

A Panoramic Virtual Reality System For Visual Navigation Studies In Solitary Foraging Ants



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Declaration page for the Masters of Research thesis

This thesis is written in the form of a journal article from *Behavioural Processes*.

Declaration

I wish to acknowledge the following assistance in the research detailed in this report:

I received assistance from Tina Peckmezian and Greg Hunsburger in installing and calibrating the Fictrac software. Tina and Greg also created the template for the virtual world inside the Unity gaming software so that I could add different panoramas into the scene. Dr. Ken Cheng provided comments and suggestions on the project and this manuscript.

All other research described in this report is my own original work.

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Abstract

Solitary foraging ant species can employ an array of navigational tools to successfully return to the nest from foraging trips, including path integration and vision-based navigation. Species inhabiting cluttered environments that are visually rich, like *Melophorus bagoti* and multiple *Myrmecia* species, rely heavily on cues from these visual landmarks and the surrounding panorama. Additionally, these ant species can utilise the sky compass to orient by detecting both the sun's position and the sky's UV and polarised light gradients. Multiple characteristics of landmarks, the surrounding panorama and the sky compass cannot be realistically manipulated in the natural environment but can be readily manipulated by using a virtual reality (VR) environment under laboratory conditions. Here we present a novel virtual reality system that projects a visual panorama using commercially available projectors. This system provides a 300° visual panorama and includes a spherical treadmill system to record the individual's movement and orientation. We present a thorough description of the virtual reality system and demonstrate the system's performance by comparing the orientation in the virtual reality system and the natural environment. Individuals in virtual reality conditions did not orient towards the nest, suggesting the VR system is missing some crucial component for navigation.

Australian Higher Ed. Graduation Statement Abstract

Solitary foraging ant species can employ multiple navigational tools to navigate, including path integration and vision-based navigation. Species inhabiting visually rich environments rely heavily on cues from landmarks and the panorama. Multiple navigational cues cannot be manipulated in the field but can be manipulated by using virtual reality. We present a thorough description of the virtual reality system and demonstrate the system's performance. Individuals in virtual reality conditions did not orient towards the nest but did correctly orient in the field, suggesting the VR system is missing some component for navigation.

Highlights:

- **Individuals were able to orient to the nest successfully after displacement in the field.**
- **Pre-test/Post-test comparison shows no effect of delay and tethering procedure on successful field orientation.**
- **Individuals in virtual reality conditions show a random distribution of headings and do not orient towards the nest, suggesting that they cannot orient within the VR system.**

Keywords:

Ant; Foraging; Navigation; Virtual Reality; Visual Panorama

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1. Introduction

Ants (Formicidae) are a diverse family of insects in terms of physiology, geographical distribution, and behaviour, making them a group of interest to scientists for centuries (Gould, 1747). One group of widely studied behaviours in ants is the array of navigational strategies employed during foraging trips in order to find food and return to the nest. Group foraging ant species utilise pheromone based chemical trails to provide guiding mechanisms to individual foragers (Wilson, 1962; Traniello, 1976). This method of navigation, termed 'route following', provides markers or sign posts in order to accurately guide individual foragers to areas with a high density of food resources and then facilitates an accurate return to the nest site. As a navigational strategy, route following requires limited demands on the individual ant's navigational abilities (Hölldobler and Wilson, 1990). Route following is by no means the only navigational tool available to group foraging ants as after multiple trips foragers are able to learn these routes from past trips and use this memory to navigate both to known resources and back to the nest-site (Collett and Zeil, 1998).

The use of chemical trails for navigation during foraging may not be an effective strategy across all environments or ecological niches, leading certain ant species to rely on alternative strategies for successful navigation. One such example is a group of thermophilic ant species that inhabit desert regions throughout the world, acting in a specialised niche due to the unique desert environment (Wehner, 1987). The genera in question include *Cataglyphis* (northern Africa, Europe, and Asia), *Melophorus* (Australia), and *Ocymyrmex* (southern Africa), and members of these groups forage solitarily without the use of pheromone trails. Thermophilic desert ants are diurnal scavengers inhabiting environments characterised by high daytime temperatures and low annual rainfall where these ants subsist on insects that have perished due to the heat (Wehner, 1987). The high ground temperature ranges of these environments would quickly evaporate any pheromone based chemical trails and the ant's scattered and random food source is not ideal for a group based foraging strategy (Wehner et al., 1983).

1.1 Desert Ant Species

Desert ant species do not forage in groups, but are solitary foragers. They are active during the hottest parts of the day when surface temperatures exceed 50° Celsius and they can remain active even in temperatures reaching close to the ant's thermal maximum (Marsh, 1985; Christian and Morton, 1992). Evidence suggests that these species have a specialised physiological response to high temperatures and are able to synthesise heat shock proteins at

higher temperatures than other species (Gehring and Wehner, 1994). The morphology and behavioural patterns of these species are also highly attuned to life in such a harsh environment. Members have long legs that allow them to achieve high speeds reducing the time spent foraging in the heat, as well as the ability to lift their body away from contact with the high surface temperatures of the ground (Sommer and Wehner, 2012). These species also may lower their body temperature during the hottest parts of the day utilising thermal refuges by climbing onto grass blades and other objects in their environment to reach the cooler air layers above the ground (Christian and Morton, 1992). These factors allow desert ant species to inhabit an ecological niche where many other insect species could not survive, thus reducing competition with other insects as well as reducing the danger of predation (Briese and Macauley, 1980; Wehner et al., 1992).

As solitary foragers, individuals are adept at navigating through their environments to find food and returning accurately to their nest. Desiccated insects are scattered in a random distribution across the desert floor, necessitating a solitary foraging strategy and navigational abilities capable of successfully returning the forager to the nest site alone and as quickly as possible to escape the high ground temperatures. Desert ants have the capacity to employ multiple navigational strategies in order to successfully make foraging trips to and from the nest depending on the available cues. Two of these navigational abilities are path integration and visual navigation using available landmarks and the surrounding panorama (Wehner et al., 1996; Wehner, 2003). While these ant species have the capacity to use either of these strategies, their use is mediated by what environment the species inhabits. In barren landmark-free environments, foragers rely heavily on path integration to judge distance and direction of the nest (Wehner and Srinivasan, 2003; Bühlmann et al., 2011; Cheng et al., 2014). Where landmark cues are plentiful, foragers rely on these landmarks and the surrounding panorama to navigate (Narendra, 2007; Graham and Cheng, 2009a). Since these mechanisms rely on visual cues that are separated in space, cues from landmarks or the surrounding panorama and the celestial compass can be experimentally manipulated to give conflicting navigational information. As a result foragers are presented with two diverging directions indicating the home vector. When these manipulations occur foragers will choose a compromise pathway between the two directions indicated by the cues (Narendra, 2007; Reid et al., 2011; Collett, 2012) or cues for the landmarks will override cues from the celestial compass (Wehner et al., 1996; Fukushi et al., 2004; Narendra, 2007).

1.2 Path Integration - Orientation by Celestial Compass

The navigational strategy of path integration is the ability to maintain a direction and vector distance pointing from the individual's current position to the nest site (Wehner, 2003). As the forager searches for food through a winding outbound path, it continually updates the vector associated with the nest. Once the forager has found food, it can return to the nest through the most direct route. To successfully recall this home vector, path integration employs a celestial compass, which differentiates distinct patterns in ultraviolet (UV) light. Ants are able to perceive polarized directions in UV light through a specialized group of receptors in the dorsal region of the eye, called the dorsal rim (Wehner, 1997). The celestial compass employs the polarisation of scattered skylight and the position of the sun itself, which allows the ant to compensate for the inherent ambiguity associated with polarised light (Duelli and Wehner, 1973; Wehner, 1984, 1987; Rossel and Wehner, 1986). Desert ants may use either of these cues in isolation to successfully navigate, but when both the sun and polarised light are presented in conflict, studies have shown the forager's navigational behaviour is governed by the polarised skylight and not the sun's position (Wehner and Müller, 2006). More recent work has contradicted these findings; instead suggesting a compromise heading is adopted between the two conflicting cues (Lebhart and Ronacher, 2014).

1.3 Path Integration - Distance by Odometer

The distance component of path integration, called an odometer, is estimated through a step counting mechanism (Wittlinger et al., 2006, 2007a). In flying insects such as honeybees, the odometer is closely associated with optic flow through the integration of flow-field cues (Esch and Burns, 1996; Srinivasan et al., 1997; Srinivasan et al., 2000; Esch et al., 2001). In ants these visual cues have been shown to have a minor role in distance estimation, as cues only play a role when the ground underneath the individual is altered and not when the lateral visual fields are altered (Ronacher and Wehner, 1995; Ronacher et al., 2000). Ants mainly rely on non-external cues to estimate the distance from the nest by employing a stride integrator or pedometer. These perceived distances have been experimentally altered by manipulating the length of the ant's legs (Wittlinger et al., 2006, 2007a). A complete understanding of the underlying physiological mechanisms of the pedometer remains elusive (Wittlinger et al., 2007b; Seidl and Wehner, 2008)

1.4 Path Integration - Coupling Direction and Distance

Together the pedometer and celestial compass allow the forager to calculate the distance of each path segment in conjunction with the forager's direction to allow the forager

the ability to return to the nest on the shortest, most direct route (Müller and Wehner, 1988). The path integration navigational strategy is heavily relied upon by desert ant species living in habitats that are landmark-free where potential visual cues are scarce, such as the widely studied north African species *Cataglyphis fortis* (Wehner, 2003; Wehner and Srinivasan, 2003; Bühlmann et al., 2011). However, it is important to mention that *C. fortis* has been shown to use landmarks to navigate when they are present. When returning foragers are displaced from near the nest site (zero vector) to an unfamiliar location, *C. fortis* can successfully navigate to the nest through the use of landmarks located near the nest, though it is worth noting that this example was an unusual nest site located near a collection of bushes which served as landmarks (Wehner et al., 1996).

1.5 Visual Navigation (Landmarks)

Another desert ant species, *Melophorus bagoti*, inhabits a very different environment in central Australia from the north African *C. fortis* and consequently relies heavily on visual navigation to return to the nest. *M. bagoti* inhabits a cluttered semi arid environment filled with bushes, trees, and grass tussocks (Cheng et al., 2009, 2014). *M. bagoti* may also employ path integration to return to the nest when landmarks are not available, but when compared to *C. fortis*, the Australian ant performs significantly worse at accurately estimating the distance to the nest (Cheng et al., 2006; Narendra et al., 2007; Bühlmann et al., 2011). The use of both beacon landmarks and en route landmarks for navigation in *M. bagoti* have been much studied (for review, Cheng et al., 2009), but *M. bagoti* can also accurately navigate using the surrounding panorama.

1.6 Panoramas

Recent research on *M. bagoti* and other ant species that rely on visual navigation has focused on the insect's use of the surrounding landmark panorama to successfully navigate not specific landmarks (Graham and Cheng, 2009a, 2009b; Wystrach et al., 2012; Narendra et al., 2013a, 2013b; Zeil et al., 2014). Two prevailing models describe what aspects of the panorama the ant utilises to successfully find the nest. The first of the models, the skyline-height model is presented and tested in Graham and Cheng (2009b). Graham and Cheng (2009b) limited the available landmark cues by replacing the landmarks of the panorama with black plastic in the outline of the skyline. *M. bagoti* was still able to successfully orient towards the nest suggesting that the limited information provided by the skyline was sufficient for navigation. The skyline-height model has been expanded from this study to suggest that the foraging ant will memorise the perceived elevation of aspects of the

panorama around the nest. When the ant is displaced from the nest and views an altered skyline, it will move towards areas of the panorama that appear lower in elevation to that of the memorised image while moving away from portions that appear higher when compared to the memorised image. By attempting to align the perceived heights of the skyline with the memorised nest-specific image, the individual is in effect moving away from objects in the panorama that are too close and moving towards objects that are too far away to align with the image taken at the nest site (Wystrach et al., 2012). Julle-Daniere et al. (2014) has recently provided direct evidence for the skyline-height model in *M. bagoti*. This capability has been shown beyond *M. bagoti* in other ant species living in visually rich environments (Fukushi, 2001; Narendra et al., 2013a, 2013b).

The second model to explain the use of panorama cues is the visual compass model and is presented in Graham et al. (2010). This model suggests that through learning walks just outside the nest entrance the ant is able to memorise snapshots of the surrounding panorama at the nest from different directions. When returning to the nest from foraging trips, the ant will compare and rotate itself so that the current view achieves the highest amount of similarity with the memorised view. The best match between the current and memorised panorama will be achieved when the ant is oriented towards the goal's location. If the current visual panorama lays in-between two separate snapshots taken at the nest, the forager can average the directions corresponding with these snapshots. This average would give the forager an intermediate direction between the directions of the learned snapshots, allowing the forager to successfully navigate to the nest with only a few learned views.

Recent research suggests that both strategies may be utilised by *M. bagoti*, and the use of these aspects of the panorama are possibly dictated by whether the ant is on a well established route (visual compass) or if the ant is released in a novel location (skyline-height model) (Wystrach et al., 2012).

1.7 Substitute Species - Other Solitary Foraging Ants

M. bagoti remains active during the summer months when temperatures are high, but begins to reduce activity as temperatures lower during March and April, which is then followed by a winter long dormancy period (Christian and Morton, 1992). *Myrmecia pyriformis*, which similarly relies on visual cues for successful navigation and has a longer period of activity, was used to complete our initial testing.

Myrmecia pyriformis are solitary foraging ants that, like *M. bagoti*, are endemic to the Australian continent and live in cluttered environments that are rich in landmark-based cues.

Unlike *M. bagoti*, *M. pyriformis* live in woodland habitats and forage in one to two nest-specific trees within 15 m of the nest where they feed on other insects and sugar secretions. Foragers do not employ pheromone based chemical trails but do have the capacity to navigate through both path integration and the surrounding panorama (Reid et al., 2011; Narendra et al., 2013a, 2013b; Reid et al., 2013). Another distinction between these species is that *M. pyriformis* is a nocturnal forager with members leaving the nest at dusk twilight, spending the entire night in the nest specific tree foraging on nectar and collecting small insects, then returning to the nest in the predawn hours (Reid et al., 2013). Evidence suggests that these long foraging trips may be dictated, at least in part, by the ant's reduced navigational abilities in low light levels (Narendra et al., 2013b). What makes *M. pyriformis* a good surrogate species for the preliminary virtual reality testing is that they primarily use visual cues such as landmarks and the panorama to navigate back to the nest (Narendra et al., 2013b). Their large size (up to 30mm) also makes *M. pyriformis* a prime candidate for future study involving neural recordings or manipulation.

1.8 Virtual Environments

The use of virtual reality (VR) has become a rapidly expanding experimental tool for the study of both behaviour and its underlying neural mechanisms (Fry et al., 2009; Takalo et al., 2012; Paulk et al., 2014; for review see Dombeck and Reiser, 2012). A VR system involves the use of a group of artificially generated, usually visual, cues that are perceived by the animal as a real environment. As the animal interacts with this environment through movement or changes in orientation, the system will update the artificial cues presented to the animal, forming a closed-loop system that convincingly mimics a real world equivalent. VR can provide researchers multiple benefits in experimental design including complete control over stimulus presentation and the ability for neural recording on a restrained individual (Dombeck and Reiser, 2012). This restraint or tethering of the animal can be a significant drawback depending on the animal model in use. Ravassard et al. (2013) found that the place cells of tethered rats moving on a track ball in a virtual world were out-of-step with the virtual environment and less precise when compared to free moving rats in the real world. These findings suggest that vertebrate models with a functioning vestibular system will encounter competing cues while restrained in a VR environment. A similar issue arises when using some invertebrate models which have sensory systems tuned to physical motion, such as the fly's gyroscopic halteres (Frye, 2007, 2009). By creating a VR system for walking insects which lack motion based sensory systems (vestibular system), it is believed that the proposed

model could hold an advantage in both recording the relevant behavioural responses of the animal and in the study of the behaviour's underlying neural circuitry.

In regard to the study of navigation, utilisation of a VR system grants us the unique ability to simultaneously control and manipulate multiple visual cues essential to navigation. Aspects of the celestial compass, landmarks, and the surrounding panorama, all visual cues critical for successful navigation in solitary foraging ants, cannot be realistically manipulated in the natural environment. Many of these cues, however, can be readily manipulated by using VR under laboratory conditions. The greater control over visual cue manipulation in conjunction with the advantages of using walking insects in VR makes the construction of such a system an endeavor that could enhance our understanding of navigation and the relevant neural mechanisms underlying these behaviours.

1.9 Research Objective

As stated above, multiple characteristics essential to navigation in solitary foraging ants are difficult if not impossible to control and manipulate in the field or in the lab. These cues may be manipulated through utilising the expanding field of VR. For this project we developed a novel VR system that has the capacity to recreate the terrestrial cues of the ant's environment along the horizon (up to 45° above the individual), which are employed to navigate to the nest successfully. Within the VR system the subject was tethered upon a polystyrene ball supported by a stream of pressurised air, a system first described in Dahmen (1980) with orientation and virtual paths of the subject being recorded using the Fictrac system, described in Moore et al. (2014). The system provides a 300° visual panorama encompassing the entire field of view of the individual. The surface the visual panorama is projected upon is penetrable by UV and polarised light allowing for the manipulation of aspects of the artificial horizon and the individual's sky compass in the virtual environment. This system's capabilities permit an abundance of manipulations of the ant's navigational cues as well as potentially allowing for future manipulations of the ant's memory capabilities. The system could grant researchers a more thorough understanding of the solitary foraging ant's navigational strategies and the mechanisms underlying these navigational abilities than can be achieved by testing these abilities in the field.

The goal of the current project was to confirm that the virtual environment the subject animal is presented within the system is perceived as similar enough to the natural environment that the subject can successfully orient itself towards the nest's location. This was accomplished by comparing the orientation and initial heading of individual foragers

both inside the virtual reality system and in the natural environment. This preliminary study conducted four test conditions on individual ants in hopes of answering questions regarding the virtual reality methodology and the study species itself. First, we tested through displacement from the nest, zero vector ants' ability to successfully orient towards the nest site to illustrate this species ability to navigate using landmarks and the panorama. The second and third tests involved comparing the initial orientations of this field test to two conditions within the VR system. In VR condition one, the individual was presented the surrounding panorama from the release site with no UV component. This condition tested if panorama alone would be sufficient for successful orientation. In VR condition two we added a rudimentary UV component to the panorama by shining UV light through the projector screen only in the sky portion of the panoramic images and not the ground portion creating a simple sky/ground distinction in panorama. This condition was meant to test if even a simple UV component was sufficient to allow for successful navigation with the green sensor channel even if the UV channel was incorrect. Our final condition involved releasing the individual back at the real world displacement site to test that the individuals could still successfully orient to the nest site after the anesthesia procedure required to tether individuals into the virtual reality system. This final condition is crucial to an explanation regarding any ability or lack thereof to successfully orient inside the VR system for these ants.

2. Methods

2.1 Study Subject

The initial testing of the virtual reality system's proof of concept was completed using the nocturnally foraging *Myrmecia pyriformis*. This ant species has many key ecological and behavioural similarities to our main target species, *Melophours bagoti*, such as solitary foraging behaviour, showing no recruitment behaviour during foraging and the use of visual cues for navigation. These similarities make them an ideal surrogate for this study. Multiple members of the *Myrmecia* genus have been shown to have enhanced visual capabilities used for both hunting and accurate navigation during foraging trips and they are able to successfully navigate back to the nest from previously unvisited sites near the nest using distant visual cues from the panorama (Narendra et al., 2011; Reid et al., 2011; Narendra et al., 2013a, 2013b). This species is also active during late August, unlike other Australian ant species known to navigate using the visual cues of the panorama. Individuals were collected and tested from three nests on the Macquarie University campus in New South Wales, Australia (33°76'96.41"S, 151°11'15.58" E).

2.2 Virtual Reality System

The virtual reality system consisted of three commercially available Dell M115HD projectors (Dell Inc.) rear projecting the virtual environment onto an Acrylic cylinder (30 cm diameter; 20 cm height; Plastix Australia Pty Ltd) as pictured in Figure 1A. Each projector contributed 100° of the horizontal visual field and together they created a 300° visual field fully encompassing the ant's field of vision. The projectors each formed $\frac{1}{3}$ of the horizontal visual field and the full panoramic skyline image was split into thirds and fed to each projector using the TripleHead2Go (Matrox Electronic Systems, Ltd.) splitter creating a 5.3:1 horizontal: vertical aspect ratio for the projected image. There was no overlap between projected images making up the panorama so that light intensity remained consistent across the display. Upon this field an image of the surrounding skyline panorama from the release point was projected. A complete 360° panoramic digital photo of each release site was recorded using a Canon G10 camera (Canon Inc., Tokyo, Japan) with a specialised convex mirror lens (GoPano; EyeSee Inc., Pittsburgh, PA, USA). The camera was placed at ground level and images were made horizontal using a spirit level. The camera and mirrored lens system produce images that encompass a 360° horizontal field and a 120° vertical field of the environment. Approximately 50° of the vertical field is below the horizon while 70° of the total field is above the horizon. The 360° image was unwrapped to a rectangular panorama using PhotoWrap (EyeSee Inc.) and the bottom 40° of the image was removed to eliminate the ground below the horizon. The top 24° was also removed so that the image fit in the correct proportions on the screen at the 5.3:1 aspect ratio described earlier (300° horizontal: 56° vertical). The image was resized to 1200×400 pixels, and all procedures match previous studies using similar panoramic images (Wystrach et al., 2013; Julle-Daniere et al., 2014). While the images used in the study were 360° by 56°, only 300° of the horizontal image was projected in the arena at one time. This allowed for a 60° section of the arena directly behind the individual (and outside of the individual's visual field) to be open for observation and recording. The simple static UV component of the VR system was created by using three light fixtures placed around the outside of the cylinder with one being positioned directly on top each projector. These lamps each illuminated one third of the image horizontally and projected the UV light directly on to the screen at a horizontal level. Each lamp contained a 20w UV bulb and the light of these bulbs was restricted to the top portion of the projection screen roughly in line with the horizon using a UV resistant screen.

The virtual world was created using the Unity game engine (Unity Technologies). The world consists of the panoramic image of the skyline as the 360° horizon and an image of the

ground for the floor of the virtual environment (Figure 1B). As the individual moved the ground texture passed underneath while the horizon would rotate when the individual turned. We believe that a static image with no 3D component to be sufficient when testing initial heading as the changes in the skyline over short distances (22cm) would be minor and the large degree of complexity and difficulty in recreating a 3D world that mimics the intricacy of the field sites.

As the projection surface was convex for each projector, a basic projection of the panorama image would expand at the edges of each projection causing a warping effect and creating distortion in the image. To compensate for this curve, and thus eliminate distortion in the image, a custom geometric correction was applied within the Unity software to have the image's width remain constant along the curving surface of the cylinder and eliminate the warping of the image.

Individuals were tethered above a spherical treadmill consisting of a polystyrene ball (Weight 1.5g, Diameter 50mm; Zart Pty. Ltd.; Figure 1C). Rotation of the ball was achieved through supporting the sphere upon a thin stream of pressurised air, which was pushed through a hemispherical recess of a 3D printed ball mount (Plastic Ink Pty.). The stream of pressurised air was fed into the ball mount using a 5mm diameter plastic tube and an air compressor (Sparmax TC630; Ding HWA Co. Ltd.).

Movement of the ball along all three axes was recorded using the FicTrac system (Moore et al., 2014). A distinct black and white pattern was created upon the surface of the polystyrene ball through the use of a felt tip pen (Sharpie, Newell Rubbermaid Inc.) as shown in Figure 1C. The sphere's position and rotation during testing were recorded at 30 Hz using a commercially available web camera (Quickcam Pro 9000, Logitech International S.A.). These images were used by the FicTrac software to create a virtual map of the sphere's surface and during testing allows the system to update the location of the tethered individual in the virtual world and to create the virtual path of the individual used for data scoring and analysis (Moore et al., 2014). FicTrac was calibrated to the size of the ball through a configure program created by the Unity programmer. The diameter of the polystyrene ball (50 mm) was input into the calibration program and this allowed the system to output a complete turn of the ball as the correct circumference (157.1 mm). Turning calibration had to be inspected manually with one complete turn of sphere corresponding with a complete turn in the panoramic image which was also accomplished by using a configure program set up in Unity by the programmer.

Individuals were tethered in place above the ball by adhering a small carbon steel piece (Length 7mm × Width 2mm × Thickness 0.05mm; Livingstone International Pty. Ltd) to the dorsal region of the individual's thorax using dental cement (Synergy D6 FLOW A3.5/B3 Nano Composite; Coltène/Whaledent Inc.) and a UV LED curing light (BA Optima 10; BA International). A small (Diameter 3mm, Thickness 1mm) neodymium magnet was glued to the top of a flat head pin (Length 26mm; Prym Group) allowing for the ability to attach the insect to the pin and remove the pin after testing. The flat head pin was fitted into a dissection pen and clamped onto a 15cm vertical rod positioning the individual over the track ball using a custom design 3D printed boss head clamp. This configuration allows for fine vertical adjustment of the individual's position over the track ball. The tether maintained the ant's ability to freely move its legs on the air-supported ball while being held in place from above by the tether (Figure 1C).

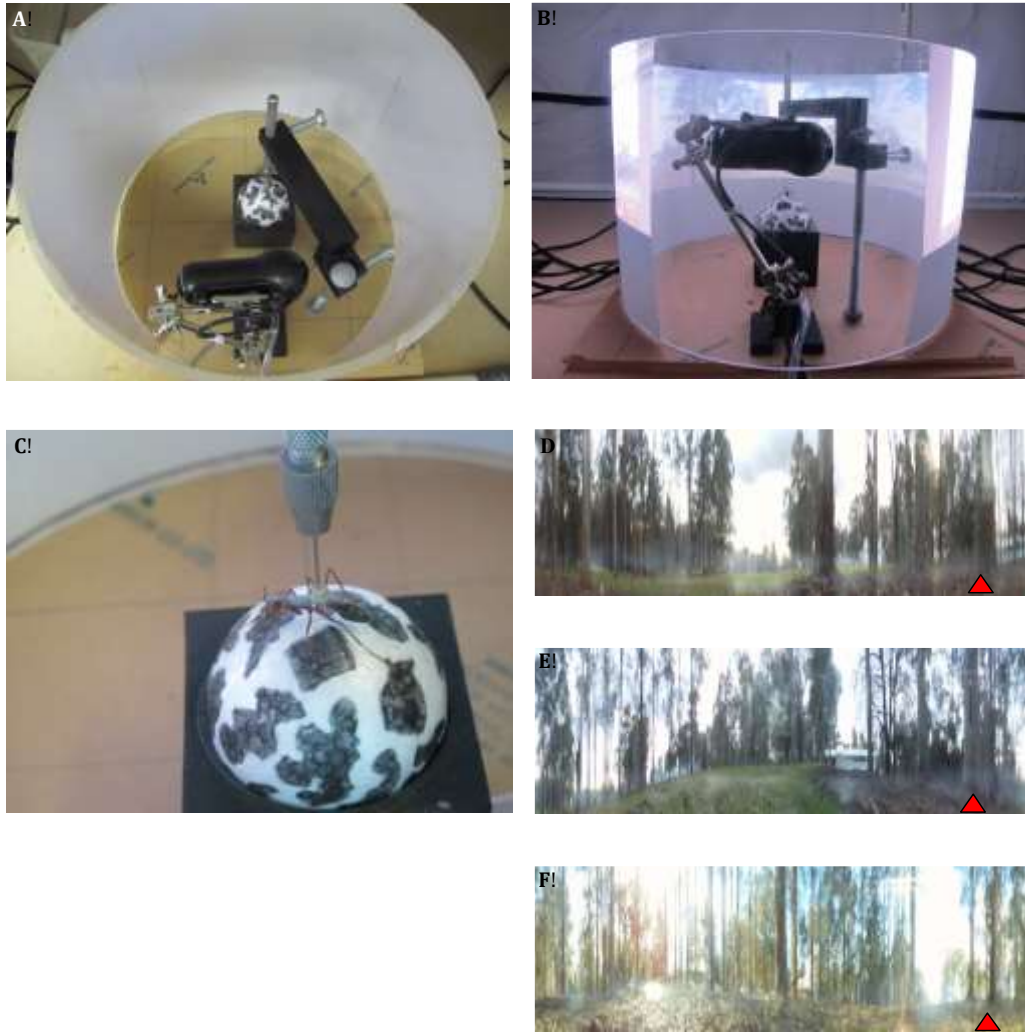


Figure 1. Components of the virtual reality system. (A) Overhead view of the system. The screen was constructed of a (30 cm diameter \times 20 cm length) plastic tube allowing for a 300° horizontal projection. (B) View of the system while operating. Three projectors were used to project the view of the panorama to the individual tethered on the spherical treadmill (track ball). A webcam was fixed in place behind the individual in order to record input to the FicTrac program from the track ball's movement (Moore et al., 2014). (C) Individual tethered in place above the track ball. A small piece of carbon steel glued to the individual's thorax and a magnet attached to a flat head pin create the tethering apparatus. The track ball mount and the adjustable tether arm were 3D printed. A small flow of air supports the track ball in the mount allowing the insect to move freely while tethered from above. (D-F) Panorama images from the displacement sites. Individuals from three nest sites were used in this experiment. Panoramic images from the three displacement sites were projected into the VR system. Red arrows denoted nest location.

The tethering procedure required that the individual be anaesthetised momentarily for the tether to be fitted properly. Individuals were cold anaesthetised at -18°C for 2-3 minutes and once the carbon steel piece and dental cement had been applied they were returned to

their tube and allowed to recuperate at room temperature for 1 hour to allow for recovery and adaptation to the adhesive and carbon steel piece.

2.3 Experimental Design

At the nest entrance, 64 individuals were captured. Of this total, 26 individuals were collected as they performed excavation and cleaning duties around the nest and 38 individuals were captured as they left the nest site during the evening twilight. Individuals collected at the start of foraging trips and those at the nest site showed no significant difference in initial heading during any of the field or virtual conditions and all individuals returned to the nest site after final release. As a result of this observation, foragers and nest site individuals were combined into a single group. Individuals were collected using a darkened plastic tube container and marked. Individuals collected at the nest site are considered zero vector ants, meaning that any acquired path integration information from previous foraging trips has been set to zero. This allows for testing the individual's ability to navigate using only visual cues present at the release site (Wehner et al., 1996; Graham and Cheng, 2009; Narendra et al., 2013). Captured ants were given a sugar solution (10%) and remained in the tube through the night. The next morning, approximately 12 hours after capture, the foragers were released at a displacement site 5 m from the nest. Foragers were released on a wooden board with a 44 cm diameter goniometer on the surface raised 5 cm off the ground. The goniometer was segmented into 24 sectors each encompassing 15° of the horizontal plane. The wooden board had a 2 cm diameter hole in the centre of the goniometer, which attached to a 4 cm long plastic tube. Foragers were deposited into the plastic tube and allowed to climb up onto the board and begin orientation. The ant's initial heading upon release was recorded at 22 cm from the release point using the goniometer and the ant was immediately recaptured for two VR testing conditions.

A total of 58 ants were successfully tethered inside the virtual reality system for testing. Six individuals had tethers break before testing and were not able to be included in any VR conditions and another 4 individuals had their tether detach during their second VR condition. Our overall dropout rates between the four conditions are discussed in greater detail in the next section. After being placed upon the track ball the individual was presented a black screen for 30 seconds to allow for adjustments of the tether and airflow. After this buffer period the individual was presented a panoramic image of the release site in a closed-loop system that would rotate corresponding with the individual's turning upon the ball on a 1:1 ratio for 250 seconds. The orientation of the panorama image presented, as well as the order of the UV presence and absence conditions, were randomised for each individual. The

virtual path during testing was recorded using the FicTrac system and an initial heading was recorded by using a goniometer overlay on the virtual path. This testing allowed for a comparison of the initial heading in the natural environment and in the virtual world created by the system. Once each individual was tested under both VR conditions, the individual was removed from the tether magnet and returned to its collection tube.

After testing the first 10 individuals with only the initial field test and the two VR conditions, we added a final field post test as we were concerned about the anaesthesia's effect on navigation. Beginning with individual 11 and continuing to the completion of the study, once VR testing was complete individuals were immediately returned to the field displacement site 5 m from their nest. Each individual was again tested on the goniometer board, using methods identical to the initial field test. After the final test condition, individuals were allowed to return to the nest. All individuals were returned to the nest site within 24 hours of collection.

2.4 Statistical Analysis

Initial headings of individual ants for the field experiments before and after VR testing were measured by goniometer crossings at 22 cm. Initial headings within the VR system conditions were measured through a virtual goniometer by scoring the individual's orientation at 22 cm from the release point. The data were analysed with circular statistics (Batschelet, 1981; Zar, 1998) using the statistics package Oriana Version 4 (Kovach Computing Services). Whether mean initial orientation at the release site was significantly directed towards the nest was calculated across all conditions using V-tests, with alpha set at $p = 0.05$. The two VR conditions and the final field condition were compared in pairwise comparisons to the initial field test for differences in mean orientation vector using Watson-Williams F-test (when conditions of von Mises distribution were met) and Hotelling's paired test (Zar, 1998). Pairwise comparisons only compared individuals who completed both conditions. Individuals were dropped from the VR conditions if their tethering piece became dislodged during one of the trials, as reattaching the tethering would have required a second round of anaesthesia. This occurred in 10 individuals where individuals completed the pre field test but were unable to complete one or both of the virtual conditions as their tether became detached from their bodies (six individuals were unable to complete the UV present condition and 10 were unable to complete the UV absent condition). The dropout rate between the initial field test ($n = 64$) and the UV absent ($n = 54$) and UV present ($n = 58$) virtual reality conditions were 15.6% and 9.4% respectively. The post field test was added as a condition after collecting data from 11 individuals in only the first 3 conditions. Four individuals died before they could be tested on

the post field test. These deaths led to a dropout rate from the initial field test ($n = 54$) to the post field ($n = 50$) of 9.3%.

3. Results

Individuals released at the displacement point on the goniometer behaved similarly to ants from previous studies navigating using visual cues (Julle-Daniere et al., 2014; Narendra et al., 2013a; Wystrach et al., 2012, 2014; Zeil et al., 2014). After emerging from the tube and onto the wooden goniometer board, the individual slowly turned in place before slowly moving off from the release point in a chosen direction.

Figure 2 shows the distributions of initial headings of individuals for the first field test (panel A), the second field test (panel B), the virtual reality condition with no UV present (panel C), and the virtual reality condition with the simple UV sky/ground contrast (panel D). Individuals in the initial field test were significantly non-uniform (Rayleigh test, $p < 0.0001$) showing a significant orientation towards the nest direction at 120° (V test, $p < 0.0001$). The second field release condition after the VR testing showed a similar distribution of initial headings which was also significantly non-uniform (Rayleigh test, $p < 0.0001$) and directed significantly towards the direction of the nest site at 120° (V test, $p < 0.0001$). Individuals showed no difference in initial heading between their two field conditions (Watson-Williams F-test, $F = 0.372$, $p = 0.543$) (Hotelling's paired test, $F = 0.641$, $p = 0.531$).

Individuals placed inside the virtual reality system initially behaved similarly to when they were in field conditions with individuals slowly turning on the ball. However, individuals did not move off in a straight line in the virtual conditions; instead their paths were circular and spiral shaped suggesting that the individual is disoriented and possibly preforming searching behaviour (Wehner, 1981; Wehner and Srinivasan, 1981). This disorientation was present irrespective of the presence/absence of the UV light. Individuals in the VR showed a uniform random distribution of initial headings in both the UV off condition (Rayleigh test, $Z = 0.95$, $p = 0.387$) and the UV on condition (Rayleigh test, $Z = 0.23$, $p = 0.792$) and were not significantly oriented towards the nest site (UV off, V test, $p = 0.865$)(UV on, V test, $p = 0.666$). The two VR conditions show no significant difference in distribution to each other as would be expected by their uniform distribution (Hotelling's paired test, $F=0.046$, $p = 0.955$). VR conditions do significantly differ from the field conditions: Field Pretest to Virtual Reality UV Absent (Hotelling's paired test, $F = 20.646$, $p < 0.0001$) Field Pretest to Virtual Reality with UV Present (Hotelling's paired test, $F = 17.279$, $p < 0.0001$)

Field Posttest to Virtual Reality UV Absent (Hotelling's paired test, $F = 6.807$, $p < 0.005$)

Field Posttest to Virtual Reality UV Present (Hotelling's paired test, $F = 3.963$, $p < 0.05$).

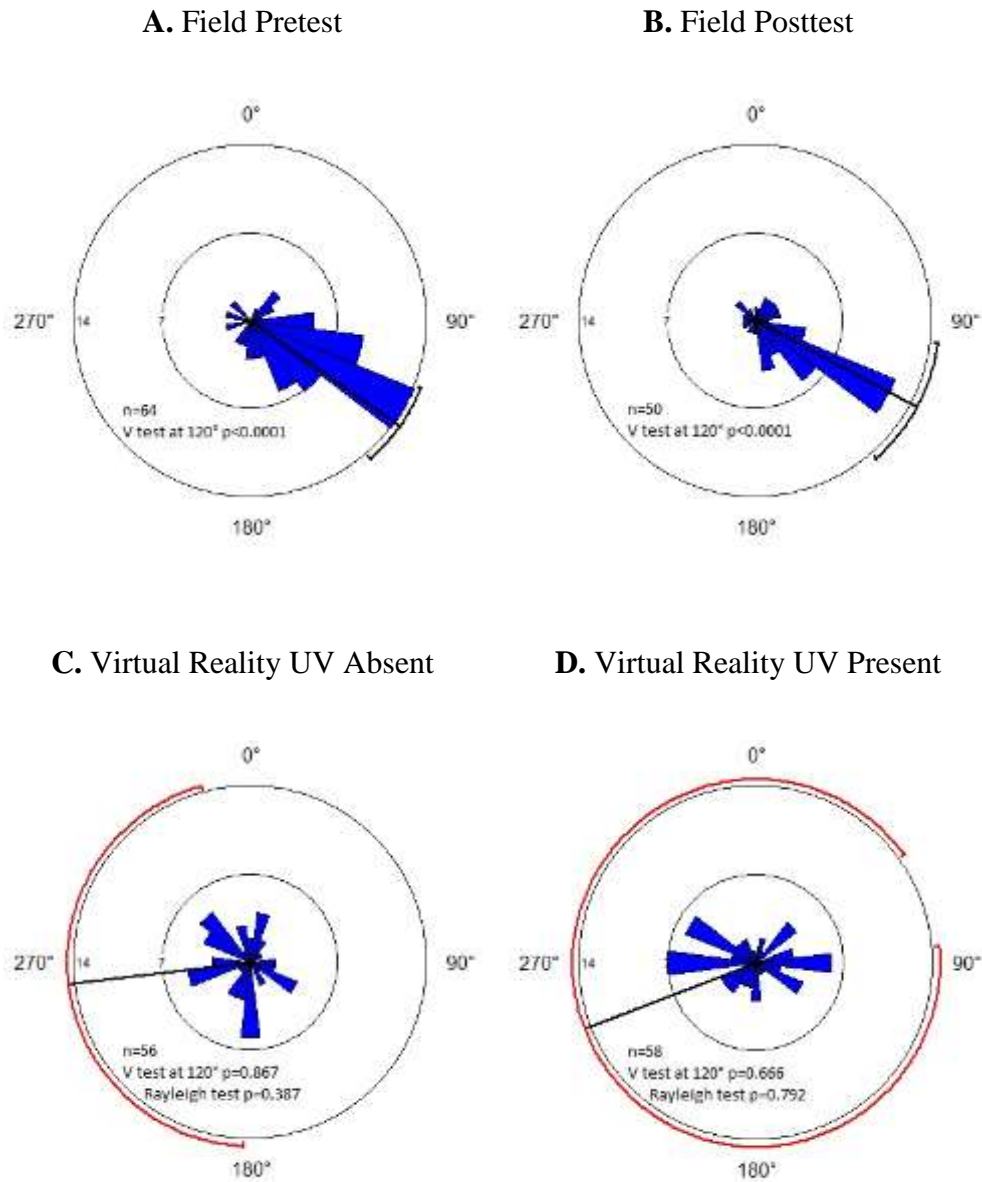


Figure 2. (A-D) Circular histograms of individual's initial heading at 22cm from release point. Black/Red bars represent mean compass bearing of the distribution with 95% confidence interval. Correct nest direction for all conditions was at 120°. Distributions A and B, the real world field pretest and posttest are significantly non-uniform (Rayleigh test, $p < 0.0001$) and directed towards the nest site heading of 120° (V test, $p < 0.0001$) while the two virtual reality conditions (C-D) meet the conditions of a uniform distribution (Rayleigh test, $p > 0.05$). Mean angles of the pretest and posttest (A-B) do not significantly differ (Watson-Williams F-test, $F = 0.372$, $p = 0.543$) (Hotelling's paired test, $F = 0.641$, $p = 0.531$). Mean angles of the two virtual conditions (C-D) do not significantly differ (Hotelling's paired test, $F = 0.046$, $p = 0.955$). All comparisons between field conditions and virtual conditions are significantly different: A to C (Hotelling's paired test, $F = 20.646$, $p < 0.0001$) A to D (Hotelling's paired test, $F = 17.279$, $p < 0.0001$) B to C (Hotelling's paired test, $F = 6.807$, $p < 0.005$) B to D (Hotelling's paired test, $F = 3.963$, $p < 0.05$).

4. Discussion

4.1 Navigation: Landmark Cues in the Field

From the results of the first displacement test, we show that members of *M. pyriformis* were able to accurately establish a correct initial heading towards the nest from the displacement site 5 m from the nest. Individuals were considered zero vector ants due to their collection at the nest site, removing any accumulation of path integration cues. The ants' correct initial headings in conjunction with this collection method suggest that it is likely that the individuals are using visual landmark cues and the panorama to navigate. These results parallel similar work on *M. pyriformis* that showed a heavy reliance on landmark-based cues when navigating after displacement (Reid et al., 2011). The woodland habitat in which *M. pyriformis* resides is well suited for this navigational mechanism, providing an abundance of landmark cues and a distinct skyline panorama to aid individual foragers traveling to the nest-specific foraging tree and returning to the nest (Reid et al., 2011; Narendra et al., 2013b).

4.2 Comparison of Pre and Post Field Tests

The field-test conducted after the VR preparation and procedure shows that individual *M. pyriformis* can still successfully orient to the nest from the displacement site. A comparison of the pre and post VR field-tests shows that when individuals were released on the goniometer board they were able to correctly orient toward the nest and performed this task as well as when they were first tested. After moving off the board, the ants continued toward the nest site and over 90% of individuals had entered the nest within five minutes of release. Two individuals were unable to find the nest after five minutes and a further two individuals were killed due to bird predation after data collection but before entering the nest. These results suggest that the 16-20 hour delay, the tethering procedure, the tethering piece, and the anesthesia procedure likely have little to no effect on the landmark-based navigational abilities of these ants. The ability to cope with a 16-20 hour delay in solitary foraging ant species is not surprising given the findings of previous studies. In both *Cataglyphis fortis* and *Melophorus bagoti*, retention experiments involving memories of visual cues associated with the nest showed no deterioration over the lifetime of the individuals (Zeigler and Wehner, 1997; Narendra et al., 2007). While a study illustrating life long memory retention of landmark-based cues in *M. pyriformis* would be a necessary step in making definitive statements for this species, for the purposes of this study's methodology memory of landmark cues associated with the nest seem to be stable across the necessary time frame.

Beyond the ability to cope with this delay, it was important to establish that the cold anesthesia, tethering procedure and the VR conditions did not inhibit the ant's navigational

abilities. If a significant reduction of navigational ability had been found in the post VR field test, it may have required adjustments to the preparation procedures. Fortunately, our comparison of the field pre-test and post-test suggests that the anesthesia procedure and the attachment of the metal tethering piece do not significantly affect the individual's ability to navigate in the field. This finding holds specific importance to any future studies with this species in the VR as it allows us to maintain the current preparation procedure. Some alterations to the procedures will likely need to be considered when testing on *Melophorus bagoti* due to size differences between individuals of the two species. *M. bagoti* may also show more sensitivity to cold anesthesia compared to *Myrmecia pyriformis*, necessitating a replication of the pre/post field tests during testing in the desert species.

Using a cold anesthesia for the tethering procedure has been commonly used in studies with honeybees in order to tag individuals but has been shown to disrupt some forms of memory and other behaviours (Erber et al., 1980; Robinson and Visscher, 1984). Paulk et al. (2014) cold anesthetised honeybees at 4 °C for 5-10 minutes for tethering in a VR system, however this procedure proved less effective with *M. pyriformis*, with individuals still showing signs of activity at 10 minutes. Once removed from the 4 °C individuals quickly (< 1 minute) became more active making tethering difficult and the procedure required alteration. After adopting the -18 °C (2-3 minutes) anesthesia procedure, individuals showed no signs of movement when removed from the chamber and regained activity after a few minutes allowing for placement and curing of the tether piece and adhesive without difficulty.

Once attached, the carbon steel tether piece seemed to have little to no lasting effect on individuals' behaviour. Once an individual recovered from the anesthesia procedure within the collection tube, some individuals attempted to remove the tether piece for 1-3 minutes before abandoning this behaviour and moving around their collection tube normally. The piece did not seem to affect movement either on the floor of the tube or during climbing on the tube's walls. The animals were able to lift their bodies off the ground and after release were able to quickly move off the goniometer board and through the woodland floor to the nest site.

4.3 Virtual Reality Tests

Initial heading results of ants placed into the virtual reality system under both conditions (UV presence/absence) along with observations of the individual virtual paths were possibly indicative of searching behaviour and initial headings were in a random distribution, however a more thorough analysis of the paths of the individuals would be

necessary to make such a statement with more confidence. Searching behaviour is characterised by looping spiral paths with increasing size. These spirals pathways are commonly interrupted by a return to the center of the search (Wehner and Srinivasan, 1981, Müller and Wehner, 1994). Ants will exhibit searching behaviour once navigational cues indicate the nest is near or if individuals are displaced to unfamiliar sites when their home vector is set at zero (Merkle and Wehner, 2008). The observed search-like behaviour while the individuals were experiencing the VR conditions combined with the random distribution of initial headings suggests that the current VR system fails to create an environment with sufficient cues to allow individuals to correctly orient towards the nest site direction. Our findings in the field along with previous research in *M. pyriformis* (Reid et al., 2011) suggest that individuals should be able to navigate to the nest by relying on the panorama presented in the VR conditions. Due to individuals' failure to accomplish this, the VR system currently must lack some cue or cues critical to successful navigation in this ant species. The most likely of these cues is the absence of realistic UV cues present only in the sky portions of the panorama.

We have previously mentioned two of the prevailing models on how ants may use landmark cues and the panorama to navigate: the visual compass model and the skyline-height model. Both of these necessitate the ability to perceive defining aspects of a landmark or the skyline as a whole through some mechanism. Möller (2002) proposed that insects are able to detect the skyline by distinguishing the ultraviolet/green contrast between the ground in the foreground and the sky in the background. The sky portion of the panorama has much higher levels of UV light compared to the UV light being reflected from vegetation and other objects below the skyline (Chittka et al., 1994). This difference would cause a distinction that ants and other insects may be able to perceive through the use of UV and green receptors in their eyes. Möller (2002) suggests that the UV/green contrast creates a reliable discrimination mechanism between ground objects and the sky that is superior to detection based only on the UV contrast due to the fixed threshold in the UV/green contrast. A distinction also arises when looking at only the separation of UV levels between the ground and sky, yet Möller (2002) believes that a purely UV-based mechanism for skyline distinction would be less likely due to a variable threshold that changes in different light conditions. Additionally, the results of Möller (2002) suggest that skyline detection would be impossible by only using green receptors due to overlap between ground objects and the sky in the green colour range. A UV/green contrast mechanism for skyline detection could not to be utilised by individuals in the current VR system due to the lack of UV cues present only in the sky portions of the panoramic image that are integrated into the closed-loop system.

The findings of Möller (2002) would indicate that our virtual reality condition with no UV cues present in the panorama would not be sufficient to allow the ants to detect the characteristics of the skyline, as only cues in the green wavelength were present. Furthermore, our addition of a simple UV skyline would not have provided the relevant cues to detect the skyline, as UV light would be projecting in all places above ground level. This would suggest that UV light levels presented to the individual would be the same in the sky portion of the panorama and in any landmarks rising from the ground. This VR condition would not have created the UV/green contrast required to allow the insect to detect skyline cues. In fact the addition of the simple UV contrast may have been a conflicting cue, as in the UV spectrum the panorama was a featureless skyline with no differences across the horizontal plain. In whole, the current panorama projection, and especially the UV component, seems to be inadequate at presenting individuals with the cues necessary for landmark based navigation.

While the lack of a realistic updating UV skyline is the most obvious cue lacking in this VR system, this is by no means meant to imply it would be the only reason that individuals did not orient correctly. There is a large array of possible issues as the environment within the VR system differs with the field in many aspects and the use of the tether is unnatural. A possible source for this disorientation may lie within the tethering system and track ball. The inertia of polystyrene ball may be too high or the size of the ball may be an inappropriate size for this species. It is also possible the tether attached to the individual and the magnet could be too intrusive and not allow for correct movement on the ball. Such issues may be difficult to untangle but it may be possible to test only the panoramic cues of the arena without the use of the tether and track ball, which we will discuss while exploring potential future directions for this system.

Another potential cause of the individuals differing behaviours in the field conditions and the environment in the VR system is temperature. The temperature within the VR system can be notably higher than in the field. This difference is due to having three projectors and a high power computer system in an enclosed space. This difference in temperature increases through continual use and may cause a reduction in motivation to navigate home. It may be advantageous to move the VR system to a larger room with better climate control to attempt to mitigate this temperature increase.

There may also be aspects of the panoramic image that may need to be altered in order to create the cues required for successful navigation. One such possibility is that the contrast of the image may be too low and would need adjustment for the individual to successfully detect the horizon characteristics. Another possibility is that there may be too much lag

between the movement of the individual and the update of the virtual world. This may break the reality for the individual and cause it to become disoriented. It is also possible that the images in their current form do not capture enough of the vertical field in order to allow the individuals to successfully orient. Such an issue would require a complete reimagining of the arena and the projected images and may be very difficult to accomplish without professional experience.

These represent a few of the possible issues that may be linked to the observed results, but through future alterations and testing of the system and the panoramic images we may be able to eliminate many of the possible confounds that led to our current results in the VR conditions.

4.4 Future Directions

As individuals could not orient correctly inside the virtual reality system using only landmark-based cues, results suggest that the UV light cues presented in the VR system will need to more closely mirror real world conditions. The current UV lamp based setup creating a simple skyline contrast will need to be replaced with a configuration in which UV is only projected into the blue portions of the panoramic image corresponding to the sky. The UV projection would also need to be incorporated into the closed-loop system of the VR, meaning the UV cues would need to update with the projected panorama image and rotate around the projection screen when the individual turns on the ball. By incorporating the UV cue into the closed-loop system and making it only appear in the sky portions of the panorama, we would create a skyline cue that is much closer to the cues that these ants use in the field and would create the UV/green contrast in skyline that Möller (2002) suggests as the mechanism underlying an ant's ability to perceive aspects of the skyline.

Creating a realistic, updating UV component for the virtual reality system would most likely need to be incorporated into the projectors themselves either through custom design or after market manipulations to the hardware that restricts the light spectrums being projected. Digital light processing (DLP) projectors have been manipulated to project in UV light when projecting in the shorter wavelengths of visible color (Blue and Violet). This modification can be accomplished by removing the infrared/ultraviolet filters within the projector's light pathway between the lamp and the lens. This projector alteration is commonly used to as a curing mechanism for UV resin in homemade 3D printers (Bogue, 2013). If we can successfully remove these filters, it may still be possible with our current projectors to create

the necessary skyline cues and the UV/green contrast ants may use to orient correctly when navigating.

If these modifications fail to achieve the desired orienting response in navigating ants it may mean that a projector based VR system is not a realistic tool for studying navigation in solitary foraging ants due to the importance of UV light in multiple of their navigational tools (Duelli and Wehner, 1973; Möller, 2002; Sommer and Wehner, 2005; Ronacher et al., 2006). It may be true that studying ant navigation in virtual reality may require the use of a light emitting diode (LED) arena incorporating UV and green diodes into the diode pixels. These arenas involve panels of LED pixels that can be programmed to display different patterns in closed-loop systems. LED based arenas are already in use in other virtual reality studies using honeybees (Moore et al., 2014; Paulk et al., 2014) and integrating UV wavelength lights into similarly designed arena panels seems possible. A UV enabled LED arena could be used to create the desired green/UV contrast in the skyline and may be the future of virtual reality testing for ant navigation.

The implementation of the magnet to tether individuals within the VR system may need to be explored further as a possible confound. Use of the magnet and tether piece was conceived to allow easy attachment and removal of the individuals from the VR system and granted the ability to test individuals across all conditions. Solitary foraging ants can use a variety of cues in order to navigate and *Myrmecia pyriformis* has previously shown a heavy reliance on landmark-based cues (Reid et al., 2011; Narendra et al., 2013b). We believe the magnet is an unlikely source for the failure in orientation, however it is possible that by using the magnet in the VR conditions the presence of conflicting cues may have contributed to the random distribution of headings that were observed. Ants have been shown to orient towards magnetic fields (Çamlitepe and Stradling, 1995; Banks and Srygley, 2003) and can use magnetic landmarks as cues to navigate to the nest site (Buehlmann et al., 2012). Before future testing of the system a study should be conducted in which individuals are released at the displacement site with a magnet attached to the dorsal region of their thorax. This condition would indicate if the magnet's presence disrupts the individual's navigational abilities and would remove this as a potential confound for the VR system.

As discussed earlier there may be some aspect of the tethering device or the track ball that is contributing to the lack of correct orientation in these individuals. A possible control experiment would be testing the individuals in an open loop setup where the individual is placed within an arena with the static panoramic image projected on the walls. This would exclude possible issues that arise through tethering and running an individual on the track

ball, as those would be excluded from this condition. Additionally this condition would more closely resemble the methodology of the field tests in our current work. Such an experiment would most likely require a larger arena to be constructed than the 30cm diameter tube in use currently to ensure the skyline heights do not change rapidly over small distances.

Another possible future analysis of the current experiment would be to develop a program that allows us to more fully analyze the walking paths of the individuals during testing. Due to time constraints the current program is designed only to allow us to retrieve the initial heading from the playbacks but the program could be further improved to allow for analysis of the shape and trajectory of the paths. This would allow us to establish if the paths have the characteristics of searching behavior and would help us determine if the paths were natural in character or if the tether or arena are causing unnatural or unreliable behaviors for navigating.

4.5 Conclusions

Though initial testing of this VR system did not fulfill the desired function of being able to reproduce the panoramic cues necessary for navigation, the use of VR still has the potential to considerably increase our understanding of a wide variety of behaviours and neural processes. VR systems grant experimenters the benefit of complete control of stimulus presentation and restraint of the subject allows for neural recordings of a variety of behaviours that would not be feasible with freely moving subjects (Dombeck and Reiser, 2012). The use of invertebrate walking insect models in VR systems in particular is ideal due to their lack of motion based sensory systems. These benefits combined make a compelling case for a continued interest in implementing a VR system for ant navigation study. VR could allow researchers almost limitless manipulations to test how ants use landmarks and the surrounding panorama that are not possible to accomplish in the field. Though hurdles still remain in creating a realistic virtual world to induce navigation in the ants, the inclusion of ultraviolet light cues through the projector system or in an LED based VR system may provide the solution. Regardless of the ultimate success or failure of the present virtual reality system on studying ant navigation, the use of virtual reality in studying a wide range of behaviours and their underlying neural mechanism questions will continue to expand and may allow key insights into how the brain works.

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