p > 0.05$; Trip 10, $F_{1,62} = 0.99, p > 0.05$).

Discussion

All displacement conditions taken together suggest that 45° represents the upper limit of the vector calibration in *M. bagoti* under our testing conditions. Furthermore, cue weighting of the inbound vector, outbound vector, and panorama cues appears to change as directional conflicts increase. At 45° , the stored inbound cues seem to dominate, with foragers showing full shifts to this direction in both the arena and at the displacement site. As conflicts increase, at 90° and 135° , the current outbound vector appears to be weighted more heavily compared to the inbound vector, as observed shifts decrease numerically and represent only 37.2% and 19.2% of the conflict, respectively, after 25 trips. These compromises were also evident at the displacement site. Unlike orientation testing within the uniform arena, at the displacement site foragers were also exposed to the presence of the panorama at the displacement site supporting the inbound vector direction. At the largest vector conflict, 180° , the current outbound vector appears to fully overpower the stored inbound vector, resulting in no vector calibration. Even with the presence of the visual panorama at the displacement site, the outbound vector still dominated.

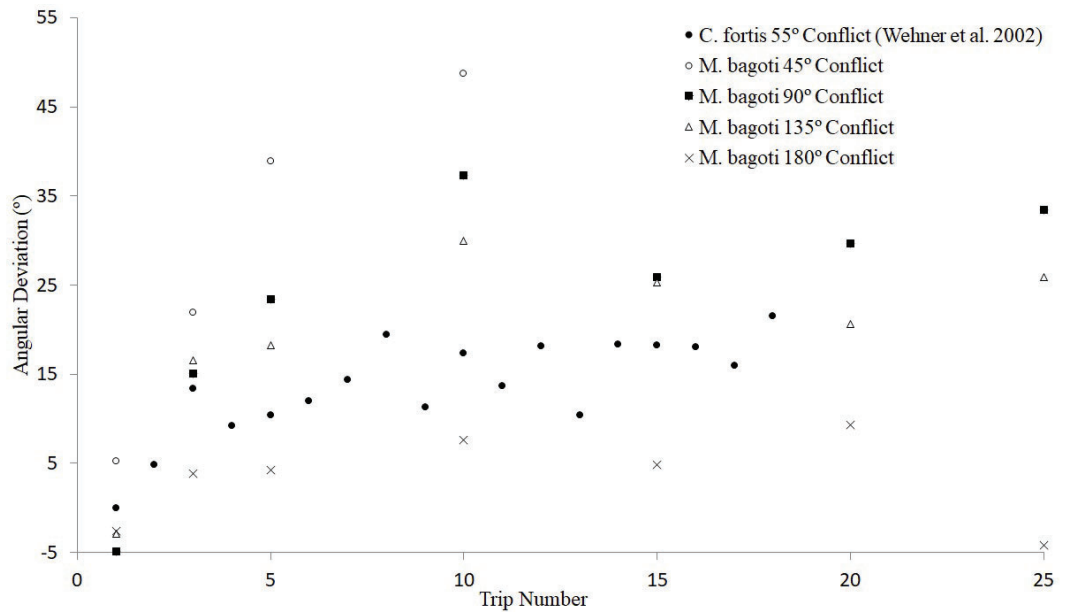


Fig. 6 Comparing vector calibration rates by trip number in *Cataglyphis fortis* with a 55° conflict (Wehner et al. 2002) and all *Melophorus bagoti* conditions in the uniform arena. Diagram depicts the angular deviation from the outbound vector direction (0°) at each recorded trip. Due to experimental differences between *C. fortis* and *M. bagoti* studies, inferential statistical comparisons would be inappropriate, but the data suggest that asymptotic calibration of the vector has been reached by Trip 10 in both species.

The full shifts to the inbound direction in the 45° condition do not align with similar tests in *C. fortis* (Collett et al. 1999; Wehner et al. 2002), where displacement training never resulted in complete shifts to the inbound vector, and *C. fortis* foragers oriented in compromise directions between the inbound and outbound vectors. While the rate of calibration between *C. fortis* and *M. bagoti* appears similar, reaching asymptotic calibration by Trip 10, the calibration magnitudes remain larger in *M. bagoti* (Wehner et al. 2002; Fig. 6). This difference may be due to disparities in the availability of panorama cues between these two species. *C. fortis* inhabits barren landscapes largely devoid of panorama cues, and returning foragers can only rely on their vector and the backup mechanism of systematic search to return home (Wehner and Srinivasan 1981; Müller and Wehner 1994). Use of both the outbound vector and systematic search are present in Wehner et al. (2002), as foragers at the displacement site show small shifts (~30%) in initial heading and appear to veer toward the nest as they move further from the displacement site. When paths differed by 55°, *C. fortis*

foragers never developed succinct inbound routes and even after 50 displacements showed evidence of search behavior when returning to the nest. In contrast, *M. bagoti* foragers can rely on a wealth of terrestrial cues in order to locate the nest entrance after displacements off-route and these foragers do return directly to the nest when paths differed by 45°. Unlike *C. fortis*, on their first displacement, *M. bagoti* foragers compromised between the vector direction and the true nest direction, suggesting the visual panorama is competing with the vector cues and helping foragers navigate to the nest (Cheng et al. 2009). By ten exposures to this site, *M. bagoti* foragers become fully oriented to the true nest direction. Moreover, observations of their homeward routes suggest they are returning straight to the area around the nest and are not engaging in directed search behavior like *C. fortis* (Wehner et al. 2002). These straight homeward routes point to the panorama, available during training, as a potential cause of the full shifts to the inbound vector we observed in *M. bagoti*. Additionally, the panorama along the inbound route at the 45° site would closely resemble that of the outbound route to the feeder, which may reinforce inbound vector memory on subsequent trips, resulting in larger shifts in the arena testing. The importance of terrestrial panorama cues for vector calibration has been previously shown in honeybees, with the presence of panorama cues during the inbound or outbound route biasing the path integrator in that direction (Otto 1959; Collett and Collett 2000a).

The distinctions between *C. fortis* (Collett et al. 1999; Wehner et al. 2002) and *M. bagoti* in the current study may, however, have several origins. As discussed, the observed differences could be ontogenetic in origin: the presence of panorama cues during training could facilitate full shifts to the inbound direction. But there could also be phylogenetic differences in these distantly related species that underlie these disparities in vector calibration. To further tease apart these potential underlying factors, the desert ant *Cataglyphis velox* would be a prime candidate for study, as it is a species closely related to *C. fortis* that yet inhabits cluttered environments like *M. bagoti* (Mangan and Webb 2012).

In the 90° and 135° arena tests, *M. bagoti* foragers showed the same rapid shift toward the inbound vector after three training trips as in the 45° condition. Yet over larger conflicts, orientation shifts remained incomplete even after 25 displacements and more closely resembled the incomplete shift directions witnessed in *C. fortis* (Collett et al. 1999; Wehner et al. 2002). In both the 90° and 135° conditions, forager orientations never reached the shifts in the 45° condition and were numerically smaller in magnitude despite the larger conflict. The observed decreases in vector calibration may be explained by the panorama cues along the inbound routes at the 90° and 135° sites (along with the 180° site) during training, which would less resemble the foragers' outbound trip and potentially result in less inbound vector reinforcement compared to the 45° displacement condition.

When tested at the displacement site, the results of Trip 1 of the 135° displacement condition resemble the results of a previous *M. bagoti* study (Legge et al. 2014) where foragers were trained to travel to a feeder and then displaced with conflicting vector and panorama cues (135°). Foragers also oriented in an intermediate direction between the cue sets, though interestingly, foragers in Legge et al. (2014) oriented closer to the direction indicated by the panorama cues (at 135°) compared to the current study (~68° vs. ~33°). This difference is likely due to differences in panorama cues around each nest, although these studies also trained and displaced individuals over different distances, which might also account for the observed differences.

As *M. bagoti* relies more on panorama based directional cues compared to *C. fortis*, a more fitting comparison on cue conflict may be with other solitary foraging ants that live in landmark rich environments. Similar cue conflict tests have been conducted on the nocturnally foraging *Myrmecia midas* and *Myrmecia pyriformis* along with the diurnal *Myrmecia croslandi* (Narendra et al. 2013ab; Freas et al. 2017bc). Unlike *M. bagoti*, *M. croslandi* and *M. midas* foragers displaced off-route with 90° cue conflicts orient in the direction dictated by the panorama, though some *M. croslandi* foragers showed evidence of

initially choosing a compromise direction between the vector and panorama direction before shifting to solely panorama guidance (Narendra et al. 2013a; Freas et al. 2017b). *M. pyriformis* foragers displaced off-route with a 60° cue conflict chose either the panorama or vector direction when orienting and did not compromise (Narendra et al. 2013b). Disparities in cue use could be rooted in differences in phylogeny, landscape makeup, light level variation, or foragers' level of experience along the foraging route.

The observed decrease in vector calibration at larger directional conflicts may be the result of the conflict magnitude itself. Similar navigational conflicts have been tested in rodents between a recently accumulated vector and a learned landmark cue. Over small directional conflicts, hamsters (Etienne et al. 1990) chose long-term landmark memories over their current short-term vector memory, similar to the preference we see in the condition with 45° conflicts with the long-term vector memory of previous trips overriding the current outbound vector. Yet this memory weighting changes as conflict size increases and at the largest conflict (180°), hamsters tended to ignore the long-term landmark memories, and showed a greater reliance on their current vector to return to the nest (Etienne et al. 1990; Etienne et al. 1996). These results are similar to those found in our current study, in which the same trends turn up both in the arena (long-term vector memory vs. short-term vector memory) and at the displacement site (long-term vector memory + long-term panorama memory vs. short-term vector memory). This suggests there may be some limit to the cue conflict size beyond which individuals switch from preferring the use of learned cues such as the panorama or stored inbound vectors, to their current vector.

Given the forager orientations at the displacement site, it is also possible that panorama cues present during training influence inbound vector cue strength. When trained at 90° and 135°, the foragers' observed bowing routes may have a self-reinforcing property. When the ants follow a bowed path and find their way home, they are rewarded, as we assume that finding home has reinforcing properties. Learning principles then suggest that the

homing ant is more likely to repeat the bowed path once more on the next trip, that is, in the same context. Given that such a path once again takes the ant home, the path is reinforced once more, making it even more likely on the next trip. Such a positive feedback loop cements a less than fully efficient path that nevertheless gets the navigator home reliably. Once the forager experiences this path and reaches the nest, it becomes the most memorable and salient route. This would cause foragers to continue to use this route on subsequent trips. When comparing the mean vector of foragers' orientations at the displacement site test with orientations in the arena tests, we found no difference on Trip 10. These results suggest the headings in displacement site and arena tests are the same after calibration. It appears that once the calibration reaches its asymptote, the availability of the panorama during displacement tests does not add much to the shift toward the inbound route.

M. bagoti is known for developing one-way routes that bend and curve around obstacles that are common in its cluttered environment (Kohler and Wehner 2005; Wehner et al. 2006; Cheng et al. 2009). Therefore, these curved homeward routes during training may reinforce the compromise orientations. At 180°, the initial movement toward the outbound vector and the inability of foragers to learn to ignore the vector in favor of the panorama cues initially may result in the stored inbound vector being weak and steadily overpowered by the outbound vector cue in subsequent arena tests. It would be interesting to collect and examine these inbound routes from all of these displacement sites, but this effort must await a warranted future study.

Conclusions

M. bagoti foragers exhibit vector calibration when their inbound and outbound foraging routes differ. These changes occur rapidly but appear to have an upward limit of ~45°. These results suggest the strength of the previous inbound and current outbound vector cues change as the conflict between them increases. Over smaller directional conflicts, the inbound vector memory of previous trips dominates. Yet as the conflict increases, the current outbound

vector becomes preferred to where at the largest conflict (180°) it completely dominates the inbound vector memory. Exposure to the surrounding panorama cues appears to aid foragers returning home, even on the first displacement. Yet we see the same trends in orientation at the displacement site as we do in the arena with heavier weighting of the current outbound vector as directional conflicts increase.

Author contributions

Experiments conceived and designed: CAF. Data collection and analysis: CAF. Manuscript production and revision: CAF and KC.

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Chapter 5. Landmark learning, cue conflict and outbound view sequence in navigating desert ants.

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Landmark learning, cue conflict and outbound view sequence in navigating desert ants.

Cody A Freas¹, & Ken Cheng¹

¹ Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Abstract

Mobile animals need to reliably find goal locations and animal navigators acquire and use multiple cue sets within their environment designating direction and distance estimates of these locations. To accomplish this challenge, foraging ants use multiple navigational tools including path integration and the learning of the landmark panorama. During landmark-based navigation, foragers first acquire the landmark cues around the nest through pre-foraging learning walks, and then learn non-nest site landmark cues along their foraging routes in order to return home. In the current study, we explore both foragers' ability to extrapolate views from around the nest to local displacement sites and landmark learning during the first foraging trips away from the nest area. During Experiment 1, foragers were given variable amounts of exposure to the nest area before being displaced 8 m away where their return trips were recorded. In Experiment 2, foragers' return trips from a site 8 m from the nest were recorded with the surrounding landmarks during either the outbound or inbound trip obstructed from view and with/without the aid of a homeward vector. Foragers were unable to correctly orient or return home efficiently regardless of the exposure level to the nest panorama, suggesting an inability to extrapolate views from learning walks and supporting recognition based navigation. Foragers were able to use experience of either the outbound or inbound view sequence to initially orient home, yet were unable to quickly return to the nest when only exposed to the inbound route. Our results suggest that exposure to the outbound foraging path is critical for efficient homeward route formation.

General Introduction

Solitary foraging requires that each forager have the ability to accurately locate goal locations, such as known resource patches or the nest entrance. Ant species that forage alone rely on two main navigational tools to accomplish this task: path integration (Collett & Collett, 2000; Wehner, 2008) and learned terrestrial landmark cues (Wehner, 2003; Collett, Graham, Harris, & Hempel-de-Ibarra, 2006; Cheng, Narendra, Sommer, & Wehner, 2009), with systematic search as a backup mechanism when other cues fail (Müller & Wehner, 1994; Schultheiss & Cheng, 2011; Schultheiss, Cheng, & Reynolds, 2015).

When employing landmark-based navigation, foragers use the cues present in the surrounding panorama for both distance and directional information while traveling to and from the nest (Wehner, Michel, & Antonsen, 1996; Collett et al., 2006; Graham & Cheng, 2009). Using terrestrial cues for navigation requires learning, and foragers first must acquire the terrestrial cues around their nest, a task they accomplish through multiple pre-foraging learning walks (Nicholson, Judd, Cartwright, & Collett, 1999; Baddeley, Graham, Philippides, & Husbands, 2011; Zeil, Narendra, & Stürzl, 2014; Fleischmann, Christian, Müller, Rössler, & Wehner, 2016; Fleischmann, Grob, Wehner, & Rössler, 2017). Foragers are able to retain these visual cues acquired around the nest entrance, and, while foraging, can compare those views to their current view in order to return to the nest (Wehner, Boyer, Loertscher, Sommer, & Menzi, 1996; Collett, Collett, & Wehner, 2001; Collett et al., 2006; Cheng et al., 2009; Wystrach, Beugnon, & Cheng, 2011a; Wystrach, Schwarz, Schultheiss, Beugnon, & Cheng, 2011b). After leaving the nest area for the first time, individuals perform a series of turn backs, orienting back to the nest site, during which they are likely learning the landmark makeup along their foraging route (Nicholson et al., 1999; Zeil, 2012; Zeil et al., 2014), retaining multiple landmark memories experienced while foraging away from the nest (Graham & Cheng, 2009; Schultheiss, Wystrach, Schwarz, Tack, Delor, Nooten, Bibost, Freas, & Cheng, 2016; Freas, Whyte, & Cheng, 2017a).

The process of comparing stored views to the present scene is referred to as a view based model of ant navigation (Collett, 2010; Zeil et al., 2014). This model suggests that the sequence in which an ant learns the homeward route would be inconsequential, as a forager would navigate based on comparisons of the currently viewed scene and memories acquired during learning walks and previous foraging trips (Kohler & Wehner, 2005; Baddeley, Graham, Husbands, & Philippides, 2012; Zeil et al., 2014). Yet the solitary foragers of at least some ant species appear unable to extrapolate views around the nest to nearby locations where scenes are similar. When foragers of two Australian species living in cluttered environments, the desert ant *Melophorus bagoti* and the nocturnal bull ant *Myrmecia midas* foraged only around the nest area, they were unable to orient correctly home after local displacements of 8 and 5 meters, respectively (Freas & Cheng, 2017; Freas, Narendra, & Cheng, 2017b). In this study, we investigate more thoroughly whether desert ants can generalize views that they learn near their nest to a location 8 meters away (Experiment 1), and begin the investigation of the process by which ants learn to use panoramic views along a route between a feeder and their nest (Experiment 2).

Experiment 1

Previous findings (Freas & Cheng, 2017; Freas et al., 2017b) indicate that the initial learning walks make up only part of a forager's landmark learning and may be insufficient to allow ants to return to the nest from unvisited locations regardless of any panoramic similarities. Foragers may need to accumulate views between the nest site and other local spatial locations through exposure to either the outbound or inbound view sequence. Yet previous work in *M. bagoti* (Freas & Cheng, 2017) was only conducted on newly emerged individuals on their first foraging trip (1 meter from the nest entrance) and may have tested foragers that had not completed their pre-foraging learning walks. The experimental set up also contained a feeder-nest-displacement site spatial arrangement that may have interfered

with learning. That is, because some ants in the set up mostly traveled directly between a feeder near their nest and their nest, they might not have learned the views around their nest thoroughly. In the current study, we further investigate the observed lack of view extrapolation in *M. bagoti* foragers reported in Freas and Cheng (2017). We restricted foragers to the nest site for one foraging trip, ten foraging trips, or 5 days of foraging to test if large amounts of exposure to the nest panorama allow for successful homeward navigation.

Methods

Field site

This study was conducted on the grounds of the Centre for Appropriate Technology in the Northern Territory, Australia, located 10 km south of Alice Springs (23°45'28.12" S, 133°52'59.77" E). The general habitat of the area is a semi-arid desert with an abundance of visual cues consisting primarily of buffel grass, *Cenchrus ciliaris* and a landscape dotted with Eucalyptus trees and *Acacia* bushes (Muser, Sommer, Wolf, & Wehner, 2005). Experiments were conducted over 3 field seasons from November 2014 to February 2017 during the Australian summer months when this species is active.

Subjects

Experiments were conducted on foragers of the desert ant species *Melophorus bagoti* or red honey ant. *M. bagoti* are thermophilic and act as diurnal scavengers that typically forage only during the hottest portions of the day (Wehner, 1987; Christian & Morton, 1992). The current study required foragers with no experience of the area beyond the nest area before testing. To identify these naïve, newly emerged foragers from the rest of the foraging force we marked all individuals outside the nest for five consecutive days on the abdomen with a small amount of paint (Tamiya™). These foragers were denoted as experienced and therefore excluded from all testing. As the average forager life span is approx. 4.9 days (Muser et al.,

2005), any foragers emerging from the nest on the sixth day was considered naïve and were individually marked with paint for testing.

Testing arena

The testing arena used in this study was similar to the arena used in Freas and Cheng (2017; Figure 1a). A plastic, square-shaped feeder ($15 \times 15 \times 9\text{cm}$) was placed in the ground at a distance of 1m from the nest entrance. This feeder was stocked daily with mealworm pieces and cookie crumbs (ArnottTM). Foragers arriving at the feeder drop inside and are unable to escape without being removed by the experimenter. Surrounding the nest entrance and the feeder we erected a smooth plastic wall 10 cm in height to create a 2m-diameter circular *nest arena*, which restricted foragers to this area while leaving the surrounding landmark panorama visually unobstructed (Figure 2). This arena was constructed to leave a 50cm space between the nest or feeder and the arena edge (Figure 1a). In the opposite direction (180°) of the feeder, we cleared all vegetation 9 m from the arena's edge. The nest arena was cleared of all vegetation and potential food sources, inducing foragers to travel to the feeder to collect food.

We marked a displacement site (Figure 2) 8 m from the nest arena wall, 180° from the nest-feeder direction and erected a 10cm high walled corridor ($8\text{m} \times 2\text{m}$) connecting the displacement site to the nest arena, using the same plastic as the nest arena (Figure 1a) and any vegetation was cleared in a 50cm area around the entire walled setup. We then stacked dirt along the wall of the nest arena within the corridor, creating an incline to allow foragers to return naturally over the wall and to the nest after being displaced.

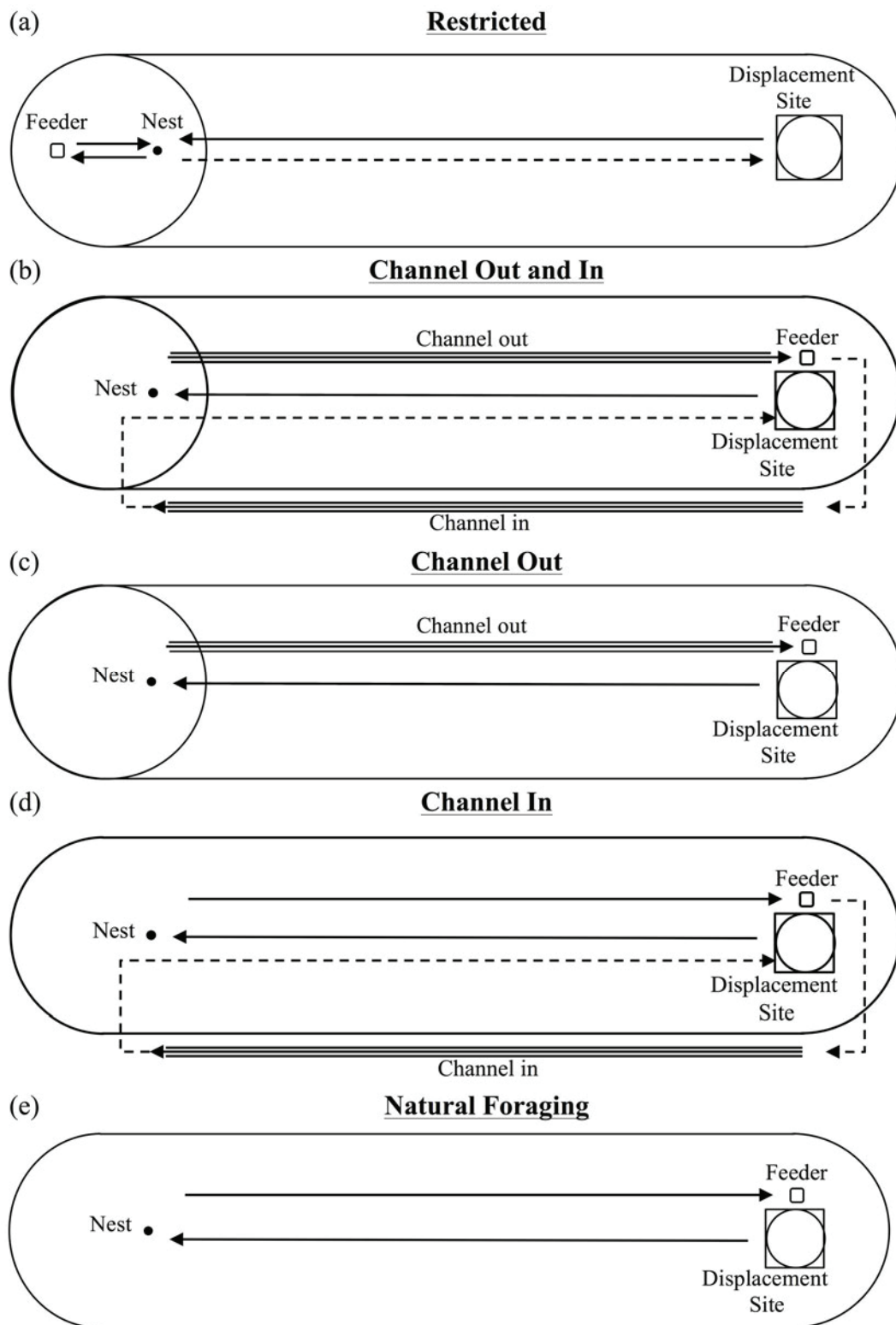


Figure 1 Diagrams of the experimental set up for Experiment 1 and 2. Bold lines indicate the foragers' movement and dotted lines show the displacements. Diagram of the experimental set up for (a) both the 3 extrapolation tests in Experiment 1 and Restricted condition, (b) Channel Out & In and Trip 11 of the Channel Out condition, (c) Channel Out condition Trips 1-10, (d) Channel In and Trip 11 of the Natural foraging condition, and (e) Natural foraging condition Trips 1-10.

Extrapolation tests

Here, foragers were only allowed access to the terrestrial cues around the nest area and were restricted from accumulating terrestrial information outside of this area (Figure 1a). During Experiment 1, newly emerged foragers were displaced after they attained one of three levels of experience within the nest arena. In the first 2 conditions, foragers were allowed to collect food from the feeder once ($n = 25$) or on their 10th full foraging trips to the feeder ($n = 23$). Foragers were manually released from the feeder after collecting food and permitted to return to the nest entrance. Each forager to be tested was collected in a darkened phial just before entering the nest to ensure they had no directional vector cues at release (as zero-vector ants) and transferred to the displacement site (Figure 1a). At the site, foragers were released onto a 60cm-diameter goniometer separated into 24 equal 15° wedges. Initial headings were measured by forager crossings at both 15 cm and 30 cm by recording the wedge where foragers first crossed. After running off the goniometer, foragers were allowed to travel back to the nest arena through the displacement corridor. Each forager's duration-to-return to the nest arena was recorded using a stopwatch. As soon as the forager dropped into the nest arena, the stopwatch was stopped. This measure eliminated variations in duration of travel due to the ease or difficulty of locating the nest entrance within the nest arena. In a third condition, the feeder was removed and food was scattered throughout the nest arena. Naïve foragers collecting food were individually marked just before they returned to the nest with a food piece. These foragers were allowed to continue foraging inside the nest arena after being marked. After these individuals had foraged for 5 days ($n = 20$), they were displaced 8 m away from the edge of the nest arena as zero-vector ants and tested identically to the previous two conditions.

Nest site panorama



▲
Displacement Site

Displacement site panorama



▲
Nest Site

Figure 2 Panoramic 360° images of the nest site and the displacement site 8.5m away. Images were taken with a HD bloggie camera with a panoramic lens (Sony). The nest site image was taken from the center of the nest arena and the displacement site image is taken from the goniometer surface.

Statistical analysis

All foragers' initial orientations were analysed using circular statistics (Batschelet, 1981; Zar, 1998) with the statistical program Oriana Version 4 (Kovach Computing Services™). To test for a uniform distribution of headings ($p > 0.05$), Rayleigh's Tests were conducted, and to test if initial orientations were significantly clustered around the nest direction at 0° , we examined if 0° fit within the 95% confidence interval (CI) of orientations (Watson tests) and with V-tests, with alpha set at $p = 0.05$. A V-test delivers a significant result when a distribution of headings is significantly clustered around a specified target direction. Only forager crossings at 30 cm are reported, as there were no differences between crossings at 15 cm and 30 cm. Trip durations were analysed using one-way analyses of variance with Post-hoc Bonferroni-Holm pairwise comparisons using JASP.

Results

In all three of the extrapolation tests, foragers did not initially orient toward the nest direction at 0° (Figure 3a-c). Foragers on their first foraging trip were significantly oriented but in a direction away from the nest direction (Table 1; Figure 3a). These foragers were also not significantly oriented to the recently zeroed vector at 180° (V test at 180°; $V = 1.598$; $P > 0.05$). After 10 trips to and from the feeder, forager orientations were distributed uniformly and not directed to the nest (Table 1; Figure 3b). Even with 5 days' worth of foraging experience in the nest arena with variable food locations, a period beyond the average lifespan of naturally foraging foragers, forager orientations were still uniform and not directed to the nest (Table 1; Figure 3c). Duration-to-return did not change regardless of experience within the nest arena (Figure 3d; One-Way ANOVA $F_{2,68} = 1.34$, $P > 0.05$).

Table 1 Statistical results for initial heading direction in all conditions in both Experiment 1 and Experiment 2. The nest direction for all conditions was at 0° with degrees increasing clockwise from the nest. All probability values are not corrected for multiple comparisons within the table

	<u>Mean Vector</u>	<u>95% Confidence interval</u>		<u>Rayleigh test</u>		<u>V test: direction 0</u>	
Experiment 1	μ (°)	Minus (°)	Plus (°)	Z	p	V	p
Trip 1	124.8	86.4	163.3	3.92	0.018	-1.598	0.945
Trip 10	51.4	137.4	325.4	0.08	0.921	0.255	0.4
5 Days	319.0	260.9	17.1	1.783	0.169	1.425	0.078
Experiment 2							
Restricted							
Trip 1	175.9	-	-	0.04	0.96	-0.03	0.62
Trip 2	323.5	305.6	342.5	15.2	< 0.001	0.50	< 0.001
Trip 10	349.35	338.2	0.49	26.8	< 0.001	0.80	< 0.001
Channel Out & In							
Trip 1	77.3	326.8	187.9	0.51	0.61	0.04	0.41
Trip 2	343.2	306.7	19.7	4.21	0.01	0.44	0.002
Trip 10	358.8	335.4	22.2	8.6	< 0.001	0.66	< 0.001
Channel Out							
Trip 1	359.0	344.6	13.3	14.4	< 0.001	0.85	< 0.001
Trip 2	352.5	337.4	7.7	13.8	< 0.001	0.82	< 0.001
Trip 10	349.5	340.2	358.9	17.4	< 0.001	0.92	< 0.001
Trip 11	5.8	352.5	19.2	15.0	< 0.001	0.86	< 0.001
Channel In							
Trip 1	356.7	348.5	4.9	18.0	< 0.001	0.95	< 0.001
Trip 2	10.4	3.1	17.6	18.4	< 0.001	0.94	< 0.001
Trip 10	12.0	4.3	19.7	18.2	< 0.001	0.93	< 0.001
Natural Foraging							
Trip 1	0.5	347.3	13.6	15.2	< 0.001	0.87	< 0.001
Trip 2	0.5	350.0	11.1	16.8	< 0.001	0.92	< 0.001
Trip 10	353.7	342.1	5.3	16.2	< 0.001	0.89	< 0.001
Trip 11	351.6	342.1	1.0	17.4	< 0.001	0.92	< 0.001

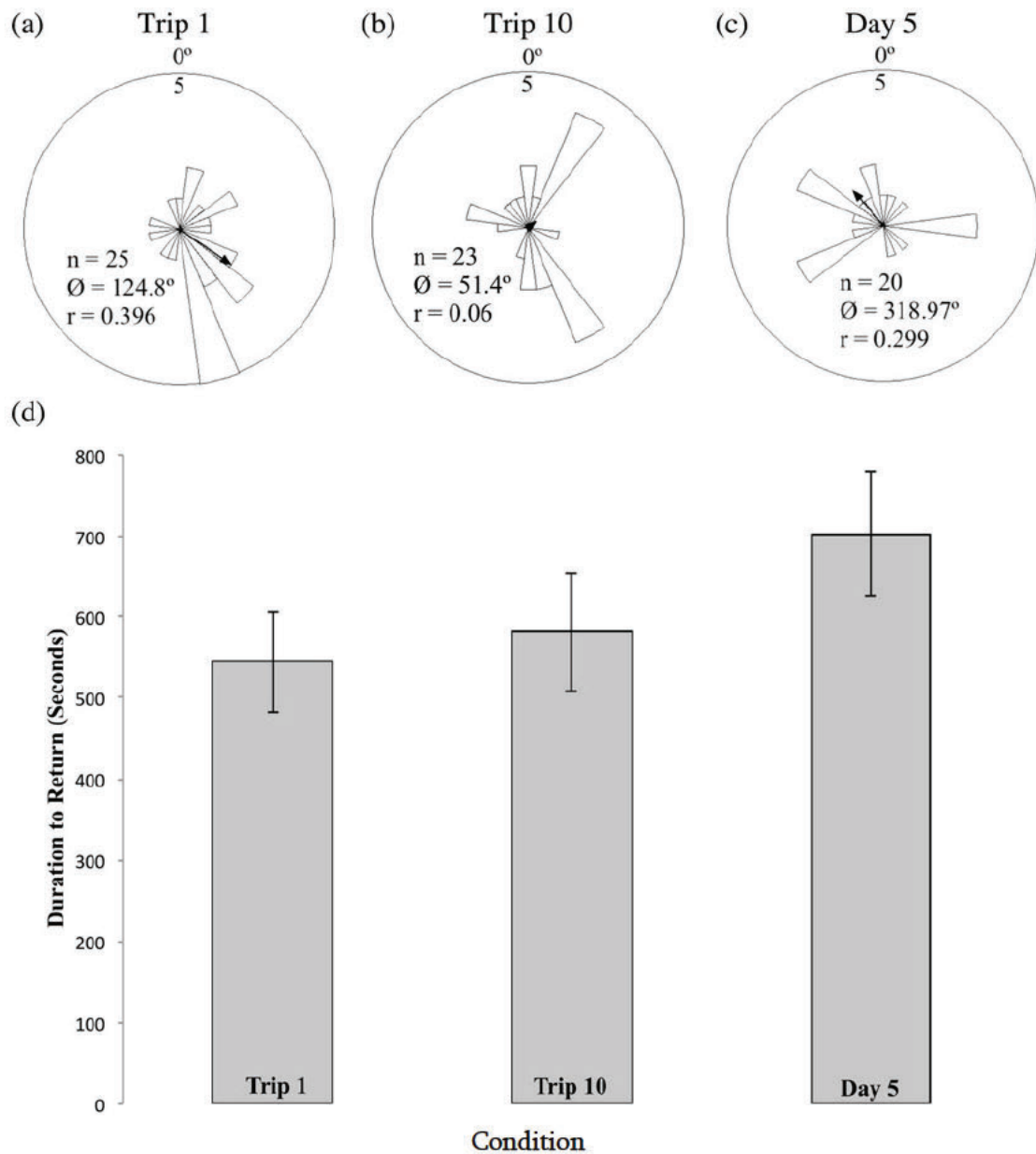


Figure 3 Circular histograms of initial headings and duration-to-return in the extrapolation tests. In the histograms, the nest direction was set at 0° . An arrow denotes the length and direction of the mean vector. Each histogram states the number of individuals (n), the mean vector direction ($\bar{\theta}$), and the length of the mean vector (r). (a) Zero-vector foragers on their first trip from the feeder. (b) Zero-vector foragers on their tenth trip from the feeder. (c) Zero-vector foragers after 5 days of foraging on scattered food within the nest arena. (d) Duration-to-return after displacement for all conditions \pm SE. Duration did not change regardless of increased exposure to the nest landmark cues (One-way ANOVA; $F_{2,68} = 1.34$; $P > 0.05$).

Discussion

Solitary foraging ants are known to learn landmark cues both around the nest during learning walks (Collett & Zeil, 1996; Müller & Wehner, 2010; Fleischmann et al., 2016) and while on foraging trips (Collett & Collett, 2000; Schultheiss et al., 2016; Freas et al., 2017a).

Previous work has shown that *M. bagoti* foragers are unable to navigate home after local displacement on their first foraging trip (Freas & Cheng, 2017) suggesting that foragers cannot extrapolate views experienced around the nest to local areas with similar panoramas. Yet these experiments were conducted only on recently emerged foragers restricted to the nest with the same feeder-nest-displacement site spatial arrangement (Figure 1a). Here we show the inability to extrapolate extends to foragers with large amounts of exposure to the nest panorama, including individuals that have spent 5 days foraging around the nest site, which is longer than a naturally foraging individual's average lifespan (4.9 days, Muser et al., 2005). We also show that the feeder-nest-displacement site set up (Figure 1a), present in both Freas and Cheng (2017) and two conditions of Experiment 1 of the current study, was likely not the cause of the inability to orient or return to the nest efficiently, as foragers with food scattered throughout the nest arena still performed poorly. Regardless of the amount of panorama exposure foragers acquire at the nest site, it appears foragers are not able to use these cues to navigate from local spatial locations without experience of that local site, resulting in reduced navigational capabilities compared to foragers that leave the nest site. As hypothesized in Freas and Cheng (2017), *M. bagoti* foragers may need at least one exposure to the panorama changes during the outbound or inbound view sequence to return to the nest successfully, even from a local site. This limitation would lend support to a recognition-triggered response model for landmark-based navigation in this species (Gaussier, Joulain, Banquet, Lepêtre, & Revel, 2000). A requirement for accurate navigation from displacement sites using this model is that the forager has previous experience of the site during the outbound portion of the current foraging trip or memories from previous foraging trips. Consequently, even large amounts of experience of the panorama around the nest site would be insufficient to successfully navigate (Gaussier et al., 2000; Möller, 2012).

These findings conflict with the results of Wystrach, Beugnon and Cheng (2012) which found that ants restricted to the nest and then displaced to a local site do orient in

the correct nest direction. One major difference between the current experiment and Wystrach et al. (2012) is that they dug a moat around the nest, whereas here, a 10 cm high wall kept the ants within about a metre of the nest. One possibility is that while the panoramic images around the nest show that the wall does not block any of the skyline at the nest site (Figure 2), it may transform the panorama when approached by the ants. Another possibility is the differences in panorama makeup between the nests. These differences could provide varied levels of navigational information to navigating ants and potentially result in the differences in successful orientation. Further study into the extrapolation of nest views to local sites is warranted.

Experiment 2

The results of Experiment 1 suggest that forager's exposure to the panorama around the nest is insufficient for efficient navigation from a local site. Foragers appear to need at least some exposure to either the outbound or inbound view sequence to successfully orient and efficiently navigate to the nest. In Experiment 2, we investigate which portions of foraging trips are important for terrestrial cue learning and homeward route formation in naïve *M. bagoti* foragers by separating the foraging route into three cue components in which learning could occur, the outbound view sequence, the inbound view sequence, and the presence of the homeward vector during the homeward route. How foragers learn a route from a displacement location 8m from the nest was tested under natural foraging conditions and in four experimental conditions when one or two of these cue components were removed.

Methods

Testing Setup

Experiment 2 required the use of naïve, newly emerged foragers, which were again identified from the rest of the foraging force by marking all individuals outside the nest for

five consecutive days and excluding these experienced foragers from testing. Beginning on day six, any unmarked naïve ants were individually marked with paint for testing. The testing arena used in this study was identical to the set-up of Experiment 1 (Figure 1a) with modifications made for separate learning conditions (Figure 1b-e).

Restricted condition

In the Restricted condition in Experiment 2, foragers were only allowed access to the terrestrial cues during the inbound portion of their foraging route and were restricted from both an outbound path to the displacement site and the presence of a homeward vector during learning (Table 2; Figure 1a). This condition tested if access only to the inbound view sequence was sufficient for foragers to both orient and navigate to the nest location. Naïve foragers ($n = 40$) on their first trip to the nest-arena feeder were allowed to collect a piece of food and exit the feeder to return to the nest entrance. Just before entering the nest, foragers were collected in a darkened phial and displaced as zero-vector ants, identical to the first (Trip 1) condition of Experiment 1 (Figure 1a). At the displacement site, foragers were released onto the goniometer where their initial orientation was recorded. After moving off the goniometer, foragers were allowed to return to the nest arena with their duration-to-return recorded. This displacement gave each forager its first experience of visual terrestrial cues of the displacement corridor but only on the inbound view sequence and without a corresponding homeward vector. The same procedure was repeated on the forager's 9 subsequent visits to the feeder, resulting in 10 measures of initial heading and duration-to-return from the displacement site.

Table 2 Conditions for Experiment 2 indicating if the panorama during the outbound/inbound path was available and the state of the path integrator at the time the ant is released.

<u>Testing Condition</u>	<u>N</u>	<u>Outbound Views</u>	<u>Inbound Views</u>	<u>Nest-ward Vector</u>
Restricted	40	✗	✓	✗
Channel Out & In	20	✗	✓	✗
Channel Out	20	✗	✓	✓
Channel In	20	✓	✓	✗
Natural Foraging	20	✓	✓	✓
Channel Out (Trip 11)	20	✗	✓	✗
Natural Foraging (Trip 11)	20	✓	✓	✗

Channel Out & In condition

In this condition, foragers ($n = 20$) were again restricted from terrestrial cue access during the outbound trip and from the presence of a homeward vector (Table 2). This condition again tested if the inbound view sequence alone was sufficient for homeward navigation and was designed to control for behaviors that might arise due to foragers being restricted around the nest, such as a potential reluctance to travel beyond experienced vector lengths. Here, foragers were allowed access to a feeder at the displacement site through a channel. The feeder was removed from the nest arena and transferred to the displacement site. Foragers were forced into the outbound channel by erecting a 2.5cm high plastic wall with a 30cm diameter around the nest connected to the sides of the channel using tape. On the outside of this 2.5cm wall, we packed a small amount of dirt to allow foragers to return to the nest naturally. This created one outward path from the nest through the channel to the feeder. Foragers travelled to the feeder through an 8.5m long channel raised 10cm off the ground (Figure 1b). This channel was made of the same plastic material as the arena and was 10 cm wide with 10cm high walls. This channel obstructed much of the terrestrial panorama during the outbound path but allowed access to the overhead celestial cues during foraging. When a forager reached the end of the channel, it would fall into the feeder and then collect food (Figure 1b). These foragers were then placed in a second identical 8.5m channel, parallel to the first, and allowed to run

off their homeward vector without access to the surrounding terrestrial cues. Once foragers had run off their vector, they were displaced back to the displacement site where they were released onto the goniometer and allowed to return to the nest arena. Both foragers' initial orientations and their trip duration to the nest arena were recorded for the first ten trips they made to the feeder.

Channel Out condition

In the *Channel Out* condition, foragers ($n = 20$) were only restricted in their access to the terrestrial cues on the outbound portion of foraging trips (Table 2). This condition was designed to test if foragers could successfully orient and navigate to the nest with only the inbound view sequence and a homeward vector. Foragers accessed the feeder in the same channel configuration as the previous condition and were collected from the feeder once they had food (Figure 1c). In this condition, foragers were transferred from the feeder directly to the adjacent goniometer and released. This process was repeated for the next 9 trips to the feeder. For this condition, we conducted a condition modification on the 11th foraging trip, where we had the forager run off their accumulated vector before being transferred to the displacement site. Each forager was released in the inbound channel and allowed to run off their accumulated 8.5m nest-ward vector before being collected again and released at the displacement site in a procedure identical to the Channel Out & In condition. Foragers' initial orientations and trip durations to the nest arena were recorded for all 11 foraging trips to the feeder.

Channel In condition

In the *Channel In* condition, foragers ($n = 20$) were allowed the terrestrial cue sequence on both the outbound and inbound portions of the foraging trips but were denied the corresponding homeward vector (Table 2). This condition was designed to explore the influence of a homeward vector on panorama learning. Here, we removed the wall separating

the nest from the displacement site and the outbound channel, allowing ants to travel to the feeder unobstructed (Figure 1d). Foragers collecting food in the feeder were placed in the inbound channel and allowed to run off their homeward vector before being collected and returned to the displacement site. At the site, they were released on a goniometer and allowed to return to the nest and both the initial orientation and the duration-to-return to the nest area of all individuals were recorded. To record duration-to-return in this condition, the removed section of the arena wall that previously marked the end of the foragers' return was replaced with a suspended string so that the displacement site to nest area travel distance was identical across all conditions.

Natural foraging condition

In the *Natural foraging* control condition, foragers ($n = 20$) were allowed both the inbound and outbound cue sequence (Table 2). Foragers were allowed to travel from the nest site, through the corridor, and to the feeder at the displacement site (Figure 1e). Once a forager had collected food from the feeder it was released onto the adjacent goniometer and allowed to return to the nest through the corridor for the first 10 trips to the feeder. On Trip 11, each forager's vector was zeroed by placing them in the 8.5m inbound channel, identical to the Channel In condition. Foragers were then collected and returned to the displacement site where their orientation and duration-to-return were recorded. Orientation and duration-to-return were collected on all 11 trips and the homeward route was collected on the first, tenth, and 11th homeward trips.

Nest-ward Paths

After completing Experiment 2, we tested more ants to collect some nest-ward paths of some individuals in all conditions. In order to record foragers' homeward paths within the displacement corridor in each condition, we erected a 9m by 2m grid of 1m squares using metal pegs and suspended string. Experimentally, it was easier to separate this laborious task

from the main experiment in which headings and durations of return were observed. For each condition we ran 10 individuals, recording the homeward paths on the 1st and 10th trips. In the 2 conditions (Channel Out and Natural Foraging) that contained a zero-vector trip 11 we also made path recordings of this final trip.

Statistical analysis

All foragers' initial orientations were analyzed using identical circular statistics (Batschelet, 1981; Zar, 1998) as Experiment 1 using the statistical program Oriana Version 4 (Kovach Computing Services™). There were no differences between crossings at 15 cm and 30 cm and only forager crossings at 30 cm are reported. Trip durations were analyzed using repeated measures analysis of variance tests with Helmert contrasts for within condition comparisons using JASP. One-way analyses of variance with Post-hoc Bonferroni-Holm pairwise comparisons were used for comparisons between conditions.

Results

During the first displacement in the restricted condition, foragers showed a uniform distribution and did not initially orient toward the nest direction at 0° (Table 1; Figure 4a). After one exposure to the inbound route, on the second displacement to the site, foragers were oriented to the nest at 0° according to a V test, but the nest was outside the 95% CI of the observed headings which ended at 342.5° (Table 1; Figure 4b). By the third displacement, both the V test and the 95% CI indicate orientation to the nest and 0°, and this orientation persisted through the rest of the 10 displacements (Trips 3-9, Supplemental Table 1; Trip 10, Table 1; Figure 4c).

Duration-to-return significantly decreased over the ten trips (Figure 5; Repeated Measures ANOVA $F_{9,351} = 20.2$, $P < 0.01$) and Helmert contrasts show that mean duration-to-return during Trip 1 (680 seconds) was significantly higher than Trips 2–10 ($P < 0.01$), which

averaged 261 seconds. Helmert contrasts of Trip 2 through Trip 10 did not significantly differ ($P > 0.05$).

Forager paths showed that on Trip 1 individuals initially moved around the displacement site before slowly expanding their paths toward the nest (Supplemental Figure 1a-e). By Trip 10 foragers initially travelled in the nest direction but still searched around the displacement site before moving toward the nest (Supplemental Figure 1f-h). On both Trip 1 and 10 foragers repeatedly returned to the area around the displacement site during their homeward trips.

Foragers in the Channel Out & In condition showed a uniform distribution and did not initially orient toward the nest direction at 0° on Trip 1 (Table 1; Figure 4d). After one exposure to the inbound route, on the second displacement to the site, foragers were oriented to the nest at 0° according to both a V test and the 95% CI of the observed headings (Table 1; Figure 4e). This statistical orientation persisted through the next 8 trips (Trips 3-9, Supplemental Table 1; Trip 10, Table 1; Figure 4f).

Similar to the Restricted condition, duration-to-return significantly decreased over the ten trips (Figure 5; Repeated Measures ANOVA $F_{9,171} = 11.4$, $P < 0.01$) and Helmert contrasts show that the mean duration-to-return during Trip 1 was significantly higher than Trips 2–10 (Helmert contrast; $t = 8.79$; $P < 0.01$). Duration-to-return during Trip 2 was also significantly higher than Trips 3–10 (Helmert contrast; $t = 3.26$; $P < 0.01$). Helmert contrasts of Trip 3 through Trip 10 did not significantly differ ($P > 0.05$).

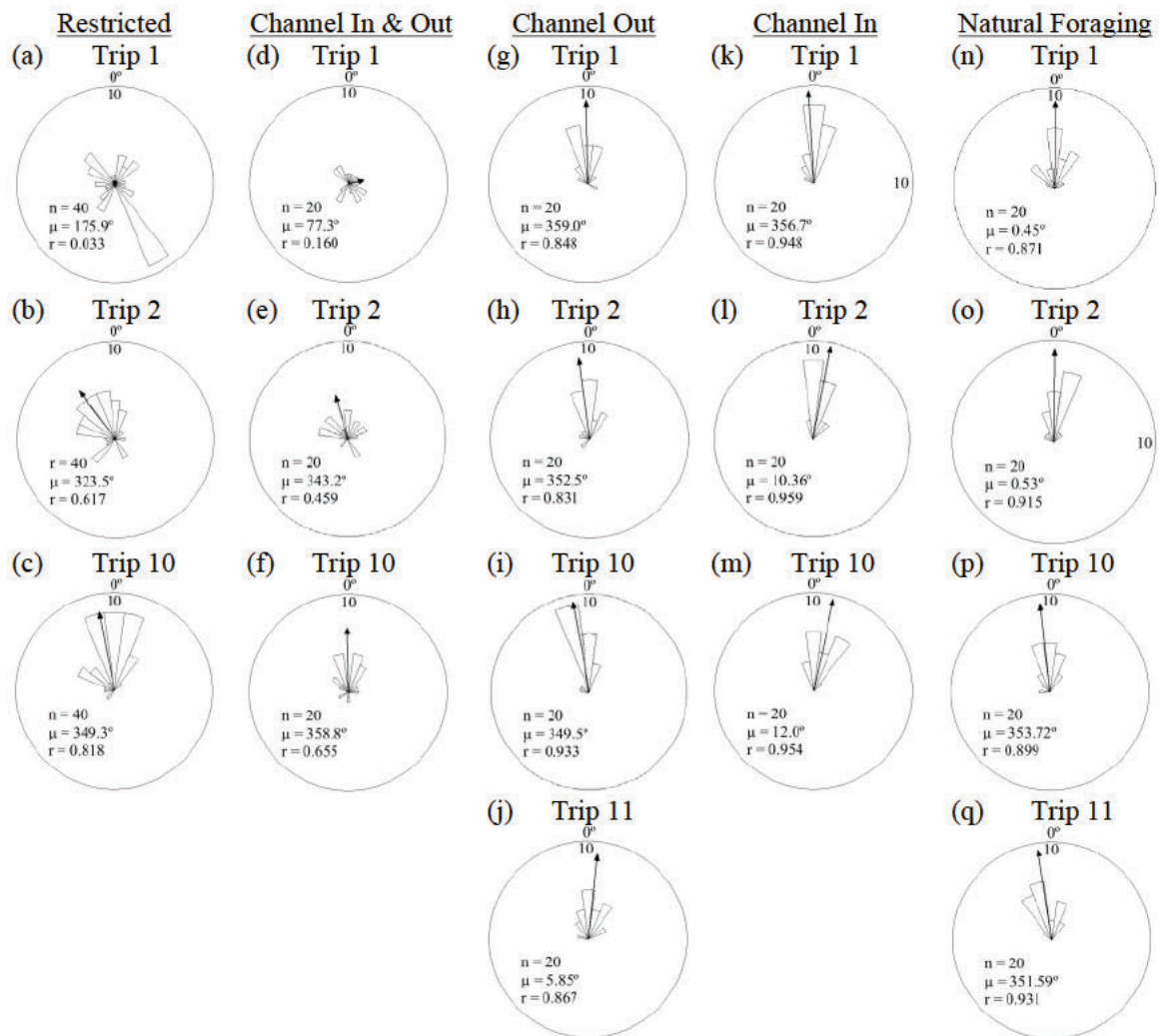


Figure 4 Circular histograms of initial headings of Trips 1, 2 and 10 in the 5 learning conditions of Experiment 2. In the histograms, the nest direction was set at 0°. An arrow denotes the length and direction of the mean vector. Each histogram states the number of individuals (n), the mean vector direction (μ), and the length of the mean vector (r). Histograms of the Restricted condition on trips (a) 1 (b) 2 (c) 10. Histograms of the Channel In & Out condition on trips (d) 1 (e) 2 (f) 10. Histograms of the Channel In condition on trips (g) 1 (h) 2 (i) 10 (j) 11. Histograms of the Channel In condition on trips (k) 1 (l) 2 (m) 10. Histograms of the Natural Foraging condition on trips (n) 1 (o) 2 (p) 10 (q) 11.

Very similar to the Restricted condition, forager paths on Trip 1 of the Channel Out & In condition showed foragers initially moved around the displacement site before slowly expanding their paths toward the nest (Supplemental Figure 3a-e). Just as in the Restricted condition, by Trip 10 foragers initially traveled in the nest direction but still searched around the displacement site before moving toward the nest (Supplemental Figure 3f-h), and both Trip 1 and 10 foragers repeatedly returned to the area around the displacement site during their homeward trips.

Foragers in the Channel Out condition showed immediate orientation to the nest direction at 0° on Trip 1 (Table 1; Figure 4g). This orientation pattern continued throughout all ten trips (Trips 2 and 10, Table 1, Figure 4h,i; Trips 3-9, Supplemental Table 1) with only Trip 9 having a 95% CI just to the left of the nest direction at 0° (CI ending at 358.9°). This orientation continued even on Trip 11 when foragers were tested without a homeward vector (Table 1; Figure 4j).

As in previous conditions, duration-to-return significantly differed over the ten trips (Figure 5; ANOVA $F_{9,171} = 7.64$, $P < 0.01$) and Helmert contrasts show that duration-to-return during Trip 1 (mean = 76.8 seconds) was significantly higher than Trips 2–10 (Helmert contrast; $t = 5.49$; $P < 0.01$), which averaged 37 seconds to return. Helmert contrasts of Trip 2 through Trip 10 did not significantly differ ($P > 0.05$).

When Trip 11 was added, duration-to-return still significantly differed across trips (Figure 5; Repeated Measures ANOVA $F_{10,190} = 24.5$, $P < 0.01$) and an a priori contrast indicated that duration-to-return during Trip 11 was significantly larger than all previous trips combined ($P < 0.01$) with an average return of 217 seconds.

On Trip 1, forager paths were directed toward the nest with individuals showing some degree of uncertainty illustrated by small segments returning toward the displacement site with winding inbound paths despite the inbound vector pointing to the nest (Supplemental Figure 4a). By Trip 10 these paths had become much straighter with fewer deviations from the straight homeward route. Forager paths in this condition do appear to be closer to the corridor sides compared to conditions (Channel In and Natural foraging) where there was no channel in the center of the corridor (Supplemental Figure 4b). During the zero-vector Trip 11, forager paths became much more scattered with foragers performing long looping paths, with foragers traveling back toward the displacement site during portions of the inbound route (Supplemental Figure 4c-e).

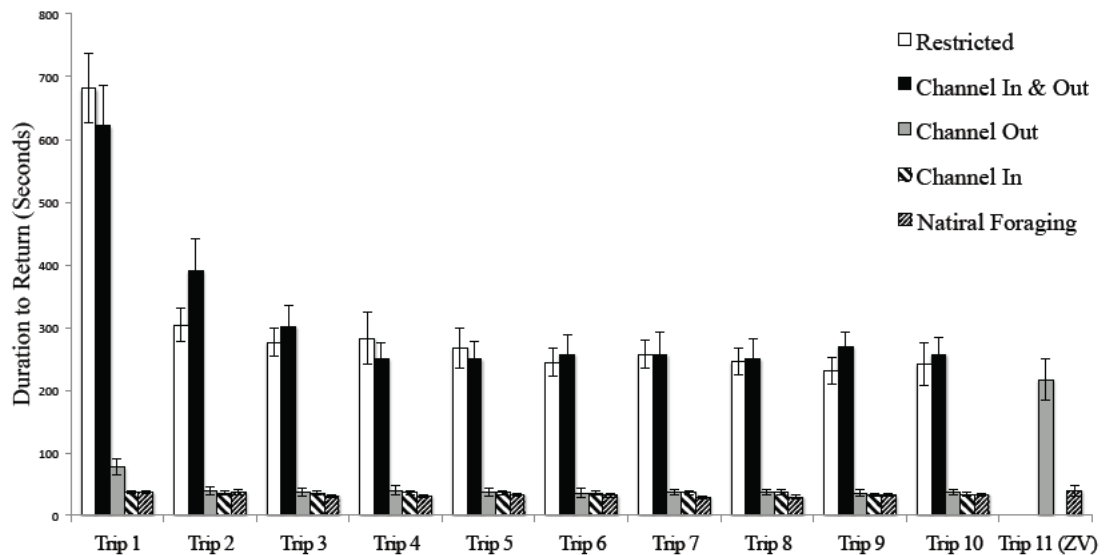


Figure 5 Duration times of repeated displacements in the Restricted, Channel Out & In, Channel Out, Channel In, and Natural Foraging conditions \pm SE. Duration-to-return values were calculated by timing individuals from the point they left the goniometer to the point they returned to the nest arena 50 cm from the nest entrance.

Foragers in the Channel In condition showed immediate orientation to the nest direction at 0° on Trip 1 (Table 1; Figure 4k). This orientation pattern continued throughout all ten trips (Trips 2 and 10, Table 1, Figure 4l,m; Trips 3–9, Supplemental Table 1) with Trips 2, 9, and 10 having the nest direction at 0° just to the left of the 95% CI (CI ending at 3.1° , 0.8° and 4.3° , respectively). Duration-to-return did not significantly change over the ten trips (Figure 5; Repeated Measures ANOVA $F_{9,171} = 0.355$, $P > 0.05$) with foragers averaging ~ 37 seconds to return to the nest across all trips. Forager inbound paths during the Channel In Condition on both Trips 1 and 10 showed foragers were directed to the nest and typically traveled quickly through the center of the channel to the nest area (Supplemental Figure 5a,b). On both Trip 1 and Trip 10 of the Channel In condition we did observe some individuals stop nest-ward movement and turned back to the displacement site momentarily. Additionally, this behavior shows up in the paths of a few individuals (Supplemental Figure 5a,b). This turning back to the displacement site in the Channel In condition only lasted a couple of seconds with foragers quickly returning to nest-ward travel.

In our natural foraging control, foragers showed orientation to the nest direction at 0° on Trip 1 (Table 1; Figure 4n) and this pattern persisted throughout all ten trips (Trips 2 and 10, Table 1, Figure 4o,p; Trips 3–9, Supplemental Table 1). Correct nest directed orientation continued even on Trip 11 when foragers were tested without a homeward vector (Table 1; Figure 4q). Duration-to-return did not significantly change over 10 trips (Figure 5; Repeated Measures ANOVA $F_{9,171} = 1.50$, $P > 0.05$) or all 11 trips (Repeated Measures ANOVA $F_{10,190} = 1.04$, $P > 0.05$) with foragers averaging ~33 seconds to return to the nest across all 11 trips. Forager inbound paths during Natural Foraging on Trips 1, 10, and 11 showed foragers were directed to the nest and typically traveled quickly through the center of the corridor to the nest area (Supplemental Figure 5a-c).

Between conditions

Duration-to-return significantly differed between the 5 learning conditions over all 10 trips ($P < 0.01$). Post-hoc Bonferroni-Holm pairwise comparisons show that in all trips the Restricted and Channel Out & In conditions were significantly higher than the natural foraging control ($P < 0.01$). Conversely, the Channel In and Channel Out conditions did not significantly differ from the Natural foraging control ($P > 0.05$) in Trip 1–10. However, duration-to-return did significantly differ during Trip 11 between the Channel Out condition (217 seconds) and the Natural foraging control (39.35 seconds) condition ($t_{38} = 5.30$, $P < 0.01$).

Discussion

Experiment 2 indicates that foragers may use any of the foraging segments (outbound view sequence, inbound view sequence, or the accumulated vector) in order to achieve successful nest orientation. Foragers with one exposure to only the outbound view sequence in the Channel In condition (Trip 1) successfully oriented and foragers with only one exposure to the inbound view sequence, in the Restricted and the Channel Out & In conditions (Trip 2),

were also able to orient correctly. Finally, foragers in the Channel Out condition (Trip 1) with only the vector cue to direct them were able to orient correctly. Additionally, individuals appear able to achieve one-trial learning of the terrestrial cues at the displacement site regardless of view sequence and the presence of a homeward vector. The results of Experiment 2, especially Trip 11 of the Channel Out condition (discussed more below), support the belief (Freas & Cheng, 2017) that the path integrator is not used as a scaffold for learning route cues. It has previously been shown that *M. bagoti* do not require a homeward vector in order to retrieve landmark cues (Kohler & Wehner, 2005). The current study suggests the vector is also not associated with landmark memory during the acquisition phase of learning.

During Experiment 2, foragers can use one exposure to either the outbound or inbound view sequence to orient correctly, yet our duration-to-return results indicate the learned view sequence (outbound or inbound) is important. Foragers take significantly longer to return home when they have never been exposed to the outbound view sequence (Restricted and Channel Out & In) compared to those conditions where the outbound view sequence was available (Natural foraging and Channel In) regardless of the number of exposures. In the Channel Out condition, when foragers had both the inbound sequence and a vector cue, foragers in the first ten trips performed as well as the Natural foraging control, suggesting the vector coupled with the inbound sequence may allow foragers to learn the terrestrial cues and the homeward route. Yet, subsequently, once these foragers did not have a current vector to guide them on Trip 11, they became significantly worse at returning to the nest compared to the control ants and were similar in performance to foragers in the Restricted and Channel Out & In conditions. This increase on Trip 11 suggests that the presence of a vector on previous trips did not help foragers learn the homeward route and that the quick return times during Trips 1-10 were most likely due to the forager using vector cues rather than exhibiting stronger route learning with the inbound view sequence. Compare this with Trip 1 of the

Channel In condition, where foragers with only one exposure to the outbound views to the feeder and no homeward vector cue returned straight home. These results show that exposure to the route in the outbound sequence allows foragers to form efficient homeward routes between the displacement site and the nest. Foragers in these conditions, after one exposure to this view sequence, reach their highest performance with foragers returning immediately to the nest and never improving across multiple trips. Foragers without the outbound view sequence, while still able to learn the terrestrial cues sufficiently enough to orient and improve significantly in duration-to-return by Trip 2, are unable to produce efficient homeward routes, exhibiting duration-to-return times over 6 times longer than the naturally foraging control.

General Discussion

Since the learning of terrestrial cues appears to occur in all conditions, with regard to both orientation and duration-to-return improvements, the question remains, what is causing the large differences in return duration? We discuss the nature of the learning in our experimental situations and two potential explanations for these findings.

With regard to what was learned, it is important to note that in 4 conditions of Experiment 2 (Restricted, Channel Out & In, Channel In, and Trip 11 of both the Channel Out condition and the Natural Forage control) zero-vector foragers are presented a cue conflict during the homeward trip. Learned landmark cues would point foragers to the true nest location at the end of the corridor (8 m away), while the vector cue would indicate the nest was at the displacement site. This conflict means that as foragers traveled to the nest they accumulated a vector cue in the opposite direction. Vector cues have been shown to increase in strength with increases in the distance traveled (Wystrach, Mangan, & Webb, 2015), meaning as the forager traveled closer to the true nest, the vector cue pointing back to the displacement site would get stronger. This conflict and potential cue uncertainty is evident in

the homeward paths (Supplemental Figure 1-5), with foragers in some conditions (Restricted, Channel Out & In) stopping homeward movement to temporarily return in the direction of the displacement site. This underlying cue conflict means that the long inbound routes we see in conditions with no outbound view sequences may not be due to any lack of terrestrial cue learning but rather the relative weakness of the learned terrestrial cues in the Restricted, Channel Out & In, and Trip 11 of the Channel Out conditions.

In the first explanation of these differences, the observed duration-to-return variation between conditions in Experiment 2 results from the differences in delay since the forager's last exposure to the terrestrial cues during testing (Ziegler & Wehner, 1997). Foragers returning from the displacement site in the Restricted, Channel Out & In, and Trip 11 of the Channel Out condition were last exposed to the terrestrial cues of the route on the previous foraging trip. In comparison, in the Natural foraging and Channel In conditions, individuals were exposed to the terrestrial cues on their current foraging trip. These exposure differences could potentially cause the variation we observe in the duration-to-return results and would be consistent with increased uncertainty over delays from the time of exposure to the cue under the temporal weighting rule (Devenport & Devenport, 1994). As previously shown in *M. bagoti* (Freas & Cheng, 2017), terrestrial cue strength is not constant, but changes due to inherent uncertainty during decision making when cues conflict. Foragers minimize uncertainty in cue reliability by discounting cue sets with increases in the time since the cue was last encountered. This discounting can cause foragers to alter navigational choices between landmark cues and the vector depending on time since last exposure to the landmark cues (Freas & Cheng, 2017). In the current context, cues experienced on previous foraging trips may be sufficiently discounted over time to result in the large durations in conditions where there is a landmark-vector cue conflict.

Findings in two previous *M. bagoti* studies, however, appear to contradict this explanation (Narendra, Si, Sulikowski, & Cheng, 2007; Freas et al., 2017a). Narendra et al.

(2007) tested foragers' long-term memory of the landmark cues around the nest by holding experienced foragers for multiple days and then displacing them with no accumulated vector 2 m from the nest. These foragers experienced delay from exposure times much longer than the current study (up to 5 days), yet returned directly to the nest and showed no evidence of returning to the displacement site. These results would indicate terrestrial cues remain strong compared to the newly accumulated vector cues over very long exposure delay periods. It is important to note that the displacement distance in this study was 1/4 the distance of the current study, making direct comparisons imperfect. But in a different study, observations during terrestrial retention tests in Freas et al. (2017a) suggest that even at distances of 8 m from the nest, there is no evidence of this cue conflict causing foragers to turn back to the displacement site. Foragers displaced 8 m from the nest after being restricted to the nest site for 5 days still oriented correctly and were observed traveling directly home after displacement with their homeward route, resembling ants in the Natural foraging condition in the current study. Additionally, no individual tested by Freas et al. (2017a) was observed exhibiting these returning behaviors described in the current study. Furthermore, the typical period between foraging trips in the current study was considerably shorter compared to the delays in these studies and the 24-hour delay in Freas and Cheng (2017), with foragers in the Restricted and Channel Out & In conditions regularly completing 3–4 foraging trips each day. While these previous findings argue against the temporal weighting explanation, it remains possible that time-dependent uncertainty plays at least some role in the current findings and the explanation remains a potential topic of future study.

In the second explanation for the observed duration differences, the sequence in which the terrestrial views between the nest and feeder are learned affects learned cue strength. Foragers given one exposure to the outbound views appear to form strong memories that subsequently overpower the conflicting vector cue during the trip home, resulting in foragers returning directly to the nest site. Foragers with exposure to only the inbound view sequence

may form weak terrestrial cue memories that, when in conflict with an increasingly strong vector cue during the homeward trip, results in higher uncertainty, more returns in the direction of the displacement site, and a longer return duration.

One explanation for the difference in memory strength between views on outbound and inbound routes concerns the nature of reinforcement learning in the acquisition of landmark cues. The basic idea is that reinforcers help to establish memories. It would appear that in these foragers, finding food (during outbound travel) acts as a key reinforcer for landmark learning rather than arriving home (inbound travel), as memories of the outbound route appear to produce stronger navigational cues. This is surprising to us, as we initially believed that successfully reaching the nest with food in tow would be the stronger reinforcer. Yet the data suggest that these inbound memories appear significantly weaker in cue strength. This means it is possible that foragers only form strong memories of sites away from the nest when they are profitable. Terrestrial memories of unsuccessful foraging routes may not be strongly learned due to the absence of food reinforcement.

It may also be that traveling out in a channel with a blocked panorama to the displacement site prevents foraging ants from performing turn back behaviours when leaving the nest. These turn backs are believed to aid the forager in learning the route panorama and may be critical to the formation of efficient route formation (Nicholson et al. 1999; Zeil 2012; Zeil et al. 2014). Further study into the importance of turn backs on the outbound trip to route learning is warranted.

These results stand in contrast to some previous findings in *M. bagoti*, most notably Wehner et al. (2006). Here, foragers were trained with different outbound and inbound routes using a forced-detour setup. When trained foragers were displaced back onto their outbound path, they were unable to return home and instead engaged in systematic search behavior. These foragers immediately returned to the nest once they crossed their inbound route during the search. These findings suggest that foragers only recognize view sequences when they

correspond with the forager's current goal, be that a food source or the nest or their motivational state (Harris, de Ibarra, Graham, & Collett, 2005). The current study seemingly conflicts with this result, as foragers with the outbound path alone appear able to orient and return home quickly. Conversely, the inbound path alone appears inadequate to create strong memories of homeward routes that can overcome conflict with vector information.

These differences in forager performance may have multiple causes, the first being the differences in experimental setup. Foragers in Wehner et al.'s (2006) study had been trained through forced detours on distinct outbound and inbound routes that, while different, might have some degree of similarity, as *M. bagoti* inhabits areas that have landmarks that would be prominent across many meters, such as trees. The current study tested individuals with dramatically different route segments where individuals may have no outbound path or an outbound segment blocked by channel walls. This may result in the difference in forager homing performance when on the inbound route where foragers in Wehner et al. (2006) navigate efficiently while foragers in the current study do not.

A second difference is that we tested initially naïve individuals on their first foraging trips, whereas Wehner et al.'s (2006) ants were not naïve, meaning that the ants might have already foraged in the test area, and their ants were trained for days before testing. This difference in experience could also be a factor in the variation in performance. Another difference is that the testing area in the current study was cleared of all local landmarks while Wehner et al. (2006) trained foragers on a more cluttered route with local landmarks such as grass tussocks which change rapidly along the route. In currently unpublished data, *M. bagoti* appears to form weaker landmark memories when only distant landmarks are available, such as in the current experiment, compared to foragers along more cluttered routes with more local landmark cues (Antoine Wystrach, personal correspondence, July 11, 2017). This study in preparation used a different test for memory strength by having ants repeatedly run segments of the route or the entire route without getting home. The weaker memory of distant

panoramas could explain the weak landmark memories in our inbound-only ants even when they were trained with a homeward vector (see Trip 11). Distant landmarks may be sufficient for successful initial orientation, as observed in our results, but may change little while the forager is moving through the environment, providing less conditioned (secondary) reinforcement to continue traveling in the chosen direction. That is, we assume that a sequence of scenes that changes substantially provides more secondary reinforcement along a route compared with a sequence of scenes that changes little. The retinal height of distant objects or landmarks changes less between two locations the further those two locations are from the object. Thus, the further away the ant is from a landmark the less discernible it is that the landmark has updated with the ant's movement in any direction. This could make navigation using distant landmarks less accurate and this lack of landmark reinforcement may be why we see these animals exhibit the large degree of turn-back behaviors, especially when near the displacement site and far from any landmarks (Figure 2; Supplemental Figure 6).

Variation in homeward route performance between our foragers with outbound view exposure and foragers released on the outbound route in Wehner et al. (2006) may have a different cause. It may be that foragers in the current study make multiple unsuccessful foraging trips before falling into the feeder, performing multiple looping foraging paths returning to the nest before reaching the feeder. We have observed such behavior in other studies in which these ants had been trained to visit a provisioned feeder. These experiences may allow foragers to learn the route using all the cues present and result in foragers returning home efficiently compared to when the outbound and inbound routes are different. Yet, it is also likely that foragers in Wehner et al. (2006) also performed unsuccessful foraging runs before becoming trained on the detour route. It is also possible that the forced detours present in Wehner et al. (2006) may have inhibited learning of the outbound route. Further study is needed to untangle these issues.

Conclusions

Regardless of experience, foragers appear unable to extrapolate landmark cues from the area around the nest to local sites, suggesting foragers must travel to the site to navigate home successfully. Foragers show one-trial learning in initial heading direction given either the outbound or inbound view sequence. Yet route duration results suggest the outbound view sequence appears to play a role in strengthening landmark memories and creating homeward routes that withstand conflict with vector information, as foragers without this view sequence took considerably longer to return home after displacement. The longer durations were caused by ants frequently turning back to the release point, behavior consistent with the accumulating of a vector through path integration.

Ethics Statement

There are no ethical requirements in Australia for research in ants. Our manipulations, moreover, were non-invasive and did not affect the tested ants or the nest in any notable way.

Competing interests

Authors declare no competing interests regarding this work.

Author contributions

Experiments conceived and designed by CAF & KC. All data was collected by CAF. CAF analysed the data. CAF & KC drafted and revised paper. Both authors have given final approval for this publication.

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Chapter 6. Compass cues used by a nocturnal bull ant, *Myrmecia midas*

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Polarized light use in the nocturnal bull ant, *Myrmecia midas*

Cody A Freas¹, Ajay Narendra¹, Corentin Lemesle¹ & Ken Cheng¹

¹ Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Abstract

Solitary foraging ants have a navigational toolkit, which includes the use of both terrestrial and celestial visual cues, allowing individuals to successfully pilot between food sources and their nest. One such celestial cue is the polarization pattern in the overhead sky. Here, we explore the use of polarized light during outbound and inbound journeys and with different home vectors in the nocturnal bull ant, *Myrmecia midas*. We tested foragers on both portions of the foraging trip by rotating the overhead polarization pattern by $\pm 45^\circ$. Both outbound and inbound foragers responded to the polarized light change, but the extent to which they responded to the rotation varied. Outbound ants, both close to and further from the nest, compensated for the change in the overhead e-vector by about half of the manipulation, suggesting that outbound ants choose a compromise heading between the celestial and terrestrial compass cues. However, ants returning home compensated for the change in the e-vector by about half of the manipulation when the remaining home vector was short (1–2 m) and by more than half of the manipulation when the remaining vector was long (more than 4 m). We report these findings and discuss why weighting on polarization cues change in different contexts.

Introduction

Arthropods are known to derive compass information using the pattern of polarized skylight [1–9]. Polarized light comprises light waves in which the wave occurs along a single plane. Light scatters after entering the earth's atmosphere and becomes partially linearly polarized. This creates an e-vector pattern in the sky arranged in concentric circles around the sun or moon's position [10,11]. The e-vector in the overhead sky remains in a stable orientation pattern perpendicular to the direction of the sun/moon. This stability makes the sky's polarization pattern a useful directional cue especially when the sun or moon's position is obscured [8,9,12–16]. Insects detect this polarized light through specialized photoreceptors that are located in the dorsal rim area of the eye [2,15–19].

Solar polarization is present even after sunset until the end of astronomical twilight when the sun's position passes 18° below the horizon [20]. During the evening or morning twilight, when the sun is near the horizon, the polarization pattern of the sky intensifies and simplifies along the North–South axis [21], making it of great interest to understand how animals that are active during twilight use this compass cue [3,5,9,12,16]. Among ants, there has been only one study [6] conducted on twilight-foraging animals [22,23]. In this study, outbound foragers of *Myrmecia pyriformis* confronted with a change in the polarization pattern by $\pm 45^\circ$ to the ambient pattern, modified their orientation, but only partially (17.96°). The authors suggested that this partial reliance was due to the extreme reliance on familiar visual landmarks that these ants exhibit [6,24]. Here, we investigate this further in a related nocturnal ant, *Myrmecia midas*, whose navigational capabilities have only recently been studied [25], in order to identify whether foragers use the pattern of polarized skylight during both the outbound and inbound journeys. We further explore whether the extent to which ants rely on polarized light changes with distance from the nest or length of the home vector during both outbound and inbound journeys.

Methods

Experiments were conducted from September 2015 to November 2016 on two *M. midas* nests located on the northern portion of the Macquarie University North Ryde campus in Sydney, Australia (33°46'11" S, 151°06'40" E). *Myrmecia midas* nests were found in habitats consisting of stands of *Eucalyptus* trees with mostly barren understoreys with the nest entrance located near the base of a tree. Nocturnal foraging activity in this species [25] required the use of red-filtered headlamps in order to observe the ants. Research in ants does not require animal ethical approval within Australia. We modified the pattern of polarized skylight by rotating a polarization filter (42 cm diameter) above the ants. The polarization filter (Polaroid HN22; figure 1) was held by a circular 2 cm thick metal ring and lifted 10 cm off the ground by four equally spaced thin metal legs. Numbers of ants tested in each condition are given in the data figures. All testing was conducted during either the evening or morning twilight when the sun's position was between -18° and 0° relative to the horizon. Evening testing began 10 min after sunset and ceased before twilight ended. Morning testing began after the beginning of twilight and ceased before dawn. Each night we obtained the sun's position at sunset and sunrise from the Astronomical Almanac (<http://asa.usno.navy.mil>) and set the ambient e-vector 180° from this direction. As *M. midas* maintain predictable nest-foraging tree route patterns in a well-defined corridor, we were able to pinpoint the orientation of the overhead e-vector and rotate the polarizer relative to that direction. We relied on a compass to locate the ambient e-vector and rotate the polarizer by $\pm 45^\circ$ from this direction. When placing the polarized filter over the forager, we rested the compass on the polarizer along the filter's polarization pattern during placement. Only after the placement was confirmed did we remove the compass.

each individual, we recorded the initial orientation, the exit orientation and their reorientation by placing small pegs in the ground (as defined in figure 1*b*). After a forager's positions were recorded, the forager was collected and marked with a small amount of enamel paint (Tamiya™, Japan) to ensure animals were not tested again. These marked foragers were then returned to the nest site.

Inbound ants at different distances from the nest

We tested inbound foragers at the same two distances (4–6 m and 1–2 m) from the nest. We followed foragers from Nest 1 travelling either 14 m (4–6 m condition) or 3 m (1–2 m condition) to their foraging tree during evening twilight. As a forager climbed the foraging tree, they were each collected in a plastic phial. Each forager was offered a small amount of honey and was then stored overnight in the dark (9 h). Each collected ant was marked with a small amount of enamel paint to exclude previously tested individuals. Foragers were released at the base of their foraging tree in the pre-dawn twilight, which corresponds to the time at which they typically return home [25]. We followed each ant as it travelled to the nest, and placed the centre of the polarizer on the ant when it reached a distance of 4–6 m or 1–2 m from the nest. Therefore, both inbound and outbound foragers were tested at the same distance from the nest. Similar to the outbound tests, the e-vector axis of the filter was oriented either $\pm 45^\circ$ relative to the dominant ambient polarization pattern. We recorded the initial orientation, the exit orientation and the reorientation of each forager in the same manner as in the outbound tests (figure 1*b*). Foragers were then followed for the remainder of their inbound path to ensure they returned to the nest site.

Conflict between home-vector length and nest location

Here, we tested individual foragers close to their nest but with a large remaining vector. We achieved this by first following foragers from Nest 1 to the foraging tree (14 m) in the evening twilight and collected them in a phial as they reached the foraging tree. Just as in previous

inbound conditions, these foragers were fed, marked with paint, held overnight and released in the pre-dawn twilight. We released the foragers on the route at the halfway point between the nest and the foraging tree (7 m). Released foragers were allowed to return to 1–2 m from the nest entrance where the centre of the polarizer was placed over the ant. As with all previous conditions, the e-vector axis of the filter was either $\pm 45^\circ$ relative to the ambient e-vector. Identical to previous conditions, we recorded initial orientation, exit orientation and reorientation for each forager (figure 1b). Foragers were then followed for the remainder of their inbound path to record their final destination.

Statistical analysis

Data were analysed with circular statistics [26,27] using the statistics package Oriana Version 4 (Kovach Computing Services, UK). As each ant had a different initial heading direction, we corrected this by designating the initial heading as 0° for each animal. The *shift magnitude* of each path was calculated by taking the mirror of the difference between the forager's initial path direction and the forager's exit orientation in each -45° condition. This calculation allowed us to compare path shifts in both directions in degrees. Foragers' shift magnitudes were compared between the $\pm 45^\circ$ and between the two distance groups using Watson–Williams *F*-tests. If shift magnitudes between the two groups do not differ, then it means both groups rely on polarized light to the same degree. A Pearson's correlation coefficient was used to test the association between the lunar phase (in per cent) and shift magnitude under the filter. Lunar phase data were obtained from calculations in the Astronomical Almanac (<http://asa.usno.navy.mil>).

Results

Outbound ants at different distances from the nest

When the polarization filter was placed on an outbound ant at both testing distances, they initially stopped moving and then slowly began to move in a chosen direction. Most foragers

would again stop as they reached the edge of the filter and performed visual scans before continuing on their chosen path. Ants did not pause after exiting the filter and continued on route towards their foraging tree.

Outbound foragers at the 4–6 m distance

When the polarizer was rotated left (-45°), the ants' exit orientations were to the left of their initial direction of orientation (mean \pm SE; Nest 1: $\theta = -26.37 \pm 4.72^\circ$; Nest 2: $\theta = -32.16 \pm 5.26^\circ$; table 1 and figure 2a(i), b(i)), and these changes were significant at both nests (Watson–Williams F-test, Nest 1: $F_{1,48} = 22.01$, $p < 0.05$; Nest 2: $F_{1,50} = 13.74$, $p < 0.05$). Conversely, when the polarizer was rotated right ($+45^\circ$), the foragers' exit orientations were to the right of their initial heading direction (mean \pm SE; Nest 1: $\theta = 17.47 \pm 5.47^\circ$; Nest 2: $\theta = 25.07 \pm 7.46^\circ$; table 1 and figure 2a(i), b(i)). These changes were also significant at both nests (Watson–Williams F-test, Nest 1: $F_{1,48} = 13.74$, $p < 0.05$; Nest 2: $F_{1,46} = 9.62$, $p < 0.05$). After exiting the -45° rotated filter, foragers reoriented significantly to the right (Watson–Williams F-test, Nest 1: $F_{1,48} = 18.25$, $p < 0.05$, mean \pm SE $\theta = 25.63^\circ \pm 5.11^\circ$; Nest 2: $F_{1,50} = 9.65$, $p < 0.05$, mean \pm SE $\theta = 23.205 \pm 6.57^\circ$; table 1 and figure 2a(ii), b(ii)). After exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams F-test, Nest 1: $F_{1,48} = 12.57$, $p < 0.05$, mean \pm SE $\theta = -19.24 \pm 5.76^\circ$; Nest 2: $F_{1,46} = 5.83$, $p < 0.05$, mean \pm SE $\theta = -26.34 \pm 5.26^\circ$; table 1 and figure 2a(ii), b(ii)). Results did not differ between nests ($p > 0.05$ for both filter exit orientations and foragers' reorientations), and shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams F-test, Nest 1: $F_{1,48} = 0.17$, $p > 0.05$, Nest 2: $F_{1,48} = 0.194$, $p > 0.05$).

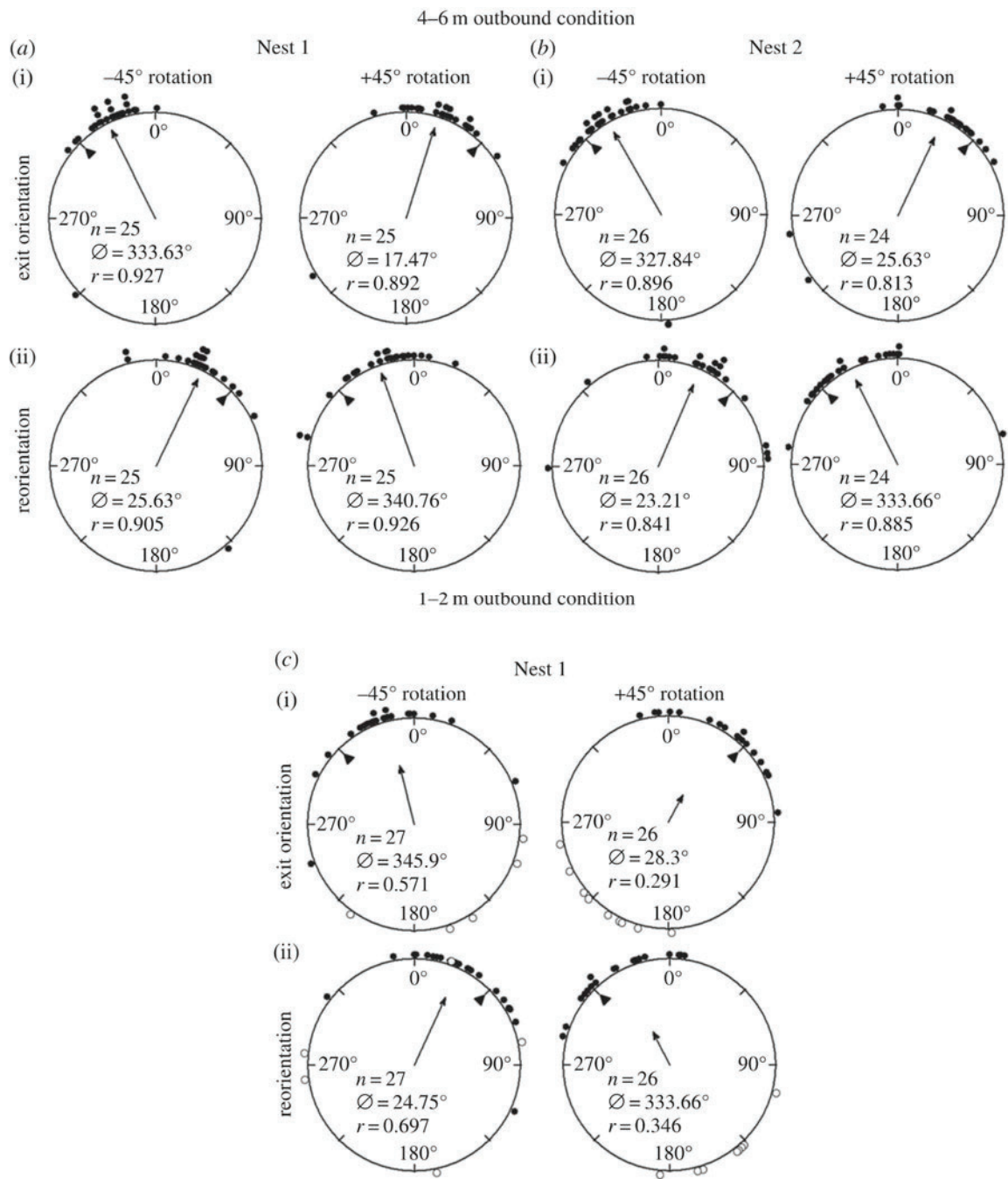


Figure 2 Circular distributions of individual *M. midas* foragers' headings during outbound conditions. Histograms show raw data of exit orientation under the filter and the reorientation after exiting the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector and the mean direction. (a) Orientations for Nest 1 during the 4–6 m outbound condition. (b) Orientations for Nest 2 during the 4–6 m outbound condition. (c) Orientations for Nest 1 during the 1–2 m outbound condition. Closed circles indicate individuals that continued on to the forging tree after testing. Open circles represent foragers that retreated once the filter was placed overhead and these individuals returned to within 30 cm of the nest entrance after testing. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Outbound foragers at the 1–2 m distance

When the polarizer was rotated $\pm 45^\circ$, individuals paused after the polarizer was placed overhead. After this short pause, most individuals continued to the foraging tree ($+45^\circ$, $n = 18$; -45° , $n = 22$; figure 2c closed circles); a minority of individuals in both conditions, however, turned back and retreated (defined as individuals that returned to within 30 cm of the nest entrance after exiting the filter) to the nest after the polarizer was placed over them ($+45^\circ$, $n = 8$; -45° , $n = 5$; figure 2c open circles). Focusing on only those individuals that continued to the foraging tree, when the polarizer was rotated 45° to the left (-45°), the foragers' exit-orientations leaving the filter were to the left of their initial path direction (mean \pm SE; Nest 1: $\theta = -18.26 \pm 6.56^\circ$; table 1 and figure 3). This path change under the filter was significant (Watson–Williams F-test, Nest 1: $F_{1, 52} = 4.31$, $p < 0.05$). When the polarizer was rotated 45° to the right ($+45^\circ$), forager exit orientations were to the right of their initial path (mean \pm SE; Nest 1: $\theta = 32.81 \pm 6.4^\circ$; table 1 and figure 3) and this shift was also significant (Watson–Williams F-test, Nest 1: $F_{1, 50} = 12.29$, $p < 0.05$). After exiting the -45° rotated filter the foragers reoriented significantly to the right (Watson–Williams F-test, Nest 1: $F_{1, 52} = 9.95$, $p < 0.05$, mean \pm SE $\theta = 26.23 \pm 6.73^\circ$; table 1 and figure 2c), and after exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams F-test, Nest 1: $F_{1, 50} = 10.79$, $p < 0.05$, mean \pm SE $\theta = -29.43 \pm 5.87^\circ$; table 1 and figure 2c). While the number of individuals who retreated was insufficient for statistical analysis, foragers' exit orientations shifted as would be expected, either to the left (mean \pm SE $\theta = -35.34 \pm 26.60^\circ$) or to the right (mean \pm SE $\theta = 37.14 \pm 9.33^\circ$) of the nest entrance direction corresponding with manipulations in the polar filter (-45° or $+45^\circ$, respectively). Shift magnitude was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams F-test; $F_{1, 51} = 0.65$, $p > 0.05$). When the -45° and $+45^\circ$ shifts were combined, the shift magnitude was also not significantly different between the outward heading ants of the two outbound testing conditions (Watson–Williams F-test, $F_{1, 151} = 0.17$, $p > 0.05$).

Table 1 Statistics of outbound and inbound forager shifts under polar filter and reorientations.

<u>Condition</u>	<u>Mean Vector</u>	<u>95% Confidence Interval</u>		<u>Mean Vector</u>	<u>Rayleigh test</u>	
	<u>$\mu(\circ)$</u>	<u>Minus()</u>	<u>Plus()</u>	<u>Length</u> <u>r</u>	<u>Z</u>	<u>p</u>
Outbound 4-6m						
Nest 1						
Exit Orientation						
-45	333.63	324.86	342.40	0.927	21.46	< 0.0001
+45	17.47	6.5	28.2	0.892	19.89	< 0.0001
Re-orientation						
-45	25.63	15.61	35.66	0.905	20.47	< 0.0001
+45	340.76	331.94	349.50	0.926	21.45	< 0.0001
Nest 2						
Exit Orientation						
-45	330.3	320.05	340.55	0.896	20.92	< 0.0001
+45	25.03	10.44	39.62	0.813	15.88	< 0.0001
Re-orientation						
-45	23.21	10.33	36.08	0.841	18.41	< 0.0001
+45	333.66	322.36	344.96	0.885	18.81	< 0.0001
Outbound 1-2m						
Exit Orientation						
-45	345.90	321.71	10.10	0.571	8.81	< 0.0001
+45	28.25	336.79	79.71	0.291	2.28	0.102
Re-orientation						
-45	24.75	6.37	43.13	0.697	13.01	< 0.0001
+45	333.66	290.04	17.27	0.346	3.11	0.043
Outbound 1-2m Non-Retreaters						
Exit Orientation						
-45	346.47	334.18	358.76	0.897	14.50	< 0.0001
+45	32.81	20.26	45.37	0.893	14.36	< 0.0001
Re-orientation						
-45	23.36	10.17	36.55	0.883	14.03	< 0.0001
+45	330.56	319.07	342.06	0.91	14.90	< 0.0001
Inbound 4-6m						
Exit Orientation						
-45	318.84	307.03	330.64	0.940	12.38	< 0.0001
+45	34.13	26.27	42.00	0.973	13.256	< 0.0001
Re-orientation						
-45	35.29	20.24	50.33	0.904	11.54	< 0.0001
+45	319.82	306.80	332.84	0.928	12.05	< 0.0001
Inbound 1-2m						
Exit Orientation						
-45	335.14	326.62	343.66	0.955	14.592	< 0.0001
+45	19.73	10.16	29.30	0.957	13.73	< 0.0001
Re-orientation						
-45	27.59	15.31	39.86	0.909	14.592	< 0.0001
+45	332.65	320.65	344.64	0.933	13.06	< 0.0001
Inbound Vector & Landmark Conflict						
Exit Orientation						
-45	324.23	313.75	334.71	0.953	12.28	< 0.0001
+45	39.42	24.20	54.64	0.902	11.40	< 0.0001
Re-orientation						
-45	36.49	24.31	48.67	0.936	12.28	< 0.0001
+45	327.12	311.87	342.37	0.902	11.39	< 0.0001

Inbound ants at different distances from the nest

When foragers were released back at the base of their foraging tree during the morning twilight, they also initially paused for a brief period and scanned the environment without translation, before travelling in the nest direction. Nest-bound foragers typically paused again once the polarization filter was placed above them, yet some individuals continued their forward movement. The same behavioural difference occurred at the filter edge, as some inbound foragers did not stop at the edge of the polarizer. After exiting the filter, all foragers continued on to the nest entrance and entered the nest.

Inbound foragers at the 4–6 m distance

When the polarizer was rotated to the left (-45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm SE; Nest 1: $\theta = -41.16 \pm 6.02^\circ$; table 1 and figure 3a), and these changes were significant (Watson–Williams F-test, Nest 1: $F_{1,26} = 39.17$, $p < 0.05$). When the polarizer was rotated to the right ($+45^\circ$), foragers' exit orientations were to the right of their initial heading direction (mean \pm SE; Nest 1: $\theta = 34.13 \pm 4.01^\circ$; table 1 and figure 3a), and these changes were significant (Watson–Williams F-test, Nest 1: $F_{1,26} = 50.57$, $p < 0.05$). After exiting the -45° rotated filter, the foragers reoriented significantly to the right (Watson–Williams F-test, Nest 1: $F_{1,26} = 29.07$, $p < 0.01$ mean \pm SE $\theta = 35.29 \pm 6.02^\circ$; table 1; figure 3a). After exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams F-test, Nest 1: $F_{1,26} = 62.51$, $p < 0.01$, mean \pm SE $\theta = -40.18 \pm 6.64^\circ$; table 1; figure 3a). Shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams F-test, $F_{1,26} = 1.12$, $p > 0.05$).

Inbound foragers at the 1–2 m distance

When the polarizer was rotated to the left (-45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm SE; Nest 1: $\theta = -24.86 \pm 4.35^\circ$; table 1 and

figure 3b), and these changes were significant (Watson–Williams F-test, Nest 1: $F_{1,30} = 23.51$, $p < 0.01$). When the polarizer was rotated to the right ($+45^\circ$), foragers' exit orientations were to the right of their initial heading direction (mean \pm SE; $\theta = 19.73 \pm 4.88^\circ$; table 1 and figure 3b), and these changes were significant (Watson–Williams F-test, $F_{1,28} = 18.59$, $p < 0.01$). After exiting the -45° rotated filter the foragers reoriented significantly to the right (Watson–Williams F-test, $F_{1,30} = 20.84$, $p < 0.01$, mean \pm SE $\theta = 27.59 \pm 4.35^\circ$; table 1 and figure 3b). After exiting the $+45^\circ$ rotated filter, the foragers reoriented significantly to the left (Watson–Williams F-test, Nest 1: $F_{1,28} = 21.25$, $p < 0.01$, mean \pm SE $\theta = -27.35 \pm 6.12^\circ$; table 1 and figure 3b). Shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams F-test, $F_{1,29} = 0.65$, $p > 0.05$). When the -45° and $+45^\circ$ shifts were combined, total shift magnitude in foragers tested at 1–2 m was significantly smaller than foragers tested at 4–6 m (Watson–Williams F-test, $F_{1,57} = 10.93$, $p < 0.01$).

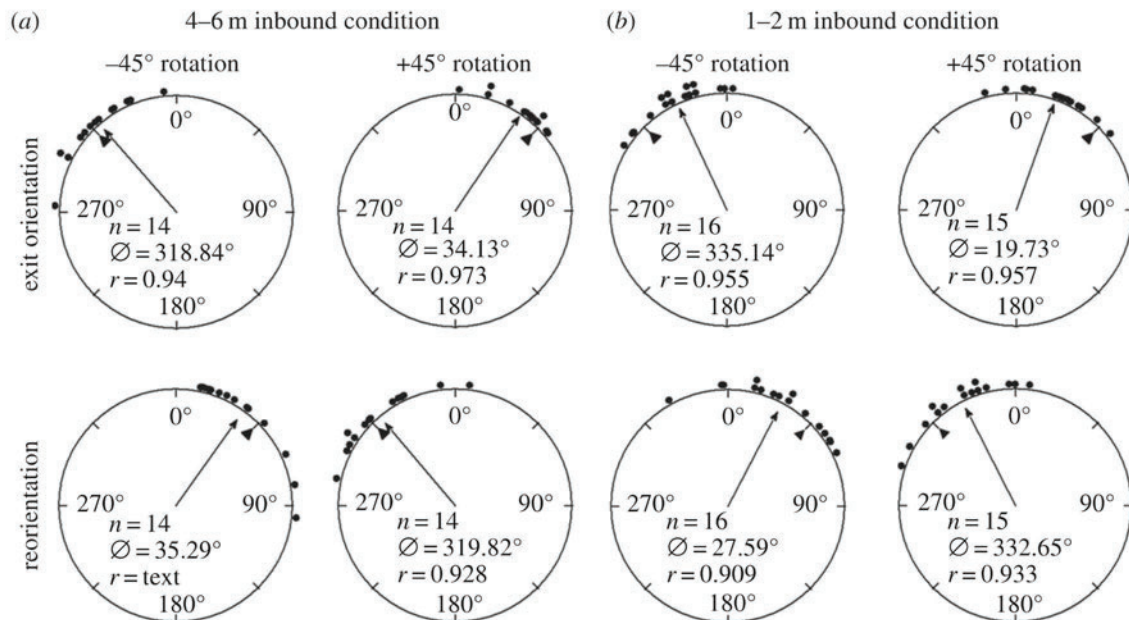


Figure 3 Circular distributions of individual *M. midas* foragers' headings during the inbound conditions. Histograms show raw data of exit orientation under the filter with the individual's initial orientation and reorientation with the forager's exit orientation under the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector and mean direction. (a) Orientations for Nest 1 during the 4–6 m inbound condition. (b) Orientations for the 1–2 m inbound condition. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Conflict between home-vector length and nest location

Inbound ants with 14 m home vectors were displaced on the route but half way home and had to travel only 7 m to find the nest. The ability of these ants to detect a change in the pattern of the polarized light was assessed at 1–2 m from the nest entrance. When the polarizer was rotated to the left (-45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm SE; Nest 1: $\theta = -35.77 \pm 5.35^\circ$; table 1 and figure 4), and these changes were significant (Watson–Williams F-test, Nest 1: $F_{1,26} = 50.78$, $p < 0.01$). When the polarizer was rotated to the right ($+45^\circ$), foragers' exit orientations were to the right of their initial heading direction (mean \pm SE; $\theta = 39.42 \pm 7.77^\circ$; table 1 and figure 4), and these changes were significant (Watson–Williams F-test, $F_{1,26} = 29.09$, $p < 0.01$). After exiting the -45° rotated filter, the foragers reoriented significantly to the right (Watson–Williams F-test, $F_{1,26} = 37.59$, $p < 0.01$, mean \pm SE $\theta = 36.49 \pm 6.21^\circ$; table 1 and figure 4). After exiting the $+45^\circ$ rotated filter, the foragers reoriented significantly to the left (Watson–Williams F-test, Nest 1: $F_{1,26} = 15.67$, $p < 0.01$, mean \pm SE $\theta = -32.88 \pm 7.78^\circ$; table 1 and figure 4). Shift magnitude size was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams F-test, $F_{1,26} = 0.18$, $p > 0.05$). Foragers in this condition showed shift magnitude size similar to that of foragers tested 4–6 m from the nest entrance (Watson–Williams F-test, $F_{1,54} = 0.01$, $p > 0.05$), and these combined shift magnitudes were significantly greater than foragers tested at the 1–2 m vector travelling from a tree 3 m away (Watson–Williams F-test, $F_{1,57} = 8.35$, $p < 0.01$).

Lunar phase

Across all conditions, lunar phase was not associated with changes in shift magnitude of foragers while under the filter (Pearson's correlation coefficient, $r = -0.127$, $p > 0.05$).

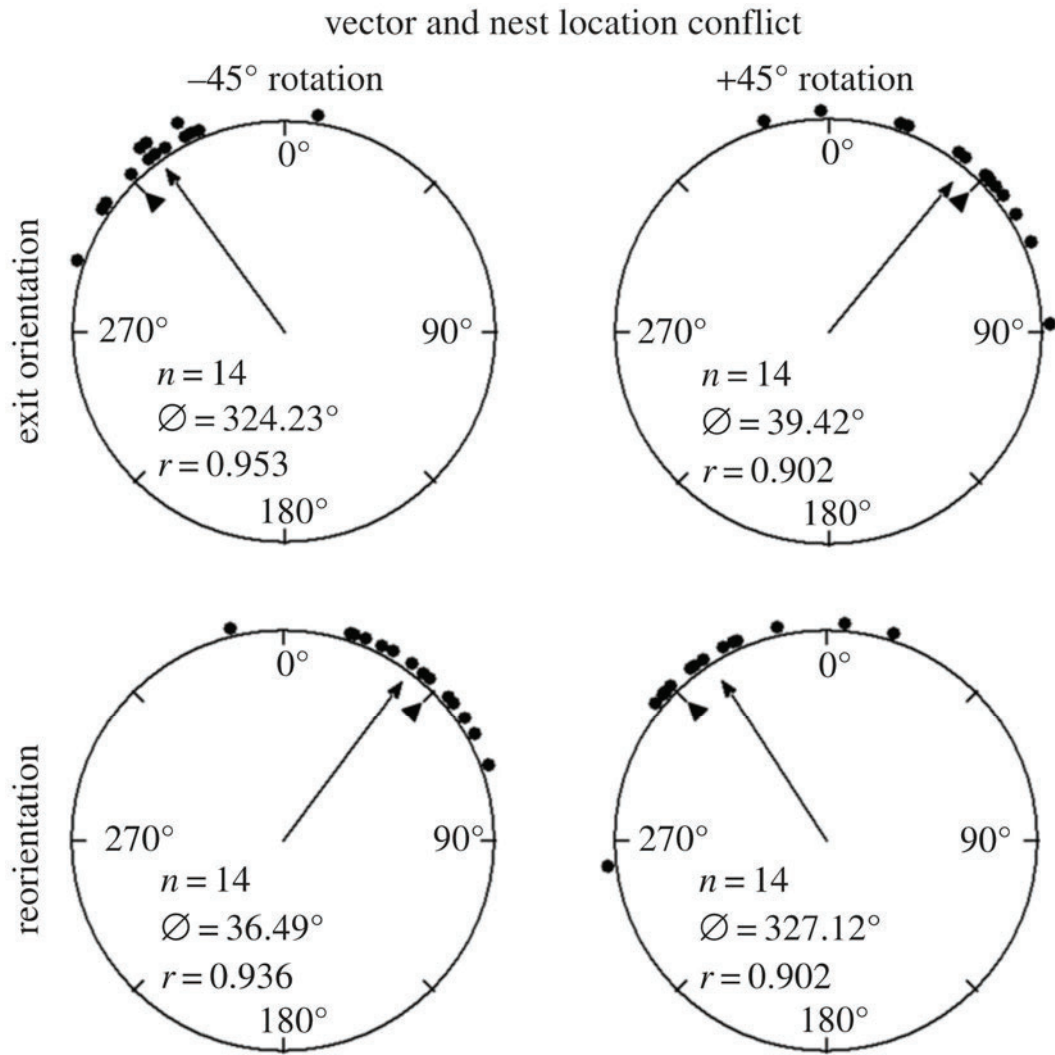


Figure 4 Circular distributions of individual *M. midas* foragers' headings during the long-vector 1–2 m inbound condition. Histograms show raw data of exit orientation under the filter with the individual's initial orientation and reorientation with the forager's exit orientation under the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector direction. *n*, number of individuals; Ø, mean vector; *r*, length of the mean vector.

Discussion

In this study, both inbound and outbound foragers changed their heading direction in response to changes in the overhead polarization pattern. In outbound foragers, we found that distance away from the nest did not influence the weighting foragers gave to this cue. Conversely, ants rely most on the pattern of the polarized skylight when they are returning home (inbound) and have a long-accumulated vector (4–6 m).

In *M. pyriformis*, use of the polarization cue was tested only in outbound ants close to the nest [6]. Here, when the polarized filter was rotated by $\pm 45^\circ$, ants changed their heading direction in the appropriate direction but by less than half of the rotation (-21.8° for -45° rotation; $+14.1^\circ$ for $+45^\circ$ rotation). In our study, the *outbound M. midas* ants at both 1–2 m and 4–6 m away from the nest (figure 2) compensated for the change in the overhead e-vector by about half of the manipulation (1–2 m group: -18.26° for -45° ; 32.81° for $+45^\circ$; 4–6 m group: -26.37° (Nest 1) and -32.16° (Nest 2) for -45° ; 17.47° (Nest 1) and 25.07° (Nest 2) for $+45^\circ$). Both species appear to choose a compromise heading direction between the celestial and terrestrial compass cues during their outbound journey, and this appears to hold true at different distances from the nest.

In the inbound condition, we found that foragers of *M. midas* tested 1–2 m from the nest compensated for the change in the overhead e-vector by about half of the manipulation in their heading (-24.86° for -45° and 19.73° for $+45^\circ$, figure 3a). Interestingly, unlike in the outbound conditions, inbound *M. midas* ants tested at 4–6 m from the nest compensated for well over half of the e-vector manipulation in their altered heading (-41.16° for -45° and 34.13° for $+45^\circ$, figure 3b). Such large compensation was also found in inbound foragers that had a 14 m home vector but were released half way home and tested close (1–2 m) to the nest (-35.77° for -45° and 39.42° for $+45^\circ$, figure 4). This shows that inbound ants respond more to a change in the pattern of polarized skylight than outbound ants. These results imply that inbound ants weight polarization cues differently: ants with a longer home vector respond more to a change in the polarization pattern.

Our results suggest that foragers use both terrestrial and celestial cues, but the weighting of these cues appears to change with the ant's foraging context. In this study, *M. midas* foragers en route appear to weight vector cues in combination with the surrounding terrestrial cues, shifting their paths significantly under an altered polarization pattern. Yet when *M. midas* foragers are displaced to a local area with a vector direction conflicting with

the surrounding terrestrial cues, individuals ignore the accumulated vector and orient using only the terrestrial cues [25]. Thus, it appears that nocturnal *Myrmecia* ants use the pattern of the polarized skylight only when the readouts of the celestial and terrestrial cues align. Furthermore, when there is a conflict between the two sources of compass cues, the celestial cues are suppressed or ignored [25]. This further implicates navigational context as a factor in celestial cue weighting. These behavioural differences may arise as foragers in the polarization experiment encounter no mismatch in cue sets before the polarized light filter, causing them to respond to the altered polarized light pattern while under the filter. Whereas after displacements off-route, foragers are presented with a mismatch between the familiar visual territory and their stored views, causing them to ignore celestial compass information when returning home [25].

The significant differences in shift magnitude in inbound foragers under different conditions were not predicted. Inbound foragers travelling from long distances (14 m, longest foraging route at this site) show larger shifts under the filter compared with individuals that forage in trees closer to the nest regardless of proximity to the nest. These disparities suggest greater weight is being placed on the polarization compass when in conflict with terrestrial cues in these foragers. It appears that the proximity of the nest tree at the test location, a potentially salient terrestrial cue, does not decrease the observed shifts in these far-foraging, long-vector individuals, implying that vector length clearly influences the weight given to the polarization pattern cue. These increases align with the hypothesis that with longer accumulated vectors, ants put more weight on these vector cues [28]. In our case, this difference in weighting persists even after a 9 h delay, with the direction of polarized light, linked to the position of the sun, having changed. These delay periods align with this species' foraging ecology as foragers typically spend this period on their foraging tree overnight [25]. Our results also align well with those from our previous *M. midas* study where only long-vector (more than 5 m) individuals show any evidence of orientation using path integration

after distant displacement [25]. It may also be possible that, as tree fidelity has not been studied in this species, there may be some other difference between individuals that forage further from the nest site and those that forage at a nearer tree. These differences could include disparities in visual scenes encountered by these two foraging groups at Nest 1 or potentially even genetic differences between foragers travelling different distances to the nest. Further study into these behavioural choices is merited to untangle these possibilities. Furthermore, it is interesting that the large shifts in long-vector inbound foragers are not seen in outbound foragers travelling to the same foraging tree. Vector memories in these outbound foragers are based on past foraging trips, whereas inbound foragers are using the vector memory of the current foraging trip [29,30]. This discrepancy may influence the weight these individuals give the vector cue. Unfortunately, as Nest 2 has since died, our field site currently has only one known nest with individuals foraging long distances (more than 5 m), making study of these differences difficult. Further study into this species and its use of celestial cues for navigation is warranted. It is worth noting that the observed heading directions in both outbound and inbound foragers could be in part due to visual changes caused by the filter, independent of the e-vector rotation. Beyond the e-vector shift, light intensity levels are reduced, and there are changes in the visibility and salience of both celestial and terrestrial cues under the filter. These changes could alter the weighting of cues in this study compared to foragers navigating under natural conditions.

Conclusion

We show that both outbound and inbound *M. midas* foragers respond to changes of the e-vector orientation. Outbound ants compensate only partially to the change in polarized light, and this holds true at different distances from the nest. Inbound foragers with a longer home vector respond almost fully to the change in the pattern of the polarized skylight.

Data accessibility

Our data are collected in the electronic supplementary material.

Authors' contributions

Experiments conceived and designed by C.A.F., A.N. and K.C. All data were collected by C.A.F. and C.L. C.A.F. analysed the data. C.A.F., A.N. and K.C. drafted and revised paper. All authors have given final approval for this publication.

Competing interests

Authors declare no competing interests regarding this work.

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Chapter 8. The view from trees: Nocturnal bull ants, *Myrmecia midas*, use the surrounding panorama while descending from trees

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Nocturnal bull ants, *Myrmecia midas*, use the surrounding panorama while descending from trees.

The view from the trees: Nocturnal bull ants, *Myrmecia midas*, use the surrounding panorama while descending from trees

Cody A Freas¹, Antione Wystrach², Ajay Narendra¹ & Ken Cheng¹

¹ Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

² Institute of Cognitive, Brain and Behavioural Sciences, University of Toulouse, Toulouse, France

Abstract

Solitary foraging ants commonly use visual cues from their environment for navigation. Foragers are known to store visual scenes from the surrounding panorama for later guidance to known resources and to return successfully back to the nest. Several ant species travel not only on the ground, but also climb trees to locate resources. The navigational information that guides animals back home during their descent, while their body is perpendicular to the ground, is largely unknown. Here, we investigate in a nocturnal ant, *Myrmecia midas*, whether foragers travelling down a tree use visual information to return home. These ants establish nests at the base of a tree on which they forage and in addition, they also forage on nearby trees. We collected foragers and placed them on the trunk of the nest tree or a foraging tree in multiple compass directions. Regardless of the displacement location, upon release ants immediately moved to the side of the trunk facing the nest during their descent. When ants were released on non-foraging trees near the nest, displaced foragers again travelled around the tree to the side facing the nest. All the displaced foragers reached the correct side of the tree well before reaching the ground. However, when the terrestrial cues around the tree were blocked, foragers were unable to orient correctly, suggesting that the surrounding panorama is critical to successful orientation on the tree. Through analysis of panoramic pictures, we show that views acquired at the base of the foraging tree nest can provide reliable nest-ward orientation up to 1.75m above the ground. We discuss, how animals descending from trees compare their current scene to a memorised scene and report on the similarities in visually guided behaviour while navigating on the ground and descending from trees.

1. Introduction

Solitary ant foragers moving on the ground are adept at navigating through their environment, both while searching for resources and when returning to their nest. Ants that forage alone show the ability to utilise multiple visual navigational systems to reach desired locations. These mechanisms include path integration using the celestial compass (Collett and Collett 2000; Wehner and Srinivasan 2003), systematic search (Wehner and Srinivasan 1981; Müller and Wehner 1994; Schultheiss et al. 2013) and landmark-based navigation (Wehner 2003; Collett et al. 2006; Collett 2012; Schultheiss et al. 2016).

Landmark based navigation has been widely studied in diurnal ants (Wehner et al. 1996; Fukushi 2001; Wehner 2003; Cheng et al. 2009; Collett 2010; Bühlmann et al. 2011; Wystrach et al. 2011, 2012; Lent et al. 2013; Narendra et al. 2013; Schultheiss et al. 2016; Freas and Cheng 2017; Freas et al. 2017a), and the current knowledge of landmark use in ants that forage nocturnally is expanding (Reid et al. 2011; Warrant and Dacke 2011; Freas et al. 2017bc; Narendra and Ramirez-Esquivel 2017; Narendra et al. 2017). What these studies have in common is that they explore navigational behaviour that occurs chiefly in two dimensions while ants are travelling to goal locations on the ground. Yet foragers of multiple species, most notably those of the *Myrmecia* genus, must travel vertically up into their foraging tree to feed and then must successfully descend to return to the nest (Reid et al. 2011; Narendra et al. 2013; Freas et al. 2017bc). Nocturnal species of this genus have the added challenge of completing this feat during the evening and morning twilight when visual cues are less salient compared to those used by diurnal species (Reid et al. 2011; Reid et al. 2013; Freas et al. 2017bc; Narendra et al. 2017).

The study of visually directed behaviour while moving vertically has been little studied outside a few vertebrates (Jeffery et al. 2013; Yartsev et al. 2013). In ant species that forage predominantly on the ground, three-dimensional research has focused primarily on the ability of the path integrator to account for the slope of the ground surface during distance

estimation (Wohlgemuth et al. 2001; Wintergerst and Ronacher 2012). Navigating desert ants appear very adept at integrating terrain slope into their homeward vector, but have not been shown to use landmark cues when foragers are not oriented horizontally. The study of three-dimensional navigation using visual landmark cues is limited to work on the neotropical ant *Cephalotes atratus* L.. This species lives in nests high in the forest canopy, and workers may jump off the trunk and direct their fall back to the same tree farther down. These ants have been shown to use landmark-based cues to direct their fall back to the tree trunk, yet appear to orient their bodies horizontally during the fall and may navigate only during this period (Yanoviak et al. 2005; Yanoviak and Dudley 2006). In the red wood ant, *Formica lugubris*, foragers have been shown to use both chemical and terrestrial cues while ascending and descending trees, yet which terrestrial cues are in use remains unknown (Beugnon and Fourcassie 1988; Fourcassie and Beugnon 1988).

Here, we investigate whether foragers of the night-active *M. midas* actively navigate while foraging vertically on a tree face. *M. midas* foragers rely primarily on landmark cues when navigating to the nest while on the ground (Freas et al. 2017b), and have also been shown to use polarised skylight pattern to compute a homeward vector while on-route (Freas et al. 2017c). However, nothing is known about their behaviour while on a foraging tree. Nests of this species are located in the ground, at the base of a tree trunk. Some individuals forage directly on this ‘nest-tree’, while other individuals navigate first along the ground before climbing up into a nearby tree’s canopy. First, we examined whether foragers displaced on the vertical tree face position themselves towards the nest direction during their descent to the ground. Next, we tested foragers’ descents when the terrestrial cues and celestial cues were in conflict. Then, we tested a subset of each nest’s foragers that forage on the nest-tree (Freas et al. 2017b). Next, to exclude the use of potential cues beyond the surrounding terrestrial cues, we blocked these terrestrial cues around the nest tree and recorded forager descents without access to the panorama. We also analysed pictures of the

visual panorama at different heights and positions on the tree to discover whether nest-oriented views stored while foragers are on the ground contain sufficient information for nestward orientation while on the tree. Finally, we describe behaviours foragers exhibit while descending the tree, which appear to be similar to the scanning behaviours previously described on the ground (Wystrach et al. 2014; Zeil et al. 2014).

2. Methods

2.1 Field site and study species

Experiments were conducted from September 2015 to October 2016 on three *M. midas* nests located in forested areas of the Macquarie University campus in Sydney, Australia (33°46'11" S, 151°06'40" E; Freas et al. 2017bc). All three nests were located within a 200m² area and foragers at each nest foraged on trees within a 15m radius (typically ≤ 5 m) of the nest entrance. *M. midas* inhabits wooded areas consisting of *Eucalyptus* trees with understories clear of vegetation. All forager collections took place during the evening twilight and all testing occurred during the next morning after sunrise for adequate visibility during testing.

2.2 Foraging tree tests

To determine if foragers travelling on the foraging tree actively navigate to position themselves towards the nest direction during their descent, we collected foragers travelling to a neighbouring foraging tree as they reached the tree base. These individuals were displaced to four sides of the tree face and their homeward paths were observed. This experiment was first conducted on 60 individuals (15 per displacement site) from Nest 1 and then the experiment was repeated on another 40 individuals (10 per displacement site) from Nest 2. During evening twilight, outbound foragers were collected just as they climbed onto their foraging tree located 3m from the nest entrance at Nest 1 and 4m from the nest entrance at Nest 2. Foragers were marked with a small amount of paint (Tamiya™, Japan) to prevent

retesting. Marked foragers were held overnight in a plastic phial with a small amount of sugar water in a darkened box. The next morning, beginning at 9am AEST and ceasing at noon, foragers were displaced to one of four sites on the foraging tree face 2m above ground level. The four displacement sites were designated on the tree face in relation to the nest location (0° , 90° , 180° , 270°) with 0° being the nest direction and increasing clockwise. Foragers were released from the phial and allowed to climb out of the phial and onto the tree. Once on the vertical tree face, foragers were allowed to return to the nest by climbing down the tree to the ground. As the forager descended the tree, its path was marked at 1m above ground level, ground level, and 20cm away from the tree, and directional measurements were recorded at these three points using a smartphone-housed digital compass. Once the forager had travelled 20cm from the foraging tree it was observed for the remainder of its path to ensure that all individuals returned to the nest entrance.

2.3 Cue conflict tests

In our second testing paradigm, we collected 30 foragers at Nest 1 in a similar procedure to the first experiment. Foragers were allowed to leave the nest and travel to their foraging tree located 4m from the nest entrance. At the base of this foraging tree, these foragers were collected, marked and stored overnight. The next morning, foragers were displaced to the tree located just above nest location (nest tree). It was assumed that these foragers have some previous experience of the panorama at this site due to the proximity to the nest. Foragers were released onto the face of the nest tree, 2m above ground level, in one of two displacement sites, designated in relation to the nest location (0° , $n = 15$; 180° , $n = 15$) with 0° being the nest direction. This testing regime was conducted on foragers with an acquired homeward vector as ants were captured 4m from their nest and our displacements put this vector in $\sim 90^\circ$ conflict with the terrestrial cues. Identical to previous tests, foragers were released from their phial and allowed to climb onto the nest tree face. Once vertical, foragers were allowed to return to the nest by climbing down the nest tree. As the forager

descended the tree, its path was marked at 1m above ground level and ground level, and directional measurements were recorded at these points. Once ants reached ground level they were observed to ensure all individuals entered the nest.

2.4 Nest tree foragers/landmark blocking experiment

The third experiment focused on a subset of ants ($n = 20$) that forage in the tree directly above the nest entrance (Nest 3). These foragers were allowed to leave the nest and travel the short distance to the nest tree (10cm). Once the forager climbed onto the nest tree at 1.5m, it was collected in a phial, marked on the gaster to prevent retesting and held overnight with food in an identical procedure to previous tests. The next morning, these foragers were displaced individually onto the nest tree but 180° from the nest direction, 1.5m from the ground. In this condition, foragers' full paths on the tree face were recorded by placing small markers just behind the forager as they travelled around the tree face and down to the ground. These markers were placed approximately 10cm apart along the path and stopped once the individual touched the ground. For each marker, we recorded the height and direction in relation to the nest entrance. Forager paths were calculated at every 10cm from the release point to the ground and these positions were used for orientation analysis. After testing, foragers were observed as they returned to the nest entrance.

The landmark blocking condition was conducted on a separate group of nest tree foragers at Nest 3 ($n = 22$). Foragers were again allowed to travel the short distance to the nest tree (10cm). Once the forager climbed onto the nest tree, they were collected, marked and fed, identical to the previous condition. Before testing, (4) 2m long tent poles were anchored into a $1.5\text{m} \times 1.5\text{m}$ square around the nest tree, $\sim 75\text{cm}$ from the tree trunk. A 2m high thick plastic screen was attached to the pole tops and then anchored to the ground using metal posts. This screen was suspended off the ground by a few centimetres to allow for ants to travel underneath. This set up blocked the surrounding terrestrial cue availability below the 2m mark on the nest tree, yet did not block the view of the canopy above or any other cues on the nest

tree itself. Additionally, nest tree foragers were selected for this condition as the nest entrance was located at the base of the tree (10cm) and was well within the enclosed square created by the plastic sheet, allowing foragers access to any cues the nest presents. After collection, foragers were displaced on to the tree face opposite the nest site (180°), and 1.5m off the ground. Foragers' full paths were recorded using the same methods as in the unblocked condition. After testing, foragers were allowed to search for the nest and upon failure after 3 min. were collected and returned to the correct nest entrance location and allowed to enter the nest.

2.5 Image analysis: information available from the foraging tree

For all three nests, we quantified the mismatch in the panoramic scenes between nest-oriented views from the ground at the base of the foraging tree and at different elevations and compass directions on the trees where the ants were tested. To accomplish this, we collected a nest-oriented panoramic image at the base of the foraging tree. We then collected panoramic images at the four cardinal directions on the tree (0° , 90° , 180° , & 270°) at both 1m and 1.75m in height. The panoramic image measured 360px width and 117px height (roughly 50px and 67px below and above horizon, respectively) and were down sampled to a resolution of 1 pixel per degree. The images were converted to grayscale by keeping the blue colour channel only. This diminishes differences between clouds and blue sky but maintains high contrasts between terrestrial objects and the sky. Rotational image difference functions (rotIDFs) were calculated by using the sum of the absolute difference in pixel intensity between the reference and test images, for all possible rotations of the test images (in one-degree steps) using custom written scripts in MATLAB (for further details, see Zeil et al. 2003; Stürzl and Zeil 2007; Zeil et al. 2014).

2.6 Scanning behaviour

In order to describe the scan-like behaviour on the tree face, individual foragers were recorded both while on the tree face after displacement and on a vertically oriented board.

Forager scans were recorded using a free held camera (PowerShot G12, Canon™). Foragers were recorded after local off-route displacement on their foraging tree.

2.7 Statistical procedure

Data from all experiments were analyzed with circular statistics (Batschelet 1981; Zar 1998) using the statistics package Oriana Version 4 (Kovach Computing Services™). Rayleigh's Tests were conducted on foragers' positions on the tree face, testing if data met the conditions of a uniform distribution ($p > 0.05$). If data were not uniform, we tested whether positioning on the tree face was significantly clustered around the nest direction using V-tests, with alpha set at $p = 0.05$. We also examined if the predicted direction (0°) fit within the 95% confidence interval of the foragers' positions during descent to further test positioning towards the nest (Watson Test). When an ant abandoned its descent to travel back up the tree (see blocking condition), only the positions of the individual's final descent were used for analysis.

3. Results

Individuals placed on the tree face at the displacement sites initially paused for a short period. After this pause, foragers typically moved a short distance (usually up the tree 10 – 30cm) away from the displacement point and then paused again and performed what we classify as scanning behaviours on the tree face (described below). Following this scanning behaviour, the forager moved along the tree face descending to the ground. During their descent, foragers typically performed at least one more scan-like behaviour before reaching the ground.

Nest 1

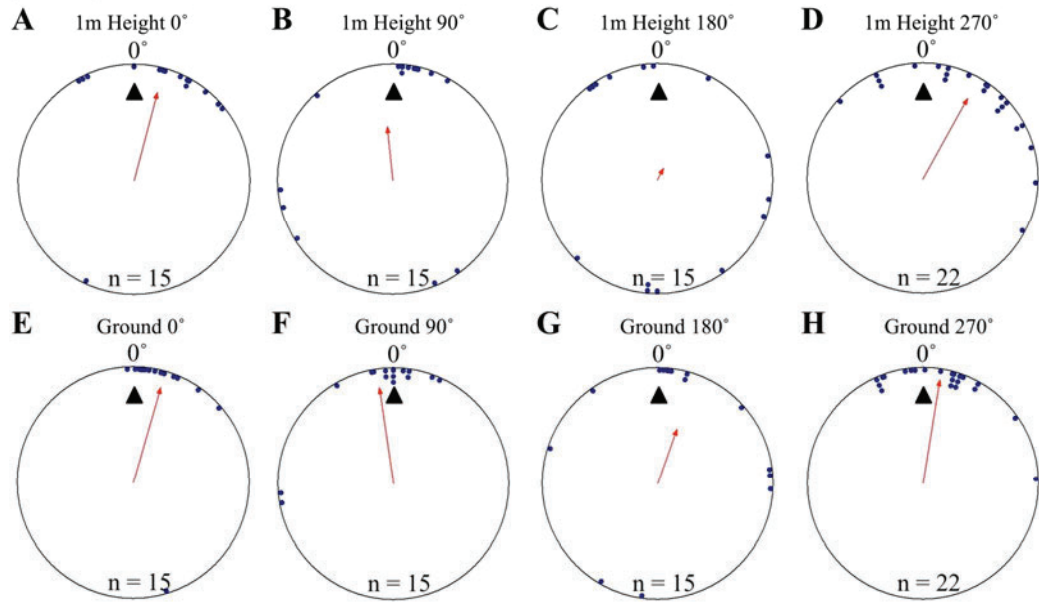


Fig. 1 Circular distributions of individual *M. midas* foragers' positions on the tree face during displacement experiments on their foraging tree. Figures show the raw data of forager positions at two heights after displacements to one of four sides of the tree at a 2m height at Nest 1. The nest direction for each figure is at 0°, labelled by a black triangle. The arrow denotes the direction and length of the mean vector. Foragers were collected at the base of their foraging tree, held overnight and then released vertically on the tree face at one of four sites (0°, 90°, 180°, 270°). (A) The position of individual foragers released at the 0° location at 1m in height. (B) Forager positions of individuals released at the 90° location at 1m in height. (C) Forager position of individuals released at the 180° location at 1m in height. (D) Forager positions of individuals released at the 270° location at 1m in height. (E) The position of individual foragers released at the 0° location as they reach the ground. (F) Forager positions of individuals released at the 90° location as they reach the ground. (G) Forager position of individuals released at the 180° location as they reach the ground. (H) Forager positions of individuals released at the 270° location as they reach the ground.

3.1 Foraging tree tests

At both the 1m height and as they reached the ground at 0m, Nest 1 foragers' positions on the tree face in the 0°, 90°, and 270° displacement conditions were significantly non-uniform and significantly clustered to the nest's direction at 0°. Additionally, in these three conditions at both heights (1m and 0m), the nest direction fell within the 95% confidence interval of the forager's positions (Table 1; Fig. 1A,B,D,E,F,H). In the 180° condition, foragers' positions when crossing the 1m height were uniform and not directed to the nest direction at 0° (Table 1; Fig. 1C). Yet as foragers in the 180° condition reached the ground, their positions on the tree were significantly non-uniform and clustered to the nest's direction at 0°. The nest direction also fell within the 95% confidence interval of the foragers' positions

at 0m (Table 1; Fig. 1G). After reaching 20cm from the tree base, forager paths in all four conditions at Nest 1 were grouped towards the nest entrance (Table 1) and all individuals immediately travelled the 3m back to and entered the nest.

At Nest 2, foragers' positions on the tree face in all displacement conditions (0° , 90° , 180° , 270°) were significantly non-uniform and significantly clustered to the nest's direction at 0° as they crossed to the 1m height marker. Additionally, the nest direction fell within the 95% confidence interval of the foragers' positions at 1m high in all conditions (Table 1; Fig. 2A-D). Nest-ward positioning continued as foragers reached the ground, with all conditions showing significant non-uniformity and significant cluster towards the nest direction. Additionally, the nest fell within the 95% confidence interval of the foragers' positions (Table 1; Fig. 2E-H). At Nest 2, once foragers had reached 20cm from the tree, all individuals were oriented to the nest direction at 0° (Table 1), travelled in a straight path to the nest entrance and entered. At the ground, foragers typically did not stop to scan again but continued on in their current direction. In all conditions foragers immediately returned to the nest entrance and entered the nest.

Nest 2

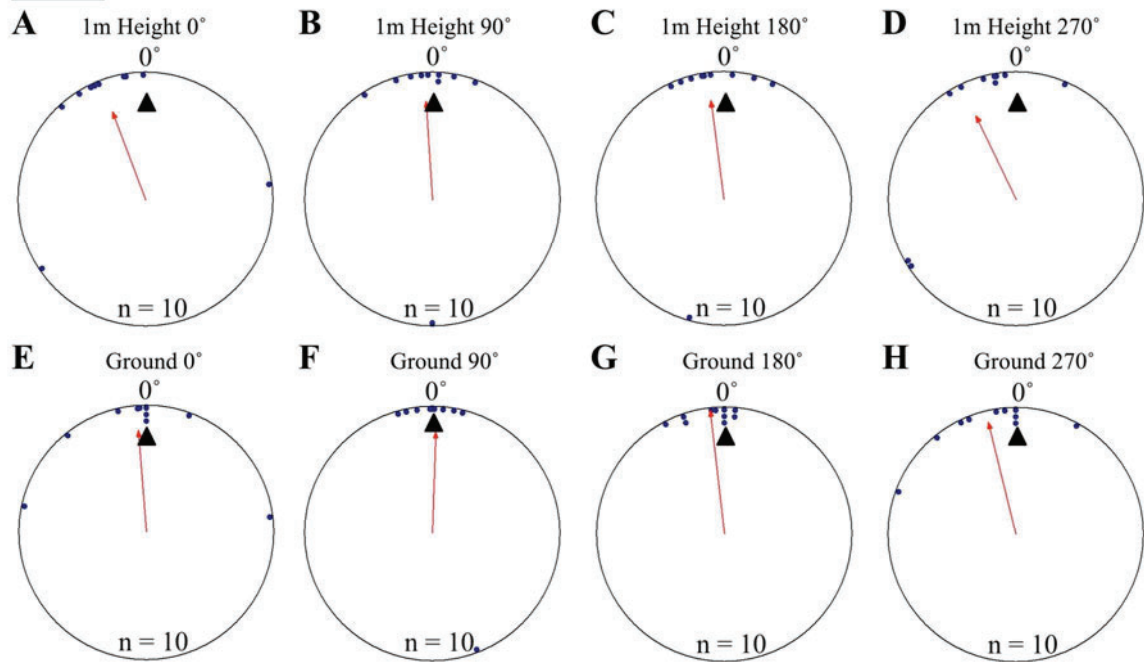


Fig. 2 Circular distributions of individual *M. midas* foragers' positions on the tree face during displacement experiments on their foraging tree. Figures show the raw data of forager positions at two heights after displacements to one of four sides of the tree at a 2m height at Nest 2. The nest direction for each figure is at 0°, labelled by a black triangle. The arrow denotes the direction and length of the mean vector. Foragers were collected at the base of their foraging tree, held overnight and then released vertically on the tree face at one of four sites (0°, 90°, 180°, 270°). (A) The position of individual foragers released at the 0° location at 1m in height. (B) Forager positions at Nest 2 of individuals released at the 90° location at 1m in height. (C) Forager position at Nest 1 of individuals released at the 180° location at 1m in height. (D) Forager positions at Nest 1 of individuals released at the 270° location at 1m in height. (E) The position of individual foragers released at the 0° location as they reach the ground. (F) Forager positions of individuals released at the 90° location as they reach the ground. (G) The position of individual foragers released at the 180° location as they reach the ground. (H) Forager positions of individuals released at the 270° location as they reach the ground.

3.2 Cue conflict tests

To test if foragers position themselves towards either the terrestrial or celestial cues during their decent, we displaced foragers off their foraging route in order to put these cue sets in 90° conflict. Individuals foraging away from the nest and displaced on the nest tree showed significant nest directed positioning on the tree face at 1m above ground level. Positions on the tree in both the 0° and 180° displacement conditions were significantly non-uniform and significantly grouped to the nest direction at 0°. This pattern continued as the foragers reached the ground, with foragers' positions being significantly directed to the nest location and non-uniform. In both conditions and at both the 1m height and at ground level

(0m), the nest direction fell within the 95% confidence interval of foragers' positions on the tree (Table 1; Fig. 3A-D). Foragers in both the 0° and 180° conditions showed no evidence of using their celestial based vector while positioning themselves on the tree (at 270°). After descending the tree, all foragers found and entered the nest (15cm from the tree). At the ground, foragers continued on in their current direction. In all conditions foragers immediately returned to the nest entrance and entered the nest.

3.3 Nest tree foragers/landmark blocking experiment

Nest tree foragers displaced to the opposite side of the tree (180°) from the nest tree at 1.5m with access to the surrounding terrestrial cues behaved similarly to foragers that travel away from the nest to forage on a different tree. Foragers initially paused at the release point, and then moved a small distance, where they performed scan-like behaviours. These continued intermittently during the forager's descent. At the 1.4m height, after a 10cm descent, foragers showed uniform positioning around the tree and were not oriented to the nest site (Table 1; Fig. 4A; Fig. 5A). This uniform distribution continued at the 1.3m, and 1.2m heights (Rayleigh test, $P > 0.05$; V test, $P > 0.05$). At 1.1m, forager positions were still uniform (Rayleigh test, $Z = 1.754$, $P > 0.05$) but were significantly clustered to the nest direction, and the nest location was within the 95% confidence interval of forager positions (V test, $V = 0.295$, $P < 0.05$). At the 1m height, forager positions on the tree face became significantly non-uniform and significantly grouped around the nest direction at 0° (Table 1; Fig. 4C; Fig. 5A). This non-uniform and clustered pattern persisted at all 10cm height measurements from 1m to ground level with foragers significantly positioned on the nest side of the tree (1m – 0m; Rayleigh test, $P < 0.001$; V test, $P < 0.001$; Table 1; Fig. 4E; Fig. 5A). At all heights between the 1m and ground level measurements, the nest direction fell within the 95% confidence interval of foragers' positions on the tree. Once foragers had completed their descent, all individuals found and entered the nest (10cm from the tree).

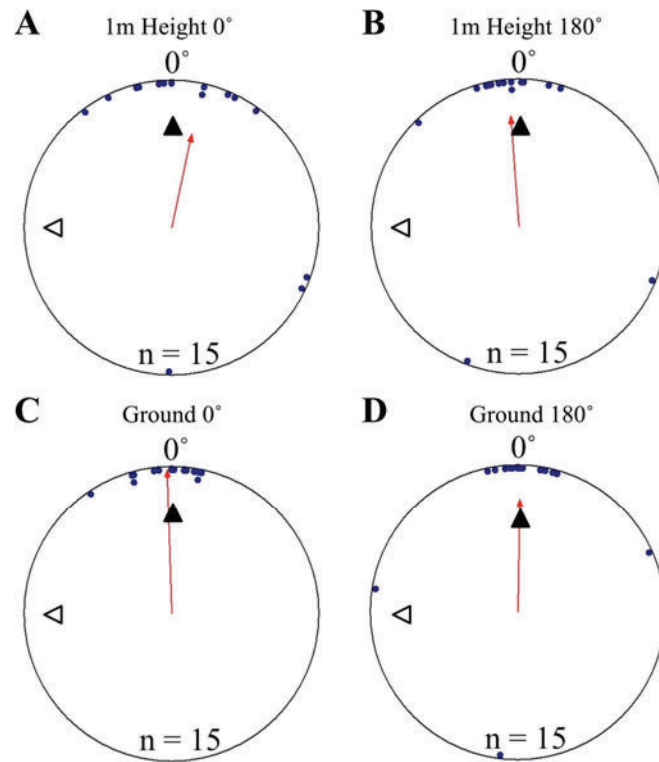


Fig. 3 Circular distributions of individual *M. midas* foragers' positions on the tree face during displacement experiments with cue conflicts. Figures show the raw data of forager positions at two heights after displacements to one of two sides of the tree at a 1.5m height at Nest 3. The nest direction for each figure is at 0°, labelled by a black triangle. The foragers' accumulated vector was at 270°, labelled by a white triangle. The arrow denotes the direction and length of the mean vector. Foragers were collected at the base of their foraging tree, held overnight and then released vertically on the tree face of the nest tree at one of two sites (0° & 180°). (A) The position of individual foragers released at the 0° location at 1m in height. (B) Forager position at Nest 1 of individuals released at the 180° location at 1m in height. (C) The position of individual foragers released at the 0° location as they reach the ground. (D) The position of individual foragers released at the 180° location as they reach the ground.

When the surrounding terrestrial cues were blocked, nest-tree foragers displaced to the opposite side of the tree (180°) behaved differently from previous conditions. Foragers typically scanned once near the displacement point. After this, half of the foragers tested ($n = 10$) travelled up the trunk above the 2m-blocked height before beginning to perform more scans. As a whole ($n = 20$), foragers did not orient to the correct nest direction at any height 1.4 – 0m during their descent (1.4m, 1m, 0m; Table 1; Fig. 4B,D,F; Fig 5B). At all heights, forager positions on the tree met conditions of a uniform distribution (1.4m – 0m, Rayleigh test, $P > 0.05$) and were not significantly oriented in the direction of their home vector at 0° (1.4m – 0m, V test, $P > 0.05$). As foragers reached the ground, they did not travel to the nest

entrance located within the landmark-blocking arena but instead performed looping paths, some even returning back up the tree. After 3min, two individuals found the nest entrance and the rest were collected and moved to the nest entrance where they willingly entered.

Focusing only on those foragers that responded to the blocked panorama by ascending the tree to 2m or higher (Fig. 5B), when foragers first descended from 2m or higher, they were positioned towards the nest site at 190cm (V test, $V = 0.745$, $P < 0.001$). This nest-ward positioning continued at all heights through 1.4m height (V test, $V = 0.578$, $P < 0.01$) until the 1.1m height where forager positions were no longer non-uniform (Rayleigh test, $Z = 0.504$, $P > 0.05$) and no longer clustered to the nest side of the tree (V test, $V = 0.203$, $P > 0.05$). These foragers' positions were uniform and not clustered towards the nest at any height between 1m (Rayleigh test, $Z = 0.559$, $P > 0.05$; V test, $V = 0.132$, $P > 0.05$) and 0m (Rayleigh test, $Z = 0.974$, $P > 0.05$; V test, $V = -0.177$, $P > 0.05$). Foragers that did not ascend above the blocking screen ($n = 10$) were not positioned towards the nest at any height (V test, 1.4m, $V = -2.827$, $P > 0.05$; 1m, $V = -1.474$, $P > 0.05$; 0m, $V = -0.862$, $P > 0.05$).

3.4 Panoramic image analysis: information available from the foraging tree

For all three nests, when comparing the nest-oriented panoramic views from the base of the tree to nest-oriented panoramic views at 1m and 1.75 on the tree, we found that at both heights on the tree, the rotIDFs showed a distinct valley of minimum of mismatch (i.e. best matching direction) that was directed towards the nest (Fig. 6A,B (green and red curves)). This shows that directional information can be recovered up to 1.75m (at least) from a visual memory acquired at the base of the foraging tree. We then analysed whether animals can recover nest oriented views from different compass directions around the tree (0° = nest). At both 1m and 1.75m on the tree, the views available at the other directions, 90° (green), 180° (black) and 270° (brown), do not generate a clear minima when compared with a view at the base of the tree (Fig. 7A,B).

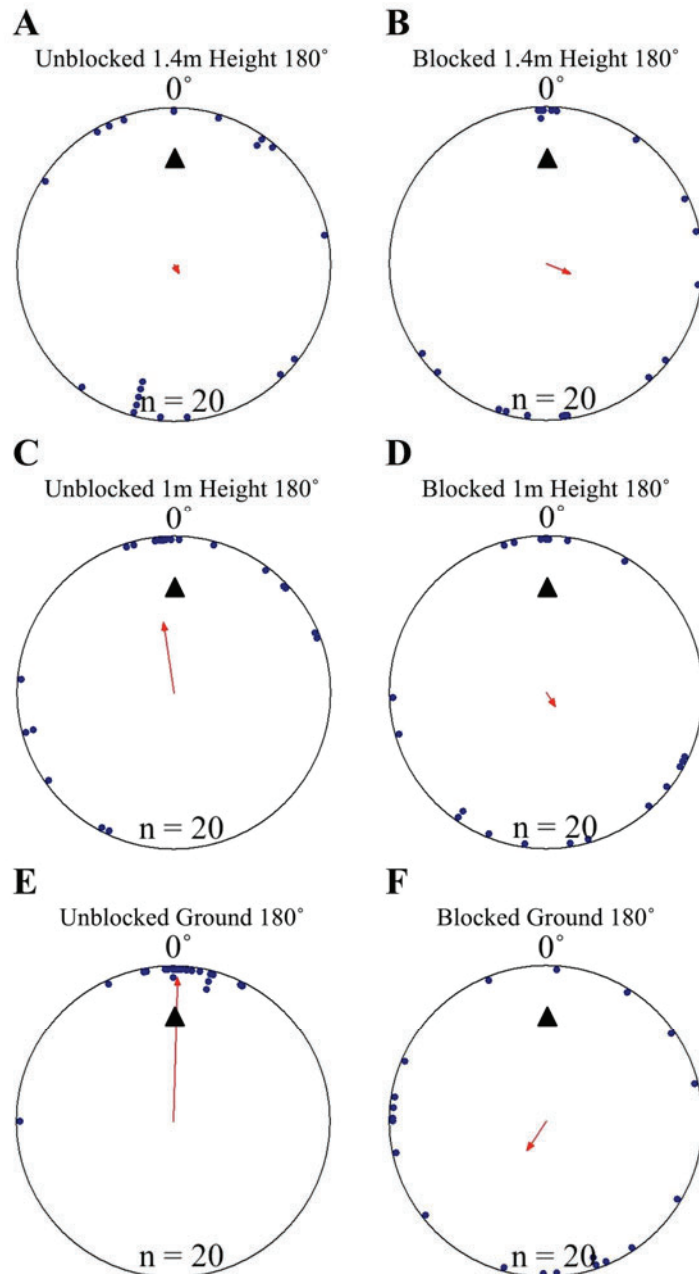


Fig. 4 Circular distributions of individual *M. midas* nest tree foragers' positions on the tree face during the landmark blocking experiments on the nest tree. Figures show the raw data of forager positions at three heights after displacements to one of two sides of the tree at a 1.5m height at Nest 3. The nest direction for each figure is at 0°. The arrow denotes the direction and length of the mean vector. Foragers were collected at the base of the nest tree, held overnight and then released vertically on the tree face of the nest tree opposite the nest entrance (180°) with the surrounding landmark panorama either unblocked or blocked. (A) The position on the tree face of individual foragers released at the 180° location as they begin their descent at 1.4m in height with the surrounding landmarks unblocked. (B) The position on the tree face of individual foragers released at the 180° location as they begin their descent at 1.4m in height with the surrounding landmarks blocked. (C) The position on the tree face of individual foragers released at the 180° location at 1m in height with the surrounding landmarks unblocked. (D) The position on the tree face of individual foragers released at the 180° location at 1m in height with the surrounding landmarks blocked. (E) The position on the tree face of individual foragers released at the 180° location as the forager reaches the ground with the surrounding landmarks unblocked. (F) The position on the tree face of individual foragers released at the 180° location as the forager reaches the ground with the surrounding landmarks blocked.

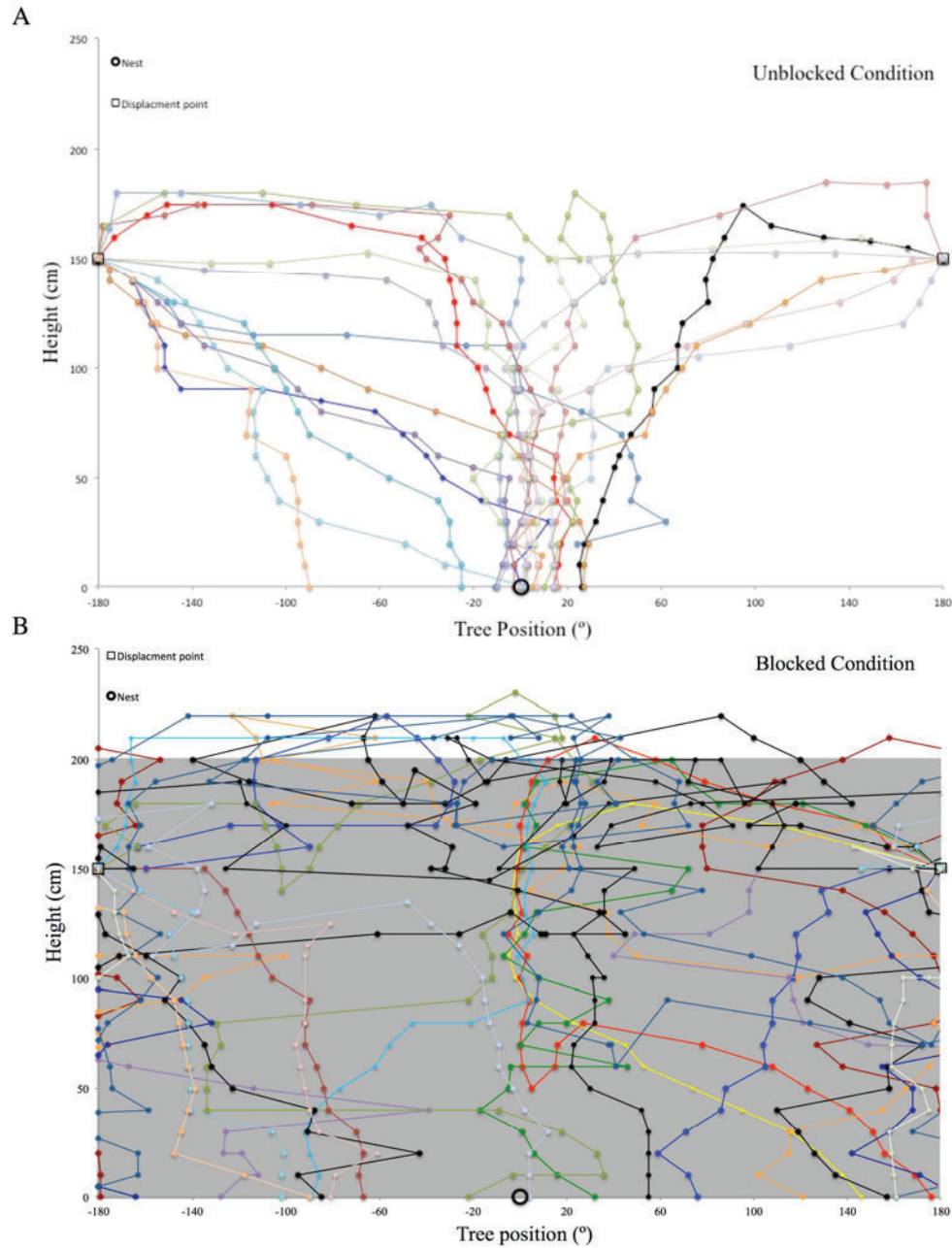


Fig. 5 Individual *M. midas* nest tree foragers' paths descending the tree face in the landmark blocking experiment. Circular positions on the tree face have been unwrapped to show individuals' paths from the 180° off-route, 1.5m high displacement site (open square) to the ground. The plots are cylindrical, with +180° and –180° being the same position on the side of the tree opposite the nest. The open circle at ground level (0 cm) denotes the nest entrance direction. (A) Forager paths in the unblocked condition with the surrounding landmarks visible. (B) Forager paths in the blocked condition with all surrounding landmarks below 2m blocked using a plastic screen. The grey area in the background signifies the blocking screen surrounding the tree from 0m to 2m.

3.5 Scanning behaviour

While ants were on the tree face, foragers exhibited several kinds of scanning behaviours, the common characteristic of which was a shift of the body and head to bring the head's orientation at or near the horizontal plane. With the head at or close to horizontal, individuals then slowly rotated their head horizontally across the field.

The first kind of scan-like behaviour exhibited by these foragers was to use a piece of the tree's structure, such as a jutting piece of bark, a knot, or burl, creating a horizontal space at the top at which individuals can orient their entire body horizontally and then slowly shift their head across the horizontal plane (Fig. 8A). This behaviour was environment-dependent and could occur at any point during the foragers' descent.

The second kind of scan-like behaviour, dubbed downward pitch scans, occurred as the individual reached the top of a bark strip or other structure and was oriented upwards. Individuals lowered the pitch of their head while the body remained vertical, allowing individuals to bring the head close to the horizontal plane (Fig. 8B). This behaviour was also environment-dependent but typically occurred during the initial portion of the foragers' route when some foragers travelled upwards from the displacement site.

The third kind of scan-like behaviour, termed head roll scans, occurred as foragers were travelling horizontally across the vertical tree face. Foragers altered their head position by rolling the head towards the tree face, bringing the tree side of their head down and positioning their head close to the horizontal plane. From here, individuals slowly moved their head across the horizontal plane to scan (Fig. 8C). This behaviour typically occurred when foragers were not yet on the nest side of the tree.

The final kind of scan-like behaviour, labelled the push up or upward pitch scan, was observed on the vertical tree face with the individual oriented down with the head positioned below the body. The individual extended its front legs, pushing its body and head away from the tree face. The individual's head pitched upward, reaching at or near the horizontal plane.

In this position, the individual would slowly move its head across the field (Fig. 8D). The upward pitch scan was usually observed as foragers reached the side of the tree facing the nest. These behaviours would continue throughout the forager's descent when on their descending route.

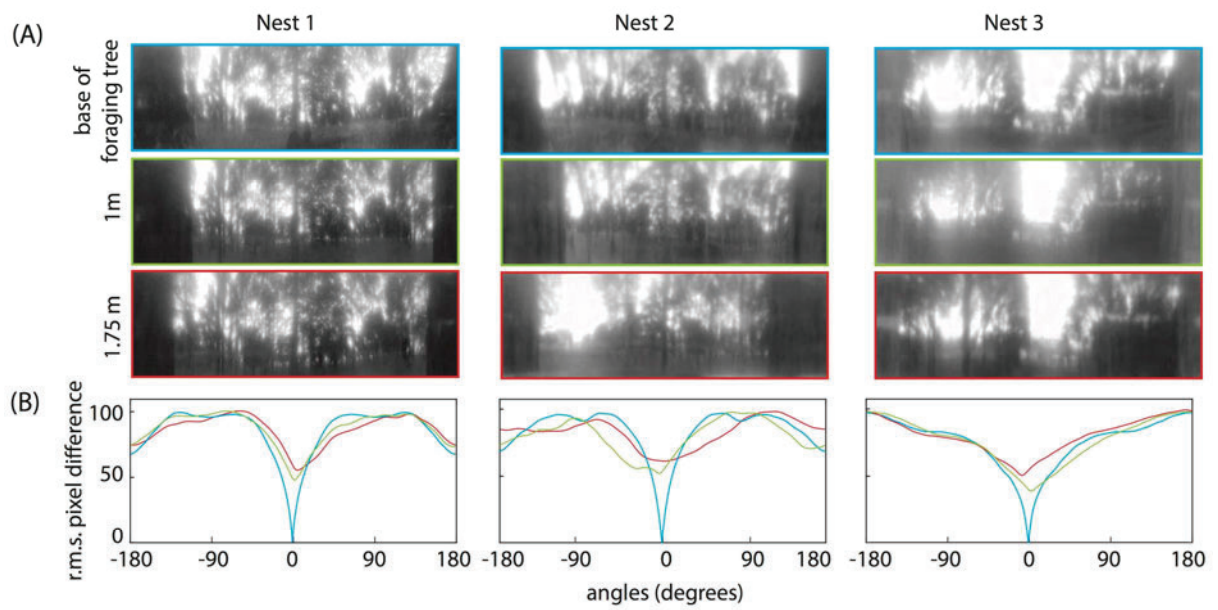


Fig. 6 Quantifying the change in the panorama at different elevations on the foraging tree at the three nests. (A) Panoramic images at the base of the foraging tree (blue), 1m in height (green), and 1.75m in height (red). Images were downsampled to 1 pixel per 1° to resemble the ant's visual acuity, filtered through only the blue colour channel and oriented with the nest centred. (B) The rotIDF compares the root mean square pixel difference between the panorama at the base of the foraging tree with itself (blue), the 1m (green), and the 1.75m (red) panoramas. The nest direction in all comparisons is centred at 0°.

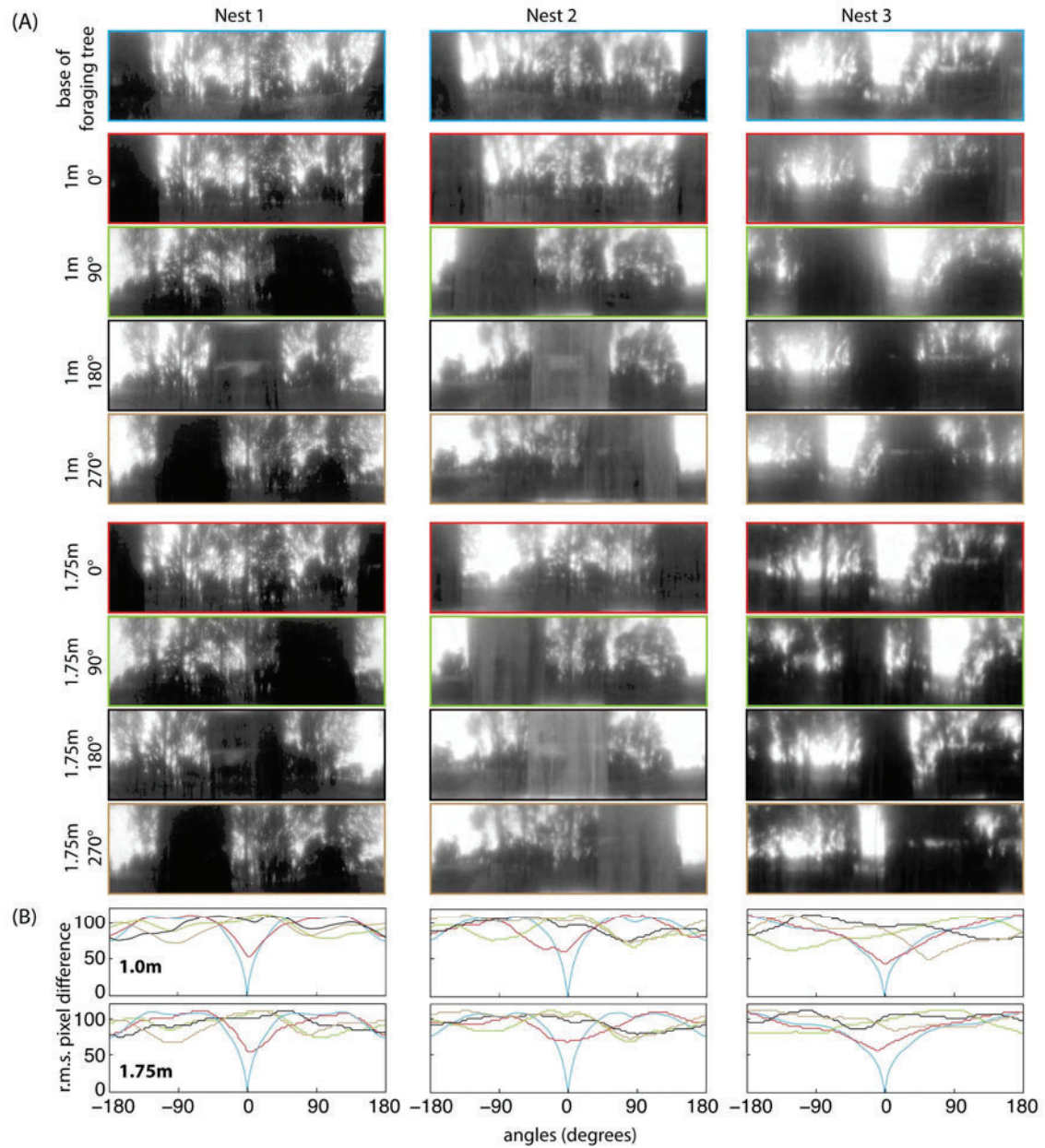


Fig. 7 Quantifying panorama changes at the 4 displacement directions and at two elevations on the foraging tree at the three nests. (A) Panoramic images at the base of the foraging tree (blue), 1m in height at 0° (red), 90° (green), 180° (black), 270° (orange) and 1.75m in height at 0° (red), 90° (green), 180° (black), 270° (orange). Nest orientation is at the centre of each image and images were downscaled to 1 pixel per 1° to resemble the ant's visual acuity, filtered through only the blue colour channel and oriented with the nest centred. **(B)** The rotIDF compares the root mean square pixel difference between the panorama at the base of the foraging tree with itself, and the foraging tree at both 1m and 1.75m at each direction. The nest direction in all comparisons is centred at 0°.

4. Discussion

In the current study, we show that *M. midas* foragers successfully orient to the nest side of their foraging tree during their descent. Correct nest directed positioning appears to occur well before foragers reach the ground, with foragers' positions grouped towards the nest direction at the 1-meter height and at ground level. This ability appears to extend beyond the forager's current foraging tree as individuals displaced from their foraging tree to the nest tree also successfully positioned themselves towards the nest direction both at 1-meter height and at ground level. Even nest-tree foragers, which show evidence of reduced navigational knowledge on the ground (Freas et al. 2017b), are able to successfully orient while on their foraging tree above the nest entrance. Visual terrestrial cues appear to be critical to this navigational ability, as when the surrounding terrestrial cues were blocked, foragers were unable to successfully orient towards the nest entrance. Analysis of the panorama at different foraging heights suggests that ants can obtain nest orientation information at both 1m and 1.75m above the ground, provided they are on the nest-facing tree face (0°). Finally, use of the surrounding terrestrial cues fits with behaviour on the tree as foragers appear to actively scan while on the tree, bringing their head orientation to or near the horizontal plane and then slowly rotating it across the field.

When *M. midas* foragers are displaced in a local environment on the ground, they are able to successfully use the surrounding landmark cues to orient towards the nest (Freas et al. 2017b). Our results suggest this ability extends to elevation-based displacements. The ability to orient to familiar landmarks after vertical displacement has been previously shown in the desert ant *M. bagoti* (Schwarz et al. 2014), a species that forages on the ground almost exclusively (Schultheiss and Nooten 2013). It is currently unknown if foragers include travelling vertically up the nest tree in their learning walks or if on their first trip onto the foraging tree they perform a vertical form of turn back behaviour as is observed with ants on

the ground (Graham and Collett 2006; Müller and Wehner 2010; Fleischmann et al. 2017) and has also been reported in bees (Lehrer 1991,1993).

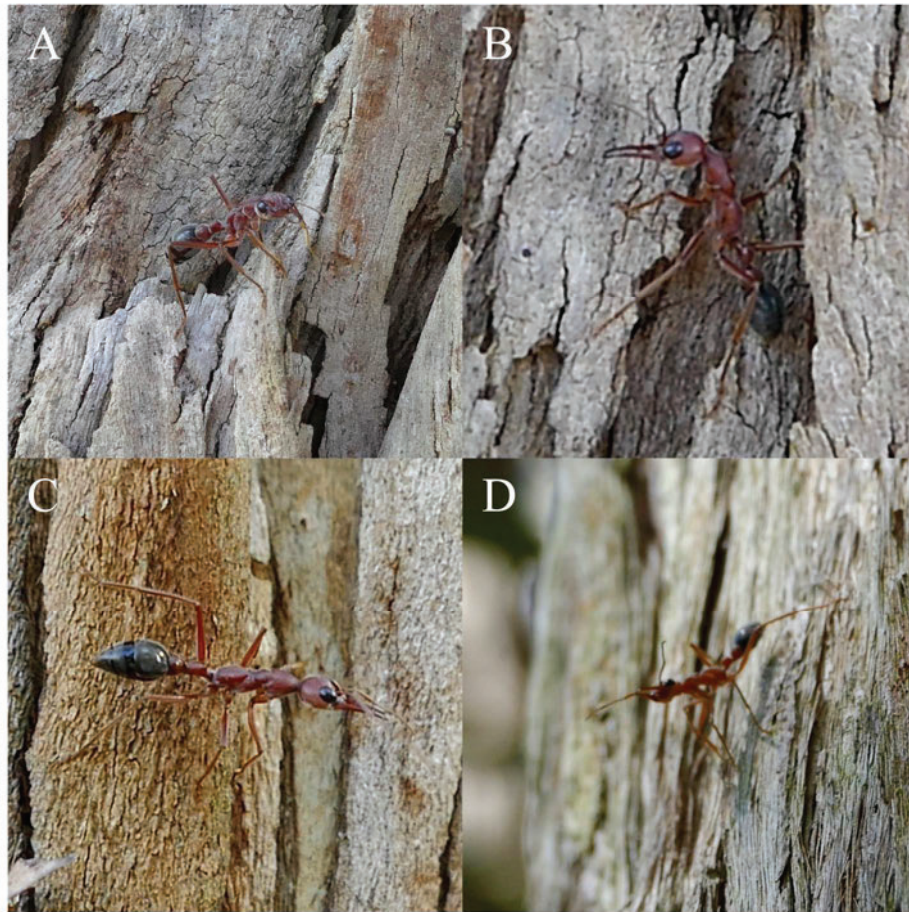


Fig. 8 The four described vertical scanning behaviours. All images were taken as foragers were descending their foraging tree after displacement. (A) The horizontal scan. (B) The downward pitch scan. (C) The head roll scan. (D) The push up or upward pitch scan.

Similar nest-ward positioning was present when foragers were displaced off their foraging route to the nest tree. Ant species inhabiting complex, landmark-rich environments typically rely heavily on terrestrial cues for navigation, with landmarks tending to suppress any accumulated vector information (Wehner et al. 1996; Narendra et al. 2007; Mangan and Webb 2012; Narendra et al. 2103a). Yet in situations where the celestial based vector and terrestrial cues conflict, some species exhibit directional compromise behaviour (Narendra 2007; Collett 2010; Legge et al. 2014; Wystrach et al. 2015; Wehner et al. 2016). This compromise between cues sets has not been observed in *M. midas* while navigating on the

ground, as terrestrial cues largely dominate in a local area (Freas et al. 2017b). Yet *M. midas* foragers have shown evidence of vector cue use and celestial/terrestrial directional cue compromise while on their foraging route during both the outbound and inbound journeys (Freas et al. 2017c). In the current study, foragers showed similar behaviour with no evidence of using their naturally accumulated celestial based vector for positioning and their behaviours were consistent with navigation through terrestrial cues. It is worth noting that the accumulated vector lengths in this test are relatively short (4m), but this distance is representative of the typical vector length by observed individuals at our field site (Freas et al. 2017b) and foragers have been shown to use celestial cues at these distances (Freas et al. 2017c).

The final unblocked condition tested foragers that travel straight up the nest tree to forage. These foragers have been previously shown to be unable to successfully orient when displaced locally on the ground (Freas et al. 2017b). It is believed that these foragers are naturally restricted horizontally to the nest site and either do not actively navigate during foraging or have reduced navigational abilities similar to *C. bicolor* digger ants, which do not forage (Wehner and Menzel 1969; Freas et al. 2017b). The results of our unblocked condition suggest these foragers do actively navigate while foraging in the nest tree as these individuals successfully orient to the nest side of their foraging tree after displacement and this positioning occurs well before they reach the ground.

Our landmark blocking condition also tested nest-tree foragers, allowing us to keep the nest entrance and any directional cues it provides within the blocking arena and accessible to the foragers. Foragers' inability to position themselves towards the nest direction in this setup corresponds with landmark blocking experiments on the ground where foragers cannot orient to the nest when the surrounding panorama is blocked (Freas et al. 2017b). These results would also appear to exclude any scent-based cue, or local visual cues on the tree surface that could be used on their own for directional information. Our results also suggest

that this species cannot use the unblocked canopy of the tree alone for directional information, at least during the final two meters of their descent.

The use of the surrounding panorama for direction information is also supported by forager behaviour in the blocking condition before descending the tree. Foragers that immediately descended the tree ($n = 10$) were not positioned towards the nest at any height as expected if foragers used the surrounding terrestrial cues to orient. Foragers ($n = 10$) that responded to the blocking screen by first ascending above 2m were positioned correctly but below 1m correct positioning ceased (1m – 0m). These findings suggest that the distant terrestrial cues are critical not only for a forager's initial positioning but are also involved in route maintenance during a forager's descent. It is possible that foragers must scan the surrounding visual panorama during their descent in order to maintain positioning on the tree. This would explain the scanning behaviour observed throughout forager descents in all conditions. Alternatively, there is the potential that some cues may provide heading information en route through optic flow, a hypothesis that could be tested by replacing the uniform blocking material we used with a striped pattern on the inside of the blocking arena in a future study. It is worth noting that optic flow has previously been shown to have only a minor role in ant navigation (Ronacher and Wehner 1995; Pfeffer and Wittlinger 2016).

Our analysis of panoramic pictures revealed that sufficient visual information is available in the scene for the ants to orient on these trees. Image comparisons revealed variability across trees and locations, but overall, the information necessary to retrieve the nest direction using a terrestrial visual compass strategy (Wystrach et al. 2011; Baddeley et al. 2012) is available. As noted earlier (Zeil et al., 2003; Schwarz et al. 2014), changes in height have little impact on the information available in these panoramic views. This stable nest-ward minimum in panorama information may also be used in bees and wasps as they ascend in height during their learning flights (Zeil 1993ab; Stürzl et al. 2016; Murray and Zeil 2017). In the case of our ants, it is worth noting that using memories from the correct side of the tree

is useful primarily when the ant is currently located on that side of the tree, as this position was where the best matches were obtained. It appears that rotIDF is not very powerful at predicting the nest direction when the ant is located on an unfamiliar side of a tree (90° , 180° or 270°), but has more predictive power when the ant is located on the familiar side (0°). Even though there were no detectable minima at the 90° , 180° or 270° positions on the tree (Fig 7B), ants were able to successfully guide themselves back towards their familiar corridor on the tree and then towards the nest. This reflects what is observed on the ground. Ants on the ground may use rotIDF only when on familiar routes and other strategies when off-route (Wystrach et al. 2012). Assuming that ants learn the scene when located on their habitual side of the tree, this would provide a gradient of familiarity that could be used to reach and stick to the nest side of the tree. Whether foragers use this gradient of familiarity (Zeil et al. 2003), the visual compass (Wystrach et al. 2011; Baddeley et al. 2012) or other visual strategies (Wystrach et al. 2012; Horst and Möller 2017), remains to be tested.

Scanning behaviour characterised by the rotation of the individual's head and body in place (Wystrach et al. 2014; Zeil et al. 2014) can be useful to exploit the familiarity of the surrounding visual scene. Ants perform more scans when their familiar surroundings have been altered or when the direction provided by terrestrial cues conflicts with celestial cues (Wystrach et al. 2014). In the current study, we show that this behaviour may extend beyond ground level, as individuals travelling vertically appear to actively scan while on their foraging tree. This potential behaviour, which is closely associated with the use of learnt visual cues, along with the results of the blocking condition and the panorama analysis, further indicate that the use of learnt visual cues is likely in use during forager descents. It has recently been shown that while on their foraging route members of *M. pyriformis*, another nocturnal *Myrmecia* species that relies heavily on the visual scene (Reid et al. 2011), attempt to stabilise their head horizontally while travelling en route on an uneven surface, as view similarity drops markedly as the view is rotated (Raderschall et al. 2016). This species has

also been shown to perform extensive scanning behaviours during learning walks around the nest indicating scan behaviours are part of the nocturnal ant's navigational repertoire (Narendra and Ramirez-Esquivel 2017). Similar behaviours seem to apply to navigation on the tree in *M. midas* where foragers appear to attempt through multiple scanning behaviours to position their heads horizontally during scanning. These scans may serve a similar function as scans displayed on the ground (Wystrach et al. 2014; Narendra and Ramirez-Esquivel 2017), and thus suggest that similar visual memories and strategies may be used when foraging both on ground and on trees. A future study on the foragers' ability to effectively scan while navigating along a vertical plane is warranted.

It is also important to note that the described behaviour of raising the head while vertical may also potentially involve the use of celestial cues, such as the sun's position, when they are available. Work on honeybee dancing in the Asian species *Apis florea*, a behaviour strongly tied to the position of the sun, has shown that when dancers are on a steep slope, these individuals rotate their head position to compensate for this slope. This compensation allows them to keep their visual field stable with the horizon while dancing (Dyer 1985; Dyer 2002). This behaviour appears similar to what we observe in the current study, albeit without the horizontal movement of the head, which we have deemed scanning behaviour. It remains possible that foragers could also be using celestial cues as well as terrestrial cues while on the tree. *M. midas* foragers typically only forage in trees within 5m of the nest and have shown no evidence of orienting to vectors of this length. In the rare case that foragers travel farther from the nest (14m), we have only observed weak evidence of orientation to a vector (Freas et al. 2017b). As such, it may be possible that the observed scanning behaviour on the tree surface also allows foragers access to celestial cues.

Finally, the extent of these vertical navigational abilities is currently unknown, as well as at what height these individuals naturally show nest ward positioning during their descent. Observations of returning foragers in the predawn twilight suggest that foragers are oriented

to the nest at heights over 3 meters, yet an analysis of this behaviour may prove difficult. *M. midas* nests at the field site are located in small stands of trees, interspersed with large tracks of grass. This habitat leads to large differences in skyline height surrounding the nest (supplemental material). These large skyline changes may not change drastically with changes in height of the viewer. Further studies into how the terrestrial cues change over larger changes in elevation are warranted.

4.1 Conclusions

The experiments in the current study show that *M. midas* actively and critically use the surrounding visual scene to orient and descend along the correct side of the tree. Image analysis of the visual scene on the tree shows that the scene provides sufficient information for these individuals to orient successfully using stored views. These foragers may extract this visual information during on-tree scanning behaviours where individuals scan their surroundings in the horizontal plane. Together, these findings suggest that visual navigational strategies and memory use may be similar between foragers navigating on the ground and on the tree.

Conflict of Interest Declaration

The authors claim no conflict of interest.

Author contributions

Experiments and Analyses were designed by CF, AW, AN, KC. CF collected all data. CF and AW analyzed the data. CF, AW, AN, KC drafted and revised the manuscript.

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Chapter 9. Summary and Potential Future Directions

Summary and Future Directions

Ants have been shown to be expert visual navigators in their use of both path integration and learned panorama cues. Furthermore, they display a remarkable ability to weight these cue systems, making navigational decisions based on the most reliable information present. While this thesis explores many of these navigational tools, our findings also open new avenues of investigation for future studies in both these species and other solitarily foraging ants.

In Chapter 2, we found that *M. bagoti* foragers retain lifelong memories of non-nest skylines that appear to be stored separately from memories of the nest panorama. Yet, we also found that retroactive interference occurs when the panorama changes at a given spatial location. It remains unknown if the retroactive interference we observed at the feeder site was caused by the similarity of the artificial skylines or the spatial set up of our experiments.

To further explore the retention of non-nest memories, the potential effects of context cues during memory retrieval should be considered, as it is believed that context cues help confirm that the correct memory is retrieved (Collett and Collett 2002; Collett et al. 2013; Cheng 2005). The most interesting of these is the potential that foragers use their accumulated vector during terrestrial memory acquisition to separate different panorama memories. During natural foraging, changes in the panorama would be associated with corresponding changes in the path integrator as the forager moves through the environment. Foragers may acquire landmark cues in association with different vector lengths, a process that may separate these views in the forager's memory and reduce interference between panorama memories at different sites. The current set-up does not test this possibility, as both skylines are experienced at the same spatial location. A future experiment could test these foragers at the original and new skylines present in the current study but change the distance from the nest so these skylines are experienced at different vector lengths. This testing would explore if the path integration system has any effect on skyline memory acquisition or retrieval. Another

test could force foragers to travel through one skyline in order to forage at the centre of the second skyline. Such a test could tease apart whether foragers can form long term memories of two similar skylines if the forager experiences these skylines at different points in the foraging trip. Another interesting aspect of panorama learning and retrieval would be to explore how both skyline similarity and the current vector state affect learning of new non-nest panoramas through the same types of manipulations we employed in Chapter 2.

Another potential avenue for further study would be testing retention over longer time periods. The short, above ground life span of *M. bagoti* limits the current retention testing set up, as the foraging life span in *M. bagoti* is only, on average, 4.9 days (Muser et al. 2005). A useful future project on the retention of skyline memories would be to test ant species with longer life spans. A clear candidate for this testing would be *M. midas*, or another *Myrmecia* species, which shares *M. bagoti*'s heavy reliance on panorama cues when navigating. The *Myrmecia* genus is much longer lived, with foragers of some species living over 2 years (Hölldobler and Wilson 1990).

In Chapter 3, we show that foragers restricted to the nest area were unable to generalize or extrapolate panorama cues from the nest to local sites. Yet these foragers show one-trial learning after only one inbound experience. Panorama cue strength appears to increase with experience as multiple exposures to the homeward route eventually override a conflicting vector. Forager cue choice between the learned panorama and a conflicting vector also appears to be mediated by cue reliability. We found that panorama cues decrease in strength as the amount of time since the last exposure to the panorama increases, a behavioural decision that is in accordance with predictions under the temporal weighting rule. Finally, through a control condition, we show that foragers do not show evidence of vector calibration at vector conflicts of 180°. The speed and limits of vector recalibration in *M. bagoti* are further explored in Chapter 4, since in Chapter 3 we found no evidence of vector calibration, but limited testing to foragers with the maximum cue conflict of 180°.

The extrapolation portion of Chapter 3 has already been expanded on in Chapter 5, where we gave foragers larger amounts of exposure to the nest panorama, though questions regarding the lack of extrapolation in these foragers remain. A potential avenue for future research would be to increase the size of the arena around the nest site, as learning walks in other desert ant species can exceed the 2 m diameter arena present in the current set up (2 m in *C. bicolor*; Wehner et al. 2004). Foragers may need to travel larger distances from the nest during learning walks to successfully generalize or extrapolate panorama cues from the nest to local areas. To test this theory, future research should expand the nest arena so that learning walks are not artificially restricted. Another possible project would be to record these learning walks in *M. bagoti* in order to explore the critical distance these foragers must travel for successful navigation from local sites.

In Chapter 4, we show that *M. bagoti* foragers show evidence of rapid vector calibration at 45° conflicts, shifting fully to the inbound vector. Over larger directional conflicts, however, the observed calibration shift shrinks and foragers initially orient in intermediate directions. At the maximum 180° conflict, foragers still showed no evidence of calibration to the inbound vector even after 25 trips.

This project faced several limitations that made collecting both the full inbound paths and the outbound orientations of these foragers unfeasible. Future work on vector calibration should record the inbound routes of foragers, especially in the 90° and 135° conditions where we observed incomplete calibration and bowing inbound paths. It would also be useful to have an observer or recording equipment at the nest site to explore calibration shifts of the outbound path which we observed but did not record in the current study. The clear species differences in calibration between *M. bagoti* and *Cataglyphis fortis* (Collett et al. 1999; Wehner et al. 2002) would also be an avenue of future work, and within the chapter we propose conducting the experiments on *Cataglyphis velox*, as this species is closely related to *C. fortis*, yet inhabits cluttered environments like *M. bagoti* (Mangan and Webb 2012).

In Experiment 1 of Chapter 5, we expand on the findings of Chapter 3 by showing that even with large amounts of exposure to the nest site panorama, foragers cannot orient or quickly navigate home from local sites. This supports our belief that foragers cannot extrapolate views from the nest and need to be exposed to the outbound/inbound view sequence to learn the correct direction to the nest from local sites, even when the panoramas are similar. In Experiment 2, we find that foragers show one trial learning of the nest direction given one exposure to either the outbound or inbound view sequence, yet were unable to quickly return to the nest when only the inbound route was unblocked. Our findings suggest that the acquisition of the views during the outbound path was critical for foragers to create homeward routes in this set up. Foragers with the outbound views blocked continually turned back and returned to the displacement site while trying to find the nest, suggesting that their path integrator was overpowering the panorama cues.

We still do not know what is the underlying cause of foragers' inability to create efficient homeward routes without the outbound views. An interesting future condition would be to manipulate the available landmarks and their distance from the arena and corridor. If closer landmarks that change rapidly during the inbound route give more reinforcement to the forager, then we may observe fewer turn backs and straighter routes to the nest despite the availability of the outbound views. It would also be interesting to test the effects of delays of panorama exposure on foragers both with and without outbound views, though given the findings of Narendra et al. (2007), Freas and Cheng (2017) and Freas et al. (2017) it is unlikely that delays in exposure are the cause of the turn back behaviour in the current set up. It remains possible that over longer delays (>24h) we may see increased hesitancy to use the panorama while accumulating large conflicting vectors.

In Chapter 6 we explore the navigational abilities of another ant species, the nocturnal *M. midas*, which also inhabits a cluttered, landmark rich environment and heavily relies on these visual cues to navigate. *M. midas* foragers were shown to orient only to panorama cues

and showed only limited use of an accumulated vector at unfamiliar sites. Panorama cues also dominated when foragers were off their foraging route but still close to the nest. Finally, we showed that foragers that forage in the nest tree are unable to orient from local displacements suggesting that they either do not actively navigate or have reduced navigational capabilities.

The lack of forager orientation to celestial cues and the degree to which the vector cues are used by *M. midas* is an interesting area for future study that we expand upon in Chapter 7, showing that *M. midas* foragers do use celestial cues while on route. Furthermore, we found that vector length plays a role in the strength of the celestial cues both during orientation (Chapter 6) and while on route (Chapter 7; Wystrach et al. 2015). We also further explore the nest tree foragers that are initially described in Chapter 6, showing that this group of foragers actively navigate using panorama cues while descending the tree (Chapter 8). This suggests that these foragers' inability to orient from local sites on the ground appears to be due to the inability for this subset to extrapolate views from the nest. The navigational abilities of these nest-tree foragers warrants further study in order to find if these foragers are restricted to the nest area for their whole life, to further understand the foraging ecology of all foragers in the nest, and to better understand how and when foragers learn the panorama of non-nest sites.

In Chapter 8, we show that *M. midas* foragers actively orient to the nest direction while descending trees in a local area. Foragers rely on the panorama to orient to the nest entrance while descending and will occasionally perform scanning behaviours while their body is positioned vertically. The panorama appears critical to orientation, as foragers cannot orient to the nest when the panorama is blocked, suggesting canopy cues or local cues on the tree itself are either not in use or are not sufficient for successful navigation. The subset of foragers that successfully oriented above two meters in the blocked condition did not stay oriented for the full descent, indicating that the panorama is critical for both orientation and maintaining a route.

In the current study, we were limited to studying the final two meters of the foragers' descent due to the added complexity of accurately recording forager positions above this height. A potential future study could examine at what height foragers successfully orient and how panorama cues change at heights over 2 meters. A thought-out methodology for accurate position measurements would need to be implemented. Another potential avenue would be to further examine and quantify the scanning behaviour described in Chapter 8. Again, there are challenges involved with getting these measurements that would require the careful creation of a methodology that accurately collects this data. One important aspect that could be interesting to explore would be to measure the extent to which foragers can bring their head to the horizontal plane while on the tree.

There is also the potential that foragers could orient with the panorama blocked. If the blocking material was not uniform, foragers might be able to use optic flow to remain on their chosen route during their descent. This hypothesis is easily testable in a future study by creating a pattern such as vertical or horizontal stripes on the blocking material and allowing foragers to orient on the tree above the blocked height.

Clearly, there are an array of avenues to explore for future research regarding both panorama learning and dynamic cue choice behaviours in *M. bagoti* and *M. midas*. The work presented within this thesis builds a solid foundation for future work in both species. Furthermore, these topics can also be explored and expanded upon in other navigating ant species providing a better understanding of how these behaviours have arisen.

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Chapter 10. Auxiliary Publications During Candidacy

In addition to these projects, I have also co-authored five scientific works covering topics both in and out of the field of insect navigation and cognition (listed below). I have chosen to only include those works (In bold) focusing on insect cognition due to the focus of this thesis.

2015

Cheng, K. & Freas, C. A. 2015. Path integration, views, search, and matched filters: The contributions of Rüdiger Wehner to the study of orientation and navigation. *Journal of Comparative Physiology A* 201(6).

Croston, R., Branch, C. L., Kozlovsky, D. Y., Roth, T. C. II, LaDage, L. D., Freas, C. A. & Pravosudov, V. V. 2015. Potential Mechanisms Driving Population Variation in Spatial Memory and the Hippocampus in Food-caching Chickadees. *Integrative and Comparative Biology*, **55**(3), 354–371.

Pravosudov, V. V., Roth, T. C. II, LaDage, L. D. & Freas, C. A. 2015. Environmental Influences on Spatial Memory and the Hippocampus in Food-Caching Chickadees. *Comparative Cognition and Behavior Reviews*, **10**, 25–43.

2016

Schultheiss, P., Wystrach, A., Schwarz, S., Tack, A., Delor, J., Nooten, S. S., Bibost, A.L., Freas, C.A. & Cheng, K. 2016. Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama. *Animal Behavior* 115, 19-28.

2018

Freas, C. 2018. Arthropod Cognition In (Eds.), Encyclopedia of Animal Cognition and Behavior. Vonk, J. & Shackelford, T.K. Springer International Publishing. Berlin, Germany. (Invited, In Review)

Cheng & Freas 2015, Path integration, views, search, and matched filters: The contributions of Rüdiger Wehner to the study of orientation and navigation.

Cheng, K. & Freas, C. A. 2015. Path integration, views, search, and matched filters: The contributions of Rüdiger Wehner to the study of orientation and navigation. *Journal of Comparative Physiology A* **201**(6), 517-532.

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Cheng, K., Freas, C.A. (2015) Path integration, views, search, and matched filters: the contributions of Rüdiger Wehner to the study of orientation and navigation. *Journal of Comparative Physiology A*, vol. 201, pp. 517–532.
<https://doi.org/10.1007/s00359-015-0984-9>

Schultheiss et al. 2016, Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

Schultheiss, P., Wystrach, A., Schwarz, S., Tack, A., Delor, J., Nooten, S. S., Bibost, A. L., Freas, C. A. & Cheng, K. 2016. Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama. *Animal Behavior* **115**,19-28.



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Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

Patrick Schultheiss^{a, b}, Antoine Wystrach^{c, d}, Sebastian Schwarz^e, Aloys Tack^a, Jeanne Delor^a, Sabine S. Nooten^{a, f}, Anne-Laurence Bibost^a, Cody A. Freas^a, Ken Cheng^{a, *}^a Department of Biological Sciences, Macquarie University, Sydney, Australia^b Research School of Biology, The Australian National University, Canberra, Australia^c School of Informatics, University of Edinburgh, Edinburgh, U.K.^d Centre de Recherches sur la Cognition Animale, Centre National de la Recherche Scientifique, Université Paul Sabatier, Toulouse, France^e Department of Psychology, University of Manitoba, Winnipeg, Canada^f Hawkesbury Institute for the Environment, Western Sydney University, Richmond, Australia

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Ants use the panoramic skyline in part to determine a direction of travel. A theoretically elegant way to define where terrestrial objects meet the sky is to use an opponent-process channel contrasting green wavelengths of light with ultraviolet (UV) wavelengths. Compared with the sky, terrestrial objects reflect relatively more green wavelengths. Using such an opponent-process channel gains constancy in the face of changes in overall illumination level. We tested the use of UV wavelengths in desert ants by using a plastic that filtered out most of the energy below 400 nm. Ants, *Melophorus bagoti*, were trained to home with an artificial skyline provided by an arena (experiment 1) or with the natural panorama (experiment 2). On a test, a homing ant was captured just before she entered her nest, and then brought back to a replicate arena (experiment 1) or the starting point (the feeder, experiment 2) and released. Blocking UV light led to deteriorations in orientation in both experiments. When the artificial skyline was changed from opaque to transparent UV-blocking plastic (experiment 3) on the other hand, the ants were still oriented. We conclude that UV wavelengths play a crucial role in determining direction based on the terrestrial surround.

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Navigating ants use a multifaceted toolkit (Wehner, 2009). Along with path integration (Wehner & Srinivasan, 2003), ants are known to use visual terrestrial cues for navigation (*Temnothorax albipennis*: Pratt, Brooks, & Franks, 2001; *Formica rufa*: Graham & Collett, 2002; Lent, Graham, & Collett, 2013; *Cataglyphis fortis*: Wehner, Michel, & Antonsen, 1996; *Melophorus bagoti*: Wystrach, Beugnon, & Cheng, 2011, 2012; Wystrach, Schwarz, Schultheiss, Beugnon, & Cheng, 2011; *Myrmecia croslandi*: Narendra, Gourmaud, & Zeil, 2013; Zeil, Narendra, & Stürzl, 2014) and as a 'back-up', they also engage in systematic searching (Schultheiss, Cheng, & Reynolds, 2015).

Some properties of the panorama have been shown to guide ants travelling on familiar routes, including fractional position of mass, matching of segments of the scene and the skyline. Fractional

position of mass refers to the amount of the visual scene to one's left versus right as one faces the goal direction. Wood ants, *F. rufa*, use this cue in some conditions in the laboratory (Lent et al., 2013). In other conditions, *F. rufa* might match a salient segment of the scene (Lent et al., 2013). The skyline is a record of where terrestrial objects meet the sky across the 360° panorama (Dyer, 1987; von Frisch & Lindauer, 1954; Graham & Cheng, 2009a, 2009b; Towne, 2008; Towne & Moscrip, 2008). Its use was demonstrated in Central Australian desert ants, *M. bagoti*, when an artificial skyline in black was created to mimic the natural skyline seen from the start of the journey (Graham & Cheng, 2009a). The ants oriented according to the artificial skyline even when it was rotated so that the celestial cues associated with the panorama did not match in test and training conditions.

Here we investigated further the nature of the sensory input used for view-based matching, focusing on the role of ultraviolet (UV) wavelengths of light in the use of the terrestrial panorama. Ants have been found to have two types of visual receptors in their compound eyes and ocelli (*Cataglyphis bicolor*: Mote & Wehner,

* Correspondence: K. Cheng, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109 Australia.

E-mail address: ken.cheng@mq.edu.au (K. Cheng).

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1980), or sometimes three (*M. croslandi* and *Myrmecia vindex*: Ogawa, Falkowski, Narendra, Zeil, & Hemmi, 2015). In these cited cases, one type is most sensitive to light in the green range, with maximum sensitivity at ca. 510 nm or ca. 550 nm. One other type has highest sensitivity in the UV range, peaking at ca. 350 nm or ca. 370 nm. Ground objects typically do not reflect much in the UV wavelengths, far less so than what is found in the sky (Möller, 2002). Theoretically, UV wavelengths are useful for segregating ground objects from the sky.

Two different ways of using UV wavelengths for delineating the skyline have been proposed. Möller (2002) proposed that UV–green contrast, sensitive to the ratio of UV irradiance to green irradiance, might be used to differentiate sky from ground, and thus delineate the skyline. An opponent-process contrast based on the UV:green ratio buys constancy in the face of fluctuating overall intensity both across time and across space. If a cloud covers the sun temporarily and drops the intensity, both the green reflectance of terrestrial objects and the UV irradiance in the sky diminish. But at the local level, the ratios stay fairly constant, as measured empirically by Möller (2002). While UV–green opponent neurons have been found (in locusts: Kinoshita, Pfeiffer, & Homberg, 2007), a proposed UV–green channel for segregating ground objects from the sky remains hypothetical. But such opponent-process systems are well known in other domains of visual processing in which constancy is important, such as colour vision (in primates: Hurvich & Jameson, 1957; in insects: Backhaus, 1991) and polarization vision in insects (crickets: Labhart, 1988, 1996). More recently, UV levels alone have been proposed in two separate studies (Differt & Möller, 2015; Stone, Mangan, Ardin, & Webb, 2014). Stone et al. (2014) used UV levels for segregating the skyline for artificial navigation, and found that it worked better than UV–green contrast. Differt and Möller (2015) also found that UV levels worked well in computational models, with UV–green contrast hardly adding any benefits.

If UV level or UV–green contrast is used by insects in segregating the skyline, light in the UV range should prove important for navigation based on the panoramic scene. Evidence for this claim is still lacking. We tested the importance of the UV wavelengths in the terrestrial scene for the Central Australian *M. bagoti* (Cheng, Narendra, Sommer, & Wehner, 2009; Muser, Sommer, Wolf, & Wehner, 2005; Schultheiss & Nooten, 2013) by using a clear plastic that filtered out most of the energy from UV wavelengths. The material cut out most wavelengths under 400 nm, as spectrometric measurements indicated. This obliterated most, although probably not all, of the sensitive range of the ant's UV receptor. It was a serious 'knock-down' manipulation, if not a total 'knock-out' one. Key manipulations consisted of surrounding the scene viewed by homing ants with a tall cylinder of this clear plastic. Overall brightness is reduced a little by this manipulation, and in some cases, for both ground objects and the sky. The greatest change in UV levels or in UV–green contrast, however, would be at the top border of the clear plastic. Because it is at a uniform height, a skyline defined in terms of either parameter would be uninformative. The necessity of the UV wavelengths for orientation was tested both in an impoverished artificial arena defining a skyline and in the natural panorama. The efficacy of UV wavelengths was tested by replicating the skyline of a training arena with an identical skyline using clear UV-blocking plastic.

METHODS

Location and Setting

Field work took place at a private property ca. 10 km south of the town centre of Alice Springs, Australia, in a region of semiarid

climate with an average annual rainfall of 282.6 mm. The field site is dominated by the invasive buffel grass, *Cenchrus ciliaris*, mixed with bushes of *Acacia* and *Hakea* genera, and tall eucalypts. Low buildings were also scattered around the premises, adding to the panoramic terrestrial cues (Fig. 1a). Experiments took place in three southern summers from November to March, from 2012 to 2015.

Test Animals

The red honey ant, *M. bagoti*, is widespread in the area. It occupies the niche of a thermophilic diurnal scavenger (Wehner, 1987), looking for desiccated arthropod remains and plant

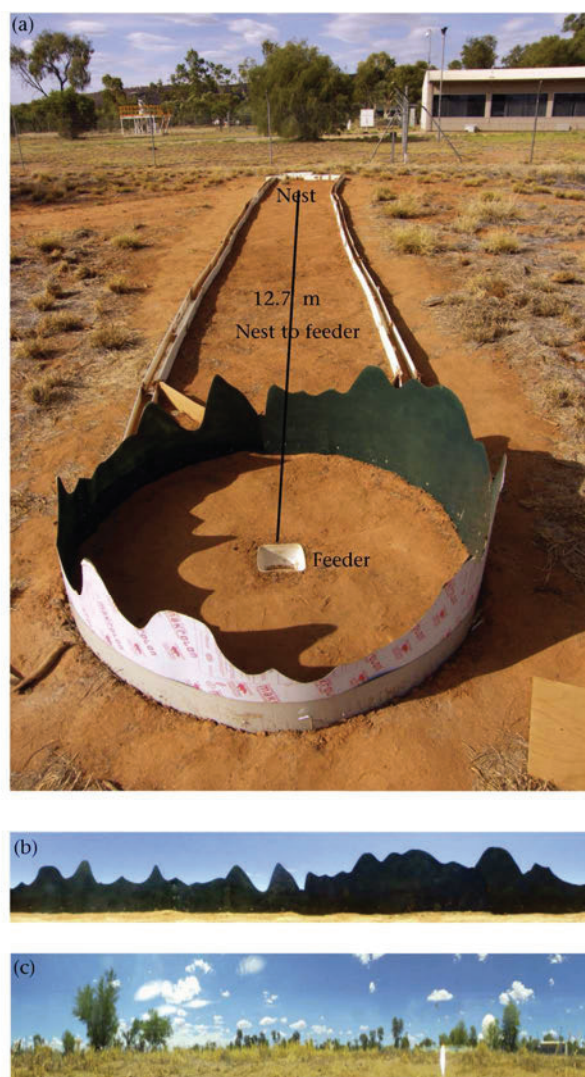


Figure 1. The set-up in experiments 1 and 2. (a) A photo of the arena used in experiment 1 with some of the surrounding scenery, which would not be visible to the ants inside the arena. An enclosure (white plastic) surrounding the nest and leading to the arena kept most of the ants foraging in the corridor and increased the number of foragers arriving at the feeder. (b) The panoramic view provided by the arena. The photo was taken with a panoramic lens and rendered into cylindrical form. The photo 'wraps around', in that the right side of the photo coincides with the left side. (c) The panoramic view at the feeder in experiment 2, with again the right side of the photo coinciding with the left side.

materials in the heat of the day during the summer (Christian & Morton, 1992; Muser et al., 2005; Schultheiss & Nooten, 2013). Ants from one nest took part in experiments 1 and 2, while ants from a different nest took part in experiment 3.

Materials and Set-ups

In each experiment, ants travelled mostly or completely over natural terrain to a plastic tub (15 × 15 and 9 cm deep) sunk into the ground as a feeder. Feeder-to-nest distance was 12.7 m in experiment 1, 5 m in experiment 2 and 10 m in experiment 3. A circular green plastic arena surrounded the feeder in experiments 1 and 3 to provide an artificial terrestrial panorama (reflectance characteristics are shown in Fig. 2b), while in experiment 2 the natural scene provided the terrestrial panorama. The arena in experiments 1 and 3 (diameter 1.4 m) had a uniform green colour but variable height (highest part 0.5 m), providing a panoramic skyline (Fig. 1). A bit of dirt was dug out to provide an entrance into the arena, under the part of the wall between the feeder and the nest.

The feeder was stocked with cookie crumbs (Arnott brand) and pieces of mealworm for the ants to forage. Slippery tape

covered the already slippery feeder walls, so that ants could not climb the walls of the feeder. During training, sticks of natural vegetation and cardboard pieces were placed in the feeder as exit ramps.

Around the route between the feeder and the nest in each experiment, we set up an enclosure of plastic or wooden boards that surrounded the nest and extended to the arena wall (Fig. 1). The materials are very hard for ants to climb over, and this increased the number of animals visiting the feeder. This enclosure was wide enough (ca. 1.2 m) so that on the route, the natural scene rose all around above the enclosure for ants travelling away from the walls, which they did most of the time.

Crucial to the study was the use of a transparent UV-blocking plastic (Makrolon brand) a material that blocks (absorbs) UV light. This material filtered out most of the energy below 400 nm (Fig. 2a). It thus blocks much but not all of the wavelengths of light that would excite the UV receptor in *Cataglyphis* ants (Mote & Wehner, 1980). This plastic surrounded the tested ant in some experimental conditions. Its dimensions were 1.4 m (diameter) by 0.61 m (height) in experiment 1, and 0.7 m by 0.63 m in experiment 2. The dimensions were chosen to cover the visible terrestrial panorama in both experiments.

Training and Test Procedures

During training, ants that arrived at the feeder were painted with nontoxic enamel paint (Tamiya brand) on the abdomen, each with a colour that represented the day of arrival. Thereafter, the ants were left to shuttle back and forth between feeder and nest for at least 2 days before testing.

On a test, an ant might be tested as a full-vector (FV) and/or a zero-vector (ZV) ant. An FV ant is so called because it possesses a vector pointing in the nest direction based on path integration on the outbound trip. Such an ant was taken directly from the feeder in a dark (opaque) vial and placed at the release point for a test. A ZV ant is so called because it has run off its vector based on path integration before being tested. We let a ZV ant run home with a bit of food, and captured it just before it entered its nest, using a small plastic enclosure to trap the ant if necessary. Then the ant was taken in the dark to be released for a test.

In testing the use of the terrestrial panorama, tests with ZV ants provide the crucial data. FV ants use the celestial compass cues as well as possible terrestrial cues, and the crucial manipulations should not affect their orientation too much. At most, the direction of their orientation might be off slightly compared with unmanipulated conditions because the UV-blocking plastic cuts out a part of the sky. The oriented behaviour of FV ants would indicate that ants were still motivated to home under the test conditions. FV test conditions were added in experiment 1 because ZV ants were not oriented in the home direction in the key experimental conditions.

On all tests, an ant was released in the centre of a goniometer consisting of a wooden board with a circle drawn on it divided into 24 sectors of 15° each. Location of testing is described in the following subsection. Only ants that held on to a piece of cookie were tested, to ensure homing motivation. We noted the sector in which the ant crossed at 15 and 30 cm from the release point, these distances being drawn on the goniometer. Each ant was tested individually only once, under one of the conditions to be described next.

Australia does not have ethical regulations concerning ants, but the manipulations used in the study are completely noninvasive. From many studies, including this one, we have noted no adverse effects on the ants.

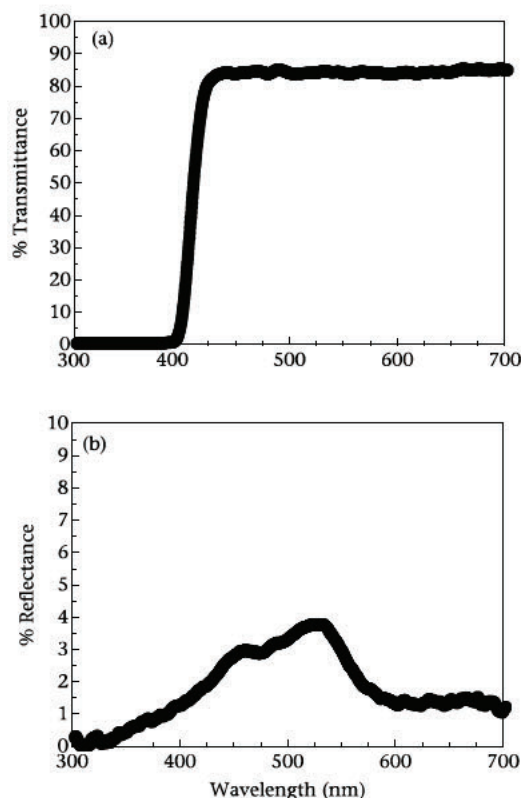


Figure 2. (a) Transmission characteristics of the Makrolon UV-blocking plastic. The photospectrometric measurements were taken with an Ocean Optics Jaz photospectrometer (Ocean Optics, Dunedin, FL, U.S.A.), with the plastic placed in front of a piece of standard white colour, and compared with the reflectance of standard white alone. Thus, in the measurements of the plastic, the light had to go through the plastic twice, to get to the standard white and then to reflect back from the standard white. Only transmittance in the range of 300–700 nm, a reliable range for the instrument, is shown. (b) Reflectance characteristics of the green wall of the arena used in experiments 1 and 3, measured with the same instrument. Note that the scale is reduced 10-fold, with maximum on graph set at 10%.

Conditions of Testing

Experiment 1

There were five test conditions in experiment 1 using the dark green arena with a skyline shape. To minimize interference with ongoing training, ants were tested in a replica of the arena of the same construction placed in the same orientation just behind the training arena from the perspective of the nest. The goniometer was placed at the centre of the test arena. In the ZV-control condition, ZV ants were tested in the replica arena, a condition that replicated training conditions. In the ZV-UV-block-inside condition, the transparent UV blocking foil, of a uniform height exceeding the maximum height of the green artificial skyline, was added on the inside of the test arena. In the ZV-UV-block-outside condition, the tall transparent UV blocking foil was added on the outside of the test arena, hugging the walls. Two conditions also tested FV ants. In the FV-control conditions, FV ants were tested in a replica of the training arena oriented in the same direction. In the FV-UV-block-inside condition, the UV-blocking foil was added inside the walls of the test arena.

Having the UV-blocking plastic both inside and outside the test arena provided more than variations on the theme. The ZV-UV-block-inside was important because it reduces the reflectance of the arena wall more than it does the irradiance of the sky. As the plastic was in front of the arena, light had to go through it to reach the wall, and go through it again in bouncing off the wall. This results in a ca. 16% reduction in transmission according to Fig. 2b. Above the wall, the transmission through the plastic is approximately 91% (square root of 84%) in the visible range, a ca. 9% reduction, but wavelengths < 400 nm were cut out as well. The brightness change of course depends on the sensory system of the ant rather than physical parameters. In this regard, data on *C. bicolor* show that their 'green' receptors (with peak sensitivity at ca. 510 nm) are more sensitive by almost two orders of magnitude than their 'UV' receptors (with peak sensitivity at ca. 350 nm; Figure 6 in Mote & Wehner, 1980). Furthermore, in ants' compound eyes, the majority (ca. 75%) of receptors are 'green' receptors (Menzel, 1972). Thus, the 'green' channel, whose contrast is at least preserved in the experimental manipulations, probably dominates brightness perception.

In both these conditions, the biggest change in UV levels, and also in UV–green contrast, was found at the upper border of the uniform transparent plastic. We expected both these UV-block conditions to affect the orientation of ZV ants adversely, while FV ants should not be adversely affected by the UV-blocking plastic.

Experiment 2

Three conditions were tested in experiment 2, all on ZV ants trained with the natural panorama. In the ZV-control condition, ants were tested in training conditions. The goniometer was placed on the feeder, so that the location of testing matched the starting point of the homeward journey on training runs. This condition was used on two replicates from the same nest but at different points in the season, one in mid-November to December and one in February. In the ZV-UV-block condition, ants were again tested at the feeder, but with a UV-blocking foil of uniform height (0.7 m diameter, 0.63 m height) surrounding them. This condition was also used on two replicates at the same two periods in the season. In the ZV-opaque condition, ants were tested at the feeder with an opaque foil (white colour, 0.7 m diameter, 0.63 m height) surrounding them. The foil effectively cut out terrestrial panoramic information, and forced the ants to use celestial sources for directional information.

Experiment 3

Experiment 3 tested the sufficiency of a clear, UV-blocking cut-out in the shape of the training arena used in experiment 1. In all conditions, ZV ants were tested, with an aim to include at least 100 test individuals in each condition. In the control condition, ants were tested in a replica of the training arena, an exact repeat of the ZV-control condition of experiment 1. In the UV-blocking-foil-cut-out condition, ants were tested in the clear cut-out in the shape of the training arena. This cut-out was placed at a distant test site ca. 143 m away, so that ants would not see a familiar scene through the transparent plastic. In the No-arena condition, ants were tested at the distant test site at which the UV-blocking-foil-cut-out condition took place, but without any arenas, as a test for orientation at that site. Based on suggestive pilot results, we predicted that the control and the UV-blocking-foil-cut-out conditions would produce heading distributions that are significantly oriented, while the No-arena condition would produce an unoriented distribution.

Data Analysis

Circular statistics based on Batschelet (1981) and one test of our own making were used for inferential statistics, calculated using Matlab (Mathworks, Inc., Natick, MA, U.S.A.). We compared headings at 15 cm and at 30 cm in all conditions, and found that in no condition across the experiments did they differ significantly in orientation or scatter. We thus restricted data analysis to headings at 30 cm. For each condition, we tested whether the distribution was significantly oriented in the feeder-to-nest direction by the *V* test (Batschelet, 1981). In addition, we examined whether the 95% confidence interval contained the predicted direction, and conducted the Rayleigh test (Batschelet, 1981) to test whether the distribution was oriented in any direction at all. We set alpha at 0.05 for these tests. Differences in scatter between conditions were tested using the Var test, a test of our own making. The absolute difference of each individual heading from the circular mean of each condition was tabulated. These absolute differences in two conditions were compared using the nonparametric Wilcoxon rank sum test (two-tailed). This test is suitable for any conditions that are oriented, for which a meaningful mean direction can be calculated. Conditions were compared against appropriate control conditions. We compared directions between a condition and its appropriate control using the Watson–Williams test (Batschelet, 1981). In cases of multiple comparisons with a group in experiments 1 and 3, we followed Holm's (1979) method for alpha correction. The first alpha was set to 0.05/*k* (number of comparisons). If the comparison with lowest *P* value is above that value, no null hypothesis is rejected (all deemed nonsignificant). If the lowest *P* value falls below 0.05/*k*, the associated null hypothesis is rejected. The next *P* value is set at 0.05/(*k*–1) to test against the next lowest *P* value, and so on.

RESULTS

Experiment 1

Ants were trained and tested with artificial panoramas in experiment 1. Results showed that the UV-blocking foil had a strong effect on the headings of ZV but not FV ants (Fig. 3, Table 1). FV ants oriented well in the nest direction with or without the UV-blocking foil (Fig. 3a), although surprisingly, control FV ants showed a leftward bias in that the 95% confidence interval did not include the feeder-to-nest direction (Table 1). ZV ants in the control condition oriented well in the nest direction (Fig. 3b, Table 1), also with a leftward bias, but ZV ants with the UV-blocking foil on either the inside or the outside of the arena were not oriented in the nest direction according to the *V* test (Fig. 3b,c, Table 1). The Rayleigh

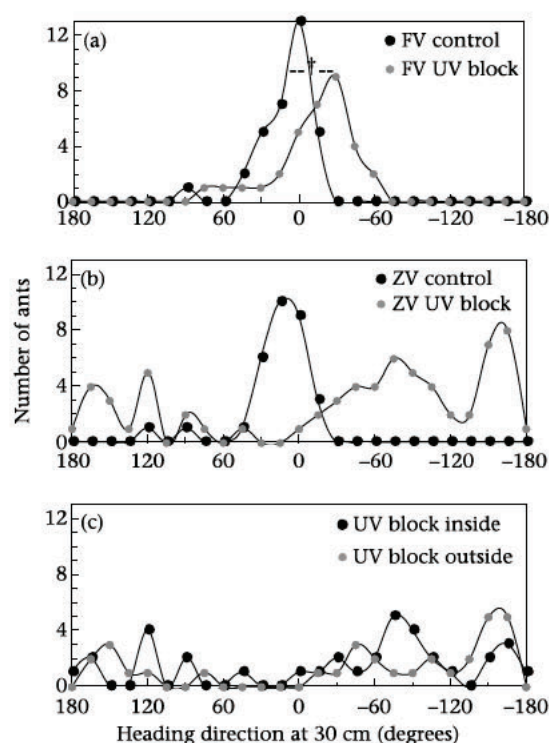


Figure 3. Results of experiment 1. Distributions of heading directions at 30 cm for (a) full-vector (FV) ants under control (training) conditions and with the UV-blocking plastic placed inside the arena, (b) zero-vector (ZV) ants under control (training) conditions and with the UV-blocking plastic placed inside or outside the arena, two conditions combined, and (c) ZV ants with the UV-blocking plastic placed inside or outside the test arena, two conditions separate. Each panel is cylindrical, with $+180^\circ$ and -180° being the same nest-to-feeder direction. Nest direction is at 0° . The line through each distribution is an atheoretical spline that serves only to help readers to visualize the data. The dagger indicates two conditions that differ significantly in mean heading direction. Inferential statistics was not conducted on the combined data in (b).

test showed, however, that these groups were significantly oriented (Table 1). That is because the ants tended to head in the opposite, nest-to-feeder direction (Fig. 3b,c). A V test for this direction showed that this tendency was not significant for the ZV-UV-block-inside condition ($V = 3.18$, $P = 0.220$), but was significant for the ZV-UV-block-outside condition ($V = 11.89$, $P = 0.001$). If the results of these two groups are pooled, the ants were significantly oriented in the nest-to-feeder direction ($V = 15.07$, $P = 0.004$). It should be noted, however, that the 95% confidence interval for either group, or for the two UV-block groups combined, did not include 180° .

In directional scatter, both ZV groups with the UV-blocking foil were more scattered than the ZV-control group (Table 2).

Comparing the FV group with the UV-blocking foil on the inside with the FV-control group, the difference in directional scatter was not significant (Table 2).

Comparing mean directions of headings of ZV ants using the Watson–Williams test, both the ZV-UV-block-inside condition and the ZV-UV-block-outside condition differed in mean direction from the ZV-control group (Table 3). For FV ants, the FV-UV-block-inside group differed significantly in mean direction from the FV-control group (Table 3).

Experiment 2

Ants were trained and tested with a natural panorama in experiment 2. In the control condition, ZV ants were clearly oriented in the nest direction (Fig. 4a), but when surrounded with a UV-blocking foil, they appeared less well oriented (Fig. 4b). The UV-block groups in both replicates, however, were in fact significantly oriented in the nest direction (Table 4). Replicate 1 of the UV-block group, however, erred to the right, with the 95% confidence interval not containing the nest direction. Directional scatter between the ZV-control and ZV-UV-block conditions were compared using the Var test. The scatter did not differ significantly for replicate 1, but did differ significantly for replicate 2 (Table 2). When the two replicates were pooled (Fig. 4c), the UV block resulted in more directional scatter in the headings of the ants compared with control conditions (Table 2). ZV ants facing an opaque surround were not significantly oriented (Fig. 4d, Table 4), and not significantly oriented in the nest direction (Table 4).

We compared the mean directions of ZV control groups against the UV-blocking groups using the Watson–Williams test. The mean direction differed for replicate 1 but not for replicate 2 (Table 3). When the two replicates were combined, ZV-control ants did not differ in mean direction from their counterparts surrounded by the UV-blocking foil (Table 3).

In addition, given the differences in behaviour between the ZV ants in experiments 1 and 2, it is of interest to compare groups across experiments in their mean direction, with the usual cautionary note needed about comparing experiments. We compared ZV control groups (two replicates combined for experiment 2) using the Watson–Williams test and found that mean direction differed significantly between experiments ($F = 6.35$, $P = 0.013$). We also compared the UV-blocking conditions (ZV-UV-block-inside and ZV-UV-block-outside combined in experiment 1 versus two replicates of ZV-UV-block in experiment 2) and found that, as expected, they differed significantly in mean direction ($F = 47.96$, $P < 0.001$).

Experiment 3

Ants in experiment 3 were trained in the artificial arena. Experimental groups were tested at a distant location from the

Table 1
Descriptive and inferential statistics for experiment 1

Condition	N	95% CI L (deg)	M (deg)	95% CI R (deg)	R	Rayleigh test		V test	
						z	P	V	P
ZV control	31	25.2	15.3	5.4	0.90	25.21	<0.001	27.04	<0.001
ZV UV block inside	34	−60.0	−106.9	−153.9	0.32	3.49	0.029	−3.18	0.780
ZV UV block outside	32	−111.1	−139.8	−168.5	0.49	7.54	<0.001	−11.89	0.999
ZV UV block, combining 'inside' and 'outside' conditions	66	−100.9	−126.3	−151.7	0.39	9.75	<0.001	−15.07	0.996
FV control	33	17.7	10.2	2.6	0.94	28.78	<0.001	30.42	<0.001
FV UV block inside	33	−2.0	−14.8	−27.7	0.87	24.79	<0.001	27.73	<0.001

The table shows results for zero-vector (ZV) and full-vector (FV) conditions, including the number of ants tested (N), mean vector direction (M), 95% confidence intervals to the left (95% CI L) and right (95% CI R), mean vector length (R), Rayleigh test results and V test results testing for significant orientation in the fictive nest direction, or exit direction according to the arena.

Table 2
Inferential statistics comparing the directional scatter of conditions in experiments 1 and 2

Experiment	Comparison	Z	P
1	ZV UV block inside versus ZV control	5.36	<0.001
1	ZV UV block outside versus ZV control	3.97	<0.001
1	FV UV block inside versus FV control	1.39	0.163
2	ZV UV block versus ZV control replicate 1	1.92	0.055
2	ZV UV block versus ZV control replicate 2	4.92	<0.001
2	ZV UV block versus ZV control, combining replicate 1 and replicate 2	5.70	<0.001

Comparisons were based on the Var test. Absolute differences of individual headings from the mean circular heading of each of two conditions are computed. The scores for each group are then compared with the Wilcoxon rank sum test, two-tailed. Different zero-vector (ZV) and full-vector (FV) conditions were compared against appropriate control groups.

Table 3
Inferential statistics comparing mean directions of conditions in experiments 1 and 2

Experiment	Comparison	F	P
1	ZV UV block inside versus ZV control	44.74	<0.001
1	ZV UV block outside versus ZV control	104.93	<0.001
1	FV UV block inside versus FV control	14.61	<0.001
2	ZV UV block versus ZV control replicate 1	9.14	0.004
2	ZV UV block versus ZV control replicate 2	3.43	0.068
2	ZV UV block versus ZV control, combining replicate 1 and replicate 2	<1	0.376

Comparisons were based on the Watson–Williams test. Mean directions of different zero-vector (ZV) and full-vector (FV) conditions were compared against appropriate control groups.

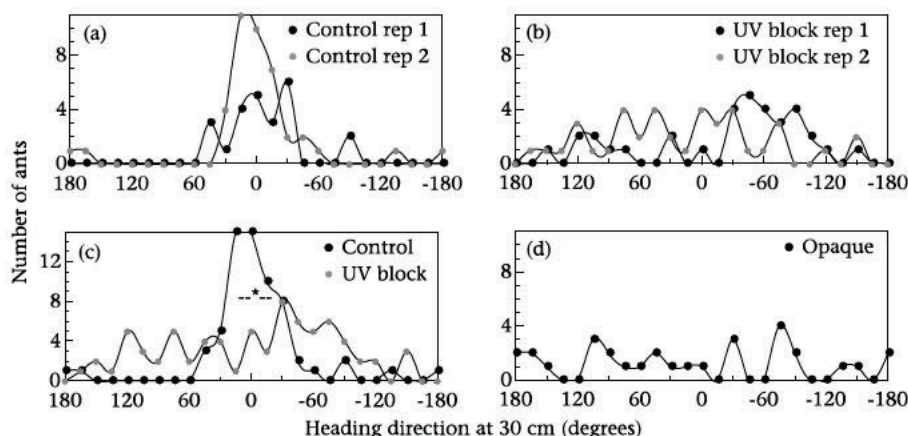


Figure 4. Results of experiment 2. Distributions of heading directions at 30 cm for zero-vector (ZV) ants (a) in control (training) conditions, separately for two replicates, (b) with the UV-blocking foil surrounding them on the test, separately for two replicates, (c) in control (training) conditions and with the UV-blocking foil surrounding them on the test, each with two replicates combined and (d) with an opaque white foil surrounding them on the test. Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualize the data. The asterisk indicates two conditions that differ significantly in directional scatter.

Table 4
Descriptive and inferential statistics for experiment 2

Condition	N	95% CI L (deg)	M (deg)	95% CI R (deg)	R	Rayleigh test		V test	
						z	P	V	P
ZV control replicate 1	24	10.0	–6.1	–22.2	0.84	16.76	<0.001	20.00	<0.001
ZV control replicate 2	40	12.0	–1.2	–14.5	0.80	25.33	<0.001	31.92	<0.001
ZV control, combining replicate 1 and replicate 2	64	7.0	–3.1	–13.2	0.81	42.00	<0.001	51.92	<0.001
ZV UV block replicate 1	34	–23.0	–54.8	–86.6	0.44	6.41	0.001	8.52	0.019
ZV UV block replicate 2	40	61.1	26.3	–8.6	0.37	5.56	0.003	13.42	0.001
ZV UV block, combining replicate 1 and replicate 2	74	17.2	–14.0	–45.2	0.31	6.87	<0.001	21.94	<0.001
ZV opaque	28	–	42.2	–	0.07	0.14	0.868	1.50	0.345

The table shows results for zero-vector (ZV) conditions, including the number of ants tested (N), mean vector direction (M), 95% confidence intervals to the left (95% CI L) and right (95% CI R), mean vector length (R), Rayleigh test results and V test results testing for significant orientation in the fictive nest direction, or exit direction according to the arena.

training site, either with a clear cut-out having the shape and orientation of the training arena (UV-blocking-foil-cut-out) or in the open at the unfamiliar site (No arena). Experiment 3 was high in power, with over 100 individuals tested in each condition. The ants (all ZV ants) appeared well oriented, somewhere in the vicinity of the feeder-to-nest direction, in the Control and UV-blocking-foil-cut-out conditions, but it is difficult to discern a clear peak in the heading distribution from the No-arena condition (Fig. 5a,b). The *V* test, however, revealed significant orientation in the nest direction in all three groups (Table 5). Both the UV-blocking-foil-cut-out group and the No-arena group erred to the left, in that the 95% confidence interval did not contain the feeder-to-nest direction. The Var test for directional scatter revealed significant differences between all pairs of groups by Holm's (1979) correction method: control condition versus No-arena condition ($Z = 5.62$, $P < 0.001$), UV-blocking-foil-cut-out condition versus No-arena condition ($Z = 3.41$, $P < 0.001$), control condition and UV-blocking-foil-cut-out condition ($Z = 2.29$, $P = 0.022$). These latter two conditions differed significantly in mean direction (Watson–Williams test: $F = 8.54$, $P = 0.004$). The No-arena condition was too scattered in heading distribution to compare with other conditions. The headings in each condition were smoothed by a running average of three bins in Fig. 5c,d. That is, the count in each bin consisted of the average of the raw count in that bin and its two immediate neighbours. These figures might show the trend of the data better, but were not used for analyses.

DISCUSSION

To summarize the experimental findings, in experiment 1, the terrestrial cues consisted of a skyline in a uniformly coloured arena, offering a form of 'pure skyline', while in experiment 2, ants homed under natural conditions. When wavelengths < 400 nm were greatly reduced at a uniform height surrounding the test ant, ants trained and tested in the arena without directional information from path integration (ZV ants) did not orient in the nest direction. Rather, they tended to orient in the opposite nest-to-feeder direction. When ZV ants homing in natural conditions had wavelengths < 400 nm knocked down at a uniform height surrounding the test ant, they were still oriented in the nest direction, but the

performance was more scattered compared with control ZV ants homing under unaltered conditions. These results point to the importance of UV wavelengths in using the terrestrial panorama to orient. Reducing UV wavelengths up to a uniform height alters the UV:green ratio and the overall UV level found in the skyline. In effect, the test skyline under such conditions would be the uniformly tall top border of the surrounding clear plastic, where the greatest change in either UV:green ratio or UV level was found. Disruption of orientation would show that one of these parameters (or both) plays a major role in defining the skyline.

In experiment 3, a clear cut-out of the shape of the training arena, made with the UV-blocking plastic foil, was placed at a distant test site. The ZV ants used this cut-out readily to home, albeit less precisely and with a distortion in the initial direction compared with controls. This shows a form of sufficiency of the contour of maximum UV–green contrast or maximum change in UV levels in the face of many changes in spectral composition, two theoretically proposed ways of extracting the skyline (Differt & Möller, 2015; Möller, 2002; Stone et al., 2014).

The most serious alternative interpretation to consider is that a slight reduction in brightness contrast, between ground objects (arena wall or the natural scene) and the sky, might have caused the ants' performance to deteriorate in the UV-blocking-foil conditions in experiments 1 and 2. The UV-blocking foil has the same physical effects on ground objects and sky in experiment 2 in the natural surround, but physiologically, the sky might show a greater reduction in overall brightness (sum of 'green' and 'UV' receptor stimulation) because it contains more intensity than ground objects in the UV wavelengths, which are knocked down by the UV-blocking foil. In experiment 1, this is compensated for to some extent because the foil reduced the intensity of the wall more (light had to pass through the foil twice in reaching the wall through the foil and then bouncing back out through the foil). It seems, however, that passing clouds covering the sun would have a greater effect in reducing intensity contrast. Such an event might change intensity levels by an order of magnitude (see Möller, 2002). Geophysically, clouds covering the sun block transmission of visible (to humans) light more so than transmission of UV wavelengths (Blumenthaler, Ambach, & Salzgeber, 1994), meaning that cloud cover tends to reduce brightness and green contrast of the skyline

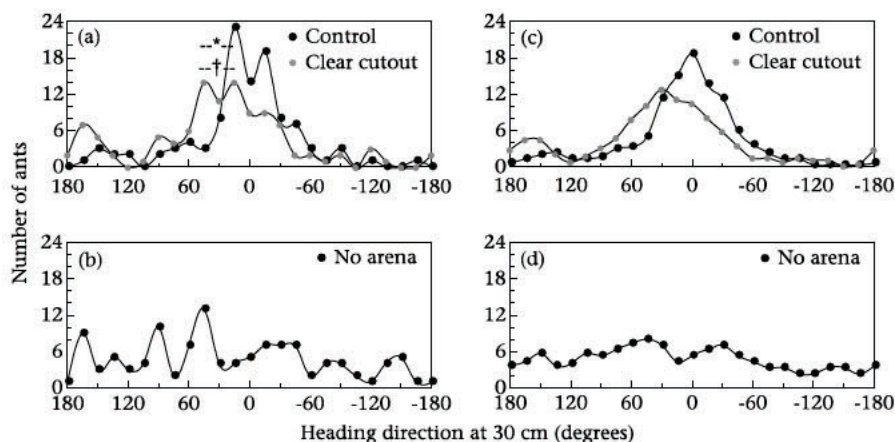


Figure 5. Results of experiment 3. (a, b) Distributions of heading directions at 30 cm for zero-vector (ZV) ants in (a) the control condition and with UV-blocking foil cut out to the shape of the training arena (clear-cutout) and (b) the No-arena condition. (c, d) Smoothed data for (c) the control condition and with UV-blocking foil cut out to the shape of the training arena and (d) in the No-arena condition. Data in (c) and (d) were transformed from those in (a) and (b) by averaging each bin with its two immediate neighbours. Each panel is cylindrical, with $+180^\circ$ and -180° being the same nest-to-feeder direction. Nest direction is at 0° . The line through each distribution is an atheoretical spline that serves only to help readers to visualize the data. The asterisk indicates two conditions that differ significantly in directional scatter. The dagger indicates two conditions that differ significantly in mean heading direction. Inferential statistics were not performed on (c) and (d).

Table 5
Descriptive and inferential statistics for experiment 3

Condition	N	95% CI L (deg)	M (deg)	95% CI R (deg)	R	Rayleigh test		V: nest direction	
						z	P	V	P
Control	108	13.0	3.0	−7.1	0.67	48.9	<0.001	72.80	<0.001
UV blocking foil cut-out	107	42.7	27.8	13.0	0.49	25.8	<0.001	15.51	<0.001
No arena	114	79.5	41.5	3.6	0.21	5.0	0.007	6.52	0.009

The table shows for each condition the number of zero-vector ants tested (N), mean vector direction (M), 95% confidence intervals to the left (95% CI L) and right (95% CI R), mean vector length (R), Rayleigh test results and V test results testing for significant orientation in the fictive nest direction.

more so than it does UV contrast and the UV:green ratio. Our observations from working with this species, albeit not formally documented, have suggested that cloud cover does not affect the orientation of ZV ants adversely. More formal investigations along these lines, however, would be illuminating and should be carried out.

In experiment 1, the ants homed in a uniformly coloured arena that proffered a skyline. The uniform coloration impoverishes spectral cues, but does not eliminate them. While the wall would have the same reflectance characteristics everywhere, the position of the sun would still provide spectral cues (Wehner, 1997). Thus, it was obvious to human observers (without a UV receptor) that one side of the arena looked brighter because the sun was shining on it. The UV-blocking plastic would not alter such a brightness gradient substantially, lowering the brightness on both the sun and the opposite sides. Polarization compass cues in the sky would also be left largely intact. The ZV ants did not orient in the home direction, but some evidence indicates that they did orient opposite the home direction. This backtracking behaviour may parallel what Wystrach, Schwarz, Baniel, and Cheng (2013) found in this species. In that study, *M. bagoti* backtracked when they were captured near their nest after homing from a familiar site (feeder) and then displaced to a distant, unfamiliar location. These ants must have been using their celestial compass to head in the nest-to-feeder direction because the distant site had no useful terrestrial information. Evidence that ZV ants of this species use celestial cues for orientation has been found in some circumstances (Legge, Spetch, & Cheng, 2010; Legge, Wystrach, Spetch, & Cheng, 2014; Wystrach & Schwarz, 2013; Wystrach et al., 2013). In our ants homing with the UV-blocking shield in place, we tentatively interpret the manipulation to have rendered the scene unfamiliar to the ants, unfamiliar enough that they too exhibited backtracking behaviour. The interpretation is uncertain because the 95% confidence interval of the mean direction did not include 180°. The distortion, if it is that, could arise because the UV-blocking foil changed the pattern of polarized light visible to the ants. The polarization compass in ants depends on UV-sensitive receptors in the dorsal rim area (Wehner, 1994). It remains possible, however, that ants in the key experimental conditions were simply disoriented.

FV ants in experiment 1 facing the UV-blocking plastic were oriented in the feeder-to-nest direction, albeit with a bias (Table 1). This shows that ants facing the UV-blocking plastic were motivated to home. Their mean direction, however, differed from that of FV controls facing the replica of the training environment. Again, changing the amount of UV wavelengths perceptible at different azimuths, compared with training conditions, might have distorted the information based on the polarization compass.

FV and ZV ants facing a replica of the training environment showed a leftward bias. Two explanations, not mutually exclusive, might account for this pattern. The first is that just to the left of the feeder-to-nest direction, the arena presented a distinctive undulating cue, a near-vertical segment (see Fig. 1a,b), which might provide a more distinct cue for approaching. This explanation assumes that well-trained FV ants use both the celestial cues and the

terrestrial panorama in orientation, and evidence for this claim has been found in this species (Legge et al., 2014). A second, perhaps related reason is that in training, only a small opening allowed exit from the arena. Some of the ants might have erred strategically to one side (and why not the more distinct side?) so as to determine the direction to turn when they arrive at the wall. These, however, remain post hoc explanations in need of confirmation.

Under natural conditions (experiment 2), obliterating UV wavelengths (<400 nm) at a uniform height did not knock out homeward orientation. Unlike the arena, the ants were both motivated to and could orient homewards, but their performance was worse, in being more scattered in initial heading. We thus conclude that UV wavelengths provide an important cue for the ants. We can only speculate at this point on what other cues are available. Assuming the UV receptor to be effectively taken out of play by the UV-blocking plastic, brightness contrast or contrast in the green channel between ground objects and sky remain possibilities. Of course, the cues linked to the sun, polarized light and spectral patterns were not blocked, and were, in principle, available as well.

In experiment 3, a cut-out made of the UV-blocking plastic mimicking the shape of the green arena was presented on the crucial test at a distant test site. Given that the plastic eliminated most wavelengths of light <400 nm, we hypothesized that the skyline defined by the cut-out would still be the top border of the arena, matching training conditions. The biggest jump in UV levels or in UV–green contrast would still be found at the top of the clear cut-out. With a sample size >100, the ants were oriented in the nest direction, although less precisely and with a deflection in mean direction compared with controls. With regard to the deflection in mean direction, one possibility is the natural panorama viewed through the clear plastic. We conducted a pixel-by-pixel comparison of the natural skyline at the test site and the skyline defined by the training arena: the best match was at about 85° (results not shown). Perhaps the ants in the clear-cut-out test perceived two skylines, one at the top of the test arena and one through the cut-out. Combining those two cues would deflect the mean direction to the left relative to controls.

In reducing substantially the UV wavelengths with the plastic, we of course changed the amount of UV light reaching the ants as well as the UV:green ratio. If either parameter is used to segregate the skyline, similar patterns of results would be found. Navigation based on a skyline defined by measuring the amount of UV light has been demonstrated in autonomously navigating vehicles (Stone et al., 2014). Stone et al.'s vehicles, however, were navigating in environments altered by humans: streets in urban neighbourhoods. Human alterations do not change the UV levels found in the sky, but make the green channel noisier, with some human-made objects reflecting little in the green wavelengths. For biological navigational systems evolving in natural habitats unaltered by humans, some form of UV–green contrast based on opponent processes may be theoretically more likely (Möller, 2002). Evidence supports such an opponent-process system in the polarization compass (Labhart, 1988, 1996). Such opponent processes buy

constancy in the face of changing overall illumination levels and alleviate the need to adjust the threshold on the basis of overall light levels, a by no means trivial problem. It would be good to carry out a similar knock-down manipulation targeting the green wavelengths as well. The UV:green ratio would also be distorted if green wavelengths are substantially reduced, and similar deficits should be found. If the ants use the amount of UV light (or stimulation of the UV receptor) for segregating the skyline, the green knock-down manipulation should have little effect.

Sensitivity to UV wavelengths serves navigation in other ways in insects. Sensory neurons sensitive to UV wavelengths in the dorsal rim of the eyes of desert ants and honeybees serve as receptors for polarized light (Wehner, 1994, 1997). Dung beetles, *Scarabaeus zambesianus*, use polarized moon light in order to roll a ball of dung away from the dung pile in a straight line (Dacke, Nilsson, Scholtz, Byrne, & Warrant, 2003). This polarization channel is also mediated by sensitivity to UV wavelengths (el Jundi et al., 2015). In the desert locust, *Schistocerca gregaria*, the polarization channel is mediated by blue receptors (el Jundi, Pfeiffer, Heinze, & Homberg, 2014), but intriguingly, UV–green opponent-process neurons have been found in the anterior optic tubercle (Kinoshita et al., 2007). These neurons are excited by unpolarized light in the green wavelengths and inhibited by unpolarized light in the UV wavelengths, or vice versa. They are thought to serve the celestial compass in locusts. Whether such opponent-process neurons can be found in circuits in insects that encode terrestrial cues remains an open question.

In sum, this study has shown that light in the UV range plays an important role in ant navigation based on the terrestrial panorama. Knocking it down by blocking UV wavelengths made ZV ants not orient in the nest direction when navigating out of a uniformly coloured arena providing a skyline (experiment 1), but instead if anything in the opposite nest-to-feeder direction. With UV wavelengths blocked, the ants did not orient as well in the nest direction under natural conditions, although they were still significantly oriented in this direction (experiment 2). With an opaque artificial arena replaced with a UV-blocking but clear arena of the same shape, the ants managed to orient significantly in the nest direction.

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Introduction

Arthropods are a widely studied animal group, inspiring interest across a broad range of scientific disciplines due to the diversity of the environments they inhabit and their broad range of ecology. This group's versatility has resulted in arthropods being the most successful animal group on the planet, with their membership including spiders, insects, and crustaceans. Arthropods are characterized as a group by their segmented bodies, appendages with joints, a hard external exoskeleton, and a lack of internal bone structures. To the layman, the behaviors of these 'lower order' organisms might appear as inflexible or hardwired and reflect the appearance of only limited cognitive abilities when compared to the cognition of vertebrates. However, there exists a wealth of scientific evidence that paints a picture of arthropods as organisms that exhibit large flexible behavioral repertoires, including the ability to perform complex cognitive functions despite their small brain size and limited neuron numbers. The cognitive processes exhibited by arthropods can be as simple as associative learning between a stimulus and a food reward, or as complex as navigation using memorized visual cues or the sophisticated communication of the honeybee dance. Within this chapter, we discuss four broad topics concerning the cognitive abilities of arthropods: associative learning, spatial cognition, social cognition, and finally, distributed cognition. Such an overview is not meant to be exhaustive but rather present an impression of the diverse range of cognitive abilities exhibited by the group.

Associative Learning

Arthropods show a surprising capacity for learning a wide range of sensory cues that may predict either reward or punishment. These learned cues can be stored over the short or long term for further use. A large portion of the research exploring the learning and memory capabilities of arthropods has focused on associative learning, a cognitive process in which an organism learns through experience to associate certain behaviors with stimuli. This type of learning includes both classical conditioning and operant conditioning and has been widely used in arthropod research to showcase an array of cognitive skills.

Classical conditioning

Classical conditioning is a learning process in which an organism learns to associate a biologically neutral stimulus with a potent, biologically relevant stimulus leading to a behavioral response when the neutral stimulus is presented alone. In the classic learning experiments conducted by Ivan Petrovich Pavlov, dogs learned to associate previously neutral

sounds such as the ticking sound of a metronome with the presentation of food. Dogs originally only salivated when the food is present, but over a number of pairings, individuals would begin to salivate at the ticking sound even in the absence of food.

Insects are commonly used subjects in classical conditioning research. For instance, during olfactory learning experiments in honeybees, a restrained bee is presented with a neutral odor, the conditioned stimulus. As this odor is presented to the bee, the unconditioned stimulus, here a small amount of sucrose solution delivered to the bee's antennae, is also presented. The bee's unconditioned response to the sucrose is to extend its proboscis, a long tubular portion of the bee's mouth, which it uses to feed (Figure 1). After multiple exposures to the odor, followed by the sucrose solution, the bee will begin exhibiting a conditioned response to the odor alone, extending its proboscis when only the odor is presented. Olfactory memories in Honeybees can be retained over long time periods when the subject is exposed to repeated presentations, whereas conditioning after only a single pairing will decay over the next 24-hour period. Repeated pairings of the odor and sucrose can produce memories of the association that can last over 7 days (Menzel, 1999; Giurfa and Sandoz, 2012).

Insert Figure 1 about here

Evidence of this associative learning using both olfactory and visual stimuli is present across the arthropod phylum. The Pavlovian salivation response can be replicated by olfactory pairing in cockroaches (Watanabe and Mizunami, 2007). Cockroaches were presented with a neutral odor and then a small amount of a sucrose solution. Just as in dogs, the unconditioned response measured was the saliva production in the cockroach's mouth. After training, conditioned cockroaches increased salivation when presented with the conditioned odor alone but not when presented neutral odor types.

Associative learning can also encompass associations beyond those revolving around attaining food rewards. In a form of classical conditioning called aversive conditioning, the unconditioned stimulus presented to the animal is negative, such as an electric shock. After pairing the shock with a previously neutral stimulus, subjects will respond by avoiding this neutral stimulus in future tests. *Drosophila* fruit flies have been shown to readily learn these associations between different odors and electric shock, actively avoiding odors that have been previously paired with the aversive stimulus (Tully and Quinn, 1985; Busto et al., 2010). Beyond insects, spiders and crabs show similar cognitive abilities, quickly learning associations between stimuli and visual cues. Hermit crabs are more likely to abandon a home shell for a new shell after experiencing an electric shock within the original. These crabs will

also spend less time inspecting a new shell before deciding to swap (Elwood and Apple, 2009).

Associative learning can be a strong driver of behavior, to the point where it can lead animals to override instinctive behavior. Certain crab species have an instinctive preference for darker environments due to predation pressures. This preference can be extinguished when bright light is paired with food rewards. Instinctive behaviors can also be paired with adverse stimuli to decrease their prevalence. Crabs can also learn to avoid dark habitats when these dark environments are paired with aversive stimuli such as electrical shocks. Crabs and crayfish will learn this association and exhibit increased avoidance behaviors such as running responses or fleeing out of the area when darkened habitats are linked with electrical shock (Barr et al., 2008; Magee and Elwood, 2013).

Operant conditioning

Operant conditioning is a learning process that involves the frequency of a behavior being controlled by the behavior's outcome. Unlike classical conditioning, during operant conditioning behaviors are not elicited by a stimulus but instead are shaped by the behavior's subsequent result, which can either be a reward or a punishment. Arthropods are adept at associative learning paradigms. This has made member species, the honeybee and *Drosophila* flies in particular, excellent models for use in learning experiments that test a number of learning parameters. Operant conditioning in honeybees has been the subject of extensive study over the last century, pioneered by work using free-flying target discrimination-tasks (von Frisch, 1914). In these tests, free-flying bees are usually given a choice between two visual targets, one of which is associated with a food reward, in an experimental design that mirrors the honeybee's natural foraging behavior. In this experimental structure, the bee chooses where to land. This behavioral action determines if it receives a food reinforcer. Such tests have allowed researchers to discover a wealth of information regarding honeybee cognition and perception, including the use of visual and olfactory stimuli, image matching, color learning, numerical cognition, and time-linked learning (Giurfa, 2015).

Drosophila flies are also commonly used in operant conditioning experiments to study a myriad of cognitive and genetic questions. An experimental instrument called a heat-box is commonly used with this species (Putz, 2002). The instrument's chamber is split, where half the area is punished and half is not. Whenever the test fly enters the punished portion of the chamber, the temperature of the space is heated. When the fly leaves this area, the temperature in the box returns to normal. Flies quickly learn this association and restrict their movement to the non-punished side of the chamber. This association has been shown to be stored in long-term memory, as trained flies will still avoid the punished portions of the heat

box hours after training. Experiments have produced similar results in jumping spiders (Peckmezian and Taylor, 2016), where specific areas of a chamber are linked to electrical shock. In jumping spiders, stimuli are typically associated with visual cues due to their high visual acuity. When electrical shocks are paired with either a black or white stimulus, spiders will respond similarly to *Drosophila* by subsequently avoiding those areas in the future (Putz, 2002; Peckmezian and Taylor, 2016).

Spatial Cognition

Animals that move through their environment need to be able to steer and find certain locations, be that a food source or the quickest path back to their nest. Survival can often depend on the animal's ability to navigate accurately, especially when their environment is harsh and prolonged exposure to the elements could mean death. Navigation requires the acquisition and application of information in the environment that can indicate the direction and/or distance of a goal location. Often, animal navigators will collect environmental cues from multiple sources. Multiple information streams provide a further cognitive challenge: the navigator must integrate these signals before making a decision on where to go.

In the Tunisian desert, specialized foraging ants wander through the scorching and featureless terrain searching for food. These ants are active during the hottest parts of the day when they scavenge for other invertebrates that have fallen victim to the intense heat. These searching ants can travel hundreds of meters from the nest in long meandering paths before finding food. Once found, however, a forager will travel in a straight line back to the nest, a small inconspicuous hole (Figure 2). How does this solitary ant find their nest in a featureless landscape when the intense heat makes chemical trails quickly evaporate? They accomplish this task using a navigational tool called path integration.

Path integration

Path integration is a cognitive process where the foraging ant acquires and combines both distance and directional cues for navigation during their search for food. When these two measurements are combined they allow the ant to keep track of their position relative to the nest's location during their trip. This is called their home vector. To measure the direction the foraging ant is facing, the ant has a sky compass that is based on cues such as the sun's location and the pattern of polarised light in the sky. In walking animals such as ants, distance estimates are collected through a step counter. These two measurements are collected, integrated and stored during the winding outbound path until the foraging ant finds food and needs to return home. This allows the ant to constantly track the nest's location while out

foraging. It then uses this collected information to accurately travel the correct distance and direction home (Figure 2; Wehner and Wehner, 1990).

Insert Figure 2 about here

Honeybees are also known to use path integration to return to their nest after foraging; though the honeybee forager needs a different solution in order to estimate how far it has traveled, as counting steps is not very useful to a flying insect. To estimate flown distances, the honeybee forager records their traveled distance using optic flow. Optic flow is a visual cue resulting from the motion of objects and surfaces across the honeybee's visual field. As the honeybee moves through its environment, objects move or “flow” past their visual field. The honeybee can then use this information to estimate how far it has traveled and combine it with the same sky-based directional cues as the ant in order to path integrate.

Even non-social arthropods use path integration to get to goal locations. Fiddler crabs need to defend home burrows and must remain vigilant for intruders even when they leave the burrow to feed on the open beach. If the burrow owner sees another fiddler crab trying to move into its burrow, it scurries back in a straight line to stop the intruder (Zeil and Layne, 2002). The wolf spider also maintains a home burrow for safety and, additionally, to ambush prey. These spiders have shown the same distance and direction estimation when returning to their burrows as ants and honeybees, indicating their use of path integration (Ortega-Escobar, 2006).

The use of path integration is generally studied as a form of working memory, a memory type involving the short-term storing of information currently in use. The distance and direction components are compiled to give the animal a continuously updated location of its position relative to their nest, both during the outbound and inbound portions of their trip. As the animal reaches and enters its nest, the path integrator is reset to zero. Yet, work in both ants and honeybees has shown that these animals can also retain long-term memories of path integration information from previous foraging trips, allowing them to use path integration cues from these trips to return to an area if there is an abundance of food or to return to the nest from a known location after long delays (Beugnon et al., 2005).

Landmarks and the panorama

Path integration is merely one of the strategies in the navigational toolkits of arthropods. Individuals can also retain long-term memories of terrestrial cues in order to navigate. Research on terrestrial cues likely brings to mind the use of landmarks, but it is now believed that animals use the configuration of multiple objects in their visual field to navigate. There is now mounting evidence that these animals use the complete landmark makeup of the

360° visual scene, termed the panorama, to navigate rather than specific elements in the scene. The predominant theory on how insects and other arthropods use the panorama for navigation is by a view-based matching strategy (Collett, 2010; Zeil et al., 2014). Here, stored images of the panorama are compared to the animal's current view while away from the goal location. To orient correctly to the goal, the navigator will attempt to match its current view with views it had previously stored. The resulting best visual match between the stored view and the current view gives the navigator the correct direction to their goal location.

View-based matching first requires that a navigator learn the panorama around its nest or other goal location. Nest panorama acquisition is accomplished in honeybees and wasps through a behavior called learning flights. Before a forager first leaves the nest area, the forager slowly flies in looping arcs while facing the nest from multiple directions. Ants and spiders exhibit very similar behaviors, called learning walks, on the ground around the nest or burrow. When first leaving their nest or burrow, they will perform multiple pre-foraging trips within the nest area in looping paths that occasionally look back toward the nest direction. It is believed that during these learning flights or walks, views of the panorama around the nest are being stored for future use. Once the panorama at the nest site is learned, foragers leave the nest area, occasionally turning back to face the nest. By turning back to the nest the individuals can learn and store multiple views of the outbound route, as well as changes in the panorama for use when they need to find their way back. These stored memories are robust, allowing foragers to navigate home even after long delays.

Certain arthropod species living in environments with access to both the sky compass and terrestrial cues have shown an additional cognitive ability called cue integration, where individuals integrate information from multiple directional cues while navigating. As sky compass cues such as the sun's position are independent of the terrestrial panorama they will not always match, leading to a cue conflict that must be resolved before a navigating animal decides which direction to go. Ants and honeybees displaced off their foraging route have been shown to average these two directional cues and choose a compromise direction between the two directions indicated by the sky compass and the panorama. This averaging ability appears to happen dynamically, as foraging ants will change how much weight they assign each cue depending on a number of factors, such as home vector length (Narendra, 2007; Freas et al., 2017).

Cognitive maps

A more complex navigational tool thought to be in use in many vertebrate species is the cognitive map. Here, animals maintain mental representations of the spatial layout of objects in the animal's environment. This allows animals to navigate successfully from locations they

have not previously visited. The presence of a cognitive map in insect/arthropod cognition remains hotly debated in the scientific community; with sceptics contending that both path integration and view based matching provide alternative, simpler explanations for the observed navigational abilities in arthropods. For further analysis of this topic and the debate surrounding its presence in invertebrates, please look to the cognitive map chapter of this text.

Social Cognition

Multiple arthropod species are highly social; a characteristic commonly associated with members of the insect order Hymenoptera, which comprises sawflies, wasps, bees, and ants. Of these, the honeybee has developed a unique behavior for communicating foraging information to hive mates in the form of dance behavior.

Honeybee dance

Foraging honeybees retain long-term memories of path integration cues after returning to the hive. Not only do they use this information on subsequent foraging trips, but they can also broadcast this information to other foragers. Upon returning to the hive, a successful forager will perform a dance behaviour, characterised by a looping figure-8 like pattern (Figure 3; von Frisch, 1967; Grüter and Farina, 2009). This dance conveys to the forager's hive mates information about both the distance and the direction of the food source they visited. The orientation of the zigzag portion communicates to observing hive mates the direction of the food relative to the sun's position, and the duration of the zigzag portion indicates the distance the food is from the hive.

This dance behavior also provides an example of associative learning in honeybees, albeit under natural conditions rather than under controlled experimental testing (Figure 1). Hive mates of the dancing forager will follow behind her during the dance and the dancer will regularly drop the scented nectar of flower patches she visited in her path (Figure 3). This behavior allows the followers to learn the scent of the flowers the dancer successfully visited.

Insert Figure 3 about here

Observational learning

The dance behavior of the successful forager and the information passed on to the following foragers provides examples of two abilities seen in honeybees: functionally referential communication and social learning. Functionally referential communication denotes signals that refer to events or objects in the world, such as the successful honeybee forager signaling where a food source can be found. Social learning is the ability of an animal to learn information or behaviors from another animal, typically a conspecific (member of the

same species). In this context, the observing honeybees are learning information about the spatial location of a profitable food source, yet a number of arthropods have been shown to observe and then duplicate behaviors of conspecifics in a range of contexts.

Social learning experiments commonly involve the separation of individuals into naive observers and experienced demonstrators. The observer, as the name suggests, observes, as the demonstrator, usually a trained or more experienced conspecific, performs a behavioral choice or test. When these observers are subsequently presented with the same behavioral choice or test, they are able to use information learned from the demonstrator in their own behavioral decisions. During mating, *Drosophila* females exhibit social learning by watching the mating behavior of other *Drosophila*, preferring to mate with males that have the same characteristics as demonstrator males that were observed to be successful at mating (Mery et al., 2009). This learning occurs even when the characteristics of the male flies have no influence on reproductive success.

Drosophila females also show social learning in their egg laying site preferences. Females who observe other females choose between two equally profitable egg-laying sites are more likely to choose the same site as the demonstrator for their own eggs (Battesti et al., 2012). In a foraging context, bumblebees learn which flowers are more rewarding by observing and then copying the flower preferences of other, more experienced foragers. In jumping spiders, when an observing spider watches a conspecific decline to attack a novel prey type, this spider will also show increased hesitation to attack this prey type when they experience them in the future.

Observers can even learn non-natural behavioral tasks demonstrated by trained conspecifics. In bumblebees, in what has been described as 'bumblebee football', researchers (Loukola et al., 2017) taught individuals to drag a wooden ball to a goal area in order to receive a food reward (example of associative learning). Once the demonstrator bee was trained, it performed this task in front of an observer bee multiple times. This observer was then presented the same test where it also successfully rolled the wooden ball into the goal. This research shows that not only are these animals capable of learning complex cognitive tasks, but they can also learn behavioral patterns that are not associated with their typical behavioral routines through observing and learning from other bumblebees.

Social learning can also extend to the individual recognition of nest mates. Remembering individual characteristics of a nest mate when living in large colonies containing thousands of members appears unlikely, but some arthropod species live in small colonies where recognizing specific nest mates may be valuable. Individual recognition can also be valuable in solitary species that regularly compete with other conspecifics for

resources. Individual recognition cues are commonly olfactory, with individuals remembering specific chemical cues associated with familiar conspecifics. Solitary hermit crabs learn the individual scents of previously encountered individuals and will link this scent with past experiences of that individual, specifically the quality of that conspecific's shell. In ants, unrelated queens learn the chemical cues of previously encountered queens and regulate their behavioral response based on this familiarity (D'Ettorre and Heinze, 2005). These recognition cues can also be visual; individuals learn the patterns or markings of conspecifics. Social paper wasps perform a form of individual recognition by learning the distinct black and yellow pattern on the front of conspecifics' faces, a visual cue associated with social dominance. When the facial patterns of a known conspecific are experimentally altered through painting, other individuals act more aggressively to the painted individual regardless of whether the paint created a higher or lower dominance signal (Sheehan and Tibbetts, 2011).

Distributed Cognition

A more recent field of cognitive study, distributed cognition, encompasses cognitive processes that reside outside of the organism's central nervous system and has been exhibited across a number of phyla, including arthropods (Cheng, 2018). One variety of distributed cognition is termed embodied cognition and covers cognition that occurs in areas of the organism's body outside the brain.

Embodied cognition

Female crickets showcase embodied cognition in their search for mates. Male crickets attract females through the production of songs, and females are attracted to males who produce the loudest of these songs. Females find their preferred mate by receiving these auditory cues through their ears, which in crickets are located on each of the front legs. These ears are connected through a tracheal tube. This tube allows the female to detect the direction of the sound's origin. Within the structure of the female cricket's ears and tracheal tube, there are a number of interneurons, which control her turning behavior. The act of identifying the loudest male's song and its direction occurs only in the structures of the female cricket's tracheal tube and the eardrums. Once these structures have processed the incoming information, the signal goes out to the motor system for the female cricket to turn in the direction of the chosen male song. This entire process occurs wholly outside the cricket's brain.

Drosophila fly larvae also show evidence of embodied cognition, through the offloading of the cognitive processes associated with locomotion to nerves along their body. *Drosophila* larvae have elongated, legless bodies, yet can crawl through the environment through coordinated body movements. These larvae are constantly searching for food using these coordinated body movements, and remarkably, input from the brain is not needed for the larvae to move. When the larvae's brain is blocked from sending signals to its body, the peripheral nerves along the body of the larvae will still coordinate the necessary movements, though directed movement to goals still requires the brain (Berni et al., 2012).

Extended cognition

Extensions of cognitive processes beyond the central nervous system can also encompass areas beyond the organism's body, including structures or objects created by the organism, a phenomenon called extended cognition (Cheng, 2018). Recent research (Japyassú and Laland, 2017) has explored extended cognition in the webs of spiders, proposing that changes in web tension represent the extension of the spider's cognitive processes into its web. As a spider's hunger level increases, it will adjust the tension level of the web's threads. When the web is tightened, smaller insects will cause disturbances that draw the spider's attention. When the spider is not very hungry, having recently eaten, it will loosen the web's threads, and these same smaller disruptions no longer draw the spider's notice. Thus the spider calibrates the web as a result of its current state, and the web in turn provides different levels of feedback to the builder based on this state. This link creates a flow of information in both directions between spider and web.

Conclusions

This chapter highlights the cognitive abilities exhibited by different arthropods. That members of this group have small brains does not limit their ability to draw upon complex behavioral repertoires across an array of contexts. Arthropods display high degrees of behavioral flexibility, with the ability to learn and retain a multitude of cues for use over both the short and long term. These attributes make this group highly valuable to researchers interested in cognition, and unsurprisingly, arthropod species such as the honeybee and *Drosophila* fly are model species for the study of learning and memory. Though some of this research has focused on modest cognitive processes such as associative learning, this chapter has also discussed more complex cognitive processes such as extended cognition, cue integration, and social learning. The study of arthropods can provide us with an understanding of how complex cognitive processes can be achieved by organisms with limited nervous

systems. Furthermore, the diversity of this phylum and the broad range of behaviors and ecology can help researchers uncover how such processes evolved.

Cross References

arthropod morphology
arthropod navigation
arthropod sensory systems
associative learning
cognitive maps
copying
Hymenoptera cognition
Hymenoptera sensory systems
insect cognition
insect sensory systems
invertebrates (Cnidaria)
learning
long-term memory
movement/Locomotion
navigation
olfactory cues
olfactory discrimination
olfactory perception
retention
short-term memory
social learning
spatial memory
working memory
visual perception
visual recognition
visual search

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Figure Captions

Figure 1 Images of proboscis extension response testing in restrained honeybees. Individual bees are immobilized inside of metal restraining tubes where only the head, mouth (proboscis) and antennae are free. During classical conditioning testing, the bee is presented a conditioned stimuli (typically a neutral odor) and then a small amount of sugar water (unconditioned stimulus) is delivered to the antennae and proboscis via a small spoon. After multiple pairings of these stimuli, the bee will extend its proboscis when the odor is presented alone. Image printed with permission from Dr. Randolph Menzel.

Figure 2 An example of a complete foraging trip in the Tunisian desert ant, *Cataglyphis fortis*, which inhabits a salt pan environment with few landmarks. The forager's journey begins at the nest location in the bottom right corner, represented by an open circle. The thin line extending from the nest location represents the winding outbound path of the forager as she searches for food, with the small black circles marking her location at 60-second intervals. The large black circle indicates the location at which the forager found food. The thick line represents the straight line inbound route back to the nest. The forager kept track of both its distance and direction from the nest during the outbound route through path integration,

allowing for an efficient return to the nest entrance. Image Reprinted from: Insect navigation: use of maps or Ariadne's thread, Wehner and Wehner (1990) (Fig. 4), published in *Ethology, Ecology & Evolution* by Taylor & Francis and reprinted with the publisher's permission (Taylor & Francis Ltd, <http://www-tandfonline-com>).

Figure 3 An example of the figure eight honeybee waggle dance with both the dancer (center individual) and follower bees (bottom four individuals). The dancer bee (1) performs the waggle portion of the dance, represented by the jagged line, and then turns to one side, here the left, and loops (2) back to the start of the waggle portion. At the start of the waggle portion (3), the dancer performs a second waggle run (4) and then typically loops in the opposite direction, in this case to the right (5). This dance provides follower bees multiple pieces of information, including the direction, distance, and quality of the potential food source. Image Reprinted from: The honeybee waggle dance: can we follow the steps?, Grüter & Farina (2009) (Fig. 1), published in *Trends in Ecology & Evolution* by Elsevier and reprinted with the publisher's permission (Elsevier Publishing, <https://www.elsevier.com/>).

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Freas, C.A., Whyte, C. & Cheng, K. (2017) Skyline retention and retroactive interference in the navigating Australian desert ant, *Melophorus bagoti*, *Journal of Comparative Physiology A*, vol. 203, pp. 353–367.

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Author for correspondence:

Cody A. Freas

e-mail: freascody@gmail.com

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Polarized light use in the nocturnal bull ant, *Myrmecia midas*

Cody A. Freas, Ajay Narendra, Corentin Lemesle and Ken Cheng

Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia

CAF, 0000-0001-7026-1255; AN, 0000-0002-1286-5373

Solitary foraging ants have a navigational toolkit, which includes the use of both terrestrial and celestial visual cues, allowing individuals to successfully pilot between food sources and their nest. One such celestial cue is the polarization pattern in the overhead sky. Here, we explore the use of polarized light during outbound and inbound journeys and with different home vectors in the nocturnal bull ant, *Myrmecia midas*. We tested foragers on both portions of the foraging trip by rotating the overhead polarization pattern by $\pm 45^\circ$. Both outbound and inbound foragers responded to the polarized light change, but the extent to which they responded to the rotation varied. Outbound ants, both close to and further from the nest, compensated for the change in the overhead e-vector by about half of the manipulation, suggesting that outbound ants choose a compromise heading between the celestial and terrestrial compass cues. However, ants returning home compensated for the change in the e-vector by about half of the manipulation when the remaining home vector was short (1–2 m) and by more than half of the manipulation when the remaining vector was long (more than 4 m). We report these findings and discuss why weighting on polarization cues change in different contexts.

1. Background

Arthropods are known to derive compass information using the pattern of polarized skylight [1–9]. Polarized light comprises light waves in which the wave occurs along a single plane. Light scatters after entering the earth's atmosphere and becomes partially linearly polarized. This creates an e-vector pattern in the sky arranged in concentric circles around the sun or moon's position [10,11]. The e-vector in the overhead sky remains in a stable orientation pattern perpendicular to the direction of the sun/moon. This stability makes the sky's polarization

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pattern a useful directional cue especially when the sun or moon's position is obscured [8,9,12–16]. Insects detect this polarized light through specialized photoreceptors that are located in the dorsal rim area of the eye [2,15–19].

Solar polarization is present even after sunset until the end of astronomical twilight when the sun's position passes 18° below the horizon [20]. During the evening or morning twilight, when the sun is near the horizon, the polarization pattern of the sky intensifies and simplifies along the North–South axis [21], making it of great interest to understand how animals that are active during twilight use this compass cue [3,5,9,12,16]. Among ants, there has been only one study [6] conducted on twilight-foraging animals [22,23]. In this study, outbound foragers of *Myrmecia pyriformis* confronted with a change in the polarization pattern by $\pm 45^\circ$ to the ambient pattern, modified their orientation, but only partially (17.96°). The authors suggested that this partial reliance was due to the extreme reliance on familiar visual landmarks that these ants exhibit [6,24]. Here, we investigate this further in a related nocturnal ant, *Myrmecia midas*, whose navigational capabilities have only recently been studied [25], in order to identify whether foragers use the pattern of polarized skylight during both the outbound and inbound journeys. We further explore whether the extent to which ants rely on polarized light changes with distance from the nest or length of the home vector during both outbound and inbound journeys.

2. Methods

Experiments were conducted from September 2015 to November 2016 on two *M. midas* nests located on the northern portion of the Macquarie University North Ryde campus in Sydney, Australia ($33^\circ 46' 11''$ S, $151^\circ 06' 40''$ E). *Myrmecia midas* nests were found in habitats consisting of stands of *Eucalyptus* trees with mostly barren understoreys with the nest entrance located near the base of a tree. Nocturnal foraging activity in this species [25] required the use of red-filtered headlamps in order to observe the ants. Research in ants does not require animal ethical approval within Australia. We modified the pattern of polarized skylight by rotating a polarization filter (42 cm diameter) above the ants. The polarization filter (Polaroid HN22; figure 1) was held by a circular 2 cm thick metal ring and lifted 10 cm off the ground by four equally spaced thin metal legs. Numbers of ants tested in each condition are given in the data figures. All testing was conducted during either the evening or morning twilight when the sun's position was between -18° and 0° relative to the horizon. Evening testing began 10 min after sunset and ceased before twilight ended. Morning testing began after the beginning of twilight and ceased before dawn. Each night we obtained the sun's position at sunset and sunrise from the Astronomical Almanac (<http://asa.usno.navy.mil>) and set the ambient e-vector 180° from this direction. As *M. midas* maintain predictable nest-foraging tree route patterns in a well-defined corridor, we were able to pinpoint the orientation of the overhead e-vector and rotate the polarizer relative to that direction. We relied on a compass to locate the ambient e-vector and rotate the polarizer by $\pm 45^\circ$ from this direction. When placing the polarized filter over the forager, we rested the compass on the polarizer along the filter's polarization pattern during placement. Only after the placement was confirmed did we remove the compass.

2.1. Outbound ants at different distances from the nest

We tested foragers at two distances, 4–6 m and 1–2 m from the nest. For the 4–6 m group, we chose foragers from two nests (Nest 1 and Nest 2), where some foragers travelled 12.8 m and 14.0 m from the nest to their foraging trees. For the 1–2 m group, we chose a separate group of foragers from Nest 1 that travelled 3 m to a foraging tree. We followed each forager and placed the centre of the polarizer over the ant when it was at 4–6 m or 1–2 m from the nest. In both conditions, the e-vector axis of the filter was oriented either $\pm 45^\circ$ relative to the dominant ambient polarization pattern (figure 1a), a method adapted from Reid *et al.* [6]. For each individual, we recorded the initial orientation, the exit orientation and their reorientation by placing small pegs in the ground (as defined in figure 1b). After a forager's positions were recorded, the forager was collected and marked with a small amount of enamel paint (Tamiya™, Japan) to ensure animals were not tested again. These marked foragers were then returned to the nest site.

2.2. Inbound ants at different distances from the nest

We tested inbound foragers at the same two distances (4–6 m and 1–2 m) from the nest. We followed foragers from Nest 1 travelling either 14 m (4–6 m condition) or 3 m (1–2 m condition) to their foraging tree during evening twilight. As a forager climbed the foraging tree, they were each collected in a plastic

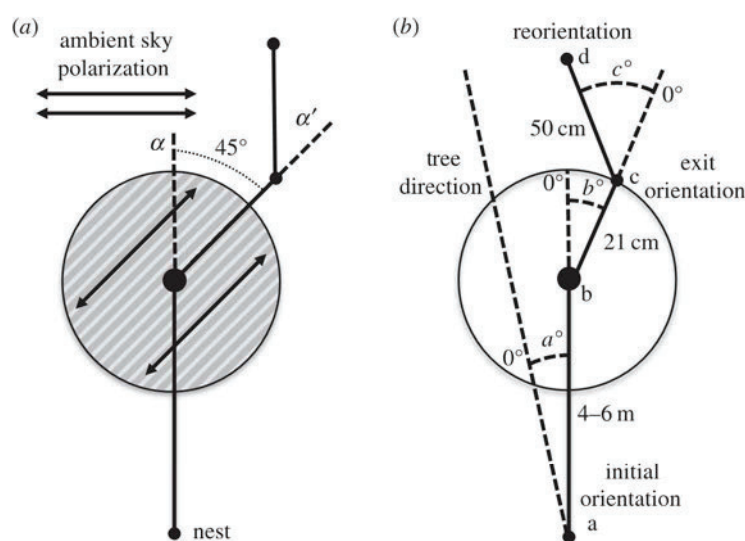


Figure 1. Schematics of the polarization filter and experimental set-up. (a) Diagram of the polarization filter. During the forager's outbound trip to the foraging tree, a polarization filter was placed over the forager with the polarization e-vector rotated $\pm 45^\circ$ of the ambient e-vector. This filter apparatus was used in a previous study [6]. (b) Diagram of measurements collected during polarization filter test. Measurements were made using a compass application on a smartphone. Initial orientation routes were measured from the nest entrance (a) to when the polarization filter was centred over the forager (b). Initial route directions (a°) were calculated with the tree direction from the nest as 0° . The magnitude of angle a has been artificially enlarged in this diagram for clarity, with angle a averaging 4.42° across all conditions during testing. Exit orientations were measured from the centre of the polarization filter (b) to the exit location of the ant on the filter's edge (c). Route directions under the filter (b°) were calculated from the forager's initial route direction. Reorientations were measured from the forager's exit location from the polarization filter (c) to the forager's path 50 cm after exiting the filter (d). Reorientation route directions (c°) were calculated from the under-filter route direction.

phial. Each forager was offered a small amount of honey and was then stored overnight in the dark (9 h). Each collected ant was marked with a small amount of enamel paint to exclude previously tested individuals. Foragers were released at the base of their foraging tree in the pre-dawn twilight, which corresponds to the time at which they typically return home [25]. We followed each ant as it travelled to the nest, and placed the centre of the polarizer on the ant when it reached a distance of 4–6 m or 1–2 m from the nest. Therefore, both inbound and outbound foragers were tested at the same distance from the nest. Similar to the outbound tests, the e-vector axis of the filter was oriented either $\pm 45^\circ$ relative to the dominant ambient polarization pattern. We recorded the initial orientation, the exit orientation and the reorientation of each forager in the same manner as in the outbound tests (figure 1b). Foragers were then followed for the remainder of their inbound path to ensure they returned to the nest site.

2.3. Conflict between home-vector length and nest location

Here, we tested individual foragers close to their nest but with a large remaining vector. We achieved this by first following foragers from Nest 1 to the foraging tree (14 m) in the evening twilight and collected them in a phial as they reached the foraging tree. Just as in previous inbound conditions, these foragers were fed, marked with paint, held overnight and released in the pre-dawn twilight. We released the foragers on the route at the halfway point between the nest and the foraging tree (7 m). Released foragers were allowed to return to 1–2 m from the nest entrance where the centre of the polarizer was placed over the ant. As with all previous conditions, the e-vector axis of the filter was either $\pm 45^\circ$ relative to the ambient e-vector. Identical to previous conditions, we recorded initial orientation, exit orientation and reorientation for each forager (figure 1b). Foragers were then followed for the remainder of their inbound path to record their final destination.

2.4. Statistical analysis

Data were analysed with circular statistics [26,27] using the statistics package Oriana Version 4 (Kovach Computing Services, UK). As each ant had a different initial heading direction, we corrected this by

designating the initial heading as 0° for each animal. The *shift magnitude* of each path was calculated by taking the mirror of the difference between the forager's initial path direction and the forager's exit orientation in each -45° condition. This calculation allowed us to compare path shifts in both directions in degrees. Foragers' shift magnitudes were compared between the $\pm 45^\circ$ and between the two distance groups using Watson–Williams *F*-tests. If shift magnitudes between the two groups do not differ, then it means both groups rely on polarized light to the same degree. A Pearson's correlation coefficient was used to test the association between the lunar phase (in per cent) and shift magnitude under the filter. Lunar phase data were obtained from calculations in the Astronomical Almanac (<http://asa.usno.navy.mil>).

3. Results

3.1. Outbound ants at different distances from the nest

When the polarization filter was placed on an outbound ant at both testing distances, they initially stopped moving and then slowly began to move in a chosen direction. Most foragers would again stop as they reached the edge of the filter and performed visual scans before continuing on their chosen path. Ants did not pause after exiting the filter and continued on route towards their foraging tree.

3.1.1. Outbound foragers at the 4–6 m distance

When the polarizer was rotated left (-45°), the ants' exit orientations were to the left of their initial direction of orientation (mean \pm s.e.m.; Nest 1: $\theta = -26.37 \pm 4.72^\circ$; Nest 2: $\theta = -32.16 \pm 5.26^\circ$; table 1 and figure 2a(i),b(i)), and these changes were significant at both nests (Watson–Williams *F*-test, Nest 1: $F = 22.01$, $p \ll 0.01$; Nest 2: $F = 13.74$, $p \ll 0.01$). Conversely, when the polarizer was rotated right ($+45^\circ$), the foragers' exit orientations were to the right of their initial heading direction (mean \pm s.e.m.; Nest 1: $\theta = 17.47 \pm 5.47^\circ$; Nest 2: $\theta = 25.07 \pm 7.46^\circ$; table 1 and figure 2a(i),b(i)). These changes were also significant at both nests (Watson–Williams *F*-test, Nest 1: $F = 13.74$, $p \ll 0.01$; Nest 2: $F = 9.62$, $p \ll 0.01$). After exiting the -45° rotated filter, foragers reoriented significantly to the right (Watson–Williams *F*-test, Nest 1: $F = 18.25$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 25.63^\circ \pm 5.11^\circ$; Nest 2: $F = 9.65$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 23.205 \pm 6.57^\circ$; table 1 and figure 2a(ii),b(ii)). After exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams *F*-test, Nest 1: $F = 12.57$, $p \ll 0.01$, mean \pm s.e.m. $\theta = -19.24 \pm 5.76^\circ$; Nest 2: $F = 5.83$, $p = 0.02$, mean \pm s.e.m. $\theta = -26.34 \pm 5.26^\circ$; table 1 and figure 2a(ii),b(ii)). Results did not differ between nests ($p > 0.05$ for both filter exit orientations and foragers' reorientations), and shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams *F*-test, Nest 1: $F = 0.17$, $p = 0.68$; Nest 2: $F = 0.194$, $p = 0.66$).

3.1.2. Outbound foragers at the 1–2 m distance

When the polarizer was rotated $\pm 45^\circ$, individuals paused after the polarizer was placed overhead. After this short pause, most individuals continued to the foraging tree ($+45^\circ$, $n = 18$; -45° , $n = 22$; figure 2c closed circles); a minority of individuals in both conditions, however, turned back and retreated (defined as individuals that returned to within 30 cm of the nest entrance after exiting the filter) to the nest after the polarizer was placed over them ($+45^\circ$, $n = 8$; -45° , $n = 5$; figure 2c open circles). Focusing on only those individuals that continued to the foraging tree, when the polarizer was rotated 45° to the left (-45°), the foragers' exit-orientations leaving the filter were to the left of their initial path direction (mean \pm s.e.m.; Nest 1: $\theta = -18.26 \pm 6.56^\circ$; table 1 and figure 3). This path change under the filter was significant (Watson–Williams *F*-test, Nest 1: $F = 4.31$, $p = 0.04$). When the polarizer was rotated 45° to the right ($+45^\circ$), forager exit orientations were to the right of their initial path (mean \pm s.e.m.; Nest 1: $\theta = 32.81 \pm 6.4^\circ$; table 1 and figure 3) and this shift was also significant (Watson–Williams *F*-test, Nest 1: $F = 12.29$, $p \ll 0.01$). After exiting the -45° rotated filter the foragers reoriented significantly to the right (Watson–Williams *F*-test, Nest 1: $F = 9.95$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 26.23 \pm 6.73^\circ$; table 1 and figure 2c), and after exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams *F*-test, Nest 1: $F = 10.79$, $p \ll 0.01$, mean \pm s.e.m. $\theta = -29.43 \pm 5.87^\circ$; table 1 and figure 2c). While the number of individuals who retreated was insufficient for statistical analysis, foragers' exit orientations shifted as would be expected, either to the left (mean \pm s.e.m. $\theta = -35.34 \pm 26.60^\circ$) or to the right (mean \pm s.e.m. $\theta = 37.14 \pm 9.33^\circ$) of the nest entrance direction corresponding with manipulations in the polar filter (-45° or $+45^\circ$, respectively). Shift magnitude was not significantly different between the -45° and $+45^\circ$

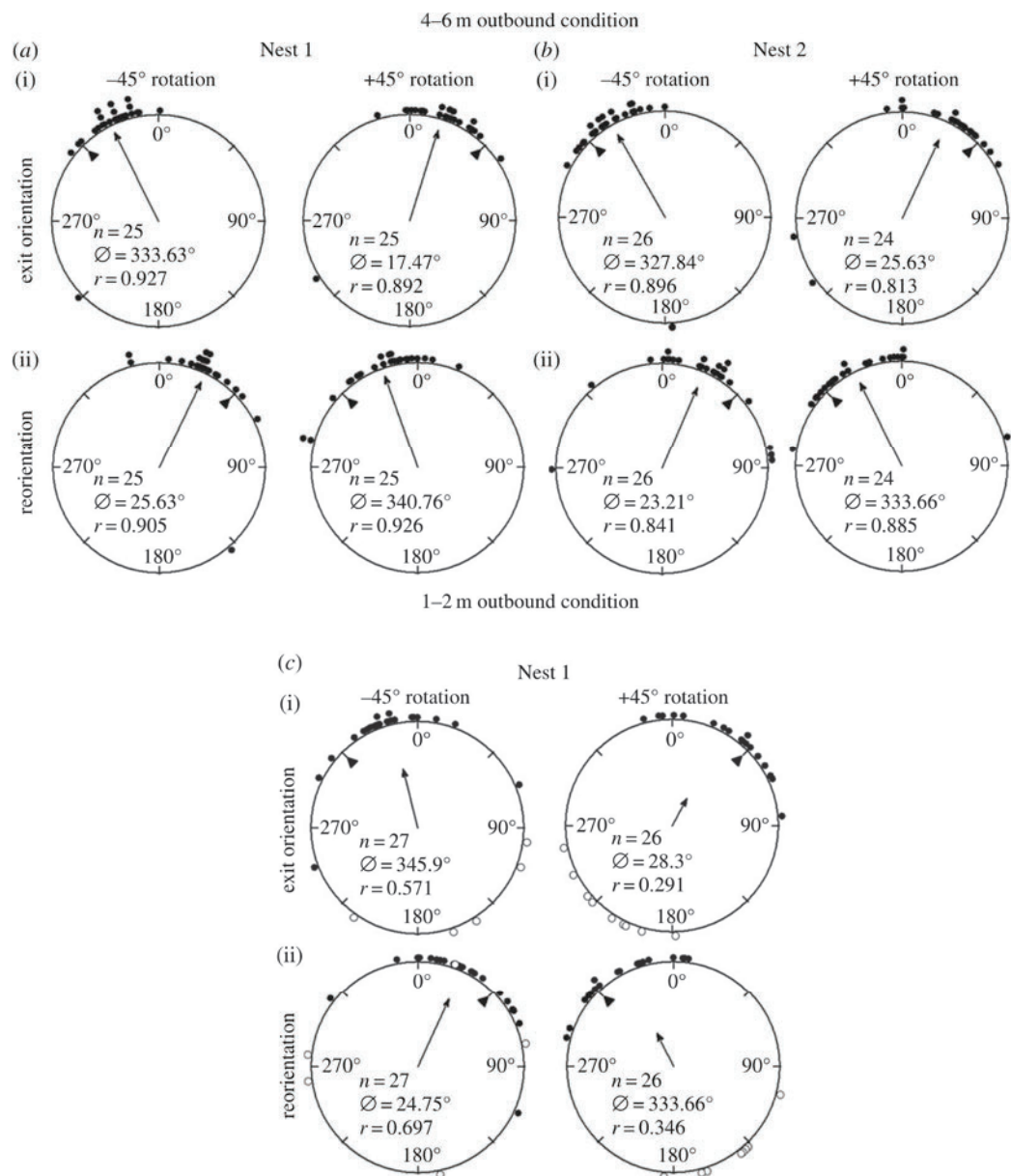


Figure 2. Circular distributions of individual *M. midas* foragers' headings during outbound conditions. Histograms show raw data of exit orientation under the filter and the reorientation after exiting the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector and the mean direction. (a) Orientations for Nest 1 during the 4–6 m outbound condition. (b) Orientations for Nest 2 during the 4–6 m outbound condition. (c) Orientations for Nest 1 during the 1–2 m outbound condition. Closed circles indicate individuals that continued on to the foraging tree after testing. Open circles represent foragers that retreated once the filter was placed overhead and these individuals returned to within 30 cm of the nest entrance after testing. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

conditions (Watson–Williams F -test; 0.65 , $p = 0.43$). When the -45° and $+45^\circ$ shifts were combined, the shift magnitude was also not significantly different between the outward heading ants of the two outbound testing conditions (Watson–Williams F -test, $F = 0.17$, $p = 0.68$).

3.2. Inbound ants at different distances from the nest

When foragers were released back at the base of their foraging tree during the morning twilight, they also initially paused for a brief period and scanned the environment without translation, before travelling in the nest direction. Nest-bound foragers typically paused again once the polarization filter was placed

Table 1. Statistics of outbound and inbound forager shifts under polar filter and reorientations.

condition	mean vector	95% CI		mean vector length	Rayleigh test	
	μ (°)	minus (°)	plus (°)	r	Z	p
<i>outbound 4–6 m</i>						
Nest 1						
exit orientation						
–45°	333.63	324.86	342.40	0.927	21.46	<0.0001
+45°	17.47	6.5	28.2	0.892	19.89	<0.0001
reorientation						
–45°	25.63	15.61	35.66	0.905	20.47	<0.0001
+45°	340.76	331.94	349.50	0.926	21.45	<0.0001
Nest 2						
exit orientation						
–45°	330.3	320.05	340.55	0.896	20.92	<0.0001
+45°	25.03	10.44	39.62	0.813	15.88	<0.0001
reorientation						
–45°	23.21	10.33	36.08	0.841	18.41	<0.0001
+45°	333.66	322.36	344.96	0.885	18.81	<0.0001
<i>outbound 1–2 m</i>						
exit orientation						
–45°	345.90	321.71	10.10	0.571	8.81	<0.0001
+45°	28.25	336.79	79.71	0.291	2.28	0.102
reorientation						
–45°	24.75	6.37	43.13	0.697	13.01	<0.0001
+45°	333.66	290.04	17.27	0.346	3.11	0.043
<i>outbound 1–2 m non-retreaters</i>						
exit orientation						
–45°	346.47	334.18	358.76	0.897	14.50	<0.0001
+45°	32.81	20.26	45.37	0.893	14.36	<0.0001
reorientation						
–45°	23.36	10.17	36.55	0.883	14.03	<0.0001
+45°	330.56	319.07	342.06	0.91	14.90	<0.0001
<i>inbound 4–6 m</i>						
exit orientation						
–45°	318.84	307.03	330.64	0.940	12.38	<0.0001
+45°	34.13	26.27	42.00	0.973	13.256	<0.0001
reorientation						
–45°	35.29	20.24	50.33	0.904	11.54	<0.0001
+45°	319.82	306.80	332.84	0.928	12.05	<0.0001
<i>inbound 1–2 m</i>						
exit orientation						
–45°	335.14	326.62	343.66	0.955	14.592	<0.0001
+45°	19.73	10.16	29.30	0.957	13.73	<0.0001

(Continued.)

Table 1. (Continued.)

condition	mean vector	95% CI		mean vector length	Rayleigh test	
	μ (°)	minus (°)	plus (°)	r	Z	p
reorientation						
−45°	27.59	15.31	39.86	0.909	14.592	<0.0001
+45°	332.65	320.65	344.64	0.933	13.06	<0.0001
inbound vector and landmark conflict						
exit orientation						
−45°	324.23	313.75	334.71	0.953	12.28	<0.0001
+45°	39.42	24.20	54.64	0.902	11.40	<0.0001
reorientation						
−45°	36.49	24.31	48.67	0.936	12.28	<0.0001
+45°	327.12	311.87	342.37	0.902	11.39	<0.0001

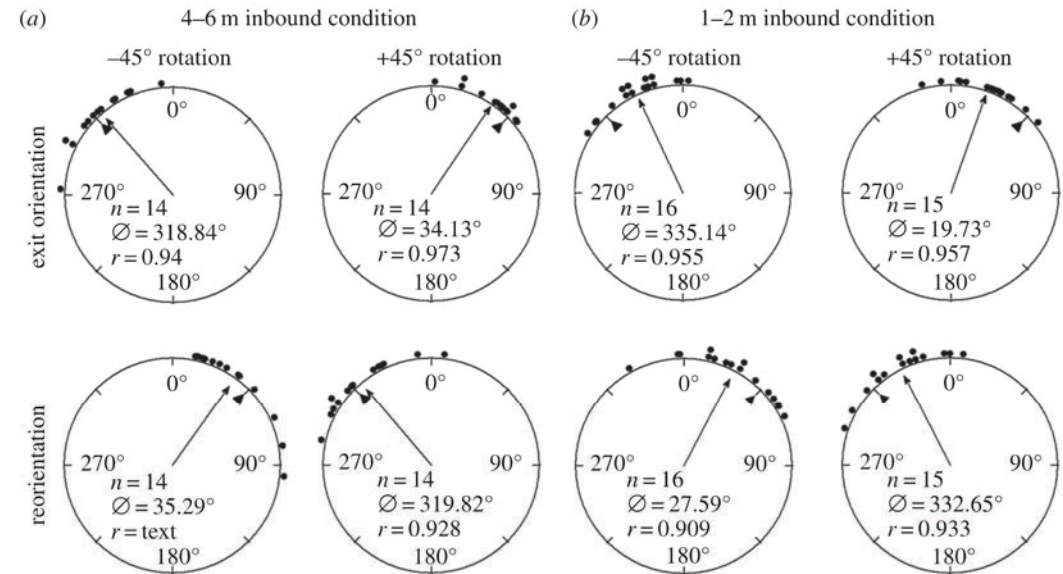


Figure 3. Circular distributions of individual *M. midas* foragers' headings during the inbound conditions. Histograms show raw data of exit orientation under the filter with the individual's initial orientation and reorientation with the forager's exit orientation under the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector and mean direction. (a) Orientations for Nest 1 during the 4–6 m inbound condition. (b) Orientations for the 1–2 m inbound condition. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

above them, yet some individuals continued their forward movement. The same behavioural difference occurred at the filter edge, as some inbound foragers did not stop at the edge of the polarizer. After exiting the filter, all foragers continued on to the nest entrance and entered the nest.

3.2.1. Inbound foragers at the 4–6 m distance

When the polarizer was rotated to the left (−45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm s.e.m.; Nest 1: $\theta = -41.16 \pm 6.02^\circ$; table 1 and figure 3a), and these changes were significant (Watson–Williams F -test, Nest 1: $F = 39.17$, $p \ll 0.01$). When the polarizer was rotated to the right (+45°), foragers' exit orientations were to the right of their initial heading direction (mean \pm s.e.m.; Nest 1: $\theta = 34.13 \pm 4.01^\circ$; table 1 and figure 3a), and these changes were significant (Watson–Williams F -test, Nest 1: $F = 50.57$, $p \ll 0.01$). After exiting the −45° rotated filter,

the foragers reoriented significantly to the right (Watson–Williams *F*-test, Nest 1: $F = 29.07$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 35.29 \pm 6.02^\circ$; table 1; figure 3a). After exiting the $+45^\circ$ rotated filter the foragers reoriented significantly to the left (Watson–Williams *F*-test, Nest 1: $F = 62.51$, $p \ll 0.01$, mean \pm s.e.m. $\theta = -40.18 \pm 6.64^\circ$; table 1; figure 3a). Shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams *F*-test, $F = 1.12$, $p = 0.301$).

3.2.2. Inbound foragers at the 1–2 m distance

When the polarizer was rotated to the left (-45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm s.e.m.; Nest 1: $\theta = -24.86 \pm 4.35^\circ$; table 1 and figure 3b), and these changes were significant (Watson–Williams *F*-test, Nest 1: $F = 23.51$, $p \ll 0.01$). When the polarizer was rotated to the right ($+45^\circ$), foragers' exit orientations were to the right of their initial heading direction (mean \pm s.e.m.; $\theta = 19.73 \pm 4.88^\circ$; table 1 and figure 3b), and these changes were significant (Watson–Williams *F*-test, $F = 18.59$, $p \ll 0.01$). After exiting the -45° rotated filter the foragers reoriented significantly to the right (Watson–Williams *F*-test, $F = 20.84$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 27.59 \pm 4.35^\circ$; table 1 and figure 3b). After exiting the $+45^\circ$ rotated filter, the foragers reoriented significantly to the left (Watson–Williams *F*-test, Nest 1: $F = 21.25$, $p \ll 0.01$, mean \pm s.e.m. $\theta = -27.35 \pm 6.12^\circ$; table 1 and figure 3b). Shift magnitude under the filter was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams *F*-test, $F = 0.65$, $p = 0.43$). When the -45° and $+45^\circ$ shifts were combined, total shift magnitude in foragers tested at 1–2 m was significantly smaller than foragers tested at 4–6 m (Watson–Williams *F*-test, $F = 10.93$, $p \ll 0.01$).

3.3. Conflict between home-vector length and nest location

Inbound ants with 14 m home vectors were displaced on the route but half way home and had to travel only 7 m to find the nest. The ability of these ants to detect a change in the pattern of the polarized light was assessed at 1–2 m from the nest entrance. When the polarizer was rotated to the left (-45°), the foragers' exit orientations were to the left of their initial direction of orientation (mean \pm s.e.m.; Nest 1: $\theta = -35.77 \pm 5.35^\circ$; table 1 and figure 4), and these changes were significant (Watson–Williams *F*-test, Nest 1: $F = 50.78$, $p \ll 0.01$). When the polarizer was rotated to the right ($+45^\circ$), foragers' exit orientations were to the right of their initial heading direction (mean \pm s.e.m.; $\theta = 39.42 \pm 7.77^\circ$; table 1 and figure 4), and these changes were significant (Watson–Williams *F*-test, $F = 29.09$, $p \ll 0.01$). After exiting the -45° rotated filter, the foragers reoriented significantly to the right (Watson–Williams *F*-test, $F = 37.59$, $p \ll 0.01$, mean \pm s.e.m. $\theta = 36.49 \pm 6.21^\circ$; table 1 and figure 4). After exiting the $+45^\circ$ rotated filter, the foragers reoriented significantly to the left (Watson–Williams *F*-test, Nest 1: $F = 15.67$, $p \ll 0.01$, mean \pm s.e.m. $\theta = -32.88 \pm 7.78^\circ$; table 1 and figure 4). Shift magnitude size was not significantly different between the -45° and $+45^\circ$ conditions (Watson–Williams *F*-test, $F = 0.18$, $p = 0.677$). Foragers in this condition showed shift magnitude size similar to that of foragers tested 4–6 m from the nest entrance (Watson–Williams *F*-test, $F \ll 0.01$, $p = 0.99$), and these combined shift magnitudes were significantly greater than foragers tested at the 1–2 m vector travelling from a tree 3 m away (Watson–Williams *F*-test, $F = 8.35$, $p \ll 0.01$).

3.4. Lunar phase

Across all conditions, lunar phase was not associated with changes in shift magnitude of foragers while under the filter (Pearson's correlation coefficient, $r = -0.127$, $p = 0.074$).

4. Discussion

In this study, both inbound and outbound foragers changed their heading direction in response to changes in the overhead polarization pattern. In outbound foragers, we found that distance away from the nest did not influence the weighting foragers gave to this cue. Conversely, ants rely most on the pattern of the polarized skylight when they are returning home (inbound) and have a long accumulated vector (4–6 m).

In *M. pyriformis*, use of the polarization cue was tested only in outbound ants close to the nest [6]. Here, when the polarized filter was rotated by $\pm 45^\circ$, ants changed their heading direction in the appropriate direction but by less than half of the rotation (-21.8° for -45° rotation; $+14.1^\circ$ for $+45^\circ$ rotation). In our study, the outbound *M. midas* ants at both 1–2 m and 4–6 m away from the nest (figure 2)

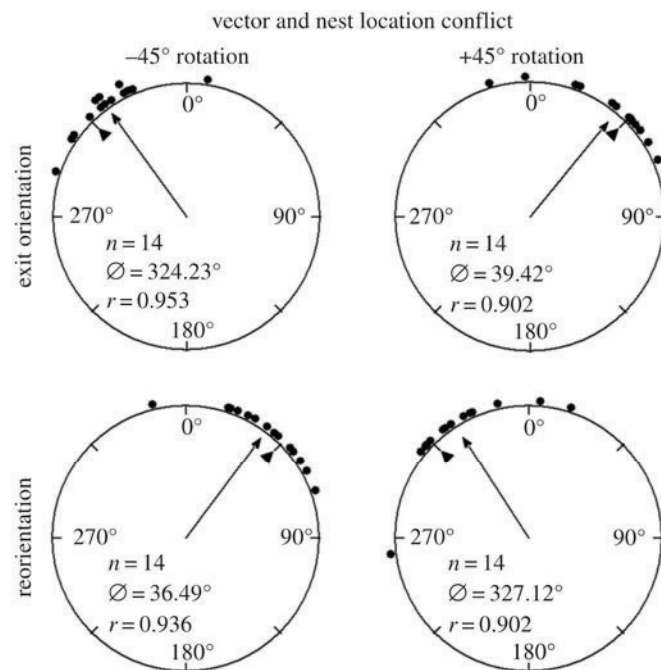


Figure 4. Circular distributions of individual *M. midas* foragers' headings during the long-vector 1–2 m inbound condition. Histograms show raw data of exit orientation under the filter with the individual's initial orientation and reorientation with the forager's exit orientation under the filter. The triangle denotes 45° in each distribution. The arrow denotes the length of the mean vector direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

compensated for the change in the overhead e-vector by about half of the manipulation (1–2 m group: -18.26° for -45° ; 32.81° for $+45^\circ$; 4–6 m group: -26.37° (Nest 1) and -32.16° (Nest 2) for -45° ; 17.47° (Nest 1) and 25.07° (Nest 2) for $+45^\circ$). Both species appear to choose a compromise heading direction between the celestial and terrestrial compass cues during their outbound journey, and this appears to hold true at different distances from the nest.

In the inbound condition, we found that foragers of *M. midas* tested 1–2 m from the nest compensated for the change in the overhead e-vector by about half of the manipulation in their heading (-24.86° for -45° and 19.73° for $+45^\circ$, figure 3a). Interestingly, unlike in the outbound conditions, inbound *M. midas* ants tested at 4–6 m from the nest compensated for well over half of the e-vector manipulation in their altered heading (-41.16° for -45° and 34.13° for $+45^\circ$, figure 3b). Such large compensation was also found in inbound foragers that had a 14 m home vector but were released half way home and tested close (1–2 m) to the nest (-35.77° for -45° and 39.42° for $+45^\circ$, figure 4). This shows that inbound ants respond more to a change in the pattern of polarized skylight than outbound ants. These results imply that inbound ants weight polarization cues differently: ants with a longer home vector respond more to a change in the polarization pattern.

Our results suggest that foragers use both terrestrial and celestial cues, but the weighting of these cues appears to change with the ant's foraging context. In this study, *M. midas* foragers en route appear to weight vector cues in combination with the surrounding terrestrial cues, shifting their paths significantly under an altered polarization pattern. Yet when *M. midas* foragers are displaced to a local area with a vector direction conflicting with the surrounding terrestrial cues, individuals ignore the accumulated vector and orient using only the terrestrial cues [25]. Thus, it appears that nocturnal *Myrmecia* ants use the pattern of the polarized skylight only when the readouts of the celestial and terrestrial cues align. Furthermore, when there is a conflict between the two sources of compass cues, the celestial cues are suppressed or ignored [25]. This further implicates navigational context as a factor in celestial cue weighting. These behavioural differences may arise as foragers in the polarization experiment encounter no mismatch in cue sets before the polarized light filter, causing them to respond to the altered polarized light pattern while under the filter. Whereas after displacements off-route, foragers are presented with a mismatch between the familiar visual territory and their stored views, causing them to ignore celestial compass information when returning home [25].

The significant differences in shift magnitude in inbound foragers under different conditions were not predicted. Inbound foragers travelling from long distances (14 m, longest foraging route at this site) show larger shifts under the filter compared with individuals that forage in trees closer to the nest regardless of proximity to the nest. These disparities suggest greater weight is being placed on the polarization compass when in conflict with terrestrial cues in these foragers. It appears that the proximity of the nest tree at the test location, a potentially salient terrestrial cue, does not decrease the observed shifts in these far-foraging, long-vector individuals, implying that vector length clearly influences the weight given to the polarization pattern cue. These increases align with the hypothesis that with longer accumulated vectors, ants put more weight on these vector cues [28]. In our case, this difference in weighting persists even after a 9 h delay, with the direction of polarized light, linked to the position of the sun, having changed. These delay periods align with this species' foraging ecology as foragers typically spend this period on their foraging tree overnight [25]. Our results also align well with those from our previous *M. midas* study where only long-vector (more than 5 m) individuals show any evidence of orientation using path integration after distant displacement [25]. It may also be possible that, as tree fidelity has not been studied in this species, there may be some other difference between individuals that forage further from the nest site and those that forage at a nearer tree. These differences could include disparities in visual scenes encountered by these two foraging groups at Nest 1 or potentially even genetic differences between foragers travelling different distances to the nest. Further study into these behavioural choices is merited to untangle these possibilities.

Furthermore, it is interesting that the large shifts in long-vector inbound foragers are not seen in outbound foragers travelling to the same foraging tree. Vector memories in these outbound foragers are based on past foraging trips, whereas inbound foragers are using the vector memory of the current foraging trip [29,30]. This discrepancy may influence the weight these individuals give the vector cue. Unfortunately, as Nest 2 has since died, our field site currently has only one known nest with individuals foraging long distances (more than 5 m), making study of these differences difficult. Further study into this species and its use of celestial cues for navigation is warranted.

It is worth noting that the observed heading directions in both outbound and inbound foragers could be in part due to visual changes caused by the filter, independent of the e-vector rotation. Beyond the e-vector shift, light intensity levels are reduced, and there are changes in the visibility and salience of both celestial and terrestrial cues under the filter. These changes could alter the weighting of cues in this study compared to foragers navigating under natural conditions.

5. Conclusion

We show that both outbound and inbound *M. midas* foragers respond to changes of the e-vector orientation. Outbound ants compensate only partially to the change in polarized light, and this holds true at different distances from the nest. Inbound foragers with a longer home vector respond almost fully to the change in the pattern of the polarized skylight.

Data accessibility. Our data are collected in the electronic supplementary material.

Authors' contributions. Experiments conceived and designed by C.A.F., A.N. and K.C. All data were collected by C.A.F. and C.L. C.A.F. analysed the data. C.A.F., A.N. and K.C. drafted and revised paper. All authors have given final approval for this publication.

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