Individual foraging careers of the Jack Jumper ant, *Myrmecia croslandi*

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A thesis submitted for the degree of Doctor of Philosophy of the Australian National University.

December, 2014
Dedicated to:
My Mum and Dad.
This would not have been possible without you.
Declaration

This thesis is an account of research undertaken between January 2011 and December 2014 at the Research School of Biology, The Australian National University, Canberra, Australia. Except where acknowledged in the customary manner, the material presented in this thesis is original and has not been submitted in whole or in part for a degree in any other university. I am the senior author and the principal contributor to all aspects of the co-authored papers within.

Piyankarie. W. A. Jayatilaka
Abstract

A solitary foraging ant needs to rely exclusively on her navigational skill set to successfully navigate to and from goals such as the nest and food sites. Of interest is how ants are able to acquire this navigational information at a young age, before they become experienced workers and how this eventually shapes them into becoming efficient foragers. Ants of *Myrmecia croslandi* are highly visual, solitary foragers that exhibit no evidence of chemical trail following or recruitment. Therefore, a forager leaving the nest for the first time, must do so completely on her own, first by deciding where to go and second by utilising the information she has acquired from the environment to journey between sites. By identifying and individually following ants, I demonstrate that ants exhibit highly individual behaviour in most tasks, from early learning, and daily foraging to navigating from unfamiliar locations.

First, I document the spatial and temporal variation in individual foraging behaviour at two nests of *M. croslandi* over a two-year period. Ants can take variable routes to the same food site and travel the longest distance when they forage on trees. Individual ants depart the nest at different times and a few ants perform multiple trips per day. Surprisingly, not a single ant foraged on consecutive days.

By examining the behaviour of inexperienced ants at the nest, I provide a detailed analysis of the learning walks of *M. croslandi*. Most learning walks take place in the morning with a narrow time window separating the first two learning walks. There are no common bearing or gaze directions between ants, however, (a) in subsequent walks ants always explore directions that they have not previously visited and (b) ants engage in a systematic, saccadic scanning behaviour. I also discuss the significant differences between learning walks of *M. croslandi* and those previously studied in two other ant species, especially in relation the ‘turn back and look’ behaviour.

In displacement experiments, I provide supporting evidence of a quick scanning behaviour that occurs as soon as ants are released. I examine the effect of a conflict in navigational information on successful homing by comparing full and zero vector ants. Zero vector ants are significantly better at navigating home, especially when released at unfamiliar sites. With the aid of the extensive individual foraging histories available to me, I show how in most cases, scene
familiarity plays a role in driving ants home from unfamiliar displacement locations and discuss in detail behaviours that are exceptions to this. Finally, I provide the first evidence of the use of artificial landmarks near the nest in this species, which increases the accuracy with which ants pinpoint the nest entrance, even though they do not appear to make use of such landmarks in the wild and discuss my findings in relation to other ants. I also document the occurrence of re-orientation walks in response to an altered visual environment which show that ants are more directed as a result of re-learning.
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Chapter 1. Individual foraging careers of the Jack Jumper ant, *Myrmecia croslandi*

1.1 Inexperienced foragers

1.1A Impressive first time navigators: learning walks

In this thesis I aimed to investigate how individually foraging ants acquire and use navigational knowledge of their environment. Social insects, particularly bees and ants, have remarkable navigational capabilities. As central place foragers, they regularly return to a central place, the nest, after outbound journeys (e.g. reviewed by Wehner and Menzel, 1990). At present, very little is known about how inexperienced individuals learn to navigate. Specifically, what mechanisms aid the first time navigation to a goal? What do inexperienced individuals learn about their local environment? There is, however, extensive literature on learning/orientation flights in wasps and bees. These flights are performed as an individual is leaving a goal, to acquire the necessary information to allow them to return (e.g. Van Iersel and van den Assem, 1964, Collett and Lehrer, 1993, Lehrer, 1993, Zeil, 1993, Zeil, 1993b, Capaldi and Dyer, 1999, Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014) (reviewed by Wehner, 1981; Zeil et al., 1996). Similarly, ants engage in ‘learning walks’ when they leave a new feeding site and when confronted with a new artificial landmark around the nest (Nicholson et al., 1999, Müller and Wehner, 2010). A common theme of both learning walks and learning flights is that the insects ‘turn back and look’ (TBL), whereby they are believed to store retinotopic views of newly discovered goals (Van Iersel and van den Assem, 1964, Wehner et al., 1992, Lehrer, 1993, Zeil, 1993a, Zeil, 1993b, Judd and Collett, 1998, Nicholson et al., 1999, Lehrer and Bianco, 2000). If enough views are stored in this manner, returning individuals may be able to match the closest snapshot with their current snapshot and gather information about the nest direction (Zeil et al., 2003, Graham et al., 2010, Wehner and Müller, 2010).
1.2 Navigation

Path integration (PI) is a core navigational system and is the only available mechanism on bare terrain when no landmarks are available (e.g. Wehner and Wehner, 1990, Schatz et al., 1999) or when there are no chemical trails (Collett and Collett, 2000). To carry out path integration an individual must monitor (a) the distance travelled and (b) the heading direction using a compass reference system such as the sun and the pattern of polarised skylight (e.g. Wehner, 1976, Horvath and Wehner, 1999, Homberg, 2004). This enables the calculation of the ‘home vector’ and when displaced from their routing paths, will cause individuals to move in the direction of the home vector towards the now, fictive nest. While much work has been done on the ‘celestial compass’ component of the path integrator, determining how walking insects measure distance for path integration has proved to be more challenging as indicated by the comparatively fewer studies on this topic. Experiments on desert ants, *Cataglyphis fortis*, were the first to provide strong evidence that ants monitor distance by using a stride integrator to measure walking distances (Wittlinger et al., 2006, 2007). Ants on stilts will overshoot the nest, whereas those with shortened legs will undershoot the nest distance (Wittlinger et al., 2006, 2007). Ants of *C. fortis* are also known to perform path integration accurately when on hilly terrains by integrating the ground distance in contrast to just the distance walked (Wohlgemuth et al., 2002). This is presumably done by measuring the gradients of the ascending and descending channels (Wohlgemuth et al., 2002) though the mechanism by which this feat is achieved remains unclear. Once both distance and directional information has been obtained using a stride counter and celestial compass respectively, the two sets of information are combined repeatedly, and the home vector is gradually updated (Müller and Wehner, 1988).

However, there are problems associated with vector based navigation. First, celestial compass cues change with the movement of the sun. Honey bees seem to circumvent this problem by using an internal, simplified guide that encompasses different polarisation patterns during different times of the day and year (Dyer and Gould, 1981, Wehner and Rossel, 1985, Rossel and Wehner, 1986; for reviews see Ronacher and Wehner, 1995, Collett and Collett, 2000, Wehner and Labhart, 2006). Another basic problem of path integration is
the accumulation of errors which increases with foraging distance (Müller and Wehner, 1988). To cope with error prone PI, insects employ systematic searches and rely on landmarks (Wehner and Srinivasan, 1981, Müller and Wehner, 1994; for reviews see Ronacher and Wehner, 1995, Collett and Collett, 2000).

A third problem with PI is that it does not allow insects to compensate for passive displacements, for example, by being driven away from their normal paths by gusts of wind (Narendra, et al., 2007a), and, less commonly, by falling into rivers when returning home (Fourcassié, 1991). The path integrator does not account for such passive shifts and therefore ants displaced from normal routing paths will normally move in the direction of the home vector towards the now, fictive nest. In these cases, ants may either (a) completely run off their path integration/home vector and engage in a systematic search when they do not encounter the nest or (b) travel half the outbound distance and then engage in search or (c) use both their path integration vector information and visual information to travel in an intermediate direction dictated by both (Wehner and Srinivasan, 1981, Müller and Wehner, 1994, Reid et al., 2011, Narendra et al., 2013, for reviews see Ronacher and Wehner, 1995, Collett and Collett, 2000).

However, the decision by individuals to use either path integration or visual information may also be determined by the reliability (Deneve and Pouget, 2004, Cheng et al., 2007, Körding, 2007), prominence of each cue in the environment or the navigational information available in the habitat (Narendra, 2007, Buehlmann et al., 2012). Furthermore, ants can also use completely different yet supplementary navigational strategies to aid their return home as shown in the *Cataglyphis* ants that use nest associated olfactory cues (Steck et al., 2009).

During the most basic landmark-based navigation mechanism, a returning individual could employ the difference between a view of the world stored at the goal location and the current view (e.g. Cartwright and Collett, 1983, Collett and Kelber, 1988, Lent et al., 2010, Philippides et al., 2011, Lent et al., 2013, Collett et al., 2014). The insect, may rotate the current view against the nest/reference views, which would allow it to (a) obtain bearing, as the direction where image differences are smallest would coincide with the compass orientation of the nest/reference view (Zeil et al., 2003, Graham et al., 2010, Baddeley et al., 2011, Baddeley et al., 2012, Zeil et al., 2014) and (b) to
gather information on its distance to the nest as image differences increase with distance from the nest view (Zeil et al., 2003, Narendra et al., 2013, Zeil et al., 2014). Experiments with artificial sky lines have shown that ants use the landmark panorama to determine the nest-directed bearing (Graham and Cheng, 2009, Reid et al., 2010, Wystrach et al., 2011). However, it is not clear at present to what extent guidance by individual landmarks and the use of the whole panorama are two separate mechanisms that serve two distinct functions in navigation (Wystrach et al., 2011). Work on *Melophorus bagoti* has demonstrated that ants on a familiar route are guided by a large area of the panoramic visual field (Wystrach et al., 2011). When a large, prominent landmark near the nest entrance was displaced and then removed, the resulting homing paths of ants were different to that expected from a purely landmark guided return, pointing to the combined use of landmarks and the distant panorama in this case, possibly guided by the level of salience of the landmark relative to other visual features. In addition, ants of *Myrmecia pyriformis* have been shown to be confused when landmark panorama is blocked in the main foraging direction and in the opposite direction (Reid et al., 2010).

Many studies on ants have investigated the use of site-specific landmarks to pinpoint a goal location via image matching strategies (e.g. Wehner and Râber, 1979, Wehner and Müller, 1985, Judd and Collett, 1998, Akesson and Wehner, 2002; Durier et al., 2003, Graham et al., 2003, Narendra, 2007). Site specific landmarks provide reliable cues for locating and pinpointing a goal. For example, ants of *Ocymyrmex robustior* perform lengthy searches to pinpoint the nest entrance in the absence of landmarks but the addition of a single landmark allows the ants to locate the entrance quickly (Müller and Wehner, 2010). Depending on the navigational information provided by landmarks in any given habitat, certain ant species may therefore rely less on path integration and more on landmark guidance (e.g. Fukushi, 2001, Fukushi and Wehner, 2004, Narendra et al., 2007, Collett, 2010, Narendra et al., 2013).
1.3 Individual foraging careers

1.3A Foraging careers: navigational mechanisms used for site and route fidelity

The paths of flying insects are difficult to follow, but a wide range of studies on ants has repeatedly shown that they follow idiosyncratic routes. This indicates that ants learn sites such as nests and food sources and the paths between these sites (Collett and Zeil, 1998). Therefore, fidelity (site and route) is a vital component of an individual's foraging career and has been reported in many ant species (e.g. Rosengren, 1971, Hölldobler, 1976, Schmid-Hempel, 1984, Fresneau, 1985, Fewell, 1990, Wehner et al., 2004, Beverly et al., 2009, Mangan and Webb, 2012). Such habits increase the reliability of navigation and enable foragers to find their way efficiently between important food sources and the nest (Collett, 2010). Collett (2010) discuss three different visual-based navigational mechanisms by which ants may guide themselves along habitual routes. The first method is beaconing, where an individual recognizes a certain landmark from its snapshot memory and then move towards the centre of this feature (Voss, 1967, Collett, 2010). If such a mechanism is used, we may expect the ants to modify their paths when encountering such landmarks (Collett, 2010, Lent et al., 2013). Second is retinal image matching, where a view along a route triggers the snapshot memory acquired at a nearby location (Cartwright et al., 2001, Collett, 2010). The individual would move until its view matched the retinal positions and sizes of the features in the remembered snapshot (Graham et al., 2004; Collett, 2010). Such retinal image matched routes may be expected to have a set of snapshot defined locations which would act as an attractor from nearby positions (Collett, 2010). Third, snapshots could act as signposts that indicate directions from the identified locations (Collett et al., 1996; Collett, 2010). The heading directions could be encoded as compass directions or in terms of the position of landscape features on the retina and such a route would then be expected to compose a sequence of directional memories (Harris et al., 2007, Collett, 2009, Graham and Cheng, 2009, Collett, 2010, Graham et al., 2010, Baddeley et al., 2011).

However, the underlying mechanisms of visual landmark guidance are not always easy to determine. Sommer et al (2008) showed that foragers of *M. bagoti* are able to learn, retrieve and employ multiple visual route memories. If
acquired successfully, learning a new route does not overwrite the former route memory, and these route memories can be retained over the entire life span of the desert ants. In a different environment to that of *M. bagoti*, *Gigantiops destructor* uses visual landmark guidance to pinpoint a goal along idiosyncratic routes (Macquart et al., 2006). These ants may need to use simpler navigation strategies (without the cognitive cost and time consumption of landmark-triggered responses) that would allow them to move along idiosyncratic routes in an environment with visual complexity (Macquart et al., 2006).

As with the workings of any social colony, route learning and selection may be based on the current local availability of food, which varies according to time of day (Harrison and Breed, 1987, Menzel et al., 1998, Collett et al., 2003). Learning may also be guided by the needs of the insect colony (Kühnholz and Seeley, 1997) and the acquisition of long-term memories. For example, wood ants, *Formic rufa* ‘remember’ spatially organised visual information for a period of seven months (Rosengren and Fortelius, 1986), *Formica polyctena*, retain fidelity to their previous routes through a period of inactivity (Rosengren, 1977) and European ants, *Lasius fuliginosus*, are able to retain trail and site fidelity for four months over hibernation (Quinet and Pasteels, 1996).
1.4 Long term study of individual foraging careers

Even though social colonies such as ant colonies are often viewed as organisation at the colony level, individual behaviour has a fundamental influence on colony function. This relationship is especially important in members of the colony that make contact with the colony’s external environment, that is, the foragers (Traniello, 1989, Biesmeijer and Tóth, 1998). Ants emerge/eclose after passing the larval and pupal stages. Following eclosion, they may either (a) engage in nest tasks and leave the nest on a later date to go out foraging for the first time or (b) if the demand for foragers increases, leave the nest to forage. In both instances, it is remarkable that ants with no previous foraging experience ‘know’ how to find food and then navigate back to the nest. Therefore, it is of great interest to examine the first time navigation to a goal, and also the processes that guide a inexperienced forager through a previously unexplored environment.

There are several challenges associated with long term studies of social colonies at the individual level. For example, most social colonies have a large work force, sometimes as large as several thousand or more. Such large numbers make the tracking of each individual an almost impossible task. More importantly, there is no precise method to determine the true experience of an individual. In addition, there are also other factors such as forager size and strategy that need to be considered. For example, larger individual foragers that do not follow chemical trails or recruit can be easily spotted on the ground. Previous studies that have individually identified and tracked ants as they become foragers have used thermophilic deserts ants such as Cataglyphis bicolor and M. bagoti that have a short foraging life span of four to six days (e.g. Wehner et al., 1983, Schmid-Hempel, 1984, Muser et al., 2005). In all these studies, experience of individuals is normally established by marking and monitoring ants for consecutive days with the assumption that from then onwards, all unmarked ants are inexperienced foragers just beginning their foraging lives. In the case of short-lived ants, this type of marking and experience determining strategy may prove reliable, even though the experience (if any) of individuals prior to the recording period remains unknown. In long-lived ants, that have low forager force turnover or low mortality, as in the
case of *Myrmecia croslandi* (see below) markings are most likely to become lost and therefore such an assumption needs to be made with care.

### 1.5 Study species

Workers of *Myrmecia croslandi* are large (11 mm in body length), monomorphic ants (Jayatilaka et al., 2011). They are more commonly known as ‘Jack Jumpers’ for their remarkable ability to execute short jumps under different circumstances (e.g. when catching prey, when the ground becomes too warm etc). These Jack Jumpers are ideal models to understand how individual foraging careers develop through time and with experience. In addition to being large and conspicuous, colonies only have a workforce of about 100-300 foragers, and workers forage individually. Therefore, by studying such a species, this study will for the first time attempt to follow individual ants as they appear above ground after months of inactivity to when they become foragers.

A central part of the study will be the analysis of ‘learning walks’ carried out by inexperienced individuals in the ants’ natural environment when they leave the nest and how they might relate to subsequent foraging behaviour. Finally, I should note here that previous attempts by myself and other researchers to train these ants to feeders have been unsuccessful.
1.6 Thesis aims

The main aims of this thesis are:

To determine the overall navigational knowledge base of individuals (chapter 2). In this chapter, I address the foraging ecology of two nests both at the individual and group level. This involved tracking of individual ants over several months to identify foraging schedules, routes and sites.

To determine what individuals do when they leave the nest for the first time and what individuals look at during learning walks (chapter 3). Early on in my thesis, I noticed that ants of *M. croslandi* performed what appeared to be learning walks before they left the nest. This chapter examines in detail the structure and function of learning walks. I also attempt here to find the relationship, if any between learning walks and the first foraging trip away from the nest.

To determine the navigational capabilities of experienced foragers returning from both familiar and unfamiliar locations (chapter 4). In this chapter, I address the question of how experienced foragers are able to return from different displacement locations, especially unfamiliar locations which individually identified ants have never previously visited. I also attempt to identify the role of learning walks and familiarity/previous experience of sites in aiding navigation from unfamiliar locations.

To determine how visual features affect the homing ability of this species (chapter 5). In this chapter, I address the effect of landmarks, both small and large on the ability of ants to pinpoint the nest entrance. As nests of *M. croslandi* frequently lack distinct, nearby landmark features, I attempt to determine if landmarks can be learnt and utilised to increase the efficiency and accuracy with which individual ants can pinpoint their nest entrance.
1.7 Acknowledgements

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1.8 References


Chapter 2. Individual foraging patterns of the Jack Jumper ant, *Myrmecia croslandi*

The results presented in this chapter have been published in:


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Publication has been approved for open public access by *Myrmecological News*.

2.0 Abstract

In ants, we know most about the foraging patterns at the colony level. We know surprisingly little about the foraging behaviour of individual foragers and how they shape the behaviour of the colony. To identify spatial and temporal variation in foraging behaviour at the individual level, we studied at two nests, the solitary foragers of the Australian jack jumper ant, *Myrmecia croslandi*. These ants are strictly diurnal and active only between October-April. Foragers of *Myrmecia croslandi* have a long life span (about a year) and we took advantage of this to determine the variation in their time of activity and foraging paths over a 2-year period. By tracking the outbound paths of foraging ants using a Differential GPS we discovered that: (a) individual ants use very different routes to reach the same destination; (b) distance travelled by foragers was longest (up to 15m) when they travelled to nest-specific *Eucalyptus* trees on which they foraged either for prey or tended to sap-sucking insects; (c) and ants made short forays (< 2m) into non-tree sectors where they exclusively hunted for prey. Individual foragers exhibited temporal fidelity based on their nest departure times, and could be classified as those active (a) all day, (b) only within 8hrs of sunrise and (c) only after 8hrs of sunrise. By monitoring individual activity for seven consecutive days we show that individual ants carry out up to 7 trips per day and rarely forage on consecutive days.
2.1 Introduction

The dynamics of an ant colony is largely influenced by its forager force (Traniello, 1989, Biesmeijer and Tóth, 1998). Foragers directly respond to changes both in the external environment and inside the colony and accordingly alter their foraging behaviour, to ensure the colony has sufficient food reserves. Typically, ants forage individually or by following pheromone trails or by following an experienced individual – a strategy known as tandem running (see Carroll and Janzen, 1973, Traniello, 1989). Regardless of the foraging strategy used by ants, all foragers face the same challenge of deciding where to forage and when to forage (Schmid-Hempel, 1984, Traniello, 1989). In solitary foraging species, this task of deciding on a foraging location and foraging time depends on the individual forager, as there are no chemical trails or other workers to guide ants from and to the nest.

Even though the relationship between an individual forager and the colony is an important one, we have little understanding of individual foraging patterns in social colonies. From honey bees, we know that foragers make individual decisions on where to forage and whether to recruit other bees depending on a range of external and internal factors (reviewed in Von Frisch, 1967, Sommeijer et al., 1983, Inoue et al., 1985, Seeley, 1995, Biesmeijer and Tóth, 1998). These decisions give rise to foraging careers, which are unique to each individual depending on the age of the worker, type of food & the frequency of foraging (Biesmeijer and Tóth, 1998). In the context of individual foraging careers in ants, we know from a few ant species that individual foragers exhibit sector fidelity, where animals forage in a small sector around their nest within which they gradually increase their foraging distance with experience (Cataglyphis bicolor: Schmid-Hempel, 1984, Pasteels and Deneubourg, 1987; Melophorus bagoti: Muser et al., 2005). The development of sector fidelity is known mostly from desert ants that are scavengers (Cataglyphis species). Foragers leaving the nest for the first time most likely exit the nest in random directions (Deneubourg et al., 1986). If ants become successful in this randomly chosen sector, they continue to repeatedly forage in this sector. If a foraging trip has been unsuccessful, the number of previously successful trips in that particular sector would determine the likelihood of continuing to foraging in that sector. Sector fidelity is high from the start of an
individual’s career in the Australian desert ant (Muser et al., 2005), whereas in the Saharan desert ant, sector fidelity increases gradually during an ant’s life. Being faithful to a particular sector, however, is not a generic trait but appears to be driven by the food encounter rates. This has been clearly demonstrated in two populations of *Cataglyphis bicolor*, one inhabiting nutritionally rich-habitat in Greece and the other occupying nutritionally-poor saltpan habitats in Tunisia (Wehner, 1987), where colonies with low foraging success or efficiency exhibited low sector fidelity.


The spatial foraging patterns and especially sector fidelity have been typically addressed in desert ants. In such habitats, the distribution of food resources is typically unpredictable, both spatially and temporally. Therefore, desert ants must scavenge for dead arthropods that have succumbed to high heat (Kohler and Wehner, 2005). In addition, these ants have a short foraging lifespan of about 6 days. Here, we study the individually foraging Australian jack jumper ant, *Myrmecia croslandi*, that nests in visually landmark-rich environments. These ants forage at temperatures well below their maximum thermal limits (Jayatilaka et al., 2011) and have a long foraging lifespan of well over a year in natural conditions. Food resources are abundant in their habitat, with ants from each nest typically visiting nest-specific *Eucalyptus* trees on which they forage (Narendra et al., 2013a). Given these striking differences in their lifestyle and
the phylogenetic importance of the ant genus *Myrmecia*, here we attempt to describe their spatial foraging patterns and with particular attention to the question whether individuals exhibit fidelity to particular sectors, routes or food sites. The long lifespan of *M. croslandi* provides an opportunity to identify temporal activity patterns at the individual level. In ants, we know very little about ‘temporal fidelity’, i.e., an individual’s preference to forage at a particular time of day. Ants are active at different times during the day and within their specific temporal niche, activity is tightly regulated by temperature (e.g. Fellers, 1989. Cerda et al., 1998. Ruano et al., 2000, Jayatilaka et al., 2011), light (Creighton, 1953, Narendra et al., 2010) competition (Schoener, 1974, Kronfeld-Schor and Dayan, 2003) and predation (Wehner et al., 1992). However, it is unknown whether individual ants are active at specific times within these temporal niches.
2.2 Methods

2.2A Study species

Ants of *M. croslandi* are monomorphic and are approximately 11 mm in body length (Fig: 2.1a). Colonies typically have a workforce of about 100-200 foragers (Piyankarie Jayatilaka, unpublished observations). The ants are strictly diurnal and are active only from Austral spring to Austral autumn (October-April) (Greiner et al., 2007, Jayatilaka et al., 2011). During this period, on days when surface temperature exceeds 35°C, ants exhibit a bimodal activity, avoiding the warmest part of the day (Fig: 2.1b). The ants are solitary foragers and show no evidence of relying on recruitment or pheromones for finding food.

**Fig: 2.1 Study species and typical activity on a warm summer day.**

(a)- A worker of *Myrmecia croslandi*. Photo has been omitted from open access of thesis. (b) - An example of the daily outbound foraging activity (solid bars) of *Myrmecia croslandi* along with corresponding surface temperature (dashed line). On warm days, ants exhibit a bimodal activity pattern. Modified after Jayatilaka et al., 2011.

Workers of *M. croslandi* feed on tree sap and hunt on both trees and on the ground for live prey, which they carry back to the nest. We studied two nests, nest A and nest B located in the Campus Field Station at the Australian National University in Canberra, Australia (35° 16’ 49.87”S and 149° 06’ 43.74”E). The nests were about 60m apart from each other. The vegetation in the area consisted of *Eucalyptus* trees such as *Eucalyptus macrorhyncha* and *E. viminalis* with very little undergrowth (Jayatilaka et al., 2011).
2.2B Recording duration

Nest A was studied between October 2011 and April 2012 with 53 days of observation. Nest B was observed between January 2011 and March 2011 and September 2011 and February 2012 with 33 and 34 days of observation, respectively. All ants that left the nest during this period were individually marked (Nest A: 43 ants; Nest B: 37) with a water-soluble acrylic paint (Citadel Colours, France). Observations were carried out throughout the day: from when the first forager left the nest until no more ants left the nest. No observations were made on rainy and overcast days. Ants were considered to have commenced a foraging trip when they crossed a reference circle of 60 cm diameter around the nest entrance. The final foraging destinations (e.g., trees) and where possible, type of prey collected were noted for each ant upon their return to the nest.

Nest exit times were monitored either by an observer at the nest or by a video camera (Canon HD Legria HFS 10) looking down at the nest. Video recordings were played back frame-by-frame in VirtualDub (Free Software Foundation Inc, Cambridge, USA) to identify individuals and their exit times.

2.2C Sector fidelity

To identify whether ants from the two nests foraged in particular sectors or visited particular sites, we opportunistically selected and tracked 32 ants from Nest A and 31 ants from Nest B. To determine at the individual level whether ants were faithful to a particular sector, we recorded three outbound foraging tracks for each marked individual. While tracks were obtained over consecutive days of recording, these may not necessarily be the consecutive foraging paths of individuals. Given several ants did only one or two trips and some were caught by spiders we were able to record three outbound paths for 21 ants (Nest A: 8; Nest B: 13). We determined the foraging direction on the first recorded trip for each ant and compared this to the foraging directions of the second and third recorded trips. Ants that foraged within 60° of the first foraging direction during two consecutive trips were considered to exhibit sector fidelity. By recording whether individuals were successful in acquiring prey during a foraging trip, we determined whether the success of capturing prey on the first trip increased fidelity to that sector during subsequent trips. We used a
Generalised Linear Mixed Model (GLM) in GenStat (VSN International Ltd, HP1 1ES, UK) to determine this relationship. Typically, foragers of *M. croslandi* head to nest-specific trees on which they forage (Narendra et al., 2013a). By determining the distance travelled by ants on the three outbound trips, we identified whether individual ants travelled distances equal to or greater than the nest-tree distance in other spatial directions. We used a two sample, unpaired t-test to analyse this relationship in GenStat (VSN International Ltd, HP1 1ES, UK). Using a Generalised Linear Model, we also tested whether ants that visited trees continued to do so, on the second and third trips. In desert ants it has been shown that as ants gain experience they travel further from the nest (Pasteels and Deneubourg, 1987, Muser et al., 1987). We hence tested whether the distance travelled by individuals in the 1st, 2nd and 3rd foraging trips differed by a one-way ANOVA test in GenStat (VSN International Ltd, HP1 1ES, UK).

2.2D Tracking foragers

Outbound, marked ants were selected opportunistically and tracked using coloured flags and a Differential Global Positioning System (DGPS) (DGPS, NovAtel Inc, Canada) (see also Narendra et al., 2013a, 2013b). The flags were placed on the ground, behind an ant as she walked across the ground. The pin-marked trail was subsequently recorded by moving the rover antenna of a DGPS system along it. DGPS consists of a stationary base station receiver (NovAtel, FLEXPAK-V2-L1L2-G GPS plus GLONASS RT-2) with a base station antenna (NovAtel, GPS-702-GG-L1/L2, GPS plus GLONASS) and a the rover receiver (OEMV-2-RT2-G GPS plus GLONASS) with a rover antenna (NovAtel, ANT-A72GLA-TW-N 532-C). The stationary base station calculates corrections for the mobile rover antenna through a radio link so that the position of the rover antenna can be determined with accuracy better than 10 cm. We monitored error estimates during recording and stopped recording when the error estimates were >10 cm. The base station was mounted on a tripod and set to integrate antenna position readings for 30 minutes before recording was started. The rover receiver was carried on a back-pack and was connected to the rover antenna at the end of a long hand-held stick that was moved along the pin trails. Data from the DGPS unit were recorded into a text file as Northing, Easting and Height along with standard deviations (in metres) for each co-
ordinate at 1s intervals using a laptop connected to the base station through a USB port. GPS coordinates and error estimates were extracted with a custom-written MATLAB program (Mathworks, Natick, United States of America) and converted to .gps files using GPSU File Converter (GPS Utility Limited, www.gpsu.co.uk, United Kingdom). These files were then registered with aerial photographs provided by the Australian Capital Territory Planning and Land Authority, ACT, Australia, using GPS Utility (GPS Utility Limited, www.gpsu.co.uk, United Kingdom).

2.2E Temporal fidelity

To determine whether ants exhibited fidelity in exiting the nest at specific times, we used ants that we observed to have left the nest at least twice (Nest A: 38, Nest B: 34) and asked whether there were changes in the daily time of forager exit.

2.2F Foraging frequency

Given that workers of *M. croslandi* are long-lived (at least a year), we hypothesised that ants do not make regular trips. To test if this was the case, we determined the number of foraging excursions individual ants made over a period of seven consecutive days (Nest A: 15 ants; Nest B: 15 ants). We used the time of exit as a measure of the number of trips ants made. We determined the foraging frequency of individual ants and also the regularity of foraging over a seven day period (Nest A: 19th January 2011-25th January 2011; Nest B: 28th January 2011-3rd February 2011). We compared differences in foraging frequency between the two nests using a two-sample unpaired t-test. We also determined the duration of foraging for three trips per ant (Nest A: 8 ants; Nest B: 13 ants). Foraging trip duration was calculated from the time a forager left the 60 cm circle on an outbound trip and returned to the nest. We compared differences in foraging trip duration between the two nests using a two-sample unpaired t-test. We determined whether the foraging duration of individuals that visited trees and individuals that did not visit trees was different by a two-sample unpaired t-test.
2.3 Results

Ants from both nests foraged on the ground and on multiple nest-specific *Eucalyptus* trees that were between 10.3-11.6 m from nest A and between 8.7-12.9 m from nest B (Fig: 2.2). At nest A, foragers visited two trees (T₁ and T₂) South of the nest whereas at nest B, foragers visited trees North-East (T₁), East (T₂) and West (T₃) of the nest. Foragers from nest A regularly returned with prey items such as aphids, spiders, crickets, moths and flies from a patch of vegetation (V) West of the nest (Fig: 2.2c).

2.3A Sector fidelity

At both nests, individuals that visited trees travelled longer distances compared to individuals that hunted for prey on the ground. Individuals that travelled shorter distances foraged in random directions around the nest. At nest A, 10 ants headed in specific directions to trees and travelled distances greater than 8m (Fig: 2.2c, 8.6±0.3m, mean±SD) compared to ants that foraged on the ground that appeared to be less directed and travelled smaller distances (Fig: 2.2c, 3.7±0.4m). At nest B, 17 ants headed in specific directions to trees and travelled distances greater than 8m (Fig: 2.2b, 9.4±0.3m, mean±SD) compared to ants that foraged on the ground that appeared to be less directed and travelled smaller distances (Fig: 2.2b, 6.0±0.9m). At nest A, sectors to the North-West (with vegetation patch) and South-West (with trees) had high forager traffic. At nest B, sectors to the North East, East and West (all with trees) had high forager traffic.
Fig: 2.2. Sector fidelity at the colony level in *M. croslandi*.

(a) - Nest A (N_A), Nest B (N_B) and the trees (T_1, T_2, T_3) and vegetation patch (V) ants visit are overlaid on an aerial photograph. (b) - Outbound paths and circular histograms of ants from Nest B. Only one path per individual is shown. Nest position: red circle; main foraging trees: red arrows. Circular histograms show bearing for: all ants (Nest B: \( \theta = 155.55^\circ, r=0.15, n=31 \)); ants that travelled less than 8m (Nest B: \( \theta = 80.16^\circ, r=0.54, n=14 \)); ants that travelled greater than 8m (Nest B: \( \theta = 225.42^\circ, r=0.46, n=17 \)). (c) - Outbound paths and circular histograms of ants from Nest A. All other conventions as in (b). Circular histograms show bearing for: all ants (Nest A: \( \theta = 241.34^\circ, r=0.41, n=32 \)); ants that travelled less than 8m (Nest A: \( \theta = 288.23^\circ, r=0.37, n=22 \)); ants that travelled greater than 8m (Nest A: \( \theta = 202.23^\circ, r=0.95, n=10 \)).
This trend of travelling longer distances to specific trees and travelling shorter distances in random directions when hunting for prey on the ground was also obvious at the individual level where we obtained three to five tracks per ant (Fig: 2.3: Nest A: 8; Fig: 2.4: Nest B: 13). At nest A, ants travelled mostly in sectors with trees $T_1$ and $T_2$ (Figs. 3a, 3b) and individuals travelled longer distances to these trees (Fig: 2.5, $9.7 \pm 0.8m$, n=7) compared to those that foraged on the ground (Fig: 2.5, $2.2 \pm 0.3m$, n=17). At nest B, ants travelled mostly in sectors with trees $T_1$, $T_2$ and $T_3$ (Fig: 2.4) and individuals travelled longer distances to these trees (Fig: 2.5, $9.4 \pm 0.2m$, n=18) compared to those that foraged on the ground (Fig: 2.5, $5.2 \pm 0.3m$, n=21). At both nests, there was a significant difference (Fig: 2.5) between the distance travelled by individuals that visited trees compared to those who foraged elsewhere (Nest A: t-test, t=-11.38, d.f=22, P<0.001; Nest B: t-test, t=-12.26, d.f=37, P<0.001).

A smaller proportion of ants from Nest A exhibited sector fidelity compared to nest B (Fig: 2.3a). This was determined by the variation in heading direction of the second and third foraging trip relative to the first foraging trip. At nest A, three foraging trips occurred within 60° of the first foraging trip direction on the subsequent recorded trip (Fig: 2.3b, 2nd trip only) and four foraging trips occurred within 60° of the initial foraging direction on the subsequent two recorded trips (Fig: 2.3b, 2nd and 3rd trips only). At nest B, 10 foraging trips occurred within 60° of the first foraging direction on the subsequent trip (Fig: 2.4b, 2nd trip only) and 18 trips occurred within 60° of the first foraging direction on the subsequent two recorded trips (Fig: 2.4b, 2nd and 3rd trips only). At both nests sector fidelity between the 1st and 2nd (Nest A and Nest B: trip 1 vs trip 2, GLM, Wald statistic=0.07, d.f=17, $P=0.02$) and the 1st, 2nd and 3rd trips (Nest A and Nest B: trip 1 vs trip 3, GLM, Wald statistic=0.03, d.f=17, $P=0.03$) was significantly different.

Most individuals developed idiosyncratic routes to different foraging locations at both nests and most ants that visited trees used similar, but not identical routes on the next trips (Fig: 2.4a, e.g., Ant 8, 9, 13).

At both nests, the success of catching prey on the first trip did not increase fidelity to that sector in the following two trips (Figs. 2.3a and Fig: 2.4a, trip 1 vs trip 2: GLM, Wald statistic=0.06, d.f=17, $P=0.79$; trip 1 vs trip 3, Wald statistic=0.11, d.f=17, $P=0.86$). Distance travelled by individuals did not significantly increase after the 1st recorded trip, over the next two trips (Nest A:
one way ANOVA, $F_{2,22}=0.20$, $P=0.80$; Nest B: one way ANOVA, $F_{2,37}=1.00$, $P=0.40$).

Fig: 2.3. Sector fidelity at the individual level in *M. croslandi* at Nest A. (a) - Three to five outbound paths of individual ants. Nest position is at the intersection of lines. Labels at the end of each track indicate whether the forager was successful in capturing prey (P), visiting a tree (T) or unsuccessful in capturing prey (NP). (b) - *Circular histograms with bearings of the 2nd trip relative to the 1st trip* ($\sigma=33.53^\circ$, $r=0.26$, $n=8$) and *bearings of the 2nd and 3rd trip relative to the 1st trip* ($\sigma=358.28^\circ$, $r=0.03$, $n=16$). First trip = 0°. Ants that foraged within 60° of the first foraging direction (area within the dashed lines) during two consecutive trips were considered to exhibit sector fidelity.
Fig: 2.4. Sector fidelity at the individual level in *M. croslandi* at Nest B.

(a) - Three to five outbound paths of individual ants. (b) - Circular histograms with bearings of the 2<sup>nd</sup> trip relative to the 1<sup>st</sup> (ø= 37.98°, r= 0.70, n=13) and bearings of the 2<sup>nd</sup> and 3<sup>rd</sup> trip relative to the 1<sup>st</sup> (ø= 38.99°, r= 0.71, n=26). First trip = 0°. Otherwise conventions as in Fig: 2.3.

![Sector fidelity at the individual level in M. croslandi at Nest B.](image)

**Fig: 2.5. Distance travelled by ants heading towards trees or elsewhere.**

Data are presented as box plots with mean (circle), median (white line), 25<sup>th</sup>, 75<sup>th</sup> percentile and the minimum and maximum values. Nest A: n=8; Nest B: n=13.
2.3B Temporal fidelity

At both nests, individuals exhibited different schedules for time of exit (Fig: 2.6 and Appendix: 2.1). At the colony level, time of forager exit could be categorised into three distinct time slots: (a) active all day, (b) only within 8hrs of sunrise and (c) only after 8hrs of sunrise. At nest A, individuals were active in all three distinct time slots (Fig: 2.6a, Appendix: 2.1a). At nest B, individuals were active only in two time slots (Fig: 2.6b, Appendix: 2.1b). Of the ants that left the nest throughout the day at nest A, most ants avoided leaving the nest between 6-8 hours of sunrise (Appendix: 2.1a, n=6). These results support previous findings of bimodal activity in this species (Jayatilaka et al., 2011) where workers avoid leaving the nest to forage during the hottest parts of the day (Fig: 2.1b). However, this bimodal activity pattern was not obvious at nest B (Appendix: 2.1b).

![Temporal fidelity in M. croslandi ants](image)

**(a)**

**Nest A**

- Proportion of outbound ants

![Graph](image)

Time after sunrise (hrs)

**(b)**

**Nest B**

- All day
- 0-8 hours after sunrise
- 8 hours after sunrise

6. Temporal fidelity in *M. croslandi* ants.

(a) - Distribution of the number of ants that exit the nest relative to sunrise time at Nest A. Sunrise time = 0:00 hrs. Departure times were grouped as: active all day; active within 8hrs of sunrise and active 8hrs after sunrise. See Appendix: 2.1 for departure times of individual ants. (b) - Distribution of the number of ants that exit the nest relative to sunrise time at Nest B. Conventions as in (a).

2.3C Foraging frequency

Not a single worker foraged for seven consecutive days at both the nests (Appendix: 2.2a). The number of days individuals were active over seven
consecutive days varied from 1-4 days at nest A (Fig: 2.7a, 1.2±0.4 days, n=13,) and from 1-5 days at nest B (Fig: 2.7a, 0.9±0.3 days, n=15,). The number of daily trips by individuals over a total of seven days varied at both nests (Figs. 7b, Appendix: 2.2b, Nest A: one trip/day=22, two trips/day=2; Nest B: one trip/day=24, six trips/day=1) with individuals carrying out an average of 3.7±0.4 trips/day at nest A and 10.7±4.5 trips/day at nest B.

At nest A, an individual on average foraged for 44.2±12.2min (n=8) whereas at nest B, an individual foraged for a longer time of 90.7±27.8min (n=13). When visiting trees, individuals from nest A, spent a shorter time foraging (32.1±5.0min, n=7) compared to individuals that did not visit trees (84.6±21.3min, n=17). We found a significant difference for foraging duration between the two types of foragers at this nest (t-test, t=2.40, d.f=22, P=0.03) but not at nest B (Nest Btrees: 81.9±19.6min, n=19; Nest Bnotrees: 65.3±18.0min, n=20; t-test, t=0.62, d.f=37, P=0.54).

![Graphs showing foraging frequency](image)

**Fig: 2.7.** Foraging frequency of *M. croslandi* ants over seven-consecutive days.

(a) - The number of ants that were active for different days over seven-consecutive days at Nest A (n=13) and Nest B (n=15). (b) - The number of daily trips by individuals over a total of seven days at Nest A and Nest B. See Appendix: 2.2 for foraging frequency of individual ants.
2.4 Discussion

We documented differences in the individual foraging patterns of the solitary foraging Australian jack jumper ant, *M. croslandi*. These ants did not forage in all directions around the nest. Individual ants typically walked long distances to head to nest-specific *Eucalyptus* trees, on which they foraged for prey and liquid food. Some ants travelled short distances in different directions around the nest to exclusively forage for prey on the ground. In the small sample of ants, for which we recorded at least three paths for individual ants, it appears that the change from foraging on the tree to elsewhere and *vice versa* did not depend on their foraging success. Foraging frequency of ants was variable both with regard to the number of trips carried out on a single day and the regularity of foraging.

2.4A Spatial foraging patterns

Spatial foraging patterns have been best described in desert ants that are primarily scavengers relying on insects that succumbed to the desert heat (Wehner et al., 1983, Wehner et al., 2004). In these ants, individuals randomly select a sector to forage in, which enables the colony to exploit their unpredictable food resource in all compass directions around the nest (see Fig: 9 Wehner et al., 1983, Hölldobler and Wilson, 1990). With their short foraging lifespan of 6 days, individual ants continue to forage in a particular sector all their life, increasing their foraging distance with age and experience. They switch foraging to a different sector only when the number of unsuccessful trips outweighs the successful ones (Schmid-Hempel, 1984). Given their short foraging lifespan it would be futile to persist foraging in unsuccessful sectors.

In contrast, in the visually complex environments of *M. croslandi*, ants appear to rely on a two-pronged approach to foraging. One, where individuals visit spatially predictable but distant food sources located on nest-specific trees, which as a consequence leads to sector and route fidelity (Figs 3 and 4). Two, where individuals hunt for scattered food resources on the ground in close proximity to the nest (Figs 3 and 4). While ants foraging on the ground were exclusively hunters, those that foraged on trees captured prey and also collected carbohydrates from sap-sucking insects and from sap produced by trees. Ants were not restricted to foraging at a particular location and regularly
switched between foraging on the tree to foraging on the ground independent of their foraging success. Individuals that foraged on nest-specific trees travelled a distance of nearly three times greater than ants that foraged on the ground. It is unclear why the hunting ants foraged only for short distances (~4m) around the nest. As shown in the earlier work on desert ants (Schmid-Hempel, 1984, Wehner et al., 1983, Wehner et al., 2004) both experience and age play a significant role in the understanding of spatial foraging patterns. Given the long lifespan of *M. croslandi*, we were unable to determine the age or the experience of individual ants in this study. Nevertheless, it is clear that the spatial foraging strategies used by these ants are remarkably different compared to the desert ants.

### 2.4B Temporal and individual foraging patterns

Our study indicates that individual foragers of *M. croslandi* exhibit temporal fidelity to some extent. How animals choose their foraging times and what information they use to maintain temporal fidelity is however unknown. For this, animals could use information about the rate of change of surface temperature (e.g., Jayatilaka et al., 2011), the presence of competitors (Carroll and Janzen, 1973), ambient light intensity (Narendra et al., 2010) or their internal circadian rhythms (Welbergen, 2008).

Both trip duration and foraging regularity varies greatly in *M. croslandi*. Only very few foragers carry out more than one trip a day. The individuals that carry out multiple trips could be those that are more efficient or have more experience. Such variation in the number of daily foraging trips has been documented in seed harvester ants, *Pogonomyrmex barbatus*, where most foragers typically make a few but long trips and only a small number of ants make many but short trips (Beverly et al., 2009). The foraging duration in these ants has been shown to be strongly related to the extent of searching by an individual and not to the distance from the nest. Our observations indicate that ants heading to trees are well directed and do not search, whereas ants that hunt for prey on the ground engage in extensive search behaviour. Furthermore, at one nest, we found that foraging durations when visiting trees are shorter compared to when foraging on the ground, which supports the argument that the extent of searching may influence the foraging duration. Over a week’s observation we found that not a single ant foraged for seven
consecutive days in comparison to the desert ants, (C. fortis and M. bagoti), where individuals foraged regularly on a daily basis for 6-10 days (pers. obs. Ajay Narendra). This intermittent activity in M. croslandi could be the direct result of the longevity of workers compared to the short lifespan of desert ants.

The differences in activity times between the two neighbouring nests could be explained by competition, colony size or different micro-climates. Nest A was located within 2m of an active meat ant nest, Tridomyrmex purpureus. Foragers of M. croslandi from nest A had to cross a trunk trail of meat ants to reach their foraging trees. At nest B, there were no meat ant trails in the ants’ typical foraging route and this could have allowed foragers of this nest to be active throughout the day. Meat ants are one of the most behaviourally dominant ants in Australia and interfere with foraging of other ant species (Greenslade, 1976, Andersen, 1997, Gibb and Hochuli, 2004). We have often observed meat ants stealing prey from foragers of M. croslandi or even killing the foragers. Forager force at nest B was significantly lower compared to Nest A, which also could have affected the activity times of the colony. Such differences in foraging behaviour between nests located close to each other highlight the need to study multiple nests to understand the foraging dynamics of ant colonies.
Appendix: 2.1. Temporal fidelity in *M. croslandi* ants.

Nest departure times of individually marked ants relative to sunrise time is plotted against the days on which they were active. (a) - *Three distinct activity times at Nest A.* Ants observed to be active all day (N=10), active only within 8 hours of sunrise (N=21) and active only after 8 hours of sunrise (N=7). Grey shaded areas indicate the warmest period of the day (6-8 hours from sunrise) when most ants did not leave the nest. (b) – *Two distinct activity times at Nest B.* Ants active all day (N=22) and active only after 8 hours of sunrise (N=9). Otherwise conventions as in (a).
Appendix: 2.2. Foraging frequency of *M. croslandi* ants over seven-consecutive days.

(a) - *The number of trips individual ants carried out over a seven-day period at Nest A.* Size of the circles correspond to the number of trips performed by each ant on a single day. Different colours are used to represent different individuals. (b) - *The number of trips individual ants carried out over a seven-day period at Nest B.* Otherwise conventions as in (a).
2.6 Acknowledgements

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2.7 References


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Chapter 3. The structure and functions of learning walks in *Myrmecia croslandi*

3.1 Introduction

Ants, wasps and bees have very impressive navigational capabilities mainly because they are central place foragers that always return to the nest after outbound journeys (reviewed by Zeil, 2012, Collett et al., 2013). In order to do this, a central place forager can employ path integration but must also form robust long-term memories of the nest and goal locations, with the aid of landmarks. In the case of inexperienced or inexperienced foragers, these memories about the location of the nest or goal location are acquired through a highly structured process of learning during learning walks and learning flights. These visual memories are then used to guide the subsequent approach to the goal.

Wasps and bees acquire necessary information to guide them to a goal (nest or feeder) during learning/orientation flights (Van Iersel and van den Assem, 1964, Collett and Lehrer, 1993, Lehrer, 1993, Zeil, 1993a, Zeil, 1993b, Capaldi and Dyer, 1999, Hempel de Ibarra et al., 2009, Philippides et al., 2013, Riabinina et al., 2014) (for reviews see Wehner, 1981, Zeil et al., 1996). For example, wasps of the genus *Cerceris*, fly in increasing arcs around the nest and turn in such a way so that the nest entrance is fixated and prominent landmarks are seen in their frontal visual field (Zeil, 1993a). Inexperienced honeybees (Vollbehrr, 1975, Becker, 1958, Capaldi and Dyer 1999) and ground-nesting bumblebees (Hempel de Ibarra et al., 2009, Philippides et al., 2013, Riabinina et al., 2014) also perform self-teaching flights when they first leave the nest.

However, much less is known about the learning walks of pedestrian insects. Namibian desert ants, *Ocymyrmex robustior* perform learning walks when confronted with a new landmark around the nest (Müller and Wehner, 2010). The learning walks are spiral-like with well-choreographed rotations along the vertical body axis (Müller and Wehner, 2010). There are short stopping phases (~ 150ms) during the rotation movement where individuals look back in the direction of the nest. It is believed that it is at these points that individuals read the current state of their path integrator and then take & store
snapshots of the view across the nest (Graham et al., 2010, Müller and Wehner, 2010). Wood ants, *Formica rufa* also engage in learning walks when they leave a newly discovered feeder and look back at landmarks associated with the feeder and not the goal itself (Nicholson et al, 1999). Backward turns where ants face the landmarks directly become less frequent as the ants become more familiar with the location and with increasing distance from the feeder (Nicholson et al, 1999). It should be noted here that in both cases, these ants are already experienced foragers and perform learning in response to a new stimulus in their familiar environment.

There are some important similarities and differences between learning walks and learning flights of insects. First, in both ants and flying insects, learning occurs as the first response to an altered visual environment (Nicholson et al, 1999, Müller and Wehner, 2010), on leaving the nest for the first time or whenever the insects have difficulties locating the goal during the previous approach (e.g. Van Iersel and van den Assem, 1964, Zeil, 1993a). In the case of ants, individuals turn back to face the goal and it is believed that snapshots are stored during these instances (Graham et al., 2010) and in the case of flying insects, the nest entrance if fixated with prominent goals kept in the frontal visual field (e.g. Zeil, 1993b). Returning individuals would then be able to match the closest snapshot with their current snapshot and determine the nest direction. Second, in both learning walks and flights, the more familiar an insect is with its surroundings, the shorter and less frequent learning flights/walks become (see Zeil et al., 1996, Collett and Lehrer, 1993, Nicholson et al, 1999, Müller and Wehner, 2010). Third, in learning flights insects move along arcs and loops that provide parallax information about the distance between the goal and landmarks (Zeil, 1993a, Philippides et al., 2013, Riabinina et al., 2014). This ‘pivotal’ parallax information is obtained by maintaining the nest stationary on the retina, while moving in arcs and loops around the nest (Zeil, 1993a, Riabinina et al., 2014). The apparent speed of objects would then partly depend on the distance to the observer, for example, closer objects would be the most visible, and is helpful when an individual needs to learn the relative positions of prominent features near the nest (Riabinina et al., 2014). In contrast to flying insects, ants do not appear to move in well-defined arcs during learning walks (Nicholson et al., 1999, Müller and Wehner, 2010) although they look back towards the nest from different distances and bearings.
In this chapter, I will examine the learning walks of individually marked ants of *M. croslandi* at the nest site. I will analyse the structure and dynamics of learning walks and will examine in detail, where and when learning walks occur around the nest and where individuals look during learning. I will also examine the relationship between foraging and learning walks, especially in relation to where individual learning walks occur relative to the very first outbound foraging excursion. To my knowledge, this will be the first attempt to study learning walks as they occur in inexperienced foragers in their natural environment.
3.2 Methods

3.2A Study species

Ants of *M. croslandi* are solitary foragers and show no evidence of relying on recruitment or trail pheromones for finding food. See chapter 2 for more detail. I studied one nest exclusively for data collection for this chapter (Nest A). The nest was located in the Campus Field Station at the Australian National University in Canberra, Australia (35° 16’ 49.87”S and 149° 06’ 43.74”E). Workers of *M. croslandi* at this nest foraged on a *Eucalyptus* tree South-West of the nest and hunted on both this tree and on the ground (West and North-West of the nest, Fig: 3.1a) for live prey, which they carried back to the nest (Jayatilaka et al., 2011, Jayatilaka et al., 2014).

3.2B Recording

The nest was observed from October 2012 to October 2013. Data on learning walks were collected from October 2012 to November 2012. During this period above-ground activity was high following cessation of foraging activity during the winter months (Jayatilaka et al., 2011). Before above-ground activity had completely resumed, I made regular checks on the nest towards the end of Austral Winter and beginning of Spring, to ensure that recording was started when the nest became first active.

Along with a field assistant, I observed the nest from 7:00 am to 7:00 pm on consecutive days. All ants that left the nest (n=74) were individually marked (Fig: 3.1b) with a water-soluble acrylic paint (Citadel Colours, France). Ants were marked using a four spot, three colour code system with the first spot and colour on the pronotum, next two spots and the second colour on the mesonotum and propodeum respectively and the final spot and third colour on the gaster. Observations were carried out throughout the day: from when the first forager left the nest until no more ants left the nest. No observations were made on rainy and overcast days as pilot studies showed ants to be mostly inactive on overcast days and completely inactive on rainy days (n=5 days when no observations were made due to unfavourable weather from October 2012 to November 2012). Learning walks were recorded in an area of 30x40cm around the nest using a Canon HD Legria HFS 10 (Canon Inc., Japan) camera at 25 frames/second (fps) with an image size of 1920 x 1280 pixels.
Twelve marked ants out of a total of 74 observed ants were selected at random and used to record learning walks and their complete foraging careers. These ants were marked as soon as they exited the nest for the first time. A complete foraging career encompassed, for each individual, data from the time an individual (a) first became active above-ground, (b) carried out learning walks, (c) departed the nest for first time foraging (i.e. after ants had carried several learning walks and travelled distances over 2m from the nest) and (d) continued above-ground foraging activity (i.e. continued to forage over consecutive days after the first foraging trip had been recorded). While I cannot be certain if these marked ants had over-wintered, using the methods described above, I was able to ensure that the ants used had not been active in the current observation period prior to marking nor foraged in the last six months.

The first 3-5 consecutive trips of each ant were tracked using pin trails and a Differential GPS device as described in chapter 2. For the purpose of this chapter, only the first foraging trip has been included.

### 3.2C Description and analysis of learning walks

Video clips were first converted to a JPEG image sequence using Final Cut Pro (Apple Inc, Australia) and then ant movements were tracked at 25 frames per second (40ms) using Digilite (Robert Parker and Jan Hemmi, The Australian National University), a custom written MATLAB program (Mathworks, Natick, United States of America). The program allowed me to extract two co-ordinates each (X and Y) for head and thorax that served to estimate gaze direction (see below). In order to estimate digitising errors due to manual digitising, some clips selected at random were digitised five times to determine standard deviations of successive, repeat, independent measurements (Fig. 3.1c and 3.1d).

Using a scale object in the video images, X and Y co-ordinates were converted to cm and after co-ordinate transformations to make the nest the origin of the co-ordinate system and North zero, the bearing, gaze direction, retinal position of the nest and walking speed was determined using a custom written MATLAB program (Jochen Zeil, The Australian National University). Histograms of bearing, gaze and retinal position of the nest were normalised to maximum.

\[
\text{Bearing (degrees)} = \text{atan2}(\text{headY}, \text{headX}) \times 180/\pi
\]

\[
\text{Gaze direction (degrees)} = \text{atan2}((\text{headY} - \text{toraxY}), (\text{headX} - \text{toraxX})) \times 180/\pi
\]
Retinal position of the nest (degrees) = 180-(gaze direction-bearing) (modulo 360°)
Walking speed (V)
Time(s) = frame number x 0.04 (25 frames per second)
\[ V(i+1) (\text{cm/s}) = \sqrt{((\text{head X}(i+2) - \text{headX}(i))^2 + (\text{headY}...\text{ect})^2)} \times \text{fps}/2 \]
\text{Where } i = 1:n-2

Visible path length and straight line distance from the nest was plotted against time taken for each learning walk. These two parameters allowed me to establish whether individuals were more directed on the outgoing or incoming leg of learning walks. This was achieved by dividing straight line distance from the nest by the visible path length of ants to yield values between 0 and 1. According to this measure, a value close to 1 may indicate an oriented (direct/straight) path and a value close to 0 may indicate a more tortuous path (Benhamou, 2004).

In addition to video recording I also noted the daily timing of learning walks relative to sunrise time and also determined for each individual, the number of learning walks, the duration of each learning walk and in cases where learning walks occurred over a single day, the time between each learning walk.

Panoramic images were recorded at the nest using a Sony Bloggie camera (MHS-PM5, Sony Corp., Japan). The images were unwarped to (1441 x 177 pixels) rectangular panoramas (using software provided by Wolfgang Stürzl, German Aerospace Centre (DLR), Institute of Robotics and Mechatronics), Gaussian, low-pass filtered to mimic 3° ant resolution using a custom written MATLAB program (Jochen Zeil, The Australian National University, see also Zeil et al., 2014). The panoramic image at the nest was shifted such that it was centred at North and was used to ask where ants were looking (gaze direction) during learning walks. I also identified points at which individuals reversed their scanning direction and asked whether particular parts of the panorama caused individuals to do so during learning walks.
Fig: 3.1.
(a) - Outbound foraging paths of different individuals at the nest. Only one path per individual ant is shown. N: Nest position indicated by a red filled circle. T: Foraging and NFT: non-foraging trees. V: A patch of vegetation that ants visit (Modified from Jayatilaka et al., 2014). (b) - A marked forager of *M. croslandi* indicating the location of head (H) and thorax (T) co-ordinates extracted from video images. (Photo credit: Ajay Narendra). (c) - Mean average gaze directions (green) for five measurements from one video clip for one ant with average (red line) ± standard deviation/SD (grey lines). (d) - Histogram of standard deviations of all repeat measurements.
3.3 Results

3.3A The structure and dynamics of learning walks

Most learning walks occurred between 2 and 6 hours after sunrise (n=30 walks from n=12 ants, Fig: 3.2a). A few learning walks also occurred much later in the afternoon between 8 and 10 hours after sunrise (e.g. Ants 2 and 5). On average, learning walks occurred 4.4 ± 2.0hrs after sunrise (mean±SD, n=12 ants, range 2.3-9.8). First and second learning walks occurred in a narrow temporal window always within 2 hours of each other, even on separate days, with the exception of Ant 1 (Fig: 3.2a, red and blue circles). Furthermore, Ant 1 was the only individual who left the nest to forage for the first time in the late afternoon.

Learning walks normally occurred over an average of 2.3± 0.6 days (mean±SD, n=12 ants, range 2-4, Fig: 3.2b). Most ants performed learning walks over two days before starting to forage. However, in a few cases ants continued to learn for up to four days (Fig: 3.2b). When learning walks occurred on a single day, the time between each learning walk while highly variable between individuals, averaged around 13.6 ± 33.6 mins (mean±SD, n=12 ants, range 0.5-164). Each learning walk within the recording area had an average duration of 56.8 ± 49.3s (mean±SD, n=12 ants, range 12.4-225.9). On average, individuals carried out 3.7 ± 1.6 learning walks (mean±SD, n=12 ants, range 2-7, Fig: 3.2c). There was no significant correlation between the duration of learning walks and the number of learning walks (regression analysis, p = 0.28, d.f =41).
Fig: 3.2. The timing and number of learning walks.

(a) - Time of learning walks relative to sunrise time per ant. First and second learning walks on same day: red and blue open circles; first and second learning walks on different days: red and blue filled circles; all other learning walks: black circles. (Inset) – Histogram of time of learning walks relative to sunrise time. All Y values have been normalised to maximum. (b) - The distribution of learning walks over different days. Values indicate the total number of 1st, 2nd etc learning walks that happen on a single day. (c) – Number of learning walks per ant.
Some learning walks were completed within the filming area around the nest (Fig: 3.3a). However, due to the small field of view of the camera (which was necessary to determine gaze directions) not all learning walk paths were fully recorded. The learning walks captured on film were therefore categorised into (a) complete loops, (b) outbound learning paths and (c) inbound learning paths. Ants covered an approximate maximum distance of 86cm outside the field of view of the camera (estimated by average walking speed of 1.2cm/s and time of exit/entry, see Fig: 3.6). Overall, my estimate is that learning walks were restricted to an area of 2m diameter around the nest.

In summary of this section, most learning walks occurred in the morning and the first two walks were separated by a narrow time window. Ants carried out several learning walks over multiple days. The duration of each learning walk was short and ants stayed close to the nest.

During learning walks, ants performed systematic, saccadic changes in gaze direction, continuing to turn in one direction before changing scanning direction. An example quiver plot demonstrates how often an animal will scan in such a manner, usually in directions away from the nest (Fig: 3.3b). This type of systematic scanning is evident when gaze direction is plotted over time (Fig: 3.3c, green lines). When ants did turn back and look at the nest (as indicated by zero crossings of the mauve line in Fig: 3.3c, retinal position of the nest), it was mostly when they were heading back towards the nest (Fig: 3.3c, red circles on mauve line and black line for distance from the nest). Successive reversals of turning direction do not appear to be clearly related (Fig: 3.3c; blue circles on green gaze direction line). A more quantitative analysis will follow.

Unfortunately, given the resolution of the camera and the need for a sufficiently large monitoring area around the nest, I am unable to further resolve head movements in more detail (e.g. Zeil et al., 2014). These types of restrictions of the current experimental setup will be examined in detail in the discussion.
Fig: 3.3. The first learning walk of ant 8.

(a) - First learning walk. Nest is at the 0,0 intersection (black solid lines) and indicated by red circle. Arrows point in direction of travel. 
(b) – A quiver plot of the first learning walk. The nest position is indicated by a red circle. Arrow heads in quiver indicate where animal is looking. Arrows outside quiver point in direction of travel. 
(c) – Gaze direction (relative to North) (green), retinal position of the nest (red) and distance travelled from the nest (black, secondary Y axis). Purple circles on gaze direction line (green) indicate when animal reverses its scanning direction. Red circles on retinal position line (red) indicate when animal looks in the direction of the nest.

Two further examples of the first learning walks of two ants, together with their second learning walk are shown in Fig. 3.4 and 3.5. These two examples further reinforce the systematic nature of head scanning movements (Figs 3.4b and 3.5b, red lines) mostly in directions away from the nest. Furthermore, it is obvious that when animals carry out these scans they frequently encounter the same views (see Fig: 3.4b and 3.5b; black horizontal line on green gaze direction line). While ants do turn and look back at the nest, this does not appear to occur with any regularity or for long stretches of the learning walks. The histograms of retinal position of the nest rather indicate that the ants spend most of their time looking away from the nest (Figs 3.4a and 3.5a, red
histograms) and it is mainly when they are returning to the nest that they do look at the nest (Figs 3.4b and 3.5b, red lines).

Fig: 3.4. The first and second learning walk of Ant 1.
(a column 1) – *First learning walk at the nest*. Nest is at the 0,0 intersection (black solid lines). North points up. Black line: outbound path; green line: inbound path. Arrows point in direction of travel. (a column 2) – *Histograms of gaze direction relative to north at zero (green), bearing (blue) and retinal position of the nest (red) for learning walk 1*. Frequencies have been normalised to maximum. (a column 3) - Same for learning walk 2. (a column 4) - *Second learning walk at the nest*. Conventions as before. (b) and (c) *Time course of gaze direction (green), bearing (blue), retinal position of the nest (red) and distance travelled (black) for learning walks one and two*. North is at 90°.
Fig: 3.5. The first and second learning walk of Ant 5.

(a column 1) – First learning walk at the nest. Nest is at the 0,0 intersection (black solid lines). North points up. Black line: outbound path; green line: inbound path. Arrows point in direction of travel. (a column 2) – Histograms of gaze direction relative to north at zero (green), bearing (blue) and retinal position of the nest (red) for learning walk 1. Frequencies have been normalised to maximum. (a column 3) - Same for learning walk 2. (a column 4) - Second learning walk at the nest. Conventions as before. (b) and (c) Time course of gaze direction (green), bearing (blue), retinal position of the nest (red) and distance travelled (black) for learning walks one and two. North is at 90°.

Figure 3.4 and 3.5 also document one of the consistent features of learning walks in *M. croslandi*. The compass directions in which subsequent learning walks occurred (their bearing) tend to cover directions that had not been previously explored (compare blue histograms in Fig: 3.4a and 3.5a, columns 2 and 3). This will be documented in more detail later.

In cases when a complete learning walk was recorded it was obvious that animals did not constantly move away from the nest, instead moving repeatedly towards and away from the nest (see Figs 3.3c, 3.4b, 3.5b; black line) and it is during these return segments that they face the nest. However, I found no difference in walking speed or path straightness when comparing
outbound and inbound path segments of learning walks (Figs 3.3c, 3.4b, 3.5b; black line, distance from the nest), although an ant returning (inbound) directly to the nest during a learning walk could be expected to be more directed than on her outbound trip and therefore also walk faster. However, this appears to not be the case in these ants. There was no significant difference between the walking speeds between inbound learning paths and outbound learning paths (t-test, p=0.097, t=1.68, d.f=74, Fig: 3.6a) and overall, the walking speed of ants during learning walks (1.2 ± 0.1cm/s, mean±SE, n=12 and n=43 learning walks, Fig: 3.6b) did not differ significantly from average foraging speed (1.4 ± 0.2cm/s, mean±SE, n=12 foraging trips, Fig: 3.6b).

![Graph showing walking speeds during learning walks.](image)

**Fig: 3.6. Walking speeds during learning walks.**

(a) - The difference in average learning walk speed for inbound learning paths (decreasing distance/d from nest), outbound learning paths (increasing distance) and average foraging speed (for first foraging trip following learning walks) for all ants. Standard errors are shown. (b) - Box plot of average learning walk speed (open box) compared to average foraging speed (filled box) for all ants. The 25th, 50th and 75th: box; mean: filled circle; maximum and minimum values: whiskers.

### 3.3B Relationship between subsequent learning walks of individual ants

As mentioned previously, during subsequent learning walks, individual ants travelled in directions they had not visited before. Successive learning walks of individual ants occurred in different bearing directions and tended to ‘fill in’ bearings that had not been visited before (Fig: 3.7). Gaze directions in turn, during subsequent learning walks, were much more uniformly distributed,
although in many cases (Ants 3, 4, 6, 7 and 9) ants looked into directions that had not been previously explored (Fig: 3.8).

As time series plots in figures 3.3-3.5 have clearly indicated, ants did not systematically turn back and look at the nest. Overall, the distributions of the retinal position of the nest throughout learning walks do show that ants mostly look out and away from the nest (Fig: 3.9).
Fig: 3.7. Histograms of bearing for 12 individual ants relative to North.

Learning walks are colour coded as follows: Red-1\textsuperscript{st} LW, Blue-2\textsuperscript{nd} LW, Green-3\textsuperscript{rd} LW, Yellow-4\textsuperscript{th} LW, Black-5\textsuperscript{th} LW, Purple-6\textsuperscript{th} LW and Orange-7\textsuperscript{th} LW. Frequencies have been normalised to maximum.
Fig: 3.8. Histograms of gaze direction for 12 individual ants relative to North.
Learning walks are colour coded as follows: Red-1st LW, Blue-2nd LW, Green-3rd LW, Yellow-4th LW, Black-5th LW, Purple-6th LW and Orange-7th LW. Frequencies have been normalised to maximum.
Fig: 3.9. Histograms of retinal position of the nest for 12 individual ants.
Learning walks are colour coded as follows: Red-1\textsuperscript{st} LW, Blue-2\textsuperscript{nd} LW, Green-3\textsuperscript{rd} LW, Yellow-4\textsuperscript{th} LW, Black-5\textsuperscript{th} LW, Purple-6\textsuperscript{th} LW and Organge-7\textsuperscript{th} LW. Frequencies have been normalised to maximum. Ants turn back and look at the nest: grey arrows.
3.3C Consistent features in learning walks across ants (group level analysis)

There was no clear pattern in where ants carried out their first learning walks (Fig: 3.10a). Most ants (9/12 ants) carried out the second learning walk in a direction of ±90° of the first learning walk (Fig: 3.10b, grey box). Taken together, learning walks occurred in all directions around the nest (Fig: 3.11a).

There appeared to be no preferred gaze directions between ants (Fig: 3.11a) although they did reverse their scanning direction most frequently when looking North or South (Fig: 3.11a). Overall, ants spend most of their time looking away from the nest, i.e. at ±180° (Fig: 3.11a). When they did look back at the nest, they did so from all directions (Fig: 3.11b). All summary plots of individuals showed that ants differed in the degree to which they covered all directions around the nest. This was the case for bearing, gaze, reversal of scanning and retinal position of the nest distributions for learning walks (Fig: 3.11a, summary plots).

**Fig: 3.10. Histograms of bearing, gaze direction, reversal of scanning and retinal position of the nest.**

(a) – Histograms of bearing for first learning walk at maximum distance from nest for all 12 ants.

(b) – Bearing of second learning walks relative to first for all ants. Bearing of ±90° relative to first learning walk: grey box.
Fig: 3.11. Individual and group analysis of learning walks.

(a) - Individual and group summary plots of bearing, gaze, reversal of scanning and retinal position of the nest. 1st and 2nd columns – Plots of bearing for 12 individual ants. Summary plots for all learning walks per ant are shown. Frequencies have been normalised to maximum.

3rd and 4th columns – Plots of gaze for 12 individual ants. Conventions as before.

5th and 6th columns – Plots of reversal of scanning direction for 12 individual ants. Conventions as before.

7th and 8th columns – Plots of retinal position of the nest for 12 individual ants. Ant turns back and looks in the direction of the nest: black arrows. Otherwise conventions as before.

Summary histograms for bearing, gaze, reversal of scanning and retinal position of the nest are also shown.

(b) - Ant positions around the nest when individuals turn back and look in the direction of nest (-10°-retinal position of the nest <+10°). Nest: intersection of black lines. Different colours correspond to different ants. All learning walks are shown.
3.3D Relationship between bearing/gaze direction/reversal of scanning and the local panorama

In order to determine if there was a relationship between prominent or high contrast features in the panorama and bearing/gaze direction/reversal of scanning direction, I aligned a panoramic image of the nest site to group histograms presented in the earlier section.

Ants do appear to be slightly directed towards the closest feature to the nest in the panorama as indicated by the distribution of bearing (Fig: 3.12, white square on panorama and open histogram; Rayleigh test of uniformity, \( p=0.041 \)). There were no clear, preferred scanning directions in response to the local panorama (Fig: 3.12, black histogram, Rayleigh test of uniformity, \( p=0.311 \)). Ants appeared to reverse their scanning directions when they faced North or South of the nest (Fig: 3.12, blue histogram; Rayleigh test of uniformity, \( p=0.248 \), note that Rayleigh test does not account for bimodal distributions).
Fig: 3.12. Panoramic images of the nest site together with histograms for bearing (open bars), gaze (black bars) and reversal of scanning direction (blue bars) for all ants.

All figures are centred on North. Histograms have been normalised to maximum. Main foraging tree: white circle; closest feature to nest: white square. Second panoramic image has been filtered to match the resolution of a typical ant eye at 3°.

3.3E Relationship between learning walks and the first foraging trip

In the final stage of my analysis of learning walks, I asked whether there was a relationship between the direction of learning walks and the first foraging trip. Ants differed in the degree to which they covered directions around the nest relative to their first foraging trip (Figs 3.13 and 3.14). Individual histograms of each learning walk showed that all ants carried out at least one learning walk in directions ±45-90° of the first foraging trip (Figs 3.15a and 3.15b). However, this
pattern was not evident in the group distribution, which showed no clear, preferred directions between ants (Fig: 3.15c; Rayleigh test of uniformity, $p=0.384$).

**Fig: 3.13. All learning walks of all ants with corresponding first foraging paths.**

Nest: black circle. Individual ant learning paths (thin lines) and corresponding first foraging paths (thick lines) indicated. Colours indicate different ants (n=12). Please see figure 3.14 for individual paths.
Fig: 3.14. Learning walks (1st and 3rd columns) of 12 ants and the GPS path for the first foraging trip (3rd and 4th columns).

Nest is at the 0,0 intersection. Learning walks are colour coded as follows: Red-1st LW, Blue-2nd LW, Green-3rd LW, Yellow-4th LW, Black-5th LW, Purple-6th LW and Orange-7th LW. Note differences in scales between LW plots and foraging path plots.
Fig: 3.15. Learning walk bearing relative to first foraging trip.

(a) - Plots of learning walk bearings relative to first foraging trip for 12 individual ants. Inset shows colour code for learning walk number. Frequencies have been normalised to maximum. (b) – Individual summary plots of bearing relative to first foraging trip for 12 ants. Frequencies have been normalised to maximum. (c) - Histogram of bearing relative to first foraging trip for all ants and all learning walks. Conventions as in (c).
3.4 Discussion

In this chapter, I aimed to understand the structure and dynamics of learning walks as well as their role, if any, in determining where an ant foraged for the first time. While no two learning walks were the same, I did observe some regularities between ants. First, most learning walks occurred in the early and/or late mornings with a narrow time window separating the first two learning walks. Second, while there were no clear shared bearing and gaze directions between ants, in subsequent learning walks ants covered compass directions that they had not been previously explored and engaged in a systematic and saccadic scanning behaviour during most learning walks. Third, even though I found little evidence that features in the panorama around the nest play a role in the organisation of learning walks, ants most frequently reversed their scanning direction when they pointed North and South of the nest. Finally, individual ants carried out at least one learning walk in a perpendicular direction to their first foraging run.

3.4A The structure & dynamics of learning walks and relationship between subsequent learning walks

Most learning walks occurred between 2 and 6 hours after sunrise with the first two learning walks restricted to a narrow temporal window of within 2 hours, even on different days. Such temporal fidelity in the timing of learning walks maybe attributed to the fact that most foragers exit this nest 4-6 hours after sunrise (see chapter 2, Jayatilaka et al., 2014). It is unclear whether this narrow learning window allows ants sufficient time to learn the solar ephemeris function, the sun’s daily pattern of azimuthal movement as has been reported in honey bees (Towne, 2008) and Cataglyphis ants (e.g. Dyer and Dickinson, 1994, Wehner and Müller, 1993). In bees and ants, this is a highly specialised and adaptive (to various locations) process guided mostly by an innate knowledge of the position of the sun, which would make the learning of the ephemeris function a time sensitive process (Towne, 2008). In the case of M. croslandi, the only ant that left the nest to forage for the first time in the evening, performed its second learning walk more than 6 hours after sunrise. Therefore, the timing of the first foraging trip may indeed guide when learning needs to occur in order to optimise sun-compass learning. At the same time, similar to
what has been reported in honey bees, ants that predominantly forage in the morning may not need to partition their learning walks throughout the day as they would only need to sample a small portion of the sun’s course to obtain a clear, initial representation of the sun-compass function which can then be later modified and adjusted according to foraging experience (Dyer and Dickinson, 1994).

Individuals carried out between 2-7 learning walks before heading out to forage for the first time. The variation in the number of learning walks between individuals is perhaps an indication of experience from previous foraging seasons. Some individuals carried out learning walks in certain sectors (e.g. ant 4) while others covered most sectors around the nest (e.g. ants 2 and 10). It is possible that those that restrict themselves to certain directions during learning walks are those that have some experience of foraging from the previous season. We know that this species ceases above-ground activity during the Austral winter months (Jayatilaka et al., 2011). Therefore, while the individuals monitored for the present analysis were not seen above-ground for several months, it is possible that those that carry out relatively few learning walks may have already been in some capacity, experienced foragers. This then alludes to the possibility that some of these ants may possess and be able to retrieve long-term memories spanning 5-6 months. It is known that the transition from interior to the outside (when ants move from within the nest to the surface to carry out learning walks), triggers massive changes in the mushroom bodies of their brains as a result of exposure to increasing light levels (Stieb et al., 2012). Furthermore, route memories have been shown to last for several months over unfavourable weather conditions in pheromone trail following ants (Formica rufa: Rosengren and Fortelius, 1986, Formica spp.: Ebbers and Barrows, 1980, Lasius fuliginosus: Quinet and Pasteels, 1996, Formica rufa: Rosengren, 1977). Therefore, it is highly likely that individuals of M. croslandi are (a) able to retain long-term memories over unfavourable weather conditions and (b) have those memories re-consolidated during learning walks.

There was a clear separation of bearing directions of subsequent learning walks by each individual. Ants covered different directions around the nest that had not been explored during previous learning walks, suggesting that (a) these ants must maintain some type of memory of visual experiences from the preceding learning walks and (b) that this memory must span at least
several days as some learning walks may occur over as many as four days. This type of memory may be similar to what has been proposed in the desert ant, *Cataglyphis fortis* (different from long-term route memories) that allows individuals to recall that they have seen a particular view before (Collett, 2014).

Ants of *M. croslandi* employed frequent, systematic and saccadic head rotations during learning walks. During these scans, an individual repeatedly encountered the same view directions, but at different locations around the nest. This pattern suggests that the ants have the ability to learn and store identically oriented views of the nest environment at different bearings, possibly together with home vector information from the path integration system.

Ants carried out a number of walks where individuals constantly moved away from and towards the nest. As there were no differences in the straightness of the inbound legs compared to the outbound legs of the learning walks or in walking speeds, it is possible that ants do not simply rely on path integration during the learning process. This type of returning behaviour is different from what has been previously described during learning walks of *O. robustior* and *F. rufa* that spiral away from the nest and feeder respectively but do not return (Nicholson et al., 1999, Müller and Wehner, 2010), but is similar to the short ‘exploration runs’ prior to foraging described in the desert ant, *Melophorus bagoti* (Muser et al., 2005). Exploration runs in both *M. bagoti* and desert ant *C. bicolor* are defined as “small-scale round trips around the nest entrance that preceded the foraging runs” (Wehner et al., 2004). Explorations runs do not serve to bring food and are different to the direct outward trajectory that is a foraging run due to their spatial structure (Wehner et al., 2004, Muser et al., 2005). Ants also move slowly during exploration runs (Wehner et al., 2004), possibly slower than during a normal foraging run. However, in the case of *M. bagoti*, two important distinctions must be made. We still do not know whether ants turn back and look at the nest during these runs and experienced foragers are also known to perform runs in direct contrast to ants of *M. croslandi*. In the case of *C. bicolor*, we know that ants do face the nest frequently during their exploration runs but they appear to be slower which is not the case with *M. croslandi* as there was no significant difference in the walking speed during foraging and learning.
3.4B Consistent features in learning walks across ants

There were no preferred bearing directions for learning walks when all walks were considered. However, a pattern emerged when the first two learning walks were more closely examined. These two walks mostly occurred in directions ±90° to each other (also see Fig: 3.10b). The gaze directions of ants were rather uniformly distributed and did not dwell on particular features of the panorama. However, ants reversed their scanning direction when they faced North or South slightly more often and spent most of their time looking away from the nest.

During learning walks, desert ants, *O. robustior* repeatedly look back at the nest and wood ants, *F. rufa* turn back, look at and move towards prominent landmarks around the goal (Nicholson et al., 1999, Müller and Wehner, 2010). Both these behaviours during learning are widely believed to be an integral part of the acquisition of views for future homing. In order to fixate the invisible nest, animals need to use their path integrator (PI) which specifies the direction of the goal (Graham et al., 2010, Müller and Wehner, 2010). As we have seen here, ants of *M. croslandi* repeatedly look away from the nest. It should be noted that previous work by Nicholson et al (1999) and Müller and Wehner (2010) does not analyse gaze directions in similar detail. Therefore, a true comparison proves difficult. Adding to this is the fact that the turn backs in *O. robustior* are very brief, which is not the case with *M. croslandi*. Nevertheless, it is important to understand how ants of *M. croslandi* might be acquiring snapshots of the local environment necessary for homing? Do ants store nest directed views and/or views with the nest in various locations as indicated by PI?

The answer to these questions might lie in a route navigation model proposed by Baddeley et al (2012). This model determines heading direction by employing scanning movements to search for familiar nest-directed viewing directions, which is equivalent to determining the minimum of rotational image differences (Zeil et al., 2003, Narendra et al., 2013). If such a strategy is used, a returning individual of *M. croslandi* may use any one of the many stored views from the learning walks to aid the return trip. This would also mean that animals using such a system for learning would not need to turn back and look at the nest frequently, as all views are stored in directions that they may encounter on a future return journey. All that is necessary then, is to make frequent scanning
movements to obtain the most familiar viewing directions tagged with nest direction and move on accordingly. As ants of *M. croslandi* have been shown to path integrate (Narendra et al., 2013), they would be able in principle, to tag views with home direction.

Other animals also do not need to acquire nest-directed views. Nocturnal desert spiders, *Leucorchestris arenicola* do not turn back and look at the nest during learning walks (Nørgaard et al., 2012). Instead, they take sideways views of the goal, in this case the burrow, with their lateral eyes much like wasps during their learning flights (Zeil, 1993a, Nørgaard et al., 2012). In order to return to the inconspicuous burrow during the night, with very little light, the spiders would need to maintain the burrow position in the overlapping visual field of their lateral eyes. While the reason the spiders need to keep overlapping visual fields remains unclear, the authors suggest that there may be neural and visual connections between the anterior and posterior lateral eyes of the spider, in which case the use of two eyes instead of one could enhance sensitivity or spatial acuity, two traits ideal for vision in dim light (Nørgaard et al., 2012).

### 3.4C Relationship between learning walks and the first foraging trip

At the group level, there was no clear relationship between the direction of the first foraging trip and learning walks. However, individuals appeared to carry out at least one learning walk in roughly perpendicular directions to the first foraging trip. Given that most ants in this study foraged on a tree in the same sector, it is possible that ants identify the closest tree to the nest by monitoring its position when viewed from different directions during learning walks.

In conclusion, learning walks in *M. croslandi* were found to be highly variable between individuals, emphasising the importance of individual behavioural analysis, especially in a solitary foraging species. Ants systematically walked in directions that had not been previously explored by preceding learning walks. Learning walks in this species are different to those that have been described before in that animals did not simply walk away from the nest. Therefore an alternative approach to learning was discussed in an attempt to shed some light on why this may be the case. There was also little evidence to suggest that dominant features of the local panorama play a particular role in guiding learning.
3.5 Acknowledgements

I am extremely grateful to Jochen Zeil for his feedback on this chapter. I would also like to thank Ajay Narendra for his assistance with experimental and fieldwork set up, Chloe Raderschall, Animesh Agrawal and Teresa Iglesias for their help with field work and data analysis. I am grateful to Hwan-Yin Joon from the Statistical Consulting Unit (SCU) for his help with statistical data analysis.
3.6 References


Chapter 4. Navigational knowledgebase of individual foragers

4.1 Introduction

Foragers of *M. croslandi* are known to path integrate and therefore walk in the direction of their home vector when displaced outside their known foraging environment (Narendra et al., 2013). However, when full vector ants are displaced to closer but mostly unfamiliar locations of 10-15m from the nest, ants are able to successfully return home from all directions, even with the apparent conflict in information provided by their path integrator and the landmark panorama (Narendra et al., 2013, Zeil et al., 2014). In this case, these ants must disregard the path integration information in order to accurately return home (Narendra et al., 2013, Zeil et al., 2014). In order to do this both full and zero vector ants are thought to possess nest directed views at different bearings from the nest (Narendra et al., 2013, Zeil et al., 2014). This ability is thought to reflect the range over which views acquired close to the nest during learning walks (or along foraging routes) provide navigational guidance (see chapter 3 for more detail). By rotating the current view against the nest/reference views, ants can in principle (a) obtain nest directed bearing information, by identifying the minimum image difference function that coincides with the orientation of the nest/reference view (Zeil et al., 2003, Graham et al., 2010, Baddeley et al., 2011, Baddeley et al., 2012, Zeil et al., 2014) and (b) location information as translational image differences increase with distance from the nest view (Zeil et al., 2003, Zeil et al., 2012). Indeed, in the particular landscape in which the two previous studies of this species was conducted, the rotation of displacement location views against nest views gives rise to a minimum in image differences that points in the true nest direction and it appears that ants may be assessing this information when homing from locations they have not visited before (Narendra et al., 2013, Zeil et al., 2014, see also Dewar et al., 2014).

In chapter 3, I analysed the learning walks of *M. croslandi* in detail and in the current chapter I will determine the range over which views encountered during learning walks can provide navigational information. More specifically, I will determine the homing abilities of identified individuals of *M. croslandi* whose learning walks and foraging paths are known, from both familiar and unfamiliar locations of up to 10m from the nest. A distance of 10m similar to that used in...
the previous studies will allow me to determine if ants are still able to home from all directions if they have not previously foraged in certain sectors and permit better comparisons between studies. The first two studies on displacement in this species only determined their homing ability from different locations. Here, I will determine how the information obtained during learning walks may influence this process. I will also explore the scanning behaviour of these ants upon immediate release on displacement platforms to ask whether ants use similar strategies for homing as suggested in the previous studies.
4.2 Methods

I studied one nest exclusively for data collection for this chapter (Nest A). This nest was located in the Campus Field Station at the Australian National University in Canberra, Australia (35° 16’ 49.87”S and 149° 06’ 43.74”E). Most workers of *M. croslandi* at this nest foraged on a *Eucalyptus* tree South-West of the nest and hunted on both this tree and on the ground (West and North-West of the nest) for live prey, which they carried back to the nest (Jayatilaka et al., 2011, Jayatilaka et al., 2014).

4.2A Recording

The nest was monitored for displacement experiments from October 2012-December 2012. Individual ants were marked using the same procedure outlined in previous chapters. A total of 12 marked ants were used for displacements. All ants were tracked on their outbound foraging trips before displacements in order to determine the locations they had previously visited. I had also recorded complete foraging careers since the start of the Spring season including learning walks (see chapter 3 for more detail) for these 12 ants.

4.2B Displacement experiment

Ants were either displaced in full vector (FV) conditions - individuals tracked and captured at the base of the tree or until when they had caught prey, which sometimes occurred before they reached the tree (with vector information) and or in zero vector (ZV) conditions - individuals picked up at the nest on their return from a foraging trip (with no vector information, having walked the full length of their path integration vectors). All full vector ants were fed a diluted solution of maple syrup (for individual nourishment) and in the case of full vector ants without prey, individuals were also provided with live prey (spiders and moths) to entice them to return home. In all cases of full vector ants, all ants carried their prey back to the nest.

Ants were allowed to feed for a maximum of 20 minutes. Feeding ants were not moved from the capture location and the vials containing ants were covered with a black cloth to prevent them from seeing the outside environment. Ants were not majorly disrupted by this feeding process as shown by pilot studies.
Zero vector ants were not fed. In some cases ants were used multiple times, first as full vector and then as zero vector ants.

Marked ants were captured, fed and displaced at random to one of six displacement locations (5 ants per location). The six displacement locations North, West and South of the nest are shown in Fig: 4.1. I was unable to use the East direction as a displacement location due to fencing in close proximity to the nest. Given that learning walks occurred in all directions, I wanted to determine the ants’ homing ability from all directions. I also wanted to test the effect of distance (if at all) on homing ability, hence the six displacement locations.

When transferring each ant from the capture site to the displacement location, the vial containing the ant was placed in a black nylon sleeve (Zeil et al., 2014) to prevent them from assessing visual information. This vial was then vertically inserted into the centre of a 40cm diameter round wooden platform placed on aluminium legs 15cm above ground.

When ready, the foam stopper securing the vial was removed to release the ant. The initial behaviour of ants exiting their vials on the release platforms were filmed with a Canon HD Legria HFS 10 (Canon Inc., Japan) camera at 25 frames/second (fps) with an image size of 1920 x 1280 pixels. Before release, the true North direction, the nest direction and a scale was recorded. Panoramic images were recorded at the nest and at the release stations using a Sony Bloggie camera (MHS-PM5, Sony Corp., Japan). The images were unwarped to 1441 x 177 pixel rectangular panoramas (using software provided by Wolfgang Stürzl, German Aerospace Centre (DLR), Institute of Robotics and Mechatronics), low-pass filtered with a 36x36 Gaussian with $\sigma = 4$ pixels to mimic $3^\circ$ ant resolution using a custom written MATLAB program (Jochen Zeil, The Australian National University, see also Zeil et al., 2014). All panoramic images of release sites were shifted such that they were in the nest direction and compared to nest views with the same orientation.

Released ants were video recorded until they left the platform. Once an ant had moved away from the platform, their subsequent paths were marked with pins. Pins were placed into the ground at roughly 20cm intervals behind the ant and the tracker maintained a distance of roughly 50cm behind the ant in order to not disturb it. When ants started searching as a result of travelling in the wrong direction to the nest, pin tracking was stopped and ants observed for
a further 20 minutes. If ants moved a distance of 50cm in 20 minutes (after start of search or when they had stopped moving altogether), pin-trail tracking was re-started. These pin trails were then recorded using a Differential Global Positioning System (DGPS) (DGPS, NovAtel Inc, Canada) as described in detail in chapter 2.

4.2C Analysis of videos and calculating rotational image difference functions

Video clips were analysed frame by frame as described in chapter 3. From X and Y positions of head and thorax, I determined head and body orientation of ants while on the platform, their bearing and the walking speed relative to the release point. Differences between time taken to leave the platform between full and zero vector conditions were analysed using a t-test. Gaze directions of ants during displacements were also compared to those during learning walks to determine if there were preferred looking directions. Rotational image difference functions were determined using the circshift function in Matlab (for details see Zeil et al., 2014).
**Fig: 4.1. Aerial view of the nest area and panoramic images at the nest.**

(a) - Aerial view shows nest, main foraging tree, hunting patch and colour coded displacement locations. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North.

(b) - The second and third panoramic images have been centered on North and blurred to match the resolution of an ant eye (3°).
4.3 Results

4.3A Behaviour of displaced ants and their homing success

In order to determine if familiarity of an area allowed animals to successfully return home from different release locations, I compared each individual’s preceding foraging trips with their displacement paths (Fig: 4.2, thin yellow paths, n=12 ants). All ants had previously visited trees South-West of the nest, a foraging corridor which overlapped with both South displacement locations (Fig: 4.2). Therefore, all full and zero vector ants should be able to easily return from these locations that they had previously travelled along. Furthermore, full vector ants caught near the tree South of the nest should not have a conflict in navigational information as the path integration vector would point in the true home direction.

As shown here, all except one full vector ant (Fig: 4.2a, ant 11) returned to the nest successfully following displacements at these South locations. When comparing colour-coded crosses in Figure 4.2 that correspond to capture locations, ant 11, travelled to the tree instead of the nest on this occasion, even though her path integration vector pointed in the true home direction (Fig: 4.2a, ant 11, black cross). In the case of zero vector ants, all ants except one (Fig: 4.2b, ant 10) were able to successfully return from the South displacement locations. Ant 10 travelled along the tree line in a perpendicular direction to the nest direction and did not return home.

Given that all ants had frequently travelled along the South bound Nest-tree corridor for foraging, the North displacement locations should be the most challenging for these ants, with the exception of those that had previously foraged North of the nest. This appears to be the case with most ants, with one full vector ant that had previously foraged North of the nest, successfully returning from the 5m North release site (Fig: 4.2a, ant 5). This individual was able to successfully navigate to the nest even with her path integration vector pointing in the opposite direction to the nest (Fig: 4.2a, ant 5, red cross). Of full vector ants that had no prior experience of locations North of the nest, only one ant (Ant 7) was able to return to the nest. Ant 7 also ignored her path integration vector direction, which now pointed in the opposite direction (Fig: 4.2a, ant 7, blue cross). However, it should be noted that in a previous trip, this ant had walked in a North-bound loop around the nest before heading South to forage,
which may have provided her with enough familiarity of scenes North of the nest to navigate from the 10m North location (Fig: 4.2a, ant 7). Interestingly, of all lost full vector ants at the North release sites, only Ant 12 followed her path integration vector direction for more than 1m (Fig: 4.2a, ant 12) that pointed in the opposite direction to the nest. All other ants were not completely guided by their path integration vector, even though these locations were truly unknown to them (Fig: 4.2a, ants 2, 8, 10 and 11).

More zero vector ants compared to their full vector counterparts, were able to navigate from the North displacement locations. This was especially the case for the 10m North release site. Interestingly, none of the ants that successfully returned to the nest had previously foraged North of the nest (Fig: 4.2b, ants 3, 7 and 9). It is unclear what sets the successful navigators apart from the unsuccessful given their foraging histories. For example, why was Ant 1 unable to return home from the 10m North displacement location compared to ants 3, 7 and 9? Even more surprising is the behaviour of Ant 3 who was able to return from the 10m North release site but not the 5m North release site. However, this ant was displaced at the 5m North site first followed by a displacement at the 10m North location on a separate day. It is possible that this sequence of displacements may have given Ant 3 the opportunity to familiarise herself with the North release sites. Finally, lost zero vector ant 12, travelled within close proximity to the nest but then engaged in a search and proceeded to travel in the opposite direction once again (Fig: 4.2b, ant 12).

Similar to the North release sites, I expected ants that had previously foraged West of the nest to be able to successfully home from the West displacement locations. This was the case for full vector Ant 1 who successfully returned home with also perhaps the additional aid of her path integration vector, which pointed in the true home direction in this case (Fig: 4.2a, ant 1, green cross). However, full vector Ants 3, 5 and 9 were also able to successfully return home from the West release sites even with no prior experience of these locations. Furthermore, out of these three ants, Ant 5 was the only individual with no conflict in navigational information, i.e, her path integration vector pointed in the same direction as the nest direction although for a much shorter distance. For Ants 3 and 9, this was not the case with their path integration vectors pointing in perpendicular directions to the true home direction (Fig: 4.2a, ants 3 and 9, green crosses). Finally, ant 12 was unable to return to the nest.
from the 5m West release site and appeared to follow her path integration vector direction, which guided her in the opposite direction to the nest direction (Fig: 4.2a, ant 12, green cross). Zero vector Ants 3, 5, 7, and 9 were all able to successfully return to the nest from the West displacement locations (Fig: 4.2b). Of these four ants, only ant 5 had foraged West of the nest in her previous foraging runs. Zero vector ants 2 and 10 were unable to return to the nest from the 10m West release site. Interestingly both ants travelled North-West of the nest, away from the release station and both ants had not previously foraged in the vicinity of this release site (Fig: 4.2b, ants 2 and 10).
For each ant, previous foraging paths are shown in yellow and displacement paths are thick lines colour coded accurately to displacement locations. (a) – Paths for 12 full vector ants. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North; nest: black star; colour coded crosses: where full vector ants were caught before displacements. (b) – Paths for 12 zero vector ants. Otherwise conventions as in (a).
4.3B Differences between full and zero vector ants

In order to investigate whether full and zero vector ants differ in their response to displacements, I asked if (a) ants were quicker to move off the release platform in a particular vector condition and (b) whether ants were able to more successfully navigate home from release locations in a particular vector condition using the number of lost ants at each site as a measure of success. In the full vector condition, ants took an average of 9.4±7.2s (mean±SD, range 1.3-21.6, n=30) to reach the edge of the platform after release which was not significantly different from ants in the zero vector condition with a time of 10.8±10.5s (range 1.4-43.4, n=30, t-test, p=0.552, t=-0.60, d.f=58, Fig: 4.3).

I wanted to also determine if full vector ants that encountered a conflict in navigational information (i.e. ants where individual path integration vectors pointed in different directions to the true home direction) were slower in moving off the platform than those with no conflict in navigational information (i.e. ants where path integration vectors pointed in ±20° to the true home direction). However this appeared to not be the case with full vector ants with conflict taking an average time of 9.2±7.9s (n=30) and ants with no conflict in navigational information taking an average time of 9.5±7.2s (n=30) to leave the platform.

In the full vector condition, platform exit times did not significantly differ from ants in the 5m West location (longest time) and the 10m South location (shortest time) (t-test, p=0.141, t=1.63, d.f=8, Table: 4.1). In the zero vector condition, platform exit times did not significantly differ from ants in the 10m North location (longest time) and the 5m West location (shortest time) (t-test, p=0.131, t=-1.88, d.f=8, Table: 4.1).

Out of a total of 30 ants at six release sites, 13 ants were unable to locate the nest in the full vector displacements (Table: 4.1, Fig: 4.4a) while a significantly lower number of ants (n=5) were unable to locate the nest in the zero vector displacements (Table: 4.1, Fig: 4.4b, binomial test, p=0.024, d.f=58). The 10m West, 5m North and 10m North displacement locations proved to be the most challenging for full vector ants with more than half the number of displaced ants being unable to return to the nest (Table: 4.1, Fig: 4.4a), in contrast to zero vector ants (Table: 4.1, Fig: 4.4b). In both vector conditions, most ants successfully returned to the nest when they returned from
the 5m South, 10m South locations (Table: 4.1, Fig: 4.4), which fell along the most commonly visited nest-tree foraging corridor (see also Fig: 4.1) and the 5m West (Table: 4.1, Fig: 4.4) displacement site which was in close proximity to a hunting patch visited by some ants (see also Fig: 4.1). There was no significant difference between the time taken by successful and lost ants to leave the platform in either vector condition (FV\text{ successful}=11.0\pm7.5s vs FV\text{ lost}=7.9\pm7.4s, t-test, \(p=2.720, d.f=28\); ZV\text{ successful}=9.1\pm8.7s vs ZV\text{ lost}=16.2\pm10.6s, t-test, \(p=0.148, d.f=28\)) nor when all successful ants were compared to all lost ants (Successful ants=10.0\pm8.6s vs lost ants=11.0\pm9.4s, t-test, \(p=0.697, d.f=58\)).

**Fig: 4.3.** Box plots showing time taken to reach the edge of the platform for 16 full (open boxes) and zero (filled box) vector ants.

Full vector ants have been divided into those with no path integration (PI) vector conflict (open box, solid lines) and those with conflict (open box, dotted lines). The 25\(^{th}\), 50\(^{th}\) and 75\(^{th}\) quartiles: box; mean: filled circle; median: line; maximum and minimum values: whiskers.
<table>
<thead>
<tr>
<th>Displacement location</th>
<th>Full Vector</th>
<th>Zero Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of lost ants/total ants</td>
<td>Average time taken to leave platform±SD (s)</td>
</tr>
<tr>
<td>5mS</td>
<td>1/5</td>
<td>9.4±8.6</td>
</tr>
<tr>
<td>10mS</td>
<td>1/5</td>
<td>6.1±5.7</td>
</tr>
<tr>
<td>5mW</td>
<td>1/5</td>
<td>14.1±6.7</td>
</tr>
<tr>
<td>10mW</td>
<td>3/5</td>
<td>8.5±5.8</td>
</tr>
<tr>
<td>5mN</td>
<td>4/5</td>
<td>9.4±7.1</td>
</tr>
<tr>
<td>10mN</td>
<td>3/5</td>
<td>8.8±6.4</td>
</tr>
<tr>
<td>Total</td>
<td>13/30</td>
<td>9.4±7.2</td>
</tr>
</tbody>
</table>

Table: 4.1. Number of lost ants and average time taken to leave the platform for 60 full and zero vector releases at all six displacement locations.

Fig: 4.4. Ant paths for 60 full and zero vector releases at six displacement locations.

(a) – *Paths of all full vector ants at six displacement locations*. Nest is indicated. Lost ants: dotted lines. (b) – *Paths of all zero vector ants at six displacement locations*. Otherwise conventions as in (a).
4.3C Behaviour on the platform

*Initial behaviour and do ants exit in specific directions?*

Upon exit from the vial holding them, both full and zero vector ants scanned the environment before deciding to move off the platform (Fig: 4.5). This decision to move off the platform was a quick one, with ants taking on average about 10s to leave the platform (Table: 4.1).

![Diagram of ant paths and gaze direction on the platform at six displacement locations for full and zero vector ants.](image)

**Fig: 4.5.** Example ant paths and gaze direction on the platform at six displacement locations for full and zero vector ants.

The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North; direction of path integration vector: colour coded arrows; nest direction: grey arrows.
Some but not all full vector ants initially moved in the path integration direction (Fig: 4.6, solid lines) and in the case of zero vector ants who experience no conflict, initially moved away in directions that differ >90° to their path integration direction (Fig: 4.6, dotted lines). At certain release sites such as 10m South, 5m North and 10m North, zero vector ants paths on the platform fell within ±40° of the true nest direction (Fig: 4.6, dotted lines).

At other displacement locations, for example 5m South, zero vector ants were able to correct the actual paths to the nest, even when they had exited the platform in a different direction to the nest (Fig: 4.6). Conversely, in some cases, ants that exited the platform in the true home direction, still failed to successfully locate the nest. This indicates that the paths on the platform do not always clearly predict the actual paths taken by ants to return to the nest as clearly shown in examples in Figure 4.7 (Fig: 4.7, ants 12 and 4).
Fig. 4.6. Ant paths and gaze for 16 full and zero vector ants at six displacement locations.

Platform paths for all full (solid lines) and zero vector (dotted lines) ants at six displacement locations. Individual ants have been colour coded and respective path integration vectors indicated. True nest direction: grey arrows.
Fig: 4.7. Example platform and corresponding GPS paths for two individual ants. Paths shown are for 5m West full vector and 5m South zero vector ants. Shown are paths on the platform (1st column) and paths after leaving the platform at increasing scales (2nd and 3rd columns). Nest: black circle; colour coded arrow: respective path integration vector.

Where do ants look before deciding where to go?

There was no clear pattern across ants in relation to where they looked before moving off the platform. The exception to this were full and zero vector ants at the 5m West location that looked East and zero vector ants at the 10m South location that looked North-West (Fig: 4.8, compare summary grey plots and individual plots). There was also a tendency for ants at the North release sites to look South. At these locations several ants appeared to agree on where to look upon release.
Fig: 4.8. Histograms of gaze direction for 16 full and zero vector ants at six displacement locations.

Frequencies have been normalised to maximum and histograms are relative to North. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North; nest direction: black arrow; colour coded arrow: respective path integration vectors. Summary plots (grey dashed line) are also shown.
**Relationship between gaze directions and the panorama**

In the next stage of the analysis, I asked whether there was any relationship between gaze direction and panorama features. Ants did not appear to agree on where to look in relation to the panorama (Fig: 4.9). However, there are on average clear differences in where ants look between release sites, especially in the case of full vector ants but these gaze directions do not appear to be related to salient features or gaps in the panorama (Fig: 4.9). This is also true for reversal of scanning directions, although at most sites, there is a tendency for reversals to occur when image differences become large (Fig: 4.10).
Fig: 4.9. Local panorama and gaze direction at six displacement locations for 16 full (filled bars) and zero vector (open bars) ants.

Top rows for each displacement location show both panoramic scenes and panoramic scenes at ant eye resolution relative to North. Third row shows summary histograms (grey boxes). Individual ants have been colour coded and respective path integration vectors indicated. Histograms have been normalised to maximum and are relative to North. True home direction: solid black line.
Fig: 4.10. Local panorama and reversal of scanning at six displacement locations for 16 full (solid lines) and zero vector (dotted line) ants. Top two rows for each displacement location show both panoramic scenes and panoramic scenes at ant eye resolution. Third row shows rotational image difference functions relative to the nest view. Colour coded bottom rows show reversal of scanning histograms where all frequencies have been normalised to maximum and are relative to nest direction. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North; nest direction: solid white line.
4.3D Gaze directions during learning walks and displacements

I finally asked whether there was any relationship between looking directions on release platforms and the views they had encountered during their learning walks. Figure 4.11 shows the learning walks of identified ants and their path on release platforms. Given that ants cover practically all directions around the nest during learning walks (see chapter 3), almost all gaze directions upon release have been experienced during learning walks (Figs 4.11 and 4.12, see also Fig: 4.2 for individual capture locations). At this level of analysis it is thus not possible to clearly link learning walk experience to differences in behaviour when released ants attempt to determine their bearing.
Fig: 4.11. Behaviour and homing success for 12 full and zero vector ants. 

For each ant, learning walks, full (solid lines) and zero vector (dotted lines) platform paths are shown. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North; nest: black star; colour coded arrows: individual path integration vector direction; learning walks are colour coded as follows: Red-1st LW, Blue-2nd LW, Green-3rd LW, Yellow-4th LW, Black-5th LW, Purple-6th LW and Orange-7th LW. See Fig: 4.2 for individual capture locations.
Fig: 4.12. Comparison of gaze directions during learning walks and displacements of 12 ants.

For each ant, the summary gaze distributions for learning walks (grey solid lines) and individual gaze distributions during full vector (solid lines) and zero vector (dotted lines) displacements are shown. Frequencies have been normalised to maximum and histograms are relative to North. The six displacement locations were: Black-5m South, Orange-10m South, Green-5m West, Purple-10m West, Red-5m North and Blue-10m North.
4.4 Discussion

In this chapter, I attempted to relate the navigational competence of individually identified ants with their learning walks and subsequent foraging experience. To do this, I displaced ants to familiar and unfamiliar locations. Overall, I found that the homing success of displaced ants cannot be fully explained by either their learning walks or foraging history. Upon release at both unfamiliar and familiar locations, ants of *M. croslandi* engaged in a scanning behaviour of the environment before moving off the platform. This result is similar to what has been previously reported by Narendra et al. (2013) and Zeil et al. (2014) in displacement experiments with this species. This decision of where to move upon release is a quick one, after brief scanning of the environment as evident by the short time (mean 10s) spent on the platform. This indicates, as has been previously suggested, that these ants are able to gather the necessary visual information from the environment and decide on a heading direction without the need for substantial translational movements (Narendra et al., 2013, Zeil et al., 2014, Wystrach et al., 2014). However, many ants do correct their subsequent heading direction after moving off the platform, indicating that the ants are constantly making navigational decisions, even after they have decided on a direction to move.

There was a significant difference between the number of lost ants at full vector and zero vector locations in perhaps the most contrasting result between the current and the two previous studies. Using lost ants as a measure of difficulty of homing from different locations, most full vector ants struggled to return from 10m West, 5m North and 10m North locations. Given that ants were tracked on their outbound foraging trips before displacements, I can confirm that these locations were unfamiliar in the current foraging season and unexplored for at least six months. Indeed, most full vector ants appeared to successfully return home from locations that they had previously visited, i.e. those covering the normal foraging range of the nest (5m South, 10m South and 5m West). This was the case even with a conflict in information provided by their path integrator and landmark panorama encountered by a full vector ant (Zeil et al., 2014, Narendra et al., 2013). Given this conflict, the North displacement locations were perhaps the most challenging, as the information from the ant’s path integrator would, in most cases direct them in the opposite direction to that
indicated by landmarks. However, it is interesting to note than when this conflict is removed in zero vector ants, a significantly larger number of ants were able to return home. Given these results, it is obvious that these ants do not completely ignore their path integration vector when homing from unfamiliar locations but suppress is at familiar locations. In several cases, paths on the platform were not necessarily true reflections of the actual paths taken by ants indicating that ants continue to make navigational decisions as they travel from different release sites. In contrast to the previous studies on this species, the current study shows that ants can still struggle to home from locations within 10m from the nest, given the state of their knowledge and familiarity.

When gaze directions of ants between full and zero vector conditions were compared, some full vector ants looked in both home and path integration vector directions and most zero vector ants looked in the true home direction. Most full vector ants at the West release site appeared to concentrate on a compromise direction between home and path integration vector. However, overall, ants did not agree where to look upon release.

Individual ants greatly differed in where they looked in the panorama and as indicated by gaze directions, ants do not appear to be paying close attention to certain features in the panorama. At this level of analysis, it is difficult to conclude if the decision to reverse scanning is related to salient features in the panorama but there is a consistent trend for ants to reverse scanning directions when image differences become larger. This result supports those presented by Narendra et al (2013) and Zeil et al (2014) who suggest a strategy of global comparison of learnt, nest-directed views and current views as seen from the different release sites in order to recognise a heading direction as indicated by a minimum of image differences.

At this stage of analysis, it is also difficult to determine if displaced ants pay particular attention to gaze directions learnt during learning walks. However, it is possible that ants store multiple views in different orientations during learning walks, which they then compare with current views via scanning at release sites, with the aim of obtaining a known heading direction (Möller, 2012). I have also shown that ants are able to return from the South release sites most likely due to their familiarity with these locations as they overlap with the ants’ most common foraging sector. However, the ants’ behaviour does not always fit a clear pattern with (a) some ants with no prior experience of the
North and West release sites being able to return to the nest and (b) while some ants with no familiarity of these locations becoming lost. It should be noted that all ants that had previously foraged North and West of the nest were able to successfully return from these locations regardless of their vector condition. These behaviours may point to prior individual experience. As described in chapters 2 and 3, it is possible that these ants may have previous season experience of certain foraging sectors before tracking was started. The long life span of these ants (up to 1.5 years, personal observations) and the fact that ants overwinter, makes it a challenging task to fully monitor their foraging careers. Furthermore, there may be individual differences in the robustness of visual memories and the way in which they are accessed.
4.5 Acknowledgements

I am extremely grateful to Jochen Zeil for his feedback on this chapter. I would also like to thank Ajay Narendra for his assistance with experimental and fieldwork set up, Chloe Raderschall, Animesh Agrawal and Teresa Iglesias for their help with field work and data analysis. I am grateful to Hwan-Yin Joon from the Statistical Consulting Unit (SCU) for his help with statistical data analysis.
4.6 References


Chapter 5. Effect of visual features on the homing ability of *Myrmecia croslandi*

5.1 Introduction

Central place foragers that revisit important goal locations are able to do so by two principal navigational mechanisms. First, by path integration where an insect computes the distance and direction travelled and continuously updates a home vector (Müller and Wehner, 1988, Collett, 1992, Cheung and Vickerstaff, 2010). Second, by landmark guidance and many studies have shown the importance of both route and site specific terrestrial landmarks in guiding an individual to an important goal (e.g. Tinbergen and Kruyt, 1938, Wehner and Räber, 1979, Cartwright and Collett, 1983, Collett, 1992, Wehner et al., 1996, Cartwright et al., 2001, Akesson and Wehner, 2002, Graham et al., 2004, Narendra et al., 2007a, Philippides et al., 2011, Mangan and Webb, 2012, Narendra et al., 2013).

Guidance through route based landmarks may take two forms, (a) ‘alignment image matching’ and (b) ‘positional image matching’ (Collett et al., 2013). The first type of image matching can be used to determine heading direction along a habitual route where an animal can align itself so the current view matches a previously stored view (Zeil et al., 2003, Collett, 2010, Graham et al., 2010, Baddeley et al., 2011, Baddeley et al., 2012, Collett et al., 2013, Narendra et al., 2013). The second type of image matching provides information on the distance from a reference location and can serve to pinpoint goals (Cartwright and Collett, 1983, Collett et al., 1986, Zeil et al., 2003, reviewed in Zeil, 2012). By comparing a snapshot memory of a target goal with the current view, it is possible to determine the heading direction in which image differences become smaller, provided the goal and current locations have enough common features in a ‘catchment’ area (Cartwright and Collett, 1983, Collett et al., 1986, Zeil et al., 2012).

Many studies on ants have investigated the use of site specific (both natural and artificial) landmarks to pinpoint a goal location via the image matching strategies discussed above (e.g. Wehner and Räber, 1979, Wehner and Müller, 1985, Judd and Collett, 1998, Akesson and Wehner, 2002, Durier et al., 2003, Graham et al., 2003, Narendra, 2007, Narendra et al., 2013). Site
specific landmarks provide reliable cues for location and serve to complement path integration, which is prone to accumulating errors with increasing distance from the nest (Merkle et al., 2006). Depending on the navigational information content of habitats, certain ant species may therefore rely less on path integration and more on landmark guidance (e.g. Fukushi, 2001, Fukushi and Wehner, 2004, Narendra et al., 2007a, Collett, 2010, Narendra et al., 2013). This has been clearly demonstrated in two species of desert ants, one inhabiting a featureless, landmark free environment and the other inhabiting a cluttered, landmark rich environment with the use of an artificial landmark corridor (Bühlmann et al., 2011). The former is more likely to use vector information during homebound runs while the latter is more likely to switch from vector to landmark guidance and to optimise the use of site/nest specific landmarks. The learning walks I have described in chapter 3 are widely believed to be crucial in allowing ants to learn the spatial layout of landmarks relative to an important goal (Nicholson et al.,1999, Müller and Wehner, 2010). Desert ants can also use artificial landmarks and channels to find specific goals such as the nest, feeder or even to establish foraging routes between the nest and food sources (Collett 1998, Heusser and Wehner, 2002, Wehner, 2003). On return to the goal location, ants employ image matching strategies which leads them to search in the now fictive nest location in altered landmark configurations as at this location the stored and current images would have the highest similarity (Wehner et al., 1996, Åkesson and Wehner, 2002, Wehner, 2003).

In the highly visual, solitary foraging _M. croslandi_, information from path integration is overridden when displaced to locations within normal foraging ranges of the nest and landmark guidance takes precedence (Narendra et al., 2013). However, beyond this distance, in completely unfamiliar environments, the ants revert back to using their path integrator, but only for a few metres (see chapter 4, Narendra et al., 2013). While we now know the effect of the landmark panorama on the homing ability of these ants, we do not know the effect of site-specific landmarks, close to the nest on the final leg of their homebound runs. That is, are landmarks near the nest able to allow these ants to pinpoint the nest location more accurately and efficiently? In this chapter, I will be asking if ants are able to use artificial landmarks in close proximity to the nest for pinpointing the nest entrance and if so, the effect of landmark manipulations on
the return paths of ants within a small area around the nest. I expect ants to be able to home more accurately in the presence of landmarks compared to when no landmarks are present. Furthermore, by displacing landmarks in several different directions to test the effect, I expect to determine if displacements in certain directions affect homing ability more than others. For example, I predict the South displacement of landmarks coinciding with their main foraging corridor to have a greater effect on homing compared to displacements in the East of West directions.
5.2 Methods

5.2A Study species

Please see chapters 2 and 3.

5.2B Recording

One nest (Nest A) was observed for the landmark experiments from December 2013-February 2014 on consecutive days when the weather permitted. Most ants that left the nest during this period were individually marked as before with a water-soluble acrylic paint (Citadel Colours, France) and only marked ants were used for the following experiments. Inbound paths of ants were recorded in an area of 30x40cm around the nest using a Canon HD Legria HFS 10 (Canon Inc., Japan) camera at 25 frames/second (fps) with an image size of 1920 x 1280 pixels.

5.2C Landmark conditions

Two different sets of landmark experiments were carried out at the nest, (a) with 2 small black cylindrical landmarks (7.5x4cm, Fig: 5.1a) at 15cm from the nest with an apparent height of approximately 24.9° as seen from the nest to test the effect of two small, landmarks close to the nest and (b) with 4 large black cylindrical landmarks (25x16cm, Fig: 5.1b) at 10cm from the nest with an apparent height of approximately 69.6° to test the effect of multiple landmarks, close to the nest. A returning ant with 3° eye resolution would be able to observe the smaller landmarks at a distance of 1.4m from the nest and the larger landmarks at a distance of 4.8m from the nest.
**Fig: 5.1 Arrangement of landmarks at the nest and manipulation schedule.**

(a) – **Two small landmark arrangement.** Nest: red circle. (b) – **Four large landmark arrangement.** Conventions as before. (c) – **Schedule of manipulations and recordings.** Uninterrupted refers to when landmarks were displayed for a period of two weeks constantly. Interrupted refers to when landmarks were displayed in a certain configuration for a couple of hours and then returned to original configuration (see below).

**Two small landmark experiment**

Ants were first exposed to a normal, no landmark condition for two weeks (see Fig: 5.1 for recording durations). Following this two small landmarks were introduced at the nest each 15cm from the nest. All inbound paths were video-recorded during control and after introduction until paths for 10-15 individually marked ants had been recorded. The landmarks were then displaced by 10cm to the South of the nest. All inbound paths of ants were recorded until 10-15 different ants had been recorded. The time taken to record enough paths
normally depended on the daily forager traffic at the nest. I showed in chapter 2 that identified foragers were not active over consecutive days with an average of 3.7 foraging trips per day by marked ants (see chapter 2 for more detail).

In instances where ants were unable to pinpoint the nest, individuals were picked up and returned to the nest once they had searched for a total of 5 minutes (both inside and outside the field of view of the camera). In these cases filming was stopped when ants first left the recording area during search. The same displacement and capture-release methods will apply to the following manipulations unless otherwise stated.

Four large landmark experiment

Four large landmarks were introduced at the nest and the same recording procedure mentioned earlier followed (see Fig: 5.1 for recording durations). All landmarks were then displaced by 10cm to the South of the nest after 5 or more ants had left the nest in the morning foraging run, which usually took between 1-5 days depending on daily forager traffic. All inbound paths of these ants were then recorded with the displaced landmark condition. Once the foragers had returned, the landmarks were re-introduced to their original position and remained so for the rest of the day. This continued until 10-15 inbound paths of different ants had been obtained for this condition.

This method ensured that the foragers would encounter a different nest environment on their return compared to their departure. Returning the landmarks to the original position after a couple of hours every day, ensured the stability of landmarks as a visual cue that would not be disregarded by the foragers in contrast to the methodology for the two landmark experiment. Ants were then allowed to re-familiarise themselves with the original landmark position. Refer to chapter 2 for more detail on foraging frequency of individually tracked ants.

Next, the landmarks were displaced by 10cm to the West of the nest as before. The landmarks were then removed completely once a sufficient number of ants (n=5 or more) had left the nest and their inbound paths were recorded. The landmarks were re-introduced and ants were allowed to re-familiarise themselves with the original landmark constellation. Next landmarks were displaced 10cm East of the nest following the same procedure outlined before.
After a month of ants being exposed to the original landmark position (no displacements), landmarks were completely removed and then the South displacement was repeated to ascertain if ants had learnt and remembered an already familiar landmark configuration. The landmarks were in slightly different positions in the original South displacement and repeat conditions over the two months of experimentation. In instances where ants were unable to pinpoint the nest, the same capture-release method mentioned previously was followed.

Ants that carried out re-orientation walks in response to these landmark manipulations were noted. Reorientation walks were similar to learning walks (see chapter 3) but were performed by experienced foragers in response to a visual environment that had changed compared to that experienced on their outbound run.

5.2D Analysis

All data provided in this chapter was gathered from frame by frame analysis of video clips at 40ms inter-frame interval at 25fps. Ant movements were tracked using Digilite (Robert Parker and Jan Hemmi, The Australian National University), a custom written MATLAB program (Mathworks, Natick, United States of America). The program allowed me to extract two co-ordinates (X and Y) for head position. Once the video-clips were digitised, a custom written MATLAB program (Jochen Zeil, The Australian National University) was used to analyse the inbound paths of individual ants with landmark conditions.

Each ant search histogram was normalised to maximum before compound histograms were determined. Ant homing and search paths for each condition were then combined into two-dimensional search density histograms. All figures were plotted such that North pointed up. Differences in North-South and East-West directions between the two dimensional, independent, non parametric distributions were analysed using a multi sample Kolmogorov–Smirnov (KS) test (Fig: 5.2).
Fig: 5.2 Cumulative distributions for the multi sample KS test.
(a) - Cumulative distribution in the X-direction (East-West) for with landmarks (blue line) and South displacement of landmarks (red line). (b) - Cumulative distribution in the Y-direction (North-South) for with landmarks (blue line) and South displacement of landmarks (red line). Inset shows inbound path plots and heat maps for all ants for the with landmarks condition (blue lines) and landmarks displaced South condition (red lines). Nest: Black and white line intersection; current/displaced landmark position: black; original position: grey.

The time taken to enter the nest after entering the recording area (30x40cm) was calculated for each condition to determine if (a) presence of landmarks reduced the time to enter the nest and (b) if the displacement and removal of landmarks increased that time. This was only done with ants that found the nest on their own. Any significant differences between various conditions were tested.
using ANOVA followed by a multiple comparisons, post-hoc (Welch’s test for unequal variances) test for significant results. In cases where re-orientation walks were observed, the time taken to enter the nest on the subsequent return was also determined.

For each four large landmark condition and re-orientation walks, I analysed the straightness index of each path by plotting the shortest/straight line distance to the nest against the path length of ants once they had entered the recording area. These two parameters allowed me to establish whether individuals were more directed in any one of the landmark conditions. This was achieved by dividing straight line distance from the nest by the visible path length of ants to yield values between 0 and 1. Values close to 0 indicate a tortuous path and values close to 1 indicate a straight/direct path. Any significant differences between various conditions were tested using ANOVA followed by a multiple comparisons, post-hoc (Welch’s test for unequal variances) test for significant results. This was only done with ants that found the nest on their own.
5.3 Results

5.3A Individual approaches to the nest and landmark manipulations

In the normal, undisturbed condition around the nest, individual ants varied greatly in how efficiently they located the nest entrance. This may be a reflection of the level of experience in each individual (Fig: 5.3). Some ants were able to find the nest relatively easily, exhibiting straight paths to the nest (Fig: 5.3, 1st row). Others however could miss the nest by a few centimetres and only found it, at times, after extensive search (Fig: 5.3, 2nd row). These results suggest that ants are unlikely to use nest defining pheromones or CO₂ plumes from the nest to pinpoint its entrance.

Fig: 5.3 Examples of inbound paths of different ants when no landmarks were present.
Nest (N): blue filled circle, direction of travel: black arrows.

This high degree of variation between individuals was also evident in all other landmark manipulation conditions. Even when landmarks were displaced, some ants had no difficulty finding the nest, while others appeared to be greatly affected by these manipulations. I document this with individual examples for all landmark manipulations in figures 5.4 and 5.5. When two small landmarks were introduced at the nest (uninterrupted for two weeks), some ants headed directly to the nest (Fig: 5.4a) while others took more tortuous paths (Fig: 5.4b). When the two landmarks were displaced South, some ants had no difficulty finding the nest (Fig: 5.4c) while others, walked straight past the nest before eventually
returning to it (Fig: 5.4d, e.g. blue line). Some ants appeared to head directly for one of the two landmarks in both configurations (Fig 5.4a, e.g. purple line; Fig: 5.4d, black line; see also Appendix 5.2 and 5.3 for more examples) but others showed no preference for either.

Fig: 5.4 Inbound paths of different ants in the two landmark condition.
Nest (N): blue filled circle; red circles: landmarks; different colours: different ants.

When four landmarks were introduced, most ants walked directly to the nest (Fig: 5.5a) while a few ants took quite long to locate it (Fig: 5.5b). When landmarks were displaced South, some ants had no difficulty locating the nest (Fig: 5.5c), while others searched near the fictive nest position (Fig: 5.5d). During the West and East displacement conditions, ants behaved similarly with some individuals taking direct paths to the nest while others took more tortuous paths with search at the fictive nest position (Figs 5.5e-5.5h). Even following the complete removal of landmarks some ants took straight paths to the nest (Fig: 5.5i). Once again, others were greatly affected by the lack of landmarks, engaging extensive searches around the nest area (Fig: 5.5j). Furthermore, some ants appeared to head directly for one of the four landmarks in some configurations (Fig: 5.5a, e.g. red line; Fig: 5.5f, red line; Fig: 5g, black line; Fig. 5.5i, red line; see also Appendix 5.5-5.10 for more examples) but others showed no such preference.
Fig: 5.5 Inbound paths of different ants in the four landmark condition.
Nest (N): blue filled circle; black circles: landmarks; different colours: different ants.

5.3B Group responses to landmark manipulations

I wanted to determine if any of the landmark conditions had an effect on the inbound paths of the ants compared to (a) when no landmarks were present and (b) when landmarks were present in the original constellation. In order to do this I compared search density histograms, time taken to locate the nest and straightness indices between different conditions. Given the highly individual behaviour of these ants, I have also included references to the appendix in this section as it shows inbound paths for all ants observed with ants that were lost and performed reorientation walks highlighted in each condition.
Two landmark experiment (search density distributions)

The search density distribution when no landmarks were present was significantly different to when landmarks were present and when landmarks had been displaced South (Fig: 5.6, Table: 5.1).

Four landmark experiment (search density distributions)

The displacement of four large landmarks caused most distributions at the group level to shift accordingly in the displaced landmark directions. The distribution of inbound paths of ants when no landmarks were present was significantly different to the distributions caused by the introduction, displacements (South, West and East) and the removal of landmarks on both X and Y directions (Fig: 5.7, Table: 5.1). The only exception to this was the repeat of the South displacement of landmarks, where distributions along the X and Y directions were not significantly different to when no landmarks were present.

When the distribution of inbound paths of ants from the with landmark condition was compared to the displacements and removal of landmarks, only the South displacement along the Y-axis was found to be not significantly different (Fig: 5.7, Table: 5.1). This result is interesting as I would have expected most search to occur along the Y direction, i.e. the direction in which landmarks were displaced.

In all conditions, some individuals walked as close as 3cm to the nest during search moving towards and away from it. In some cases ants were still unable to locate the nest (Fig: 5.7b). This type of unsuccessful search within close proximity to the nest suggests the lack of chemical or pheromone trails to guide the entry of ants to the nest.
Fig: 5.6 Inbound paths for two landmark manipulations for all ants.

*Inbound path plots and heat maps for all conditions.* Nest: Black line intersection (rows 1,3,5); white line intersection (rows 2,4,6); current landmark position of landmark conditions: red; original position: grey; main foraging direction: grey arrows.
**Fig: 5.7 Inbound paths for four landmark manipulations for all ants.**

(a) - *Inbound path plots and heat maps for all conditions.* Nest: Black line intersection (rows 1,3,5); white line intersection (rows 2,4,6); current landmark position of landmark conditions: black; original position: grey; main foraging direction: grey arrows. 

(b) – *Example plot of shortest distance from the nest vs visible path length.* Nest is at 0. For clarity only 2 example from 2 landmarks conditions shown. Four landmark South displacement: blue; four landmark removal: purple; 5cm distance from the nest: grey line. Inset shows corresponding inbound paths.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Comparison condition</th>
<th>KS test (X-direction, Y-direction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No landmarks</td>
<td>With two landmarks</td>
<td>$p_X&lt;0.001; p_Y&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>South displacement of two landmarks</td>
<td>$p_X=0.001; p_Y&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>With four landmarks</td>
<td>$p_X=0.001; p_Y=0.001$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced South</td>
<td>$p_X=0.001; p_Y=0.041$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced West</td>
<td>$p_X&lt;0.001; p_Y=0.001$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced East</td>
<td>$p_X&lt;0.001; p_Y&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Removal of four landmarks</td>
<td>$p_X=0.002; p_Y=0.008$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced South repeat</td>
<td>$p_X=0.068; p_Y=0.053$</td>
</tr>
<tr>
<td>With four landmarks</td>
<td>Four landmarks displaced South</td>
<td>$p_X=0.001; p_Y=0.109$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced West</td>
<td>$p_X&lt;0.001; p_Y=0.004$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced East</td>
<td>$p_X&lt;0.001; p_Y&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Removal of four landmarks</td>
<td>$p_X&lt;0.001; p_Y&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Four landmarks displaced South repeat</td>
<td>$p_X&lt;0.001; p_Y&lt;0.001$</td>
</tr>
</tbody>
</table>

Table: 5.1 Table showing statistical results from the KS test comparing two-dimensional distributions from different landmark conditions.


Two landmark experiment (time taken and straightness index)

Ants exhibited a large variation in the time taken to pinpoint the nest entrance (Fig: 5.6 and Appendix: 5.2 for individual plots). There was no significant difference in the time taken between no landmarks, with landmarks and the South displacement conditions (Table 5.2, $p=0.11$, ANOVA, d.f=35). There was also no significant difference in the straightness of paths between no landmarks, with landmarks and the South displacement conditions (Table 5.2, $p=0.31$, ANOVA, d.f=35).
Four landmark experiment (time taken and straightness index)

During the four landmark experiment as a whole, there were significant differences in the time taken between the different manipulations (Table 5.2, \( p=0.001 \), ANOVA, d.f=79). Among the manipulations, the significant differences were between, with landmarks & removal of landmarks (Table 5.2, \( p=0.04 \), ANOVA, d.f=15), and the East displacement & removal of landmark conditions (Table 5.2, \( p=0.03 \), ANOVA, d.f=24). Ants that were not exposed to any landmarks took the longest time to find the nest entrance and those exposed to the East landmark displacement took the shortest time. There was no significant difference in the straightness of paths between the different manipulations of the four landmark experiment as a whole (Table 5.2, \( p=0.53 \), ANOVA, d.f=79).

During the South displacement, out of a total of 17 ants, two ants were unable to pinpoint the nest (Appendix: 5.6, red plots). Paths of ants that approached the nest from the South (their normal foraging direction) were highly tortuous and had an average straightness index of 0.08 ± 0.02 (mean±SE, n=8 ants). In contrast, paths of ants that approached the nest from North-West were less tortuous/more directed and had an average straightness index of 0.59 ± 0.13 (mean±SE, n=5 ants). This difference between the inbound path approach direction was significant (\( p=0.014 \), t-test, d.f=11, \( t=-4.00 \)).

Out of a total of 19 ants, two ants were unable to pinpoint the nest and five ants carried out reorientation walks in response to the West displaced landmarks (Appendix: 5.7 red and green plots respectively).

During the East displacement of landmarks, one ant was unable to pinpoint the nest (out of a total of 10) and five ants carried out reorientation walks in response to these displaced landmarks (Appendix: 5.8, red and green plots respectively).

When landmarks were removed, out of 18 ants, seven ants were unable to locate the nest (Appendix: 5.9, red plots). In contrast to the first South displacement, all 15 ants tested were able to pinpoint the nest entrance in the repeat of the South displacement condition.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Time taken to enter nest (s)/AV±SD/number of ants (n)</th>
<th>Straightness index/AV±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No landmarks</td>
<td>50.6 ± 46.7s, n=15</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>With two landmarks</td>
<td>19.2 ± 23.3s, n=13</td>
<td>0.46 ± 0.09</td>
</tr>
<tr>
<td>Two landmarks displaced South</td>
<td>28.6 ± 20.9, n=10</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>No landmarks</td>
<td>14.8 ± 11.6, n=10</td>
<td>0.42 ± 0.09</td>
</tr>
<tr>
<td>With four landmarks</td>
<td>8.6 ± 5.2s, n=15</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>Four landmarks displaced South</td>
<td>49.2 ± 70.6s, n=15</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>Four landmarks displaced West</td>
<td>17.7 ± 11.3s, n=13</td>
<td>0.44 ± 0.09</td>
</tr>
<tr>
<td>Four landmarks displaced East</td>
<td>9.4 ± 6.7s, n=6</td>
<td>0.58 ± 0.11</td>
</tr>
<tr>
<td>Four landmarks removed</td>
<td>39.5 ± 27.1s, n=11</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>Four landmarks displaced South</td>
<td>16.0 ± 70.2s, n=16</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>After re-orientation walks</td>
<td>4.7 ± 2.6s, n=10</td>
<td>0.79 ± 0.06</td>
</tr>
</tbody>
</table>

Table 5.2 Table showing time taken to enter the nest and straightness indices for all conditions. Ants that did not find the nest on their own or ants that performed re-learning walks have been excluded from the table.

Reorientation (re-learning) walks during four landmark manipulation experiments

Ten ants in the landmark manipulation experiments (total n=121) performed reorientation walks (Fig: 5.8a) when presented with a different landmark condition to that they had experienced on their outbound foraging run (only during the West and East displacement conditions). As clearly shown with examples in Figure 5.8a, ants take more directed paths to the nest following reorientation walks (Fig: 5.8a, compare dotted lines to dashed lines). The average time taken by ants to pinpoint the nest entrance after reorientation walks was significantly reduced (Tables 5.2 and 5.3). Compared to all other
conditions ant paths after reorientation walks appeared to be the least tortuous and significantly different to paths of ants that did not perform reorientation walks (Fig: 5.8b, also compare Fig: 5.8b to Fig: 5.7b, Tables 5.2 and 5.3).

![Diagram of reorientation walks](image)

**Fig: 5.8 Reorientation walks in response to landmark configurations.**

(a) - *Examples of three reorientation walks performed by three different ants in response to landmarks.* Learning walks: solid lines; inbound path before learning walk: dotted line; inbound path after learning walk: dashed line.

(b) - *Plot of shortest distance from the nest vs path length for 10 ants that carried out reorientation walks in response to four large landmark manipulations.* Nest is at 0. Also compare with paths in Fig: 5.7.

**5.3C Nest relocation**

On the final day of the landmark manipulation experiment, I observed the identified ants dragging other ants out of the nest. The subsequent tracking of these ant pairs revealed that individuals were being led to a new nest location about 2m South of the original nest. Over the next couple of days, all ants
relocated to the new nest. It is unclear if the constant manipulation of landmarks around the nest caused the ants to relocate their nest in such a manner. The original nest was situated in an open area with established foraging corridors to healthy food sources and had been under observation for the last three years.
5.4 Discussion

Ants of *M. croslandi* live in open grassy woodlands and in *Eucalyptus* forests giving rise to visually cluttered environments with prominent visual landmarks. They are also highly visual, solitary foragers (Greiner et al., 2007) that rely primarily on visual guidance for navigation (Narendra et al., 2013, Zeil et al., 2014). I have shown here that these ants are able to use site-specific landmarks to fine-tune their homebound navigation, i.e. to pinpoint the nest entrance with more efficiency and accuracy.

5.4A Two small landmark manipulation experiment

The introduction and subsequent South displacement of two small landmarks did not indicate either a significant change in the time taken to locate the nest entrance or the straightness of inbound paths. When landmarks were displaced after uninterrupted exposure for two weeks, I expected individuals to search at the fictive nest position as indicated by the current landmark configuration. However, I did not observe searches skewed towards the fictive nest area. The lack of search at the expected nest location and significant differences in the other variables mentioned above may be attributed to the fact that ants were exposed to these landmarks for a period of two weeks without any disruptions. Therefore, ants may have already built snapshot memories associating the nest position with the displaced configuration. In contrast, in the four large landmark experiment described below, ants were presented with a different landmark configuration to that experienced on their outbound journey.

5.4B Four large landmark manipulation experiment

There was a clear effect of the introduction, East displacement and removal of the four large landmarks on the inbound paths of ants near the nest. Ants took the smallest time to locate the nest entrance when landmarks were presented in the original configuration and took the longest time when no landmarks were present (different to removal of landmarks). There was also a significant difference in time taken between the East displacement and removal conditions.

The South displacement of landmarks caused ants to search at the fictive nest position as indicated by the shifted landmarks. Similar results have
been reported in desert ants that have been exposed to altered landmark configurations on their homebound runs (Bisch-Knaden and Wehner, 2003, Narendra, 2007b). Interestingly, individual analysis showed that ants approaching the nest from South were more likely to search at the fictive nest location and therefore more likely to have less direct paths. In contrast, all ants approaching the nest from North-West did not search at the fictive nest location. Ants that returned from North-West first encountered the nest area before reaching the displaced landmarks. Whereas, ants that returned from South reached the displaced landmarks and then the actual nest. Similar results have been reported in desert ants. Ants of *Cataglyphis fortis* searched at the fictive nest location when they approached the nest from a similar direction to that of their training session but searched at the original nest position when they approached the nest from alternate directions (Akesson and Wehner, 2002). Ants of *M. croslandi* that approach the nest from North-West may also be able to decouple compass information from their remembered snapshots in a similar manner. This would allow individuals to locate the original nest position without having to search at the fictive nest location.

While individual ants still searched in the area of the fictive nest as indicated by the West and East shifted landmarks, this behaviour was not obvious at first at the group level. It is possible that ants start dissociating the landmarks with the nest with more disruptions to their visual environment in a short period of time. Indeed, after the original landmark configuration, ants took the least time to pinpoint the nest entrance in the East landmark displacement condition which followed after the South and West manipulations.

Search at the expected nest location (sometimes as close as 3cm to the nest) during these experiments and the repeated moves towards/away from the nest, strongly indicate that these ants use a snapshot or image matching navigation strategy to pinpoint the nest entrance similar to bees and other ants (Cartwright and Collett, 1983, Akesson and Wehner, 2002). It is believed that as ants approach the nest they match a series of snapshots (taken during the outbound journey) to the current retinal image and move towards the location of ‘best fit’, depending on the saliency of these visual stimuli (e.g. Graham et al., 2004, Lent et al., 2010, Lent et al., 2013). However, as the visual environment is now different to that of the outbound journey, ants are inaccurately guided to a
fictive nest location. When no nest is encountered at the predicted nest location, ants start searching for the actual nest entrance as described here.

**Removal of landmarks**

In the absence of landmarks, the ants performed searches that covered a wide area. Similar results have been observed in honey bees where the removal of landmarks causes bees to perform elaborate search flights (Fry and Wehner, 2005). Systematic search for the nest also occurs in desert ants when individuals are displaced to distant and therefore unfamiliar visual environments (e.g. Wehner and Srinivasan, 1981, Schultheiss and Cheng, 2011). Eight ants were unable to pinpoint the nest entrance resulting in the greatest number of lost ants across all conditions (data which were excluded from the time taken and straightness index analyses). This result once again confirms that individuals have learnt the relationship between these landmarks and the nest location and therefore support the conclusion that site-specific landmarks have an effect on the homebound navigation in this species.

**Reorientation (re-learning) walks during four landmark manipulation experiments**

When exposed to the West and East landmark displacements, some ants carried out reorientation walks. These walks were termed such as they were carried out by already experienced foragers in response to an altered visual environment as opposed to naïve, first time foragers. Ants that performed reorientation walks and returned to the nest took less than 5s to pinpoint the nest entrance and exhibited more direct paths to the nest. Desert ants have been known to perform learning walks when presented with an artificial landmark near the nest in an otherwise featureless environment (Müller and Wehner, 2010) and wood ants have been known to fixate artificial landmarks near a feeder during learning (Nicholson et al., 1999). However, it is unclear as to why only some ants perform reorientation walks and why these walks only occurred during the West and East displacement conditions. It is possible that re-orientation walks are influenced by previous return success of individual ants in a similar manner to what has been previously reported during orientation.

Analysis of individual paths showed that some preferred one of two landmarks while others seemed to show no preference for either landmark. The direct approach of one or two landmarks in a set by some ants indicates that these individuals might be using beacon navigation. Beacon navigation increases the speed and accuracy at which a goal is approached and is likely to increase foraging efficiency (Collett, 1992, for review see Collett, 1996). Ants of *M. croslandi* may also use a similar strategy when faced with novel landmark configurations in order to increase their foraging efficiency. Future landmark experiments with this species could perhaps document preceding learning walks as this may also shed light on why some ants choose to search near particular landmarks in certain directions on their return (see chapter 3 for more on learning walks).

### 5.4C Nest relocation

After the cessation of the landmark experiments, ants relocated to a new nest site. Major environmental disturbances (e.g. flooding) (e.g. Yamaguchi, 1992, Gibb and Hocuhuli, 2003) and predation (e.g. Yamaguchi, 1992, McGlynn et al., 2004) are known to cause ants to relocate nests. However, it is unlikely that ants of *M. croslandi* relocated due to competition or food quality as they continued to forage on the same tree and insect patches at the new nest. Furthermore, predation is not likely to have played a role as these ants were rarely predated upon and were most vulnerable when ants crossed a gravel path to the same foraging tree. The new nest location also brought these ants even closer to a behaviourally dominant and highly active meat ant (*Iridomyrmex purpureus*) colony. Conversely, as foragers had to travel a shorter distance to their foraging tree, the new nest location may have optimised foraging efficiency (e.g. Holway and Case, 2000, and increased access to food (e.g. McGlynn et al., 2004, Buczkowski and Bennet, 2009). Nevertheless, it should be noted that these ants may be sensitive to prolonged visual disturbances around the nest.

In conclusion, I have shown here that ants of *M. croslandi* are able to use multiple, large, site-specific landmarks near the nest to increase the accuracy at
which they pinpoint the nest entrance. In most cases, the daily displacement of landmarks, ensuring sufficient visual mismatch between outbound and inbound journeys, causes these ants to search at the expected nest location which is most likely predicted by an image matching navigational strategy to some extent. The removal of landmarks after they have been learnt, cause a significant difference in the time taken to locate the nest entrance and also cause more ants to be completely lost. Certain landmark configurations cause some individuals to perform reorientation walks but factors that guide these walks remain unclear.
5.5 Appendix figure legend

**Appendix: 1-10.** Inbound paths (columns 1 and 3) and heat maps (columns 1 and 4) of individual ants under different landmark manipulations. Current two landmark position: red circles; current four landmark position: black circles; original position of landmarks before removal: grey circles; red plots: lost ants; green plots: ants that carried out re-orientation walks.
Appendix 3

Distance from the nest (cm)

0.10m

Distance from the nest (cm)
Appendix 6

Distance from nest (cm)

0.10m Distance from nest (cm)
5.6 Acknowledgements

I am grateful to Jochen Zeil for his comments on this chapter. I also thank Ajay Narendra for his advice on initial experimental set up, Teresa Iglesias for her help with data analysis and Hwan-Jin Yoon for his assistance with statistical analysis.
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Chapter 6. The life of a solitary foraging Jack Jumper ant – *Myrmecia croslandi*: Summary discussion and Outlook

### 6.1 Summary

One of the main challenges faced by a solitary foraging ant is knowing where to go and then safely returning home. In order to achieve this, individual foragers must use a set of navigational skills to acquire and use information about the environment that surrounds goals such as their nest and food sources. The success of social ant colonies is also often attributed to strict organisation at the colony level, although individual organisation and behaviour is just as significant for the efficient running of a colony. This relationship is particularly important in members of the colony that make contact with the environment outside the colony, the foragers (Biesmeijer and Tóth, 1998). It is thus important to ask the question how inexperienced individuals become foragers and how the subsequent development of individual foraging careers allows them to navigate to and from goals.

Developed from this fundamental question, the main aim of this thesis was to identify the movements and determine the navigational knowledgebase of individual foragers over their foraging life-time.

The Jack Jumper ants, *M. croslandi*, were attractive models for such a project for several reasons. First, they are non-trail following, solitary foragers. Therefore, in theory, each forager could be tracked individually over time. Second, Jack Jumpers are large (11mm in length), relatively slow moving and conspicuous against the ground while they forage, so that their foraging behaviour can be easily monitored. Third, Jack Jumper colonies consist of a small work force and only a few foragers leave the nest at any one time, making individual tracking a more manageable task.

With Jack Jumpers ideally suited for specific monitoring of foraging careers, in chapter 2, I determined the spatial and temporal variation in their individual foraging behaviour. First, I showed that foragers were highly individualistic in the routes that they took to the same foraging site characterised by a two-pronged approach to foraging (Jayatilaka et al, 2014). One is where ants visited distant but spatially predictable trees leading to sector and route fidelity. A second location consisted of closer but less spatially
predictable hunting patches on the ground where ants exploited dispersed food resources. Depending on where ants foraged, individuals captured prey, collected carbohydrates or did both. Ants were highly flexible in where they foraged, regularly switching between foraging locations and were not influenced by previous foraging success (Jayatilaka et al., 2014). The foraging strategies mentioned above sets ants of *M. croslandi* apart from the more commonly studied desert ants, *Cataglyphis* who are scavengers that initially pick a foraging sector at random, restrict foraging to a certain foraging sector in their short life span and only switch between sectors when they have been previously unsuccessful in acquiring food (e.g. Wehner et al., 1983, Schmid-Hempel, 1984). Desert ants also rely heavily on path integration (e.g. Müller and Wehner, 1988) and therefore do not necessarily ‘need’ to know their visual environment.

In terms of temporal variation, ants left the nest at different times but rarely foraged on consecutive days (Jayatilaka et al., 2014), possibly as a result of their longevity which may eliminate the need for foraging on a daily basis. The lack of daily foraging by individuals added an unforeseen limitation on my ability to track ants and will be discussed in more detail later. As trees provide a spatially stable source of carbohydrate, ants that foraged on trees were more directed in their routes exhibiting shorter foraging durations compared to ants that hunted on the ground. This indicates that the degree of searching for live prey may play a major role in foraging duration. The high degree of inter-individual differences in foraging behaviour led me to ask the next question of how ants may have acquired the navigational information necessary to forage to and from goals and how the organisation of their learning walks may have played a role in shaping this behaviour.

In chapter 3, I explored the learning walks of these ants; a highly systematic and structured behaviour exhibited by inexperienced foragers before they leave the nest for the first time to forage. Once again, in keeping with their individuality, learning walks were found to be highly variable with no shared bearing or gaze directions between ants and no strong evidence linking learning walks to features in the panorama or the direction of subsequent foraging trips. Nevertheless, I was able to examine some regular features of learning walks shared between ants. First, most learning walks occurred in the morning with the first two learning walks taking place in a narrow time window. Given the
temporal fidelity exhibited by ants in relation to the initial learning walks, the timing of the first foraging trip may guide when learning occurs with the possibility of enhancing sun-compass learning. Second, subsequent learning walks of individuals covered directions not previously explored, with ants systematically scanning the visual environment around the nest. The former behaviour points to the preservation of some type of visual memory from the preceding learning walks, spanning several days as some learning walks may occur over as many as four days. The latter scanning behaviour suggests that these ants have the opportunity to learn and collect identically oriented views of the nest environment at different bearings, coupled with some form of home vector information from their path integration system. I have also shown that ants perform re-learning walks in response to an altered visual environment which suggests that ants continuously monitor the visual scenes against their memories. As previous work on honey bees has shown the relationship between the ephemeris function and landscape to be adaptive (Towne, 2008), it is reasonable to assume that *M. croslandi* ants may also possess the ability to update and revise sun and landscape linked memories. Finally, ants looked away from the nest most of the time and only looked at the nest when they were returning to it. In an attempt to explain this behaviour, I discussed that regular turning back and looking is not necessary for learning to occur. For example, if ants determine the heading direction by employing scanning movements to search for familiar nest-directed viewing directions (Baddeley et al., 2012), comparable to determining the minimum of rotational image differences, a returning individual of *M. croslandi* can ideally use any one of the many stored views from the learning walks to aid the return trip. As ants of *M. croslandi* have been shown to path integrate (Narendra et al., 2013), they would be able in principle, to tag views with home direction. In addition, ants may store multiple views in different orientations during learning walks, which they then compare with current views via scanning at release sites, with the aim of obtaining a known heading direction (Möller, 2012).

In chapter 3, I discussed several similarities and differences between learning flights and learning walks. Here, I will make comparisons between learning flights/walks of insects and the learning walks of *M. croslandi*. First, in both ants and flying insects, learning occurs on the first exit from the nest, either in response to an altered visual environment or whenever the insects have
difficulties locating the goal during the previous approach (e.g. Van Iersel and van den Assem, 1964, Zeil, 1993a). This is also the case in *M. croslandi* with learning taking place when ants are inexperienced foragers who had not foraged for at least six months as well as in response to changes in their familiar visual environment. In the case of ground nesting wasps, learning flights occur daily and in the morning, before wasps head out to forage (Zeil, 1993). I have shown in chapter 3 that this is not the case with *M. croslandi* as not all learning walks occur in the morning nor do they occur daily. Second, flying insects turn back to face the goal, while *M. croslandi* frequently engages in saccadic scanning away from the nest. In ants, to the best of my knowledge, only two studies have explored gaze directions as they occur during learning walks in already experienced foragers (wood ants: Nicholson et al., 1999, desert ants: Müller and Wehner, 2010). However, the lack of detailed gaze analysis in these studies makes a comparison to *M. croslandi* difficult. Both the wood and desert ants occasionally turn back and look at the goal as they move away from it in contrast to *M. croslandi*. Finally, during learning flights insects move along arcs and loops (Zeil, 1993a, Nicholson et al., 1999, Riabinina et al., 2014) but ants do not (Nicholson et al., 1999, Müller and Wehner, 2010). Ants of *M. croslandi* do not simply move away from the nest, instead they do repeatedly return to it. Thus, the learning walks I have described here constitute a unique learning process that occurs in inexperienced foragers of *M. croslandi* (who have not foraged for more than 6 months in their natural environment), with some differences which sets them apart from the learning behaviours of wasps, bees and other ants. The learning walks of ants were most similar to learning flights of wasps and bees in that they occurred in inexperienced foragers in their natural environment.

Even though the Jack Jumper ants must perform learning walks in order to acquire the necessary information to aid the return trip to the nest, it is still unknown how these ants first find and agree on nest-specific trees. For example, at the current nest site, how do ants choose between their foraging *Eucalyptus* tree and the large non-foraging tree (see chapters 2 and 3)? I have shown that ants exhibit route and sector fidelity to nest-specific trees. This eliminates the possibility of these trees being picked at random as has been the case with other ants (e.g. Wehner et al., 1983, Deneubourg et al., 1986). One possibility is that ants pick the closest tree to the nest when they first head out.
to forage. Indeed at both nests observed for the entire duration of this study, ants foraged on the closest trees which lie between 8-13m from the nest. These distances appear to be typical for this species as foragers at other nest sites, for example, those studied by Narendra et al (2013) and Zeil et al (2014), also foraged at trees located between 6-15m away from the nest (Narendra et al., 2013). These nearby trees may serve as large and salient features in the panorama, distinguishable from other features in the visually complex environment of *M. croslandi*. It is known that panoramic features in the main foraging direction play the largest role in guiding navigation along with other parts of the panorama in *M. pyriformis*, the night active counterparts of the Jack Jumper ants (Reid et al., 2011). This may also extend to first time navigation to a tree. However, guidance by prominent features in landmark panorama alone cannot explain how ants first decide where to go upon leaving the nest as I have provided evidence that ants do not appear look to distinct features in the panorama during learning walks or when released at foreign sites. To clearly identify the navigational strategies in use, I will present some ideas for future experiments later on in this chapter.

Once individuals had become experienced foragers, in chapter 4, I determined the individual navigational abilities of ants by asking if they were able to successfully home from both familiar and unfamiliar locations. All ants engaged in a brief scanning behaviour on the platform in a similar manner to that previously reported by Narendra et al (2013) and Zeil et al (2014). The initial scanning on the release platform may indicate that snapshot memories need to be compared with what the ants currently see (Zeil et al., 2014). I also showed that the vector state of the ants indeed influenced their homing ability with more ants being able to successfully navigate home when they had no vector information and therefore no conflict in navigational information provided by their path integrator and landmarks. Furthermore, careful analysis of the behaviour of each individual ant upon displacement in relation to its foraging history within the current season indicated that prior knowledge of sites aided the return journeys of most displaced ants at several displacement sites. However neither previous foraging experience/lack thereof nor the organisation of learning walks were able to truly explain the behaviour of a few ants. For example, some individuals were still able to return from sites that they had not
previously visited. These results make the use of a map-like spatial memory (Menzel et al., 2005) unlikely in these ants (Narendra et al., 2013).

To my knowledge, only one other study has provided information on individual foraging histories to such a level of detail (Wystrach et al., 2012). By effectively constraining nest and route based knowledge in desert ants, Wystrach et al (2012) suggest that ants are able to use a mix of navigational strategies depending on the navigational task at hand. The observations that (a) ants of *M. croslandi* constantly monitor their environment to update their navigational decisions upon release at both familiar and unfamiliar locations and (b) show a range of flexible homing behaviours from path integration to landmark based navigation, suggest that this may very well be the case with these ants.

As *M. croslandi* are predominantly visual navigators, in the final chapter, I asked how visual features affected their homing ability by using artificial landmarks near the nest. The introduction of four large landmarks near the nest increased the accuracy at which ants located the nest entrance compared to when no landmarks were present. The visual mismatch between outbound and inbound journeys that occurred as a result of the daily displacement of landmarks caused these ants to search at the expected nest location pointing to the use of an image matching navigational strategy to some extent. I also provide evidence of reorientation walks that occur in response to landmark manipulations, which significantly increase the accuracy at which ants pinpoint their nest entrance on following return journeys. These results are similar to those reported by Müller et al (2010), in that both the current and previous studies have demonstrated that (a) the introduction of large landmarks near the nest entrance brings about learning walks and (b) that these walks allow ants to return to the nest directly, with increasing straightness.
6.2 Outlook

My work on the individual foraging careers of *M. croslandi* identifies several avenues for further research.

One of the main limitations of this study was the inability to resolve head movements for a more detailed analysis of gaze during both the examination of learning walks and the displacement experiment. Future studies will need to film at a higher resolution in order to better resolve head movements over a large area with perhaps the use of several cameras that record smaller but different areas around the goal. This would also allow the recording of full learning walk paths which was not always possible in the current study.

As previously mentioned, the lack of daily regular activity made continued individual tracking and the determination of true forager experience a difficult task. In the current study I can only be sure that the ants had no experience foraging above-ground in the current season and that they had not foraged for at least 6 months before. Future experiments with *M. croslandi* will need to first restrict access to certain foraging sectors (and therefore foraging knowledge) in a similar manner to Wystrach et al (2012) and ideally take place over a longer time period (over two years) to accurately establish an individual's movements and foraging history. In order to determine the experience of their ants, Wystrach et al (2012) used a simple strategy of marking ants for consecutive days and then classified all emerging unmarked ants as inexperienced foragers. In short-lived ants with high mortality, this strategy of associating markers with foraging experience may indeed prove reliable. However, in the case of *M. croslandi*, age-based forager experience will need to be established using other methods as marks will wear off over seasons and as ants continue to forage over several months. A more feasible approach to determining the relationship between learning walks and navigational competence is perhaps the disruption of learning walks before they can be fully completed followed by the immediate displacement of ants. This would eliminate the need for prolonged monitoring and ensure that ants are truly inexperienced. Screening off close foraging trees in a similar manner to Reid et al (2011) but before learning and above-ground foraging has occurred may help determine the role played by nearby trees on guiding leaning walks and first time navigation from the nest. It should also be noted here that previous
attempts by myself to train *M. croslandi* to feeders have been unsuccessful as ants do not respond to training. Therefore, this type of experiment will also eliminate the need for training.

Finally, level of satiation is known to influence the acquisition and development of long term memory in honey bees (Wright et al., 2007). In Chapter 4, I artificially fed full vector ants but not the zero vector ants. While my results show no effect of this feeding on the homing capability of ants (more artificially fed full vector ants were unable to successfully find the nest compared zero vector ants), it is worthwhile feeding both vector state ants to completely eliminate satiety as an influencing factor in future studies.
6.3 Acknowledgements

I am extremely grateful to Jochen Zeil and Eliza Middleton for their comments on this chapter which helped to greatly improve it.
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