Regulation of building behaviour relating to nest space and transport in leaf-cutting ants

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0.0 -Thesis Abstract

Ants build structures that facilitate the life of the colony. The structures are created in a highly variable environment by workers that lack centralised communication or a fixed plan. Instead, they use a system of self-organisation where each worker responds to local cues in such a way that a coherent structure emerges. How this process occurs can be both complex and specific to a species and therefore presents significant opportunity for study. One group of ants that creates particularly large, complicated, and sophisticated nests are the leaf-cutting ants. Leaf-cutting ants cut and carry leaf fragments, sometimes over great distances, and use them to grow a fungus garden that serves as a source of protein and nutrients for the colony's brood and adults. In this thesis I examine the regulation of nest construction in leaf-cutting ants: specifically construction of nest space and transport infrastructure, with a focus on nest tunnels and overhead clearance on foraging trails. A literature review places the nest construction behaviour of leaf-cutting ants into the context of the larger group to which they belong, fungus growing ants. It examines what is known and unknown about the construction mechanisms underlying the creation of tunnels, chambers and trails. Subsequently, I asked whether ants could regulate their digging rate in response to the tunnel space they initially encounter. Upon finding that they display higher excavation rates when presented with shorter tunnels than longer tunnels, I tested a prominent hypothesis that a 'digging pheromone' was a key component of the regulatory mechanism. The evidence suggested that it was not and that therefore a new understanding of digging regulation was required. In order to contribute to this new understanding I used the automated tracking system idTracker to follow the movement of ants as they extracted soil from a tunnel. This revealed the importance of interaction rate and arousal to the regulation of tunnel length and I integrated these factors into a conceptual model that could explain digging regulation. As a next step, I examined trail construction in leaf-cutting ants. The overhead method that leaf-cutting ants adopt in transporting leaves causes them to have an elevated clearance. If trails have overhanging obstacles, then the collision of the laden ants with these obstacles can slow their progress, requiring the removal of the obstructions for the sake of efficiency. I showed that the presence of laden ants on the trail is necessary for unladen ants to recognize the need to clear trails to a height suitable for laden ants. Furthermore, I showed that this mechanism is not triggered by the visual cue of an obstruction. These results show the importance of the dynamics of self-organised behaviour in creating large, sophisticated structures and point the way to understandings that could apply to industries as diverse as communication and micro-robotics.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Publications during enrolment

Bruce, A.I. (2016). It is not all pheromones: No evidence that pheromones affect digging face choice during ant nest excavation. *Behavioral Processes, 122*, 12-15.

Thesis including published works General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in peer reviewed journals. The core theme of the thesis is self-organisation of ant nest construction. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of Martin Burd and Tomer Czaczkes.

In the case of chapter 4 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent (%) of students contribution
4	It is not all pheromones: No evidence that pheromones affect face choice during ant nest excavation	Published	90%

* e.g. 'published'/ 'in press'/ 'accepted'/ 'returned for revision'

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: ████████████ **Date: 5/01/2016**

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student and co-authors' contributions to this work.

Main Supervisor signature: ████████████ **Date: 5/01/2016**

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"And here we shall have great occasion to renew our wonder, and admire the wise laborious ant."

William Gould, "An account of English ants," 1747

Table of Contents

1.0 -Thesis Introduction

Social insects are the only animals to have reached a pinnacle of social organisation similar to that of humans: cities that house millions, long distance transport links, effective waste transportation, efficient communication networks, crop farming, animal herding, etc. Indeed, social insects were removing and burying their waste and dead to promote hygiene while it piled up in the cities of medieval Europe, causing misery and sickness (Forgeng & Singman, 1999). Twelve-thousand years ago, when human agriculture began (Barker, 2006), fungus growing ants had already been planting, fertilizing, and weeding highly selected crop species for 50 million years (Schultz & Brady, 2008). Social insects have been protecting and cultivating livestock for at least 44 million years (Wheeler, 1914), while humans only discovered that animals were better raised than hunted 11 thousand years ago (Vigne, 2011). Mass human societies are formed from institutions that require the presence of sophisticated abilities such as elaborate cheater detection, shared intentionality, and complex language (Aktipis, 2015; Powers, van Schaik, & Lehmann, 2015). By contrast, social insects are composed of distributed units without the sophisticated, centralised communications abilities required for central control, and so utilise a process of self-organisation (Seeley, 2002). Therefore learning about the processes of self-organised behavioural regulation leads to a greater understanding of the potentials and limitations social systems.

Self-organisation was originally developed to explain phenomena in physics and chemistry (Karsenti, 2008) before being applied to biology (Serugendo, Irit, & Karageorgos, 2006). It is the process where limited units, working with only local information, organise themselves to form patterns and new structures without external guidance (Camazine et al., 2001; Seeley, 2002). The concept of self-organisation has been revolutionary for the study of social insects and considerable work has been done. However, small differences in self-organised processes can lead to large

differences in outcome, leading to highly idiosyncratic systems that have many details to explore. The mechanisms that underlie these processes allow relatively limited units to function as a coherent whole that can meet the reproductive needs of a colony. The benefit of these mechanisms for ultimate reproductive success, such as homeostatic temperature regulation and foraging, are well understood. However, their functioning is still largely to be investigated.

In this thesis I have investigated the mechanisms that underlie the construction and maintenance of the internal and external transport infrastructure of leaf-cutting ants: tunnels and trails. The focus is on the factors that regulate the construction of nest space in the form of tunnels and that regulate trail height clearance in order to facilitate trail flow. Tunnels and trails are the focus of this thesis because of their importance in the movement of colony workers through their underground and aboveground environments, and ultimately in directing the flow of information and resources. These systems of colony infrastructure raise questions of efficiency, such as how they can be constructed with minimum effort and energy and yet allow resources to be transported with minimum effort and risk. Furthermore, tunnels and trails are basic units of nest architecture which can reach enormous scale, but the mechanisms by which they are regulated have not had considerable attention.

To begin with, I performed a literature review that elucidated what is known and what is not known about the organisation mechanisms that underlie nest construction of fungus-gardening ants and embedded the experimental work within a larger background of knowledge (chapter 2). The literature review focused on fungus-gardening ants, a larger group to which leaf-cutting ants belong, rather than focusing exclusively on leaf-cutting ants. For example, the literature review reveals that the mechanisms that facilitate the creation and direction of tunnels remain to be explored, and that while the shape and size of leaf-cutting ant chambers have been well explained, other fungus gardening species have different kinds of chambers that do not fit into the established paradigm.

Finally, the literature review revealed how few of the proximate mechanisms for foraging trail construction have been elaborated upon, particularly the systems by which trail width and trail height are determined. Some of these matters were the subjects of this thesis' experimental work.

Leaf-cutting ants were chosen for the experimental part of the thesis because they create large and complex city-like nests. Particularly, they invest in transport facilitating infrastructure, trails and tunnels, that other hymenopterans, bees and wasps, do not. This allows the examination of behaviours that may be both complex in themselves and are also capable of scaling up to a consequential size. Furthermore, leaf-cutting ants are ecologically (Farji-Brener & Illes, 2000; Moutinho, Nepstad, & Davidson, 2003; Verchot, Moutinho, & Davidson, 2003; Zanetti et al., 2014) and economically significant (Cherrett & Peregrine, 1976; Della Lucia, Gandra, & Guedes, 2014; Montoya-Lerma, Giraldo-Echeverri, Armbrecht, Farji-Brener, & Calle, 2012; Nickele, Filho, Oliveira, & lede, 2012; Zanetti, Zanuncio, Leite, Jaffe, & Oliveira, 2003), thus increasing the importance of insight into their regulation of behaviour.

How do leaf-cutting ants regulate and allocate labour into nest construction tasks? The investigations took the form of experimental tests of ant behaviour in both the laboratory and in the field. To answer the question of how ants regulate the construction of tunnels, I used specialised tunnel digging arenas which allowed the ants to respond to different nest properties by varying their excavation rate.

The first question that was asked is how do ants respond to different lengths of pre-excavated tunnels (chapter 3)? That is, do they reduce their digging rate when they are presented with large tunnel spaces, as would be expected if they regulate their effort efficiently? I then examined how the ants respond to shortening of the tunnel space that they have already excavated in order to discover how ants would respond to a form of 'open feedback loop'.

Next, I tested the hypothesis of whether a 'digging pheromone' that attracts excavation effort is involved in the regulation process by presenting ants with a choice of two sites, one pheromone rich and another pheromone poor, in which they could dig (chapter 4).

Finally for tunnels, I used detailed analysis of movement and behaviour in a tunnel digging environment to more precisely enumerate the mechanism which regulates the excavation of tunnel space (chapter 5).

The thesis then broadened to examine another part of the colony network of infrastructure: foraging trails. This part of the thesis focused on how leaf-cutting ants construct their trails to have sufficiently high clearance for laden ants to pass underneath unhindered (Chapter 6). This is a question that also applies to tunnels, but is far more easily examined for trails, due to the lack of visual obstruction or need for excavation, and in the field due to the large size of a natural trail. I addressed this issue using experimental treatments that induced an asymmetry of information and capability between laden and unladen workers. Laden workers in these experiments were hindered, and therefore had immediate information about poor trail condition but, being laden, had no capacity to clear, while unladen workers were not hindered but, despite having the capacity to clear, had no direct information about its necessity.

The answers to questions of self-organised regulation may seem specific to particular problems and circumstances, but they can result in wide application. For example, ant colony optimisation (Dorigo & Maniezzo, 1996) was based on the self-organised, emergent foraging strategies of ants and has been applied to thousands of different tasks, in such diverse fields as communications network management (Di Caro & Dorigo, 1998), vehicle routing (Bullnheimer, Hartl, & Strauss, 1999), water distribution (Zecchin, Simpson, Maier, & Nixon, 2005), and manufacturing (Wang, Liu, & Zhong, 2005).

The complexities of social organisation will not entirely yield to one experiment, one thesis, or even one lifetime. Rather, we must build our scientific knowledge one step at time before aggregating it into fuller understanding. The above described chapters are one such step, and combine to advance the understanding of leaf-cutting ant nest biology and social organisation in general.

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2.0 - The organisation of nest construction in fungus-gardening ants

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2.1 - Introduction

Animals will often build nests to help manage their homeostatic needs and these nests can be some of the most amazing and ecologically significant structures in the natural world (Hansell, 1993). Nests, even if they appear simple, must simultaneously fulfill multiple criteria (Hansell, 1984). They must provide sufficient space for occupation and movement while satisfying requirements of temperature (Bollazzi, Kronenbitter, & Roces, 2008; Jones & Oldroyd, 2007), security (S. Powell & Clark, 2004; Swartz, 1998), and airflow (Bollazzi & Roces, 2007; Kleineidam & Roces, 2000) while being economical to create (Lee, Bardunias, & Su, 2008). Therefore, in the process of building a nest, the animal in question must have the ability to weigh each of these factors and decide where to allocate labour. This is interesting in a solitary animal, but it is particularly intriguing in social insects. Social insect nests must meet all the above requirements while being constructed by multiple, sometimes multitudinous, animals working together without central coordination and with different information. This review will focus on a particular kind of social insect, the fungus gardening ants, and the phenomena that allow them to construct their nests. It will address the current state of knowledge of the behavioural mechanisms that facilitate the construction, primarily by soil excavation and deposition or removal of obstructions, of three features of fungus growing ant nests: tunnels, fungus chambers, and cleared foraging trails.

There are approximately 230 species of fungus gardening ants and they mostly occupy the tropics of the New World, but range as far as New Jersey in the north and the deserts of Argentina

in the south (Hölldobler & Wilson, 2011). They cultivate a symbiotic fungus (*Leucocoprineae* and *Pterulaceae*) (Schultz & Brady, 2008), some species of which produce a specialised swollen hyphae (Quinlan & Cherrett, 1978). These hyphae are the sole source of food for the developing larvae (Mueller, Schultz, Currie, Adams, & Malloch, 2001; Quinlan & Cherrett, 1979; Weber, 1972) and provide the protein that the larvae require for growth as well as key nutrients to adults (Bass & Cherrett, 2008). In most cases the fungus cannot grow without ants (Quinlan & Cherrett, 1978) and all attine ants are obligately dependent on their fungus (Schultz & Brady, 2008).

All the attine ants harvest resources from the environment to feed their symbiotic fungus but may do so using different methods. Some attines use lower agriculture whereby they feed the fungus with detrital matter, such as degraded plant material or insect faeces, which do not require further processing before the fungus can feed upon it (Hölldobler & Wilson, 2011). By contrast, leaf-cutting ants (*Atta* and *Acromyrmex*) use a form of higher agriculture which involves collecting fresh dicotyledonous or gaminoid leaves and processing them to create a suitable substrate (Schultz & Brady, 2008; Weber, 1972). Fresh leaves and grass are far more abundant than suitable detrital material and so this ability provides leaf-cutter ants with a much greater pool of resources that they can exploit for food compared with lower agriculture, and thus allows their colonies to grow much larger. Furthermore, fluids from cut plants are also consumed by leaf-cutting ant workers for energy, increasing the utility of leaf material (Bass & Cherrett, 2008; Farji-Brener & Alejandro, 2001; Pintera, 1983; Urbas, Araújo, Leal, & Wirth, 2007). These advantages are an important factor in the ecological success of leaf-cutting ants (Hodgson, 1955) and allow them to create very large nests.

Ant nests are generally constructed underground by removing soil (Hansell, 1984; Theraulaz, Bonabeau, & Deneubourg, 1998a; Tschinkel, 2005). However, digging costs energy (Camargo, Lopes, & Forti, 2013a) and energy that is spent digging is not available for reproduction

(Lee et al., 2008). Furthermore, digging can damage an ant's mandibles, decreasing the efficiency with which worker ants can cut leaves (Schofield, Emmett, Niedbala, & Nesson, 2011) and is generally physiologically costly and can reduce an ant's lifespan (Camargo, Forti, Fujihara, & Roces, 2011). This means that nest construction would be expected to be economical and balance cost, efficiency and robustness (Buhl et al. 2004; Nakagaki et al. 2004; Bebber et al.2007).

Group-level coordination of behaviour is key in forming a cohesive whole (Johnson & Linksvayer, 2010); so the challenge is to understand the basic behavioural mechanisms that allow the ants to interact to produce a result that is greater than the sum of their individual actions without outside or central guidance (Cole & Cheshire, 1996). The nests of ants are far larger than individuals and so group level coordination is required for any structure to emerge (Buhl, Gautrais, Deneubourg, & Theraulaz, 2004). Group level coordination consists of behavioural patterns and mechanisms governing individuals that, when combined together, create a coherent structure (Emerson, 1938). However, coordination systems face many constraints. Animals that produce a structure require them to function as an effective colony phenotype (Odling-Smee, Laland, & Feldman, 1996; Turner, 2000). They need to operate in a context of environmental variation and poor performance from some individuals (Theraulaz, Gautrais, Camazine, & Deneubourg, 2003), while not exceeding the physical, cognitive, and energetic limitations of the animal. When these considerations are put together, the need for an elegant and appropriate behavioural regulation system is clear. For example, the construction of an ant nest could easily fail if the effort of one individual was completely undone by that of another. Indeed, insect colonies can be prone to work against themselves (Seeley, 2002; Theraulaz et al., 2003). The rules and mechanisms that guide the behaviour of nest construction are central to understanding the functionality of nests and how they come to exist.

The structure of an ant nest can be complex (Cassill, Tschinkel, & Vinson, 2002; Sudd, 1967; Verza, Forti, Lopes, & Hughes, 2007) and intuition would suggest that therefore they must be the result of complex rules (Deneubourg & Franks, 1995). However, complexity can also emerge from simple rules (Odell, 2002; Wolfram, 2002) and the complexity of ant nests is indeed thought to emerge from simple rules (Stickland & Franks, 1994). On the other hand, we should also remember that insects are sophisticated actors in themselves, being able in certain instances to process and evaluate multiple signals and cues (Czaczkes, Grüter, & Ratnieks, 2015; Seeley, 2002) using highly sophisticated senses (Kleineidam & Tautz, 1996) that demonstrate their sophistication as individual units. Therefore, we must keep in mind the complexities that can arise from sophisticated, variable individuals, even as we search for simple rules to elegantly account for the social organisation of insects.

Ant colonies are composed of distributed units without sophisticated communications abilities required for central control and so they utilise a processes of self-organisation (Seeley, 2002). The conventional definition of self-organisation was provided by Camazine et al. (2001):

> Self-organization is a process in which pattern at the global level of a system emerges solely from the numerous interactions among the lower-level components of the system. Moreover, the rules specifying interactions among the system's components are executed using only local information, without reference to the global pattern (p. 8).

This fits the common understanding of nest construction in social insects arising from interactions between individuals and the environment, without an overall blueprint (Deneubourg & Franks, 1995).

However, more recently De Wolf and Holvoet (2004) recognised two components to this process, separating self-organisation from emergence, though acknowledging that the two will often be combined. Their definition of self-organisation is:

Self-organisation is a dynamical and adaptive process where systems acquire and maintain structure themselves, without external control (p. 7).

This suits ant colonies where workers must build nests and distribute themselves to different tasks without an overseer (Seeley, 2002). De Wolf and Holvoet (2004) go on to define emergence as:

... when there are coherent emergents at the macro-level that dynamically arise from the interactions between the parts at the micro-level. Such emergents are novel with regards to the individual parts of the system (p. 3).

Emergence is the result of multiple agents on the micro-level interacting to produce a coherent whole greater than the sum of the parts on the macro-level (Munoz & Castro, 2009; Odell, 2002). It is robust, that is, if the failure of single individual does not prevent functionality (Munoz & Castro, 2009). In practice it may often be difficult to distinguish self-organisation and emergence clearly, since the processes will often be combined. However, splitting the definition into two is to be preferred, as it allows for a deeper and more precise discussion of concepts. In the case of ant nests, the self-organised component is the lack of outside control over the actions of individual workers, such as from a precise genetic program or an environmental template, while the emergent component is the functional nest itself, being something that does not arise 'additively' from the presence of ants. That is, the nest is considered novel, in the sense of being inexplicable or unpredictable with only information of the subparts or individuals of the system (Hampel & Oppenheim, 2008; Munoz & Castro, 2009).

What behavioural regulation systems allow fungus gardening ants to satisfy competing needs through structure? Some key elements are described below.

- A cue is anything that provides useful information but has not been shaped by natural selection and can include the unintentional byproducts of activities. By contrast a signal has been shaped by evolution (Johnson & Linksvayer, 2010; Llyod, 1983; Seeley, 1989).

- A response threshold is the magnitude of the stimulus that is required to trigger a particular behaviour (Detrain & Pasteels, 1991; Robinson, 1987). Such thresholds are important to the organisation of behavior, particularly the creation of specialists (Detrain & Pasteels, 1991; Robinson, 1987).

- Stigmergy is the tendency for ants to respond to changes in the environment that were made by their nest-mates. In the case of building, individuals are interacting through the medium of the nest structure or environment (Grassé, 1959; Theraulaz & Bonabeau, 1999)

- Positive feedback is the amplification of a process that, being fulfilled, will lead to further amplification (Theraulaz et al., 2003), while negative feedback will dampen a process (Ramaprasad, 1983). Positive feedback often leads to the creation of structures, while negative feedback, prevents them being pursued to infinity (Theraulaz et al., 2003).

- Short range activation and long range inhibition are opposing processes that involve amplification close to the stimulus but reduction with greater distance (Gierer & Meinhardt, 1972).

- Templates are patterns that already exist in the environment that can structure social insect behaviour (Camazine et al., 2001).

In normal circumstances multiple mechanisms will be combined, such as when a termite queen is used as a template in a self-organised process that also creates pillars and walls (Bonabeau et al., 1998; Theraulaz et al., 2003). These combinations are layered together to serve the needs of the colony by allowing the coordination of action across large spatial and temporal scales and by

producing specific results to meet specific needs. Unfortunately, the multilayered nature of behavioral mechanisms makes understanding them more difficult as they must be untangled in experiments that manipulate one factor, in what are often artificial scenarios. This is compounded by the fact that it is not possible to directly test behavioural rules, but only to test the consequences of those rules using both modeling and experimentation (McCreery & Breed, 2014). However, despite the ambiguities, applying these ideas to the construction of leaf-cutting ant nests is both enlightening and compelling. This is particularly so because of the very advanced nature of these fungus gardening ants (Wirth, Herz, Ryel, Beyschlag, & Hölldobler, 2013).

The nests of fungus gardening ants can range in size from relatively modest, with only a few hundred ants (Hölldobler & Wilson, 2011), to vast and elaborate cities with millions of individuals (Jonkman, 1980a; Moreira, Forti, Andrade, Boaretto, & Lopes, 2004; Moreira, Forti, Boaretto, et al., 2004). The most sophisticated of these nests have a large external mound with many entrances, connected by tunnels to domed chambers in which the ants store their fungus (Bollazzi, Forti, & Roces, 2012; Jonkman, 1980b; Lopes et al., 2011; Moreira, Forti, Andrade, et al., 2004; Moreira, Forti, Boaretto, et al., 2004; Verza et al., 2007). The chambers can be found deep underground arranged in a complex structure (Jonkman, 1980a; Moreira, Forti, Boaretto, et al., 2004; Moser, 2006; Navarro & Jaffe, 1985). Nests can also have long foraging tunnels, which facilitate the safe passage of workers and resources to the nest, and can be up to 2 m deep (Moreira, Forti, Boaretto, et al., 2004). In addition to foraging tunnels, leaf-cutting ants also create cleared trails for up to several hundred meters (Hodgson, 1955). These trails facilitate the transport of leaf-material by removing obstructions from the path of leaf-carrying ants (Rockwood & Hubbell, 1987).

2.2 -Tunnels

Tunnels are long, narrow excavations of soil and may terminate at a surface entrance, a chamber, or in a blind ending and have species specific patterns in fungus growing ants (Klingenberg, Brandão, & Engels, 2007; Tschinkel, 2003) much as in other species of ants (Sudd, 1970b). However, unlike other species of ants, the width of fungus gardening ant tunnels can vary widely (Verza et al., 2007). As well as the normal requirements of ant tunnels, such as access and space, fungus gardening ant tunnels must meet additional requirements. They must facilitate the transport of leaf fragments (at least in leaf-cutting ants) (Burd & Howard, 2005; Moreira, Forti, Boaretto, et al., 2004), soil (Verza et al., 2007), fungus and brood (Römer & Roces, 2014), and waste material (Bot, Currie, Hart, & Boomsma, 2001; A. G. Hart & Ratnieks, 2001), while providing suitable ventilation (Kleineidam & Roces, 2000). However, they cannot be over-enlarged because otherwise nest entrances could allow the ingress of rain (Kleineidam & Roces, 2000), parasites (Herrera & Valenciaga, 2011), and army ants (LaPolla, Mueller, Seid, & Cover, 2002; S. Powell & Clark, 2004; Sánchez-Peña & Mueller, 2002; Swartz, 1998). Studies on the harvester ant (*Pogonomyrmex barbatus*) have shown that tunnels are dug wide enough to allow ants to pass each other (Espinoza & Santamarina, 2010), and width was linked to head width in fire ants (*Solenopsis invicta*) (Gravish et al., 2012). However, studies that examine tunnel construction in fungus gardening ants, either on the large scale or the small scale of proximate mechanisms, have yet to be done.

Tunnels are not simply access paths, they also provide nest space. An efficient and effective nest requires that sufficient nest space is dug but that energy is not wasted. Here, the concept of individual distance, developed by Hediger (1941), is important. The theory is that ants will dig enough space per individual. However, while this effect has been shown in chambers (Franks, Wilby, Silverman, & Tofts, 1992; Pielström, 2013) and whole nest conditions (Buhl, Deneubourg, Grimal, & Theraulaz, 2005; Buhl et al., 2004; Buhl, Gautrais, Deneubourg, Kuntz, & Theraulaz,

2006; Deneubourg & Franks, 1995; Rasse & Deneubourg, 2001), it has not been shown to apply when ants are restricted to a tunnel. Investigations as to whether ants can adjust their digging rate to the available tunnel space are available in chapter 3. How they go about this adjustment requires a mechanistic explanation. A hypothesis put forward for termites, that rates of contact between individuals may lead to an estimation of nest density and thus the need or otherwise of digging (Su & Lee, 2009), has also been applied to ants with the additional proposal that a digging pheromone may serve as a stimulatory cue as part of the regulatory process. This digging pheromone is hypothesised to stimulate excavation near its location but as nest space grows and it is applied less often the pheromone evaporates. This lowers the stimulus to dig, thus leading to a reduction in digging and a corresponding fit between ant number and nest size (Deneubourg & Franks, 1995). Buhl et al. (2005) supported this idea via a model that corresponded to experimental data. As further supporting evidence, red fire ants were found to dig preferentially in sand treated with gland extracts (Chen & Zhang, 2013). Furthermore, pheromones have been shown to have a role in leaf-cutting ant digging behaviour. Pielström and Roces (2013) have showed that freshly dug pellets, but not 1hr old pellets, would stimulate digging. This indicates that an evaporative substance, possibly a pheromone, has a role in digging organisation. The existence of a pheromone that promotes digging on the tunnel digging face is tested in chapter four, and an examination of the relationship between movement, encounter rates, and digging rate in tunnels can be found in chapter five.

To create a tunnel ants must focus their digging into a narrow area (Pielström & Roces, 2013). It is likely that this focus of activity is achieved through multiple mechanisms. Primarily ants are attracted to locations where other ants have already been digging or are digging (Sudd, 1970a) or where there are surface irregularities (Pielström & Roces, 2012). One signal that has been found to aid in the focusing of digging effort is stridulation (Pielström & Roces, 2012). It is interesting to note that stridulation plays a role in worker rescue (Markl, 1965, 1973), recruiting workers to leaf-cutting (Roces & Hölldobler, 1996; Roces, Tautz, & Hölldobler, 1993), and recruiting minor

workers to leaf-riding (Roces & Hölldobler, 1995). Given that the same signal has different roles in many different situations, all of which serve to attract a workers attention to something, it may be that stridulation serves as context dependent attractive signal to workers to direct them to tasks (Hölldobler, 1999; Roces & Hölldobler, 1996; Wirth et al., 2013).

The removal of waste soil from tunnels and chambers is a crucial function; any soil from the digging face must be removed all the way to the surface lest it refill the nest. In *Atta vollenweideri* it has been shown that soil removal is accomplished in multiple steps (Pielström & Roces, 2013). The first step is that soil is extracted from the digging face itself and formed into a pellet. The pellets are then taken and dropped around 9.5 centimeters from the digging face by the excavator, which returns to digging. New ants will then retrieve the pellets and transport them over a short distance. The pellets are then retrieved for a final time and are carried to the entrance of the nest by a long distance carrier (Pielström & Roces, 2013). Should unused space be closer, pellets can also be deposited there (Römer & Roces, 2015). This division of labour in soil transport follows an intriguingly similar process (Pielström & Roces, 2013) to that found in the cutting and transportation of leaves in *Atta vollenweideri* (Röschard & Roces, 2003) and *Atta cephalotes* (Hubbell, Johnson, Stanislav, Wilson, & Fowler, 1980). Furthermore, it is also similar to the partitioned transport behaviour seen in waste (A. G. Hart & Ratnieks, 2001). This raises the question as to whether the leaf-cutting and transport and waste transport behaviour evolved from the soil excavation and transport behaviour, which would have preceeded the evolution of fungus-farming ecology.

The reason that these transport tasks have been divided up has not been precisely determined. It may enhance information transfer about forage quality (Bollazzi & Roces, 2011; Hubbell et al., 1980; Roces & Bollazzi, 2009; Röschard & Roces, 2003), information about whether an optimal number of ants have been allocated to a task (Ratnieks & Anderson, 1999), improve the

match between the size of the ant and the size of the load (Rudolph & Loudon, 1986), or enhance transport speed through specialisation for that part of the trail or tunnel (Dobrzańska, 1966; Pielström & Roces, 2013). Furthermore, fresh deposits of soil in a tunnel from the multi-stage transport process may act as stigmergic cues to prompt digging, similarly to stridulation but with a much wider signal range (Pielström & Roces, 2012; Römer & Roces, 2014). Whatever the benefit, task partitioning is more effective in large colonies because there is less waiting time between individuals (Jeanne, 1986) when there are more individuals, and leaf-cutting ant colonies might particularly benefit from this mechanism because they can grow very large (Anderson & Ratnieks, 1999). It would be interesting to see if these mechanisms also exist in fungus growing species with only small colonies or in young colonies of leaf-cutting ants that have only a small number of individuals. An examination of when these mechanisms come into play during colony ontology might reveal something about their function. Finally, it should always be remembered that some elements of task partitioning may be epiphenomenon of deeper behavioural rules (Anderson, Boomsma, & Bartholdi III, 2002).

Compared to foraging tunnels in fire ants, which are cylindrical (Tschinkel, 2011b), leaf-cutting ants will often build foraging tunnels that are elliptical in shape. This form of tunnel is much wider than other tunnels, allowing many ants to pass at once and high enough to allow the free passage of leaf-laden ants (Mintzer, 1979; Moreira, Forti, Andrade, et al., 2004). The depth of these tunnels can range from 30 cm (*A. mexicana*) to 2 m (*A. bisphaerica*) below the surface and may serve to reduce the exposure of ants and the leaf-fragments they bear to dry conditions, temperature extremes, parisitoids, and predators (Mintzer, 1979; Moreira, Forti, Andrade, et al., 2004; Rabeling, Vehaagh, & Engels, 2007). However, little work has been done concerning the mechanisms that facilitate their construction. For example, there must be an interesting tradeoff between investing effort into widening a foraging tunnel (Mintzer, 1979) and the possibility of productivity bottlenecks being caused by large leaf fragments being carried through insufficiently large tunnels

and causing blockages (Burd & Howard, 2005). Other questions remain regarding specific aspects of the architecture. For example, how is it that the ants know how high to make their ceiling? It is known that ants cut leaf-fragments into smaller pieces in response to unalterable height restrictions such that there is an increase in the rate of return (Dussutour, Deneubourg, Beshers, & Fourcassié, 2009), but how they modify their tunnel architecture to maximise leaf-return is unknown. Are they responding to reductions in ant flow and being thereby triggered to clear offending substrate? Perhaps there is a visual cue to which they are responding? The questions here are similar to that for trail height clearance, which is addressed below.

The directional properties of ant digging has also attracted limited interest. Gravity is known to be an important guide to tunnel digging (Sudd, 1972) and ants will follow roots or irregularities within the soil phase as a form of template (Espinoza & Santamarina, 2010). Aside from these two factors, further work remains to be done into what determines the directions in which ants dig tunnels. For example, it has been shown that *Atta colombica* is sensitive to magnetic fields (Banks & Srygley, 2003) and that this ability is removed when they are denied access to soil (Riveros, Esquivel, Wajnberg, & Srygley, 2014). It might be that this aids directional construction in tunnels, especially in long foraging tunnels where ants will not have access to external visual cues, but this possibility remains to be investigated. A magnetic element to tunnel direction would be very useful as it could operate underground without the necessary access to polarised light or other visual cues.

2.3 -Chambers

Ants frequently create spaces within their nests that do not have the simple structure of tunnels. Round, flat, empty spaces within nests have been found to emerge in homogenous soil as an emergent property of digging dynamics (Toffin, Paolo, Campo, Detrain, & Deneubourg, 2009). However, Römer and Roces (2014) have suggested that a differentiation be made between spaces

which are dug with storage items present, which should be called chambers, and those which are dug with them absent, which should be called cavities. This distinction allows the conceptual separation of functional space created from that created as an unrelated epiphenomenon. However, this is a teleological differentiation that does not follow from an immediate characteristic of the structure, leading to classification difficulties when the conditions under which they are dug are unknown. Furthermore, it may be that some spaces are created with no storage items present, but are used for storage later on. These ambiguities could be resolved by more studies on the creation of chambers/cavities in different species, potentially isolating key differences in the behavioural mechanisms of their creation.

The chambers of fungus gardening ants are roughly spherical, oval, or elliptical (Jonkman, 1980a; Römer & Roces, 2014; Wheeler, 1907) with one or two connecting short tunnels, called peduncles (Jonkman, 1980a; Moreira, Forti, Andrade, et al., 2004), although the chambers can also be connected directly to tunnels (Verza et al., 2007). The vast majority of workers can normally be found in fungus garden chambers (Moser, 2006). The fungus garden can be situated on the floor of the chamber (Diehl-Fleig & Diehl, 2007) or be suspended from the ceiling in some species (Rabeling et al., 2007).

The difference between the flat chambers of most ants (Tschinkel, 2003) and the spherical shape of fungus growing ants requires an explanation. In the leaf-cutting ants *Acromyrmex lundi* and *Atta sexdens rubropilosa*, chambers do not appear in nests which only contain workers i.e. without brood, fungus or a queen (Camargo, Fujihara, & Forti, 2011; Fröhle, 2010). However, when a fungus garden is present a space is excavated around it (12.5mm for *A. lundi)* that allows garden maintenance and transit across the surface of the garden to the entrance (Fröhle & Roces, 2009). Thus, as it grows, the garden acts as a 3D template for the shape and size of the chamber (Camargo, Lopes, & Forti, 2013b; Fröhle & Roces, 2009; Römer & Roces, 2014). The use of fungus and brood

as a template for chamber construction can be compared to the use of nest-mates as a template in *Temnothorax albipennis* (Franks et al., 1992) or of queens as templates in termites (Bonabeau et al., 1998). Furthermore, brood relocation has been hypothesised to serve as a seed in the process of new chamber creation. The brood would be relocated to a new location and then the cues associated with brood attracts workers and the increase in worker density would lead to digging at that location (Römer & Roces, 2014). Fungus and also more brood would then be added, further stimulating digging and attracting yet more fungus and brood until a fully fledged fungal garden is complete. However, what would trigger the first transportation of brood or fungus away from a fungus garden to seed a new chamber is not yet known. Candidates include high carbon dioxide levels, garden structural integrity, and humidity and temperature cues.

However, the explanation of fungus as a template for chambers, satisfying as it may be for most fungus gardening species, does not hold in the case of *Mycocepurus sp.* In Rabeling et al. (2007) the excavations of fungus growing (but not leaf-cutting) colonies showed that chambers were formed around fungus gardens that did not closely match the form of the fungus garden. In these cases, it is likely that the fungus chamber was dug before the fungus was imported into the chamber. This is because freshly dug chambers (as signified by an absence of an accumulation of rubbish on the floor of the nest) were full sized despite only having a few strands of fungus and newly excavated nest chambers (as indicated by signs of current excavation) were fully formed despite an absence of fungus (Rabeling pers. comm.). A further example was reported for *Cyphomyrmex morschi* and *Mycetophylax simplex* in Klingenberg et al. (2007) where the fungus chambers only filled two thirds of the chamber volume. However, it is possible that seasonal variation could account for this (Klingenberg pers. comm.). Therefore, while a satisfying explanation of chamber development has been developed and supported for some fungus gardening ants, new explanatory mechanisms are required for the above mentioned species.

Within the larger context of the nest, fungus chambers tend to be concentrated in a central region (Moreira, Forti, Boaretto, et al., 2004) and found at a variety of depths, from close to the surface down to around 6 m for larger species (Jonkman, 1980a; Moser, 2006; Navarro & Jaffe, 1985) – with the number of chambers diminishing with depth (Moreira, Forti, Boaretto, et al., 2004). Fungus growing ants can have many small chambers or a single large one in a species typical pattern (Weber, 1972). The chambers themselves vary among species from 2cm to 90cm and can also vary in size within species (Diehl-Fleig & Diehl, 2007; Moser, 2006). Chambers seem to have maximum sizes, possibly caused by diffusive gases needing to reach the center of the fungus garden (Römer & Roces, 2014). However, work verifying this and identifying the causes of species typical patterns remains to be done.

Sometimes chambers are also found as empty or sand-filled cavities with just a few workers (Moreira, Forti, Andrade, et al., 2004; Moser, 2006). In most cases, separate chambers are dug for waste but sometimes old fungus chambers can also be used for waste storage (Autuori, 1942; Klingenberg et al., 2007). However, in some "lower" species (*M. simplex* and one nest of *C. morschi*) waste has been reported as being inside the fungus chamber (Klingenberg et al., 2007).

Nest structure is important to fungus garden humidity and temperature management. The fungus gardens in the chambers require specific humidity and temperature conditions (20-25 degrees – above 30 degrees the fungus dies) for optimal growth (R. J. Powell & Stradling, 1986; Quinlan & Cherrett, 1979). Different soil layers provide varying humidity and temperature conditions and so vertical stratification of chambers provides different chamber conditions and therefore give ants a choice as to where to locate fungus for better microclimate conditions (Klingenberg et al., 2007; Mikheyev & Tschinkel, 2003). For example, the deeper chambers of *Acromyrmex rugosus rugosus* were found to have higher humidity (Verza et al., 2007). Fungus growing ant workers move brood and fungus to the best available conditions for humidity (Navarro

& Jaffe, 1985; Roces & Kleineidam, 2000) as a form of fine tuning (Bollazzi & Roces, 2002; Roces & Kleineidam, 2000). This same principle applies to temperature, with chambers becoming cooler at increasing depth (Moser, 2006). Leaf-cutter ants have very acute temperature detection (Ruchty, Roces, & Kleineidam, 2010). This leads to a seasonal movement of the fungus and brood according to where the microclimate is most suitable within the architecture of the nest (Jesovnik, Sosa-Calvo, Lopes, Vasconcelos, & Schultz, 2013; Klingenberg et al., 2007; Lopes et al., 2011; Moser, 2006; Mueller et al., 2011; Navarro & Jaffe, 1985; Rabeling et al., 2007).

2.4 -Trails

Leaf-cutting ants need to feed their fungus gardens with large amounts of leaf-material and the fixed nature of both the nest and the food source means that leaf-cutting ants face the restrictions of central place foraging, with costs increasing with distance from the central nest (Rockwood & Hubbell, 1987). Therefore they create cleared trails that stretch from leaf-sources to the nest in order to speed the progress of workers. The trails have two primary components: a pheromone trail (Viela, Jaffé, & Howse, 1987), much like the pheromone foraging trails of other ants, and a physically cleared path that allows the leaf-carrying ants to move unhindered (Farji-Brener et al., 2010; Farji-Brener et al., 2012; Rockwood & Hubbell, 1987; Weber, 1972). The pheromone trail has a volatile recruitment pheromone and less volatile orientation cue (Hölldobler & Wilson, 2011). The pheromone component of the trail is primarily maintained by minors (Evison, Hart, & Jackson, 2008), whereas the physically cleared part of the trail is largely maintained by ants between 2.2-2.9 mm head width (Howard, 2001). Ants clearing trails are significantly larger on average than ants carrying leaves but there is a considerable overlap in sizes and any ant that was clearing was equally likely to be carrying leaves 24 hrs later (Howard, 2001).

The cleared trails reduce the costs of transportation of leaf-fragments across long distances, allowing increases in speed four to ten times that over uncleared trails (Rockwood & Hubbell, 1987). More highly cleared trails are associated with increased transportation of leaf-material back to the nest (Fowler, 1978). Furthermore, faster movement along trails also allows the faster transfer of information (Farji-Brener et al., 2010), prevents leaves from drying out (Rockwood & Hubbell, 1987) and minimises the time by which workers are vulnerable to attack by phorid flies (Röschard & Roces, 2002). The trails are bidirectional (Wirth et al., 2013) and individual trails can extend for 250 m (T. Lewis, Pollard, & Dibley, 1974) with the total trail system reaching up to 300 meters in length (Howard, 2001). However, trail length has a strong negative effect on foraging efficiency, ultimately limiting how far the network can expand (Bruce & Burd, 2012). Leaf-cutting ants forage

on trails in a generally diurnal pattern (Lutz, 1929) but can switch unpredictably (T. Lewis et al., 1974).

Trails direct scouting behaviour (Farji-Brener et al., 2007; Kost, De Oliveira, Knoch, & Wirth, 2005; Shepherd, 1982) and serve as an external "memory" of past resource locations (Shepherd, 1982; Wirth et al., 2013). Leaf-cutting ants are under selective pressure to reduce costs and maximise return through the design of the trail network (Farji-Brener et al., 2015; Farji-Brener et al., 2012). The nearly inverse relationship between trail length and foraging rate makes this a particularly important factor (Bruce & Burd, 2012). Farji-Brener et al. (2015) showed that the branching angle of trails will adjust relative to the clearing required in order to trade off clearing effort against travel distance relative to the risk of desiccation to workers. Furthermore, trails can straighten significantly with time to provide a direct path (Fowler & Robinson, 1979). Fowler and Robinson (1979) hypothesised that this was done by pheromone deposition, but more testing needs to be done to verify this. Access to pioneer plant species appears to be key in determining the shape of foraging trails and more pioneers means more trail branches and a more complex system (Silva et al., 2013).

Trails require an optimal width in order to maximise foraging returns (Dussutour, Beshers, Deneubourg, & Fourcassié, 2007; Farji-Brener et al., 2012; Fourcassié, Dussutour, & Deneubourg, 2010). They should not be too narrow or congestion will slow leaf-fragment return (Burd & Aranwela, 2003; Farji-Brener et al., 2011). Nor should they be too wide or reduced inter-ant contacts may lower the stimulus provided by returning fragments and therefore reduce cutting rates (Burd, Archer, Aranwela, & Stradling, 2002; Dussutour et al., 2007). Trail edges were found to have an unexpectedly strong negative effect on leaf-transport rates (Bruce & Burd, 2012), which was found to be due to the effect of the trail edge slowing down leaf-carriers via a higher number of collisions with the edge (Farji-Brener et al., 2012). This leads to trails being wider than might

otherwise be expected. For example, the sum of widths of two trails post-bifurcation was an average of 40% higher than the width of the trail that led to them (Farji-Brener et al., 2012). Narrow trails have lower construction costs but instead have higher congestion costs whereas a wider trail has a higher maximum speed yet also higher construction costs (Farji-Brener et al., 2012).

The multi-dimensional nature of these trails, along with their ease of study (being open to the air their examination requires no excavation) has made them the subject of considerable study. Resource flows (Burd et al., 2002; Burd & Howard, 2005), crowding (Dussutour et al., 2007; Dussutour, Beshers, Deneubourg, & Fourcassié, 2009), information transfer (Burd & Aranwela, 2003; Farji-Brener et al., 2010; Roces, 2002; Röschard & Roces, 2002), and load selection (Burd, 2000; Burd & Howard, 2005; O. T. Lewis, Martin, & Czaczkes, 2008; Roces & Hölldobler, 1994; Röschard & Roces, 2002; Rudolph & Loudon, 1986) have all been well studied. They also present an interesting opportunity to study a system that is analogous to a human transportation system, yet for an animal that is very different from humanity (Bruce & Burd, 2012). However, while much is known of the functioning of trails, the mechanisms underlying the construction of these trails have not had so much attention. Howard (2001) estimated that $4.2\pm1.4\%$ of a colony's foraging ants were involved in clearing. Random actions of individuals have been considered enough to clear the trail (Weber, 1972). However, any mechanism that governs their creation must choose an optimal balance between construction cost and width whilst also facilitating information flows, as discussed above. Burd et al. (2002) hypothesised that there is a density dependent mechanism that regulates clearing. On the other hand, it is possible that interactions between response thresholds and encounter rates with obstacles on the edge of the trail are responsible for regulating trail width. To support this, Howard (2001) found that workers that had been marked during trail clearance were more likely to clear obstacles in the future. This indicated that a part of the trail population may have lower response thresholds or that this response threshold is variable and lowered by performance of the task. Whether this is correct and if thresholds change with colony size, foraging

context or other factors, is currently unknown. Investigations into the regulation of the clearance of trail height such that it allows the free flow of laden ants can be found in chapter six.

2.5 -Conclusion

The purpose of this review has been to discuss what is known and unknown about the creation of fungus gardening ant nests and trail infrastructure. Many of their goals and functions have been well explored and thoroughly documented, such as the relationship between nest size and ant number or the transfer of information along trails. However, many of the regulatory mechanisms that lead to these functional results remain to be studied. I suggest that this is the next step in this field and that investigations into this realm contribute not only to our understanding of these impressive systems but may also lead to a more fundamental understanding of social organisation and the functioning of self-organised and emergent systems. This is becoming increasingly relevant in our highly networked world where distributed systems, human and machine, will require heterachical rather than hierarchical regulation.

2.6 - References

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3.0 -The digging dynamics of leaf-cutting ant tunnels: tunnel length and digging rate

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3.1 -Abstract

The underground nests of ants are created by excavating soil so as to form tunnels and chambers, and removing the soil to the surface. However, digging is costly and so might be expected to be regulated so as to avoid wasteful investment in excess space. Some evidence of this process has been found in nests and in chambers. In this paper I examine this question in relation to tunnels. I found that ants do significantly reduce their digging effort over time in long tunnels (80cm) when compared with short tunnels (5cm), but that this effect disappears when the ant numbers are increased tenfold, from 10 to 100 workers. Attempts to disrupt feedback from tunnel length by removing previously dug tunnel length by means of a gate system lead to increased digging rates when compared to an undisturbed control. I hypothesise that this was a result of forcing ants to switch tasks and thereby causing social disruption. Shortening the tunnel by this method did not lead to increased digging rates. The implications of experience and the social organisation of nest excavation, including the nature of response thresholds are discussed.

Keywords: nest - digging - tunnel - excavation - leaf-cutter ants - *Acromyrmex lundi*

3.2 -Introduction

Many ant species construct underground nests, which can be both large and sophisticated, in order to protect their colony from inimical environmental variation (Jones & Oldroyd, 2007) as well as from predators (S. Powell & Clark, 2004). This can lead to ants being important soil engineers (Jouquet, Dauber, Lagerlöf, Lavelle, & Lepage, 2006) that affect their local environment by changing soil properties such as runoff (Cammeraat, Willot, Compton, & Incoll, 2002), altering plant recruitment dynamics (Bieber, Oliveira, Wirth, Tabarelli, & Leal, 2011), or enhancing soil

nutrients (Shukla, Singh, Rastogi, & Agarwal, 2013). While nests have also been shown to have a role in behavioural organisation (Franks & Tofts, 1994; Pless et al., 2015), the rules that facilitate their construction are not well understood. These rules are thought to produce self-organised collective action (Buhl et al., 2005), and therefore to operate without central control. Instead, organisation is thought to be regulated by such mechanisms as response thresholds (Theraulaz, Bonabeau, & Deneubourg, 1998b), local communication (Pielström & Roces, 2012), and various feedback processes (Czaczkes, Grüter, & Ratnieks, 2013).

Nest construction costs worker time, energy, and wear-and-tear (Mikheyev & Tschinkel, 2003), which could be invested elsewhere. Given this, it might be expected that ants would carefully tailor their nest size to their space requirements in order to minimize costs whilst still meeting the needs of the colony (Halley, Burd, & Wells, 2005). Indeed, Rasse and Deneubourg (2001) have found that nest volume is adapted to group size in the ant *Lasius niger*. Pielström (2013) found that equal numbers of the leaf-cutting ant *Atta vollenweideri* will dig less in a large chamber than in a small one. These results suggest that a behavioural mechanism exists to regulate the expansion of nest and chamber size during nest excavation so that it is not enlarged more than necessary. However, the space in an ant nest is made up of both tunnels and chambers (Tschinkel, 2004), which differ in shape, size and orientation, and may require separate self-organized mechanisms to regulate their construction. Attention, however, has been focused on chamber and whole nest excavation, while the behavioural digging dynamics underlying tunnels have not yet been examined. In the absence of a fungus garden, leaf-cutting ants will only dig tunnels and no chambers (Camargo, Fujihara, et al., 2011; Fröhle, 2010). Tunnels contribute volume to the nest and also serve as the connecting ducts among the different parts. In the case of leaf-cutting ants, tunnels also connect entrances to the fungus gardens, allowing the essential movement of workers, fresh leaves, and fungus. However, the relationship between behavioural organisation and tunnel space remains to be investigated.

This paper aims to examine the role of space, specifically in terms of tunnel length, in influencing digging rate in tunnels. I investigated whether workers can use the length of a tunnel as a cue to regulate their excavation. I first exposed workers of the species *Acromyrmex lundi* to tunnels of different initial lengths and measured the corresponding digging rates. Digging was constrained within a tube to a perpendicular digging face, essentially reducing digging opportunities to one dimension. This was done in order to remove variables that might occur with free excavation, such as changing geometry, division of digging effort into two places, altered surface area/volume ratio, etc. I then altered the length that ants had already dug in order to determine to what extent feedback from the length of available tunnel affects further digging effort.

3.3 -Methods

3.31 -Study species

Five colonies of *Acromrymex lundi* were collected in 2007 from Argentina, fed with rose and blackberry leaves as well as water and honey water, and maintained in a 12 hour light/dark cycle at a constant temperature of 25°C. All experiments were conducted at 25°C.

3.32 -Basic set-up

To begin an experiment, workers were removed from the foraging box of their nest, counted out, and placed into the experimental arena. The experimental arena (fig. 1) consisted of a 9.5 cm by 9.5 cm by 5.5 cm transparent plastic box with a transparent plastic lid, in which sugar water and tap water were provided *ad libitum* in two small feeders placed on two platforms. Feeding platforms were raised on pillars in order to prevent them from being immediately contaminated by excavated soil. Damp tissue paper was used to humidify the air. Despite the food, water, and humidity, ants would die on occasion. Any replicates that suffered a mortality rate greater than 30% were not

included in the analysis. The lid was taped down and the rim of the box was lined with paraffin oil in order to facilitate easy feeding without ants escaping (though the paraffin was not used for the '100 ants -initial length' treatment). A plastic tube (1 cm inner diameter) was attached to the box through a hole in the side and a small plastic 'bridge' (half a plastic tube) was installed in order to provide the ants with easy access. The tube was filled with a mixture of 70%, 15% clay, and 15% water by weight. This mixture was the substrate that workers excavated. The high sand content allowed rapid extraction (Espinoza & Santamarina, 2010), while the water and clay were sufficient to allow pellets to be formed for rapid transport. The rapid excavation allowed the behavioural signal of digging rate to be amplified. Digging progress was measured as the maximum distance that the ants could be observed to have dug. Initial tunnel length was varied and excavated length was manipulated in two separate experiments, as follows:
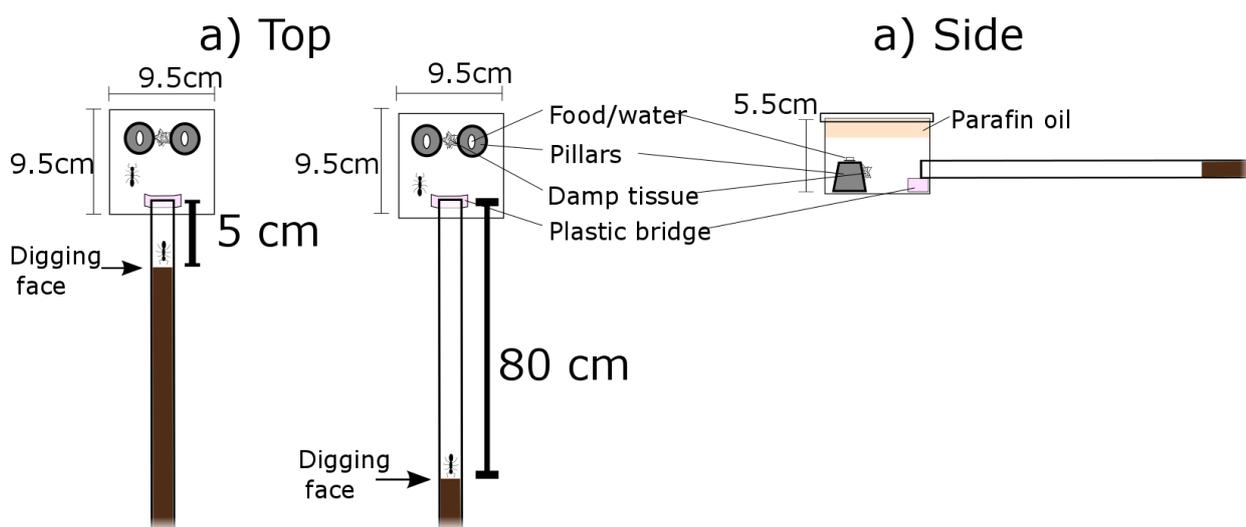

Figure 1. Experimental setup from the a) top and b) the side (not to scale).

3.33 -Experiment 1: manipulation of initial tunnel length

This experiment examined whether ants vary their digging rate in response to initially encountered available tunnel length. One of two kinds of tubes were connected to the box: one presented the ants with a short length of empty tunnel in front of the substrate, and the other

presented a long empty length (fig. 1). Groups of either 10 ants or 100 ants were tested so as to examine the relationship between space, digging rate, and group size. The 10 ant 'short length' treatment used ants from colony 1 for two replicates, ants from colony 3 for one replicate, and ants from colony 4 for two replicates. The 10 ant 'long length' treatment used ants from colony 1 for two replicates, ants from colony 4 for two replicates, ants from colony 6 for two replicates, and ants from colony E for one replicate. The 100 ant 'short length' and 'long length' treatments both used ants from colonies 1, 3, 4, 5, and 6 for two replicates each. For both the 10 ant and 100 ant group sizes, the 'short length' treatment had 5 cm of empty tunnel length followed by 55 cm of soil-filled tube. The 'long length' treatment had 80 cm of empty tunnel length followed by 55 cm of soil-filled tube for the 10 ant treatment, and 75 cm of empty tunnel length followed by 80 cm of soil filled tube for the 100 ant treatment. In all cases different lengths of tubes were joined together using silicone tube joiners that provide an airtight seal. Ants were allowed to dig for 4 days. In cases where ant excavation approached the end of their tube, extra soil-filled tube length was added using silicone tube joiners.

3.34 -Experiment 2: Manipulation of excavated length to create an 'open feedback loop'

This experiment aimed to investigate the role that feedback from tunnel length plays in determining digging rate. The intention was to change the ratio of available tunnel length and group size by removing significant portions of tunnel length rather than by increasing the number of ants. The ants would be presented with a tunnel that would not increase in length in proportion to their digging effort, and therefore the feedback that they would receive from tunnel length would be eliminated and 'open'. I wanted to do this with minimal disturbance in order to see whether a newly shortened tunnel length would lead to increased digging rates. To do this I used a gate system and a return system. The gate system allowed me to switch the ants from digging in one tunnel to digging in another (fig. 2). The rationale behind using a gate was that the disturbance from the switch from one treatment to the other would be brief, restricted to a small location, and not involve direct

handling of the ants. After initial excavation, the ants were switched to either a sham control or the 'length reduced' treatment. The sham control (fig. 2a) had empty tubes cut to match the length already dug by the ants. The floors of these tubes had been odour marked for at least 2 days (more often 3 and sometimes 5) by a sub-colony matching that of the experimental ants. It should be noted that tunnels excavated by ants would often not be completely empty (varying amounts of pellets and unexcavated soil remaining), while the new tubes were completely empty. Therefore, while the length of the old tunnels and new tunnels were matched, the volume presented may not have been exactly equivalent. The 'length reduced' treatments presented a digging face 8 cm from the gate (fig. 2b), and so prevented the ants from ever having access to very long tunnel lengths such as were available in the control treatment. The digging face for both the control and the 'length reduced' treatment were 'digging marked' by a matching sub-colony so that odour and physical markings would be appropriate for an actively dug tunnel. This was done by attaching boxes containing a number of ants from the same colony and allowing the ants to dig overnight. Immediately prior to being attached to the experiment, the tubes were detached and the ants were allowed to escape. The tube was then trimmed so that only 3cm of empty tube remained before the soil began. The tube was not cut closer than 3 cm in order to prevent damage or distortion of the digging face. The digging tubes were then ready to include into the experiment.

The return system was intended to remove the ants from the old, long, excavated tunnel to the new shorter tunnel. It consisted of a series of tubes from the gate to the box that was intended to allow ants to walk from the old digging tube to the box with minimal disruption while simultaneously preventing them from returning to the old tube so that over time all ants would transfer to the new tube (fig. 2). Leaf-cutting ants have been shown to be able to successfully use one-way systems (Ribeiro, Helene, Xavier, Navas, & Ribeiro, 2009). This one way direction was achieved by coating the inside of two tubes with fluon so that ants could slip down but not return. The first fluon section did allow the ants to scramble back up but the effort involved discouraged

them from doing so. This was intended to speed their movement towards the second fluon section, which prevented ant return altogether. This tube was replaced every day in order to prevent moisture from causing the fluon to fail. The return system was colony odour marked by appropriate sub-colonies for approximately 24 hours prior to the beginning of the experiment. Some ants would not return to the box but would remain in the old digging tube. These ants were removed from the experiment when the old tube was replaced. The sham control treatment used ants from colony 4 for 10 replicates and from colony 3 for 4 replicates. The gate length reduced treatment used ants from colony 4 for 5 replicates, and ants from colony 3 for 4 replicates.
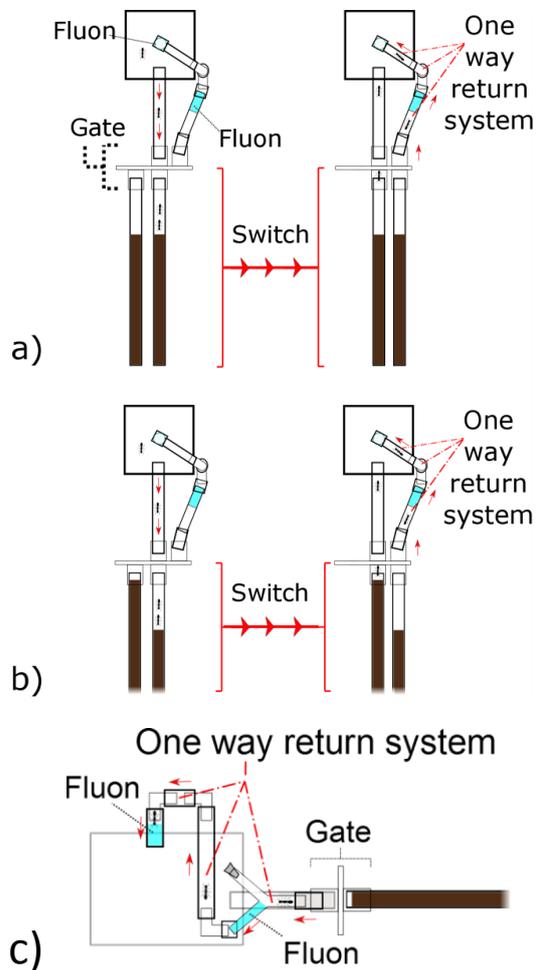

Figure 2. Diagram of the gate system and return system for a) control, b) length reduced, and c) the side. Both new tubes and the return system were exposed to matching sub-colonies prior to use in the experiment. New tubes in both treatments were previously excavated by matching sub-colonies in order to produce a typical and matching digging face.

### 3.35 -*Statistical Analysis*

The 'initial length' and 'length removed' experiments were both analysed by ANCOVA. The number of hours from the start of an experiment (the initial exposure of ant groups to their excavation opportunities) was the independent variable, and the length treatment (short or long empty tunnel; sham control or length reduced tunnel) was the qualitative factor. The dependent variable was the length of tunnel excavated. The slopes in these analyses are excavation rates with dimensions of length of tunnel excavated per unit time. I looked for significant differences in the excavation rates between treatment levels by examining first the time × treatment interaction and then for significant differences in the treatment levels. All analyses were performed with R v. 2.15.2 (2012).

4.0 -Results

Groups of 10 ants presented with 80 cm of empty tunnel length dug significantly less over time than groups of ten ants presented with 5 cm of empty tunnel length (fig. 3a: Hours*Treatment: $F(1,91)=4.13$, $p=0.044$). However, groups of 100 ants presented with 75 cm of empty tunnel length did not dig significantly differently from groups of 100 ants presented with 5 cm of empty tunnel length (fig. 4a: Hours + Treatment: $F(1,136)=0.22$, $p=0.639$). Surprisingly, the use of the gate seemed to promote digging: groups of 100 ants presented with new, colony marked tunnel length equal to their dug tunnel length by means of a gate, dug significantly more than groups that are not exposed to switching by a gate (fig. 5a, Hours + Treatment: $F(1,189)=15.56$, $p<0.001$). By contrast, groups of 100 ants that have their dug tunnel length shortened by means of a gate do not dig significantly differently over time from ants presented with empty tunnel length equal to their dug tunnel length by means of a gate (fig. 6a: Hours + Treatment: $F(1,181)=0.10$, $p=0.751$).
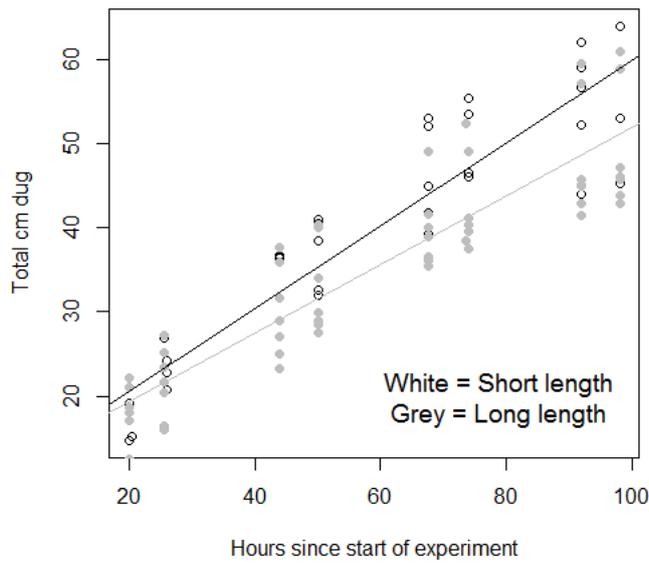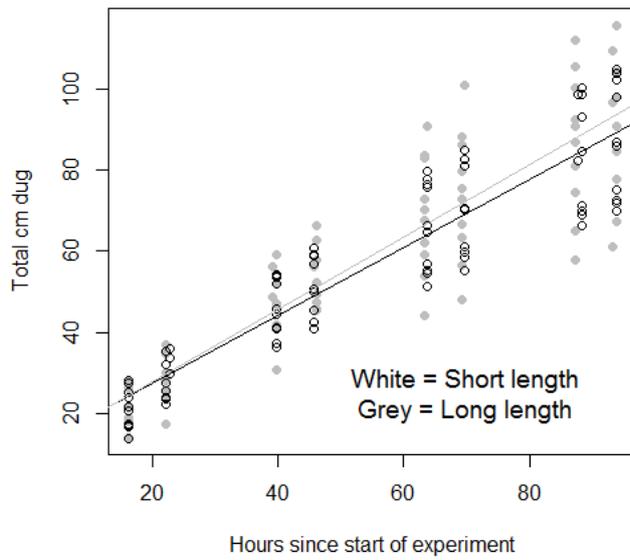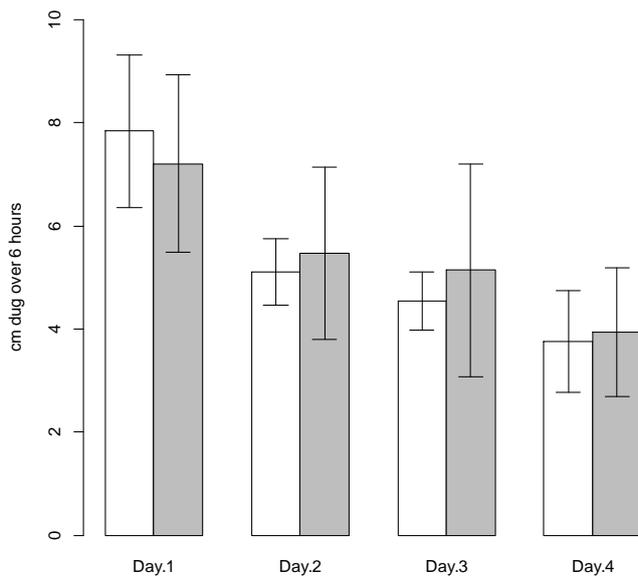
a)
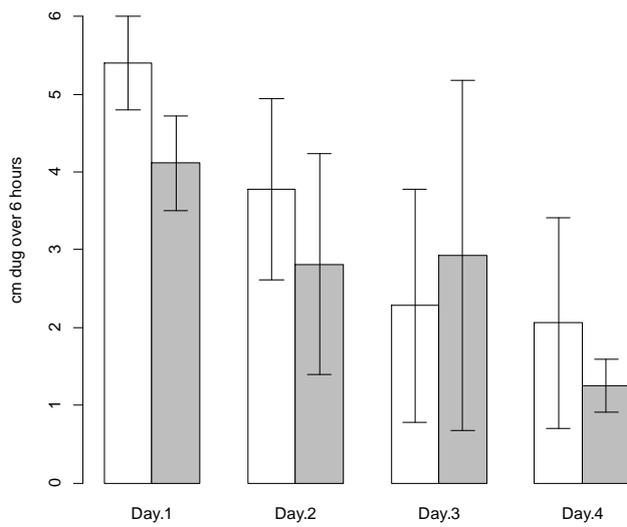
b)

Figure 3. a) Total cm dug over time for 10 ants for short tunnel length (5 cm initial tunnel length, n=5) and long tunnel length treatment (80 cm initial tunnel length, n=7). There is a significant interaction between hours and treatment (Hours*Treatment: $F(1,91)=4.13$, $p=0.044$). b) Centimeters dug over 6 hours for 10 ants for the same data as above (white = short length, grey = long length). Bars represent means, whiskers represent 1 SD.
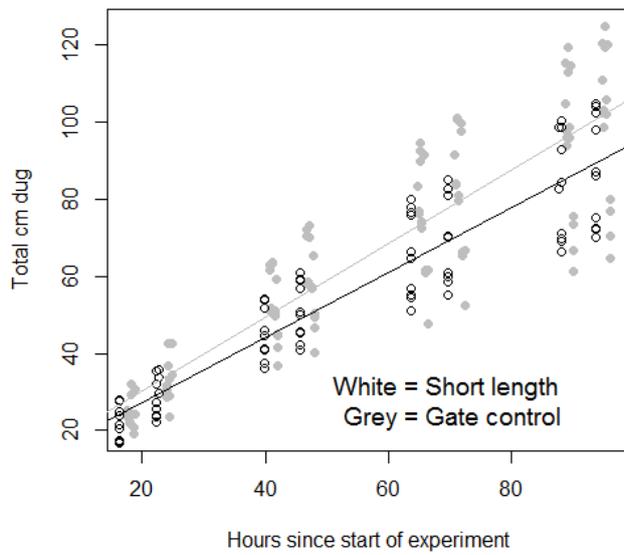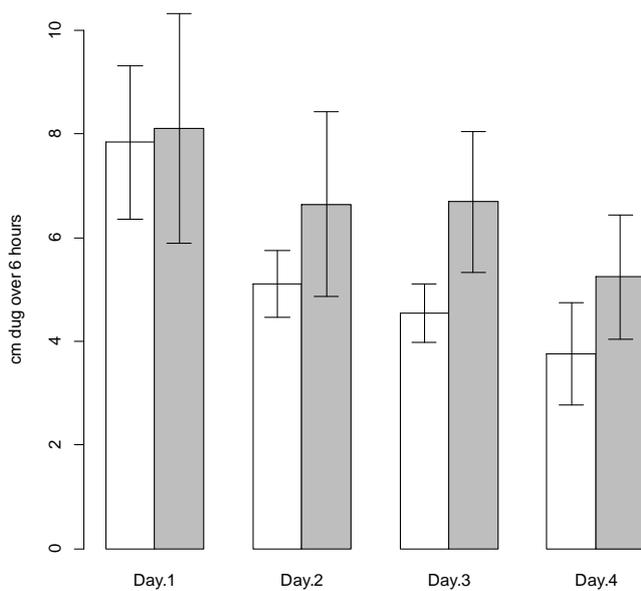
a)

b)

Figure 4. Total cm dug over time for 100 ants for short tunnel length (5 cm initial tunnel length, n=10) and long tunnel length (75 cm initial tunnel length, n=10). There is no significant difference between the two treatments (Hours + Treatment: $F(1,136)=0.22$, $p=0.639$). b) Centimetres dug over 6 hours for 100 ants for the same data as above (white = short length, grey = long length). Bars represent means, whiskers represent 1 SD.
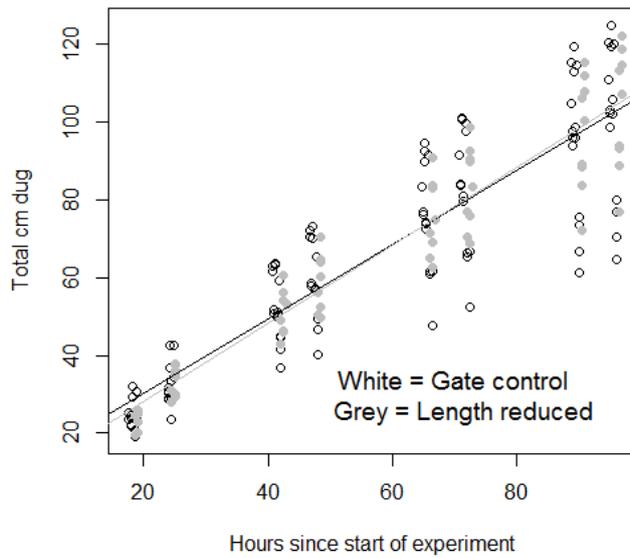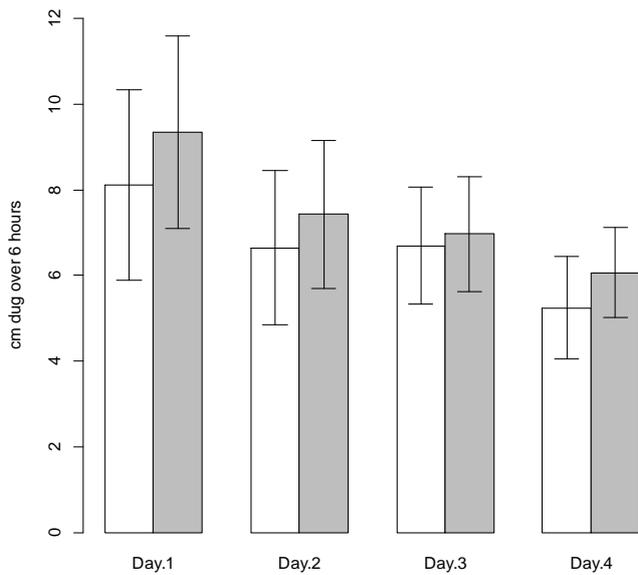
a)

b)

Figure 5. a) Total cm dug over time for 100 ants for short tunnel length (5 cm initial tunnel length, n=10) and the gate control (no tunnel length reduction, n=14). There is a significant difference between the two treatments (Hours + Treatment: $F(1,189)=15.56$, $p<0.001$). b) Centimetres dug over 6 hours for 100 ants for the same data as above (white = short length, grey = gate control). Bars represent means, whiskers represent 1 SD.

a)

b)

Figure 6. a) Total cm dug over time for 100 ants for gate control (no tunnel length reduction, n=14) and gate length reduced (tunnel reduced to 18 cm, n=9). There is no significant difference between the two treatments (Hours + Treatment: $F(1,181)=0.10$, $p=0.751$). b) Centimetres dug over 6 hours for 100 ants for the same treatments as above (white = gate control, grey = gate length reduced). Bars represent means, whiskers represent 1 SD.

5.0 -Discussion

These results show that there is a significant interaction between time and initial length treatment for the digging rate of 10 *A. lundi* workers (fig. 3a), and that this interaction disappears with higher ant numbers (fig. 4a). This suggests that the relationship between ant number and tunnel length is a key element in determining digging rate. Previous results have shown that space can affect digging rate in whole nests (Rasse & Deneubourg, 2001) and in chambers (Pielström, 2013) and this study shows that available space can also affect digging rate in the constrained conditions of a tunnel. The similar dynamics present in the three different forms of space (nests, chambers, and tunnels) suggest that the underlying mechanism could be the same for all three. Assuming that this is the case, this suggests that this effect is not the result of the specific geometrical properties of a nest or a chamber. While this experiment does not elucidate the precise mechanism underlying this effect, it is likely that the increased tunnel length reduces the concentration of ants in that space. This in turn reduces the encounter rate of ants with digging and pellet transporting ants, as well as the digging face. Given the known importance of encounter rates in regulating colony work patterns (Czaczkes, Grüter, Ellis, Wood, & Ratnieks, 2013; Czaczkes, Grüter, & Ratnieks, 2013; Gordon & Mehdiabadi, 1999; Gordon, Paul, & Thorpe, 1993; Greene & Gordon, 2007), longer tunnel length would have the effect of lowering digging rates. By contrast, in the 100 ant treatment the higher number of ants in the same space is likely to be saturating those key areas to such an extent that the empty tunnel length no longer has an effect.

My use of a gate to modify existing tunnel length significantly increased digging rates compared to treatments without a gate (fig. 5a), despite my intention that it should not. It is possible that the effect of being transferred via the gate from a familiar tunnel to an unfamiliar return system, despite the familiar colony odour markings, confused and disturbed the ants enough so that the digging rate was thereby increased. This raises the possibility that ants might be using some form of spatial awareness to navigate the internal structure of a nest and the disturbance of this mechanism

caused the increased digging rate. However, below I suggest that the increase in digging rate was more likely to have been caused by the social disruption to the digging and pellet transport system (Pielström & Roces, 2013).

In this experiment and elsewhere digging rates have been reported to drop over time (figs 3-6,b; Rasse & Deneubourg, 2001; Pielström, 2013). It may be that an ant that has been digging for some time may have a higher behavioural threshold (and lower digging rate) than ants that have not been digging. Then, as the individual ants cease digging the digging rate of the group declines. This would explain the effect of the gate system, that by removing the established 'excavators' from the system I was encouraging pellet 'carriers' to switch to digging. That is, the gate removes the established diggers and subsequently transporters become diggers. Then new and fresh ants encounter an empty digging face and are encouraged to dig, thereby causing a commensurately higher digging rate. Further investigation into both internal nest spatial awareness and forced task switching may provide new insight into both excavation mechanisms and response threshold systems.

There was no significant difference between the 'gate-control' treatment and the 'gate-length reduced' treatment (fig. 6). However, as previously stated, the effect of the gate may be masking any effect of removing tunnel length. Therefore, the most that can be claimed from these data is that, if there is an effect from removing length, it is not larger than that caused by the disruption from the gate.

As previously noted, for all the discussed experiments (figs 3-6, b; Rasse & Deneubourg, 2001; Pielström, 2013) there is a reduction in digging rate over time in the absence of otherwise stimulating factors. This stands in contrast to the usual conception of response thresholds, which is that experience in performing a task reduces the threshold (increasing the performance of the behaviour) (Theraulaz et al., 1998b). For digging it may be the opposite: the longer the duration of

task performance the more the behavioural threshold increases, reducing the performance of the behaviour. It may be that the balance of costs and benefits to a colony determine how a behavioural threshold functions. That is, when a behaviour has a low cost to the individual or if it has highly positive benefits to the colony (Westhus, Kleineidam, Roces, & Weidenmüller, 2012), then performance of the task is likely to lead to future increases in the behaviour. By contrast, if the task is costly and the immediate colony pay off is low, as is the case in digging, then performance might lead to reductions in the behaviour.

This process may play a role of preventing the colony, or subsections of the colony, from becoming entrained on costly processes at the expense of other tasks. I hypothesise that behavioural thresholds for tasks with high penalties, such as energy cost or wear and tear, will rise more quickly than activities with low penalties. How behavioural thresholds operate on an individual basis is currently poorly understood. This, and the operation of competing thresholds, deserves further investigation.

Digging in tunnels seems to show similar dynamics to that seen in other nest structures or in whole nests, indicating that the process involved may be common to all. While I cannot say for sure to what extent ants may receive feedback from tunnel length, disruption to the digging process, whether in the form of orientation or social disruption, can amplify digging rate. Furthermore, it is apparent that ants are capable of regulating their digging effort in response to initially larger tunnel lengths. The dynamics of nest excavation remains a fertile field of study.

Acknowledgements

6.0 -References

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4.0 -It is not all pheromones: No evidence that pheromones affect digging face choice during ant nest excavation

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4.1 -Abstract

Ants create nests of a size that is tailored to the number of individuals in a nest via a self-organized process. It is not yet clear how they accomplish this. Deposition and evaporation of pheromones at the digging face has been hypothesised by Deneubourg and Franks (1995) and Buhl et al. (2005) to be part of the nest construction process, with models being presented to support this contention.

This hypothesis was tested by allowing groups of 5 *Acromyrmex lundi* workers to choose between two excavation sites, one that was freshly exposed to digging and one where digging had ceased an hour previously. It was expected that if pheromones played a role in stimulating digging, then ants would show a preference for digging in the "fresh" sites rather than the "aged" sites where the putative digging pheromone had decayed.

No significant difference in digging activity between "fresh" and "aged" sites was detected. It is therefore likely that, while digging pheromones may play other roles in other parts of the digging system, they do not play an important role in regulation of soil excavation at the digging face.

Keywords: nest - digging - tunnel - excavation - leaf-cutter ants - *Acromyrmex lundi*

4.2 -Introduction

Nests are a key element in the success of eusocial insects (Wilson, 2008). They provide physical protection from predators and enemies (Herrera & Valenciaga, 2011; Theraulaz et al., 2003), help to regulate temperature (Bollazzi et al., 2008; Farji-Brener & Tadey, 2012; Jones & Oldroyd, 2007; Korb, 2003), moisture and humidity (Bollazzi & Roces, 2007, 2010; Hölldobler & Wilson, 1986), and carbon dioxide concentration (Kleineidam, Ernst, & Roces, 2001; Lüscher, 1961), as well as play a role in task allocation and colony activity (Gordon et al., 1993; Hölldobler & Wilson, 1990; Kwapich & Tschinkel, 2013; Stickland & Franks, 1994; Tschinkel, 1999a). However, the mechanisms by which social insects organize their nest construction to achieve the correct functional form and size for their species and state of colony growth are not fully understood. In this article, I investigate the hypothesis that ants regulate their nest construction through pheromone deposition on the surfaces of the growing nest structure.

The nests of most ant species are subterranean and are constructed by excavating soil from tunnels and chambers and transporting it to the surface (Sudd, 1967). Ants would be expected to avoid unnecessary excavation, and so a simple but fundamental design principal should be that nest size is adjusted to the needs of the colony. Specifically, nest volume should be tailored to the number of ants present in the colony. Such matching of form to function has been documented in several ant species by Tschinkel (1999b), Mikheyev and Tschinkel (2003), Tschinkel (2004), Tschinkel (2005), Tschinkel (2011a), L. M. Hart and Tschinkel (2012), and Murdock and Tschinkel (2015).

Deneubourg and Franks (1995) and Buhl et al. (2005) have proposed a simple mechanism to explain how a self-organised process of digging might be regulated using pheromones. Suppose that ants deposit pheromones on soil particles at the digging face of a tunnel and that this pheromone prompts other ants to dig. As the excavation progresses, the increased size of the nest means that

ants, moving at a constant speed, are less likely to be recruited to dig due to the decreased chance of encountering a pheromone laden site. As nest structure expands and the pheromone evaporates the impetus for continued excavation declines. Eventually work declines to near zero and the nest attains its final size relative to the population of ants. Models of this process predict logistic growth of nest size over time, and some experimental data conform approximately to this pattern (Buhl et al., 2005; Halley et al., 2005).

In addition, support for the proposed pheromonal mechanism was provided by Chen and Zhang (2013), who found that *Solenopsis invicta* would choose to dig significantly more in sand treated with mandibular gland extracts over sand without the treatment. Additionally, Pielström and Roces (2013) found that freshly excavated soil pellets guided the location of digging by *Atta vollenweideri* leaf-cutting ants, while pellets that were one hour old did not, suggesting that chemical cues that stimulate excavation may have evaporated from the older pellets.

Although the pheromone hypothesis has some empirical support, a behavioural effect of pheromones that the ants have themselves deposited has not been demonstrated. The present experiment investigates the possibility that fresh pheromones on the digging face of tunnels might significantly attract worker digging behaviour.

4.3 -Methods

Five colonies of *Acromyrmex lundi,* collected in Argentina in 2007 and maintained at the Biocenter of the University of Würzburg, Germany, were used for this experiment. Colonies were maintained at 25°C and 50% humidity under a light/dark cycle of 12 hr/12 hr. The experimental setup allowed groups of five workers from each colony to choose between two tubes from which to excavate a mixture of 75% clay and 25% water by weight. For each trial, the tubes were attached to opposite arms of a plastic T-junction and the ants were introduced through the stem (fig. 1).

The experiment compared the quantity of soil excavated by groups of 5 *A. lundi* workers given a choice between two digging locations: a fresh tunnel face that recently had ants digging at it, and one that had not been exposed to excavation for one hour. The procedure did not measure pheromones directly, but used time since the end of excavation as a proxy for putative pheromone quantity based on the effect noted by Pielström and Roces (2013). Significantly greater excavation in the "fresh" than the "aged" soil face would therefore be consistent with stimulation of digging by pheromones, but would not directly confirm their presence. A negative result would be inconsistent with the digging pheromone hypothesis.

Because temperature can influence digging performance in this species (Bollazzi et al., 2008), digging tubes were sealed and left overnight to bring them to ambient temperature. Digging tubes were attached for two hours to boxes containing a large number of workers (more than 30) of *A. lundi* from the same colony that supplied workers for the experimental trial itself. Excavation usually occurred during this time, and if it did not, the tube was not used in any trials. Ants from all parts of the experiment were used only once.

The setup for one of the two tubes was timed so that it could be left for at least one hour before commencement to allow evaporation of any pheromone present on the digging face of the clay substrate. This was the "aged" condition. The other tube was used immediately after excavating ants were removed. This was the "fresh" condition.

Because excavation had occurred at the surfaces in both experimental tubes, ants in a trial had to choose between two digging faces that were physically similar but might differ in the degree of pheromone evaporation. Water as well as pheromones could evaporate from the clay surface, and soil moisture differences can affect ant digging choices (Pielström & Roces, 2014). Therefore, in order to prevent physical changes to the digging face as a result of water evaporation, "aged" tubes were attached to a source of humidified air for an hour before a trial. In order to demonstrate that

the humidified air did not bias digging behaviour, a control provided ants with the same choice between two tubes of clay substrate, but, unlike the experimental trials, without the pre-exposure to digging ants. All other conditions were the same.

In both the control and experimental trials the choice tubes were weighed (Kern ABT 120-5DM, accuracy 0.01 mg) before the experiment and attached to the T-junction. Five ants were then introduced into the T-junction (fig. 1). The setup was directionally randomised and covered in black photography cloth to prevent bias. An average of 1.43 min (n=8,SD=6.5 sec) elapsed between the end of preparatory exposure of tubes to digging ants in the "fresh" treatment and the insertion of the five experimental workers. It then took an ant an average of 15.9 seconds (n=16,SD=10.0 sec) to encounter one of the digging faces.

Ants were left to dig in the setup for one hour. Preliminary tests had shown that excavation would begin well within the hour. Further measurements described below confirm this. Ants tend to dig where other ants are already digging (Sudd, 1970b) and so it was expected that the initial choice of an ant to dig at a location would be amplified by other ants from the group also choosing to dig there. At the end of the hour, the ants were removed from the tubes and both tubes were weighed. The difference between the initial weight and the subsequent weight was taken to be the amount of soil excavated from that tube.

A separate test was performed to measure the lag times from introduction to the start of digging. The control protocol was used for these measurements, but without the black cloth so that the ants' activity could be observed. Each replicate was checked once each minute and the time of first digging was recorded.

In total, 103 trials of the control setup and 96 trials of the experimental setup were performed. Paired Wilcoxon tests were used to compare the amount of soil removed from the "fresh" and "aged" tubes. Results from each of the five colonies were tested separately to detect

possible colony differences. To compensate for the inflation of Type 1 error by multiple testing, a Bonferroni-corrected probability value of 0.01 was used. When no significant effects within colonies were found, the data were pooled across colonies and again tested with a paired Wilcoxon test. Confidence intervals were formed for these tests via bootstrapping. Statistical calculations were performed with R (R Development Core Team, 2012).
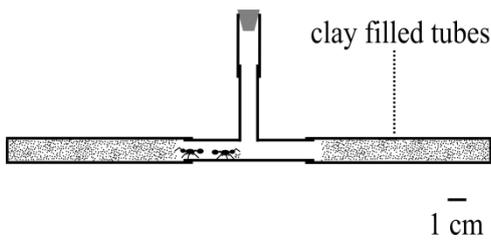

Figure 1. Digging face choice setup. One side was recently exposed to digging ("fresh") while the other was also exposed to digging at least an hour before ("aged"). The "aged" tube was attached to an air pump and water bubbler to keep the surface of the clay from drying out. Tubes had a 10 mm inner dimension. The T junction had an 8mm internal diameter, 7 cm length, and 3.5 cm stem height.

4.4 -Results

The first digging commenced after an average of 11 minutes (n=10, SD=4:03). Therefore, the total time from removal of the preparatory ants to the start of digging by the experimental ants was an average of approximately 12.4 minutes.

No colonies showed a significant difference between "aged" and "fresh" tubes for either the control or experimental condition (table 1). Therefore, the results were pooled for further examination.

As expected, ants showed no preference between the two tubes of the control trials. On average, they removed 0.19 g (sd=0.23) from control "fresh" tubes that had not been exposed to digging, and 0.23 g (sd=0.19) from control "aged" tubes that received the sham treatment of attachment to the humidifying apparatus for an hour before the trial (fig. 2a). This difference was not significant (Wilcoxon test, p=0.211, n=103). Workers also showed no discrimination between tubes in the experimental trials. They excavated an average of 0.18 g (sd=0.18) from "fresh" tubes and 0.17 g (sd=0.25) from "aged" tubes, a non-significant difference (fig. 2b; Wilcoxon test, p=0.523, n=96).

Table 1. Paired Wilcoxon tests for "fresh" and "aged" treatments for individual colonies. N indicates number of trials involving each colony. To account for Type 1 error inflation due to multiple testing, the criterion of significance has been Bonferroni-corrected to 0.01.

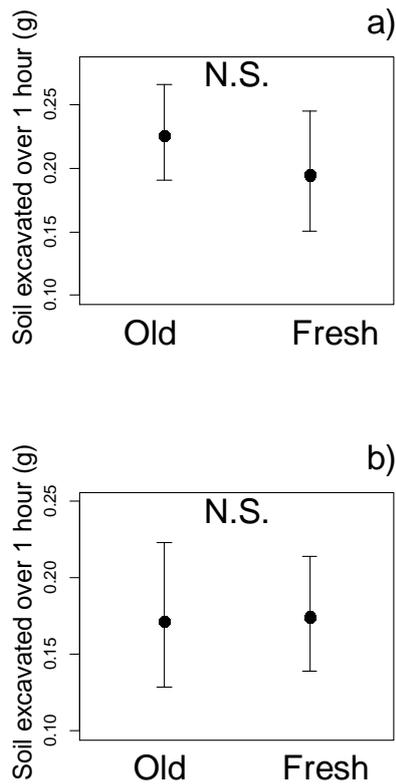| Experiment	Colony	N	P-value
Control	One	29	0.1579
	Two	15	0.1251
	Thee	30	0.1681
	Four	15	0.4263
	Five	14	0.8753
Experiment	One	26	0.6384
	Two	11	1
	Thee	29	0.6812
	Four	15	0.04126
	Five	15	0.1914

Figure 2. a) Control: mean weight of extracted soil removed over 1 hour (y axis) with bootstrapped 95% confidence intervals for "aged" and "fresh" treatments when ants were exposed to tunnel faces that had never been previously excavated (n=103). b) Experimental: mean weight of extracted soil removed over 1 hour (y axis) for with bootstrapped 95% confidence intervals for "aged" and "fresh" treatments when ants were exposed to pre-dug tunnel faces (n=96).

4.5 -Discussion

Current leading theories of ant nest excavation regulation give a large role to pheromones; this has been supported by both modelling (Buhl et al., 2005) and experimental evidence (Chen & Zhang, 2013; Pielström & Roces, 2013). Surprisingly, my results show no evidence that pheromones on digging faces significantly influence subsequent digging activity.

There are multiple possible explanations for the lack of observed reaction (discussed below), but the most likely explanation is that no pheromone with the relevant characteristics was present on the digging face. This seems probable because the exit of the mandibular gland of the ants is

situated below the mandibles and therefore is mostly exposed to soil that becomes part of the pellet. This may explain why Pielström and Roces (2013) observed differences in the behavioural reaction to fresh and aged pellets, whereas I did not find a commensurate reaction to fresh and aged digging faces.

There are additional possibilities as to why this study did not show an effect. It could be that the ants were excited and therefore were not responding to the digging pheromones of their nest-mates. However, many natural situations might require an ant to respond appropriately to a digging pheromone signal while excited, such as during an emergency nest relocation. Furthermore, Chen and Zhang (2013) also examined ants in a disturbed situation and detected significant pheromone effects.

A further possibility is that evaporation reduced the pheromone signal in the "fresh" treatment below the ant's response threshold by the time digging commenced. However, the ants had the opportunity to encounter the digging face very quickly, and began digging at around 11 minutes. Buhl et al. (2005) used a digging pheromone with a half-life of 20 minutes for their model, so a substantial proportion of the pheromone would have been available for the ants under their modelled conditions.

A final possibility is that the half-life of the digging pheromone is so long that there was insufficient decay during the one hour wait, leading to a roughly equal pheromone quantity for "aged" and "fresh". However, under these circumstances it is unclear how pheromones could regulate digging, due to the accumulation of persistent, long half-life pheromone.

Pheromones are undoubtedly important in ant nest construction and further work is required to specify their role in particular locations. However, a new understanding of digging regulation needs to be developed that does not require a digging pheromone. Examining the roles of encounter rates, movement, and context dependent response thresholds may be the way forward.

Acknowledgements

4.6 -References

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5.0 -Ants, movement, encounters, and nest space: tracking solutions

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5.1 -Abstract

Space inside an ants nests correlates with the number of individuals present. However, the mechanistic process by which this is achieved has never been fully described. In particular, the role of worker movement during excavation within a nest, and especially within an expanding tunnel system, has not be examined. Here I use the automated tracking system idTracker to follow the movements of ants as they excavate a tunnel. It was found that digging rate was significantly correlated with speed and that the proportion of time during which the ants maintained a close proximity to each other was significantly greater than expected under random movement. The clustering of workers and spacing among clusters appears to have an important effect on encounters with the tunnel digging face. I integrate these and other factors into a conceptual model explaining the process regulating excavation of tunnels and possibly other structures.

Keywords: nest - digging - tunnel - excavation - leaf-cutter ants - *Atta colombica* - self-organisation

5.2 -Introduction

Nest architecture can have strong effects on the organization of activity within an ant colony (Buhl et al., 2004; Burd, Shiwakoti, Sarvi, & Rose, 2010; Stickland & Franks, 1994), and tunnels play a central role in nest architecture. Tunnels can vary widely in design among the nests of different species. Tunnel designs include long, straight corridors of uniform width; helices; tunnels that widen and narrow and change direction; and funnel shapes that descend from a nest entrance to an initial chamber (Forti, Camargo, Fujihara, & Lopes, 2007; Tschinkel, 2003). The regulation of excavation behaviours to achieve the appropriate design is imperfectly understood, especially for one of the most basic design elements: nest size in relation to colony size. It has been observed that ants gradually reduce their digging rate as they excavate subterranean chambers and nest space (Buhl et al., 2005; Buhl et al., 2004; Pielström, 2013; Rasse & Deneubourg, 2001). In this way, further excavation is inhibited in response to the expanding nest space, eventually bringing nest size to an approximate plateau at which nest space matches the number of individuals present in that nest (L. M. Hart & Tschinkel, 2012; Mikheyev & Tschinkel, 2003; Murdock & Tschinkel, 2015; Tschinkel, 1999b, 2004, 2005, 2011a). The details of the regulation mechanism behind this pattern remains uncertain, however. The size of tunnels, like the size of chambers, is an element of nest design that should be regulated to match the colony's needs, so that excavation effort is neither insufficient nor wasted. Tunnel excavation, however, has received even less attention than nest chamber construction (Buhl et al., 2006). The different shapes and functions of these architectural features might entail different regulatory mechanisms. Here I explore tunnel elongation by teams of *Atta colombica* leaf-cutting ants and the interaction patterns that may serve as regulatory cues for tunnel excavation.

Much of the activity of any ant colony is organized by patterns of encounters between workers (Bouchebti et al., 2015; Czaczkes, Grüter, & Ratnieks, 2013; Gordon & Mehdiabadi, 1999; Gordon et al., 1993; Greene & Gordon, 2007; Pratt, 2005). The rate at which ants encounter nest-

mates performing various tasks provides information about current activities and about the needs of the colony. Patterns of encounters and responses are a possible mechanism by which the construction of tunnels may be regulated. Depending on context or recent experience, ants may switch tasks or increase their fidelity to a current task in response to the pattern of encounters they have with others (Bouchebti et al., 2015; Gordon & Mehdiabadi, 1999; Greene & Gordon, 2007; Pless et al., 2015).

To evaluate whether such a mechanism is at play during tunnel construction, I first establish a null expectation for encounter rates. If individual ants moved randomly and independently within a tunnel, the average time they spent in proximity to each other would vary inversely with tunnel length (see Methods for details). By monitoring their contact time with nest-mates, individuals could assess current tunnel length. In a short tunnel, total contact time among a fixed number of workers would necessarily be high, and if ants react to contacts by seeking opportunities for excavation, the tunnel would expand until it is long enough that encounter rates fall below a threshold level. By contrast, if the tunnel is long and worker density is low, random motion will produce encounters more rarely and digging will not be stimulated.

An alternative theory also depends on movement dynamics (Buhl et al., 2005; Buhl et al., 2004). In this case, ants moving about the nest encounter points of high concentration of digging pheromone and therefore are more likely to dig at that point. Evaporation of pheromones leads to an absence of positive feedback and therefore to a reduction in digging. In the previous chapter (Bruce, 2016) I demonstrated that such a digging pheromone is unlikely to exist in the leaf-cutting ant *Acromyrmex lundi*. However, even if it does not exist, ants might be stimulated to dig by local cues at particular sites, such as a tunnel face, if the local trigger to dig comes from physical characteristics of the digging site. Some possibilities are the presence of digging ants, tunnel geometry or scrape marks on the digging face.

The chance for an ant to encounter a stimulatory digging site will also be dependent on how far and how fast an ant moves. Arousal can strongly affect ant movement (Dobrzańska, 1978) and may be modified by such things as motor displays by nest-mates (Cassill, 2003; Möglich & Hölldobler, 1975; Stuart & Alloway, 1983; Traniello, 1977), satiation (Wallis, 1962), stimulation by hostile organisms (Detrain & Pasteels, 1992) and has been included in behavioural models (Sumpter & Pratt, 2003). Just as arousal is affected by many things, it is likely to affect many aspects of ant behaviour including movement and needs to be examined for digging regulation.

In order to determine how nest and tunnel digging is regulated, it is necessary to examine how digging rate is related to worker movement. Tunnels are a good medium for examining this behaviour as they expand at only one point, rather than all over their circumference as in a chamber, thus simplifying the analysis. In the following experiment I tracked the movement of the leaf-cutting ants *Atta colombica* in an artificially restricted tunnel system and recorded their digging behaviour.

5.3 -Methods

5.31 -Experimental setup

Experiments were performed during March and April, 2014, at Barro Colorado Island, Panama. For each replicate, ten laden ants were taken from the active foraging trail of a single large *Atta colombica* colony and introduced into an experimental tunnel digging arena (fig. 1). The single source colony may cause limited external validity, but there is no indication that this colony is not representative of other leaf-cutting ant colonies. The use of laden foragers required task switching but ensured that the experimental ants were relatively uniform in behavioural caste. All experimental groups successfully took up tunnel excavation in the apparatus. The digging arena contained a small, open atrium with a circular entrance tube leading to a gallery system with fixed

plastic walls defining a potential tunnel width. The gallery system was filled with fine white, moist sand (10% water by weight). The tunnel was arranged with a series of 180° U-turns so that a long total length was compactly presented within the viewing frame of a camera. The apparatus used a clear Perspex roof to allow ants to been seen and the progress of tunnel excavation to be recorded from above. The experiment was conducted under constant florescent lighting, although some indirect sunlight was present. While the intrusion of light was not a natural condition for excavating *Atta* ants, this setup was necessary to allow the video recording of their behaviour. The experiments were carried on at ambient temperature that varied slightly over day-night cycles and at natural humidity that was high enough (67%, SD=3.9) that the ants could live comfortably.
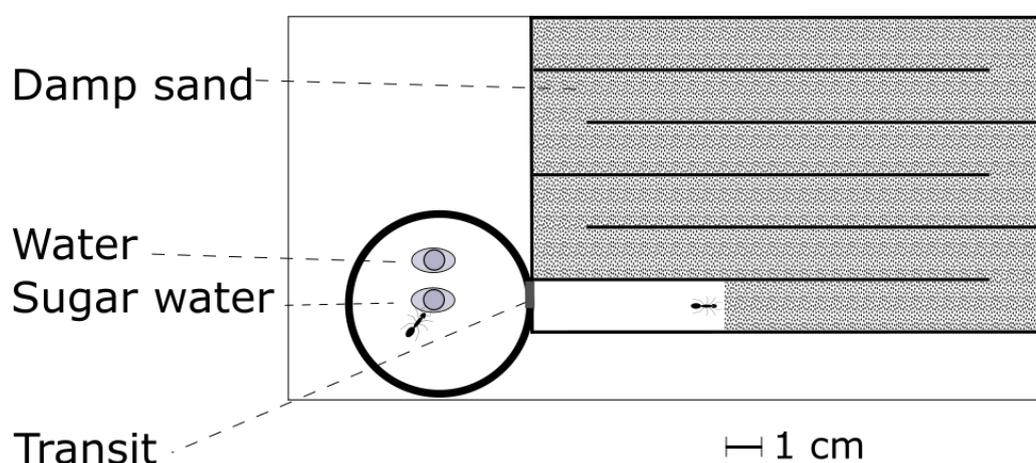

Figure 1. Diagram of the tunnel digging arena from above. The tunnel section is sealed with transparent plexiglass but the round entrance is open to the air; ants can transit from one to the other. The round entrance is a high cylinder that is coated with baby powder to prevent climbing and escape. The damp sand was excavated by the ants and transported to the entrance and periodically removed by the experimenter.

5.32 - *Video analysis and data extraction*

Video recordings of 6 replicates of tunnel excavation were made using a 70D Canon camera with a 50mm lens in video mode. Magic Lantern software was used to enable the camera to record 30 min of high definition video (1080p) at 25 frames s^{-1} every 6 hours for 72 hours, (two replicates continued for 80 hours). Post-processing with Adobe Premiere Pro CC was used to removed

shadows and dark patches from the recording. The high contrast nature of the video recording allowed us to use the tracking program idTracker (Pérez-Escudero, Vincente-Page, Hinz, Arganda, & Polavieja, 2014) to characterize ant movements during the course of excavation. Condensation on the perspex cover prevented idTracker from reliably identifying and following individual ants. However, the tracking did allow collection of important variables on worker movement that did not require unique identification of individuals.

The variables measured were: mean inter-ant distance (cm), close-proximity duration, mean speed (cm per second), tunnel length (cm), and mean excavations per minute. The first three variables were extracted using idTracker. Close proximity duration was defined as the duration in which the centres of two ants were within a set minimum distance from each other. This minimum distance was determined by taking the mean of the distance from the centre of each ant to the tips of its antennae. The logic behind this decision was that if the antennae of one ant could reach the centre of the other ant, then they were likely to be in some form of contact or aware of the other. Speed could be extracted only from identified ants and sometimes, due to the aforementioned tracking problems, identities of ants would randomly switch. To control for such erroneous switching, all movement of more than 25 pixels in one frame was excluded. While the process may have had some inaccuracies, the use of automatic tracking greatly expanded the quality of locomotion data, and as this project is concerned with changes in relative rather than absolute speed, any inaccuracies should not affect the conclusions drawn from the data. Mean inter-ant distance (cm), close-proximity duration, speed (cm per second), and mean excavations per minute were averaged over the half hour of each replicate. Tunnel length was measured digitally from the video and mean excavations per minute was determined by sampling a random one minute section per half hour replicate and counting every digging event, consisting of bite, rake, and move as a 'grab'.

5.33 -Model of random movement of ants in tunnels

As tunnels are excavated and their floor area increases, one would expect that a fixed number of ants moving independently of each other would spend less and less time in proximity with each other. The null expectation under random, independent motion of a fixed number of ants is that contact time would vary inversely with tunnel length. Consider a tunnel of length l and width w—thus area lw—and a focal ant at a given position in the tunnel. Suppose an ant can detect the presence of another ant within a radius r from its center, so that an interaction can be defined as the occurrence of a second ant within a circle of area πr^2 centered on the focal ant. If the second ant is placed randomly on the floor area of tunnel, it therefore has a probability $p = \pi r^2/lw$ of being in contact with the focal ant. As the contact radius r and the tunnel width w are fixed, this probability is an inverse function of tunnel length l. (Some adjustment must be made if the focal ant is closer than r to the wall of a tunnel, as the circle of potential contact around the ant is then censored by the wall, so that its area will be less than πr^2. Given my tunnel widths of 13 mm and an estimated value of $r = 6.11$ mm, the average area of the censored circles for randomly placed focal ants will be only about 80.1% of the maximum πr^2. However, this merely means that the adjusted probability that a second ant occurs within contact of a focal ant is $p' \approx 3.204\pi r^2/lw$, and p' remains an inverse function of l).

If N ants move independently in random walks within the tunnel, they will occur at random positions at any moment. The number of contacts, k ($0 \leq k \leq [N^2 - N]/2$), among N randomly positioned ants will follow a binomial distribution with a probability p' of "success," and the mean of the distribution will be Np'. As p' is an inverse function of length l, the total number of contacts under random motion should be an inverse function of l. If every contact lasts a mean of s seconds, then the total contact time among N randomly moving ants remains an inverse function of tunnel length l.

5.34 -Statistics

I used linear regressions to establish the relationship between excavation rate, or proximity duration, and tunnel length, or elapsed time. Model II regressions were used to compensate for the error in both axes of the data. Statistical analyses were performed with R (R Development Core Team, 2012).

5.4 -Results

Results of the statistical analyses are provided in the figure captions. Tunnel excavation occurred initially at a high rate but declined over time, so that tunnel length increased rapidly at the start of the experiments but eventually reached an approximate plateau (fig. 2). The decline in digging rate over time is therefore the pattern that must be explained. There is a strong and significant positive linear relationship between digging activity and ant walking speed (fig. 3) and a significant negative linear relationship between walking speed and elapsed time (fig. 4). The contact duration among the workers, as a percentage of the total possible contact time, was statistically constant (slopes not significantly different from zero) over time (fig. 5) and with respect to tunnel length (fig. 6). Evaluated against the null model of random movement, this constancy of the summed contact duration was significantly different from the expected inverse relationship between contact and tunnel length. An inverse relationship would produce a slope of −1 between the logarithms of these variables, but the observed slope was significantly greater (fig. 7, *p*<0.001).

Digging at the tunnel face was high at the beginning of experiments and dropped rapidly over time (fig. 8), while digging at locations other than the tunnel face was relatively constant over time (fig. 9). This indicates that the reduction in excavation rate over time comes less from an overall reduction in digging than in the reduction of digging specifically at the tunnel face. Mean

inter-ant distance increased over time as tunnel length expands (fig. 10). Ants would spend most of their time at the entrance, at one of the turns, or at the digging face (fig. 11).
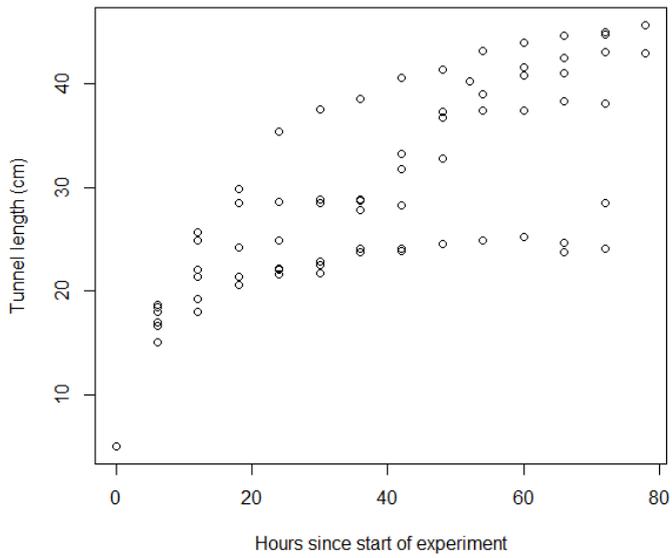

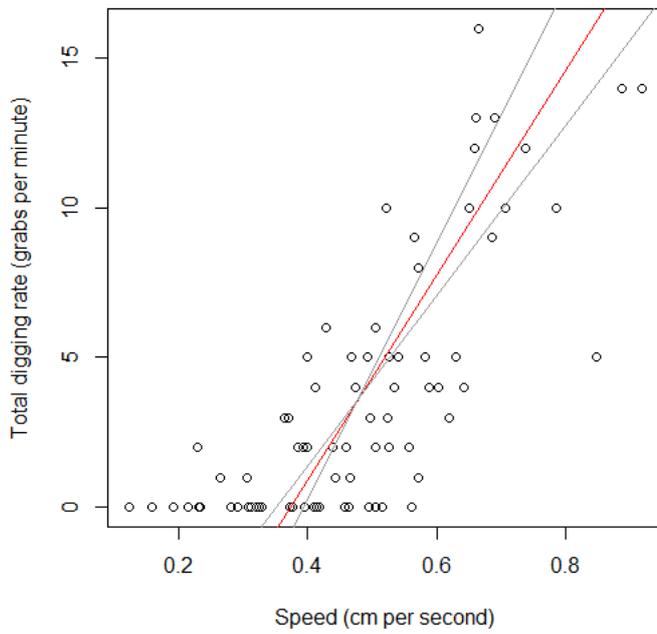Figure 2. Hours since the experiment commenced vs tunnel length (cm).

Figure 3. Relationship between ant speed (cm per second) and digging rate (digging events in one minute) ($p<0.001$, $R^2=0.57$, df=74, CI=95% of slope).

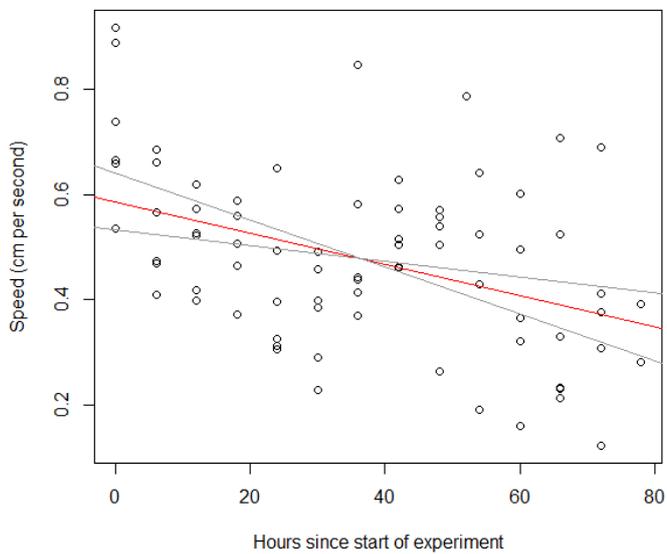Figure 4. Relationship between hours since the experiment commenced and speed (cm per second) ($p<0.001$, $R^2=0.18$, df=74, CI=95% of slope).

Figure 5. Relationship between proximity duration (the percentage of contacts among all possible pairwise distance distance comparisons) and hours since the experiment commenced (p=0.81, R^2=0.0008, df=74, CI=95% of slope).
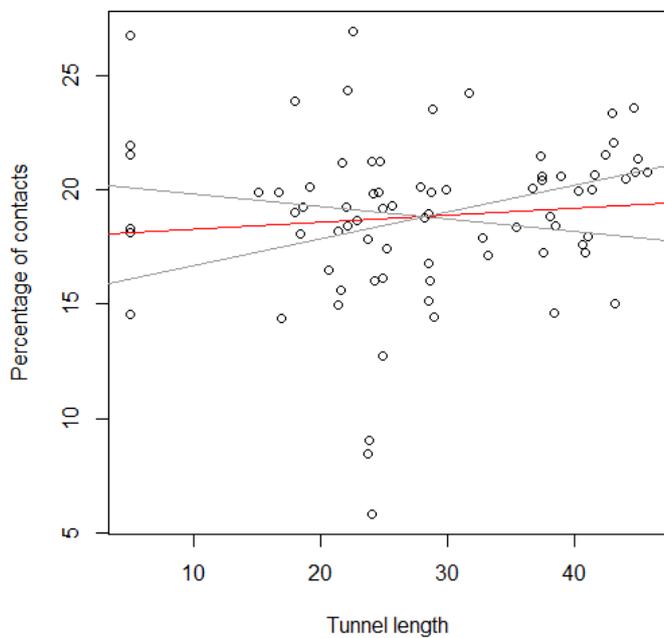
Figure 6. Relationship between proximity duration (the percentage of contacts among all possible pairwise distance distance comparisons) and tunnel length (p=0.47, R^2=0.007, df=74, CI=95% of slope).
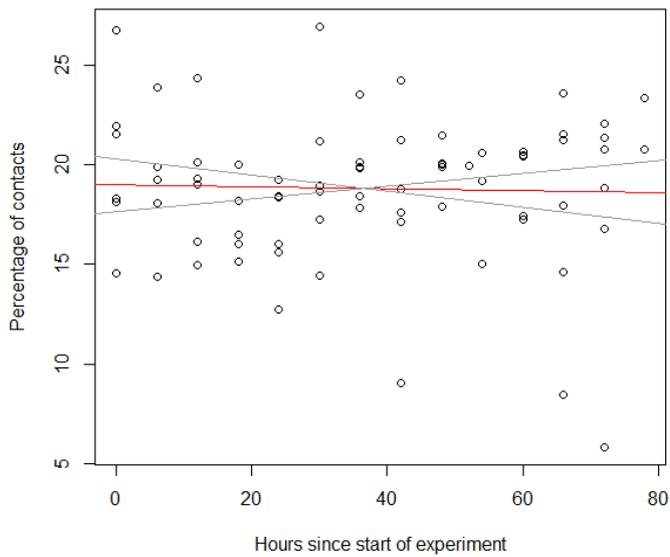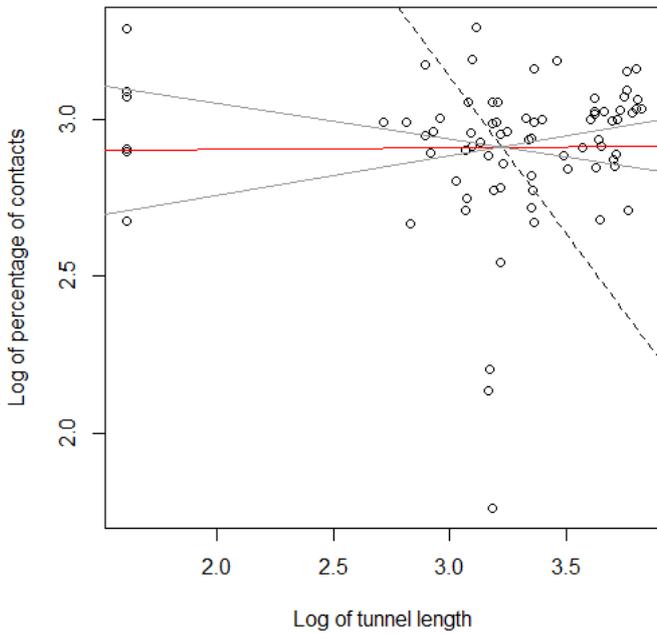
Figure 7. The slope for the relationship between log proximity duration (the percentage of contacts among all possible pairwise distance distance comparisons) and log tunnel length (middle red line, CI=95% of slope) differs significantly from random indipendent movement (broken line) ($p<0.001$).
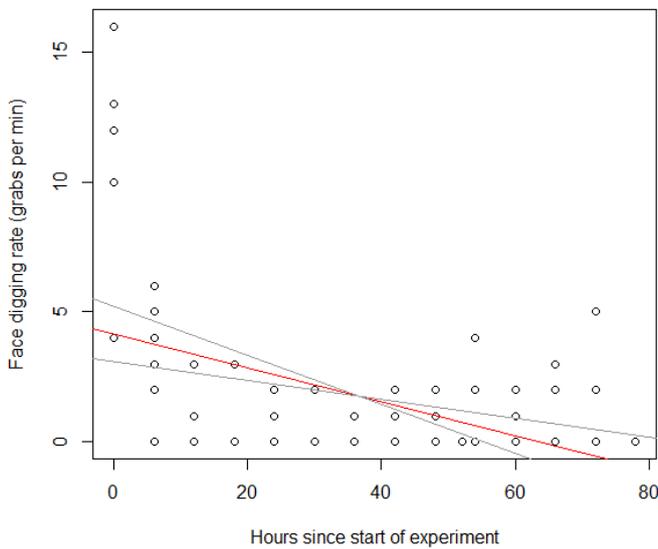
Figure 8. Relationship between digging rate on the tunnel digging face and hours since the experiment commenced ($p<0.001$, R^2=0.22, df=74, CI=95% of slope).

Figure 9. Relationship between the digging rate in the tunnel but not at the digging face and hours since the experiment commenced (p=0.86, R^2=0.0004, df=74, CI=95% of slope).

Figure 10. Relationship between mean inter-ant distance (cm) and hours since the experiment commenced (p<0.001, R^2=0.183, df=74, CI=95% of slope).

Figure 11. An example of a heatmap showing ant residency times. Red indicates more time that an ant or ants stayed at a location. It has been straightened out to 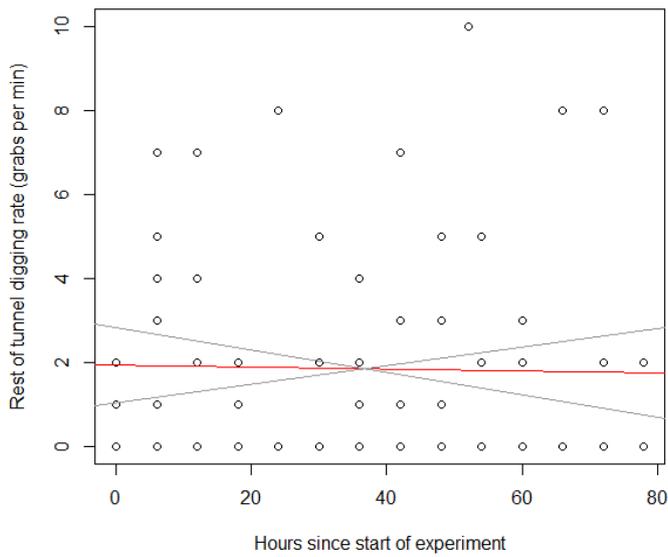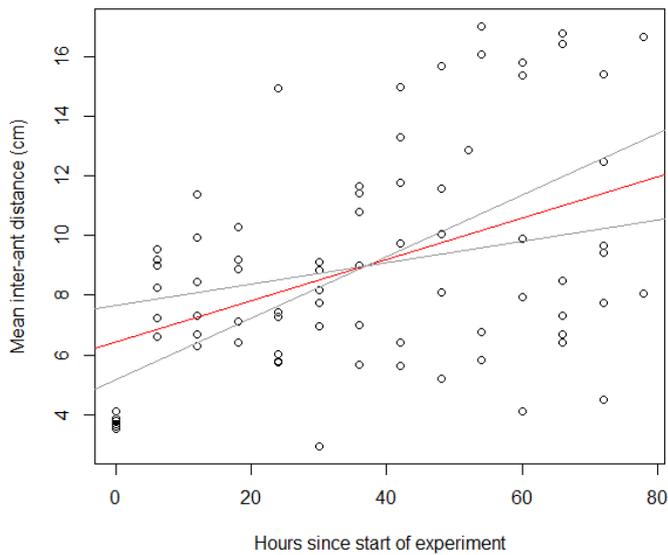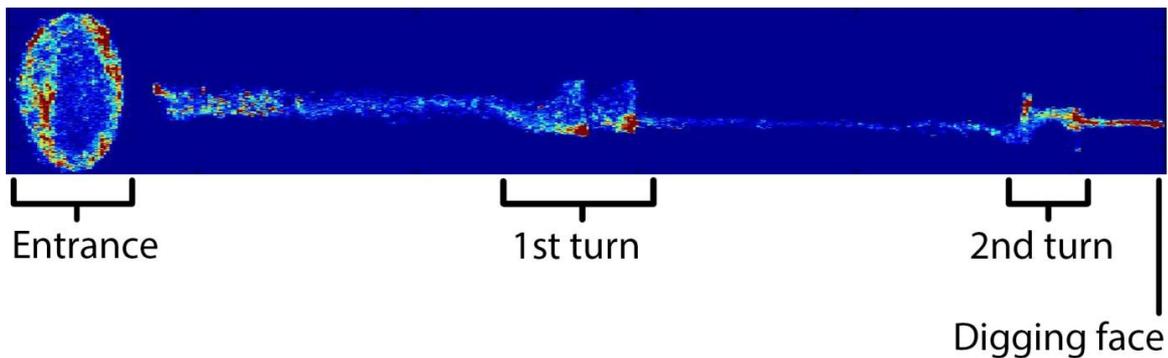represent travel distance of an ant restricted to a tunnel and to allow the determination of mean inter-ant distance. It can be seen that ants resided primarily in 3 places: outside in the circular entrance and the tunnel entrance, at the first turn, and at the second turn and digging face.

5.5 - Discussion

The association of excavation rate with mean walking speed (fig. 3) and the decline in mean walking speed over time (fig. 4) might lead to the implication that the digging rate is modulated by this decrease in speed. However, it is unlikely to be that walking speed as such is responsible for the regulation of digging rates. Rather, I propose that speed is the most easily quantified manifestation of arousal levels, and that arousal levels began high and declined with time. It has previously been shown that ants will dig at a high rate immediately following disturbance (chapter 3, fig. 6a, Buhl et al., 2005), even creating excess space that is later refilled (Römer & Roces, 2015). It is likely that removal from the trail and imposition of new surroundings were a sufficient disturbance to cause a highly aroused state among the workers at the beginning of the experiment and thus their high digging rate.

However, if arousal was the only factor affecting digging rates then we would not expect to find that ants dig less in tunnels that were initially longer rather than initially shorter, as shown in chapter 3 (fig. 3a). Therefore, a consistent explanation requires a mechanism that would result in the ants reducing their excavation activity in longer tunnels. Many authors have noted the tendency for

ants to aggregate and regulate their rate of encounters with other ants (Deneubourg, Lioni, & Detrain, 2002; Depickère, Fresneau, & Deneubourg, 2004, 2008; Gordon et al., 1993; Jeanson, Deneubourg, Grimal, & Theraulaz, 2004). If walking speed and other variables remain constant, ants would be less likely to encounter a distant tunnel face if they had a tendency to aggregate in any location other than the tunnel face. Consistent with the work of Gordon et al. (1993), I found that ants maintain a consistent level of proximity to one another over time (fig. 5) even as tunnels grow longer and the ants have more space available to them (fig. 6), resulting in a pattern of contact that is significantly different from random expectations (fig. 7). The decline in excavation that occurs specifically at the tunnel face (fig. 8 & 9) is likely due to a reduction in the number of ants encountering that point due to their aggregating behaviour. Therefore, multiple factors, primarily arousal and the tendency of ants to aggregate, interact to determine digging rate. I outline a conceptual model of such the regulation of digging in figure 12. Specifically, multiple factors such as disturbance and overcrowding determine arousal; arousal state then determines speed; and speed and the tendency of ants to aggregate determines how much space they tend to traverse. This movement influenced by aggregation determines how often they encounter the digging face. Finally, the probability that ants begin digging when they encounter the digging face is also likely to be influenced the geometry of the tunnel, scrape marks, and by arousal. As a consequence, when the tunnel length expands and arousal decreases, digging rate also decreases.

This raises the question of what factors affect arousal. It is quite likely that an overcrowded nest, i.e. one where the inter-ant contact rates are too high, increases arousal. Nest disturbance is certainly capable of heightening arousal. As we have seen, tunnel length can be long but if arousal is high then the ants will continue digging at a high rate (chapter 3, fig. 3a). It would be interesting to determine whether other factors that might not directly be related to nest construction could also raise arousal and thus increase digging. For example, if a colony was briefly exposed to an enemy colony, would subsequent digging rate be raised? If it were, we could conclude that there is a

general arousal system with results that are context dependent. If it were not, we could determine that arousal systems correspond to specific separate threats, such as nest damage or enemy ants.

A complicating question is how it can be that ants can have increased inter-ant distances over time (fig. 10) while proximity duration remains relatively constant (fig. 5). One might expect that as inter-ant distance tended to increase, proximity duration would also tend to decrease. The answer is likely to be that ants seek out a minimum level of contact with others and this causes the formation of small groups, each of which provides the others in its group with sufficient contact but which may be out of immediate contact with the other subgroups. This can be seen in the clumpiness of ant groups (fig. 11). In reality, ants are likely to transit from group to group and area to area. Previous research suggest that these subgroups are likely to be important for the removal of pellets from the tunnel face to the entrance via task partitioning (Pielström & Roces, 2013).

By comparing fig. 2 of this experiment with fig. 3, a of chapter 3, we can see that the workers have excavated a roughly comparable amount over the same time period in two experiments. However, *A. colombica* are larger ants than *Acromyrmex lundi* and therefore would be expected to dig more. It is likely that this is partly because of the switchback nature of the tunnel design interfering with the walking dynamics of ants, particularly their tendency to walk in a roughly straight line. It would be desirable to perform the same tracking experiments in long, largely straight tunnels. Indeed, comparing digging rates between straight and switchback tunnels and examining the effects of the different treatments on digging rate would be highly enlightening as to how nest geometry can affect digging rate and movement. For now, any effect of the switchback tunnel design only emphasises the importance of movement dynamics to the digging regulation processes of ants.

Here I have resolved details of how it is that ants regulate their nest size through a combination of multiple mechanisms. From this I hypothesise that arousal and the tendency of ants

to aggregate with each other leads to the self-regulating reduction of digging at a digging face as the nest expands. It may be that differences in aggregation, movement patterns, and context specific responses help to explain species typical differences in nest construction. Further investigations into these properties may contribute to the improving our fundamental understanding of self-organised and emergent systems.
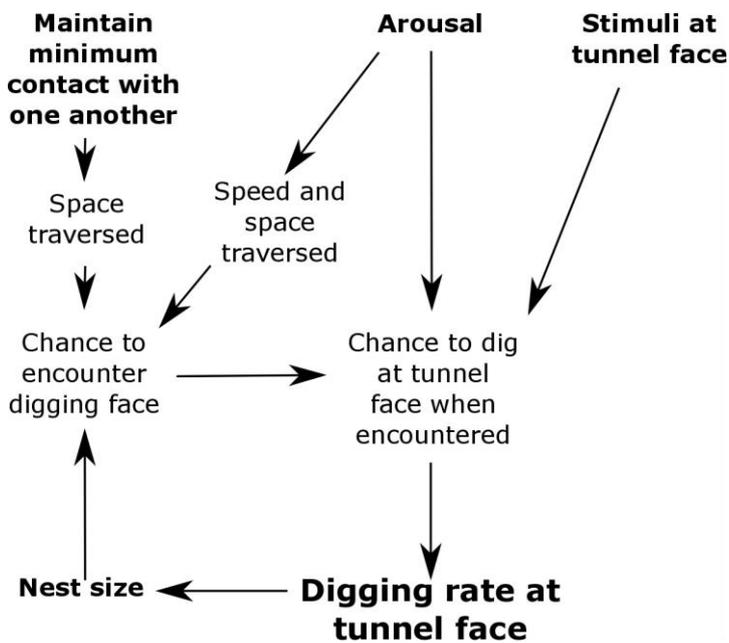

Figure 12. Conceptual model of proposed digging regulation hypothesis. Arrows indicate direction of influence but magnitude would be highly context dependent.

Acknowledgements

5.6 -References

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6.0 -Tall trails: Ants resolve asymmetry in information and capacity in collective maintenance of infrastructure

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6.1 -Abstract

The cleared trails of leaf- cutting ants are a key part of their physiology, facilitating the smooth transfer of leaf fragments and information. However, their construction and maintenance requires the redress of an asymmetry: that the ants laden with leaf-fragments have the information about the adequacy or otherwise of the cleared trail, but that the unladen ants are the ones with the capacity to repair the trail. In order for obstructions to be cleared from the trail in a responsive manner a mechanism is required to resolve this asymmetry. The experiment tested three hypotheses: that unladen ants will attack any object in their path, that the unladen ants are stimulated to clear by visual cues, or that unladen ants are responsive to delays of laden ants. The experiment presented *Atta colombica* trails with four treatments: a control that did not obstruct laden ants, a wire that delayed laden ants but not unladen ants, an overhead visual stimulus at the same height as the previous treatment but with no delay to laden ants, and all laden ants being removed. It was found that there were significantly more clearing attacks in the Laden Ant Obstruction treatment than in any other treatment. This indicates that trail clearing is sensitive to the presence of laden ants and responsive to their obstruction, but is not from visual stimulus. It is speculated that unladen ants are sensitive to the rate at which they encounter laden ants, and will increase their trail clearing

behaviour when they perceive an elevated rate of encounters with laden ants that indicate a blockage.

Key words: Foraging trails – construction – leaf-cutter ants – behavioural regulation – self-organisation

6.2 -Introduction

Trails are a key feature of an ant colony's physiology and whose primary role is communication. The cleared foraging trail networks of leaf-cutting ants (*Acromyrmex* spp. and *Atta* spp.: Attini, Formicidae) are particularly sophisticated and multi-dimensional. They facilitate the transfer of leaf resources (Bruce & Burd, 2012; Burd & Howard, 2005; Hölldobler & Wilson, 1990; Moreira, Forti, Andrade, et al., 2004), recruits and defenders (Jaffe & Howse, 1979; S. Powell & Clark, 2004), and information (Bollazzi & Roces, 2011; Farji-Brener et al., 2010), through a colony's territory to its nest. The trail systems can be vast, reaching 300 meters in length and requiring a substantial investment of worker time to clear debris (Howard, 2001). Smooth transport on trails is important because seemingly minor obstructions may cause congestion that could impose disproportionately large costs on the colony.

How do leaf-cutting ants make good investment decisions regarding their infrastructure? Leaf- cutting ants trails feature laden ants, returning inbound to the nest with a leaf-fragment, and unladen ants that may be either inbound or outbound. Information on whether a trail is sufficiently cleared to allow a smooth flow of laden ants returning with resources will reside largely with the laden ants themselves, as they experience the trail conditions including any obstructions. However, since laden ants are occupied with their burden, the capacity to clear an obstruction does not reside with them. Instead, unladen ants are the ones that clear a foraging trail (Howard, 2001). Therefore in order for the trail system to function as a coherent whole, one group of ants, unladen ants, are

required to adequately prepare the trail for the benefit of another group of ants, laden ants. Therefore, the rapid resolution of trail problems and the optimal regulation of investment into infrastructure requires the acquisition of information that can resolve the asymmetry between those ants that directly experience the problem and those that can solve it.

The problem of information asymmetry is a general one. In humans an asymmetry of information is often presented as an opportunity for one party to exploit another (Akerlof, 1970; Dawson, Watson, & Boudreau, 2010; Leland, 1979; Mocan, 2006). The struggle in coordinating human groups is in aligning interest, while the available communication and cognition is sophisticated and plentiful. In contrast, the eusocial nature of ants means that their interests are already aligned, but communication systems and cognition systems are comparatively limited and therefore are required to be efficient.

Leaf-cutting ant colonies do not invest indiscriminately in trail clearance (Shepherd 1982). For example, colonies of *Atta cephalotes* and *Atta colombica* appear to balance the travel time for laden ants against the costs of trail maintenance via the geometry of access trails from clear trunk trails to new harvesting sites (Farji-Brener et al., 2015). A geometry that allows reduced travel time prevails in exposed habitats where desiccation of workers is a danger, while reducing trail clearance is a higher priority for trail geometry under a forest canopy where desiccation in unlikely but leaf litter is copious. Howard (2001) estimated the time and energetic costs for the establishment and maintenance of cleared trails in an *Atta cephalotes* colony in Panama and concluded that the costs were very small in comparison to the foraging traffic carried by a cleared trail. Despite the apparently hugely favourable cost-benefit ratio, the above mentioned sensitivity of *Atta* trail geometry to the cost of trail maintenance (Farji-Brener et al., 2015) indicates that colonies regulate their investment in trail clearance.

The clearance of a trail to a particular height is a particularly important feature of infrastructure investment for leaf- cutting ants because they transport leaf-fragments above their heads (fig. 1), greatly increasing their requirement for vertical clearance. The increase means that overhead obstacles such as grass, leaves and small sticks can delay their movement (T. Lewis et al., 1974). However, clearing a trail up to a height suitable for leaf-laden ants poses a special challenge for organisms relying on self-organization for collective decision-making: how is information about the need for clearing aligned with the capacity to clear? In most cases of trail construction, as in other self-organized activities (Camazine et al., 2001), ants respond to cues they directly experience concerning the need for a given task. In leaf-cutting ants, most trail clearance occurs when a worker that encounters an obstacle in its path, such as a fallen leaf, attacks the obstacle by cutting a fragment and removing it from the trail. But overhead obstacles that do not obstruct the trail surface are different. An unladen worker may pass under such an obstruction without impediment, while the passage of a laden nest-mate is hindered. Laden ants rarely put down their loads to attack obstructions unless the traffic is very highly blocked (pers. obs.). This creates an asymmetry in information and capacity between laden ants, which most immediately experience the need for trail clearing but have little capacity to remove obstructions, and unladen ants, which have ready capacity to do the clearing but have no information from their own experience as to its necessity. It is possible that unladen ants are clearing obstacles that block their *own* progress and that any benefit that accrues to laden ants is coincidental, rather than tailored. Do *Atta* ants therefore resolve the asymmetry between information and capacity? The answer to this question is unclear at present, as the mechanisms that regulates trail clearance, and the adaptive principles the mechanisms serve, are not fully understood.

Investigation into the regulation of trail and tunnel height are limited. Dussutour and Deneubourg (2009) examined how leaf-cutting ants responded to a 1-cm high obstruction that delayed the progress of leaf-laden ants. They found that workers cut smaller leaf fragments in order

to facilitate smooth transport and compensated for the smaller loads by increasing the number of fragments returned. This work did not explore an alternative strategy that is normally available to leaf-cutting ants: in a natural situation ants can remove obstructions to ensure smooth trail flow rather than reduce the size of leaf-fragments (fig. 1).

Adapting leaf fragment size to the height of trail obstructions and clearing the overhead trail to accommodate optimal fragment sizes are both feasible strategies. However, one might expect that investment in trail height would be the more profitable than adjusting fragment size to the constraints of the trail because a smaller number of ants can clear a trail for their nest-mates but each fragment needs to be reduced individually. Furthermore, leaf-fragments are normally highly tuned for efficient carriage (Burd, 2000; Burd & Howard, 2005; O. T. Lewis et al., 2008; Röschard & Roces, 2002; Rudolph & Loudon, 1986) and imposing height restrictions would impede this process. Therefore, a small investment in construction work is likely to have a very large payoff in foraging efficiency. This effect is also likely to be present in tunnels, explaining why foraging tunnels are both wide and high enough to allow unrestricted flow of leaf-carrying ants (Burd & Howard, 2005; Moreira, Forti, Andrade, et al., 2004).

Here I ask: How do the unladen ants clear a trail or tunnel to a height appropriate for laden ants? I test several hypothetical mechanisms: firstly, it may be that anything on or above the trail that is in the path of an unladen ant will serve as stimulus for attack and removal, even if it is not delaying laden ants. Alternatively, ants may be responding to visual cues that the trail height is too low and use their individual judgement to assess the situation and clear the trail to an appropriate height. Finally, it is also possible that the clearing ants are stimulated by delays to their laden colony mates to seek out, attack, and remove obstruction. The following experiment tests these three possibilities.
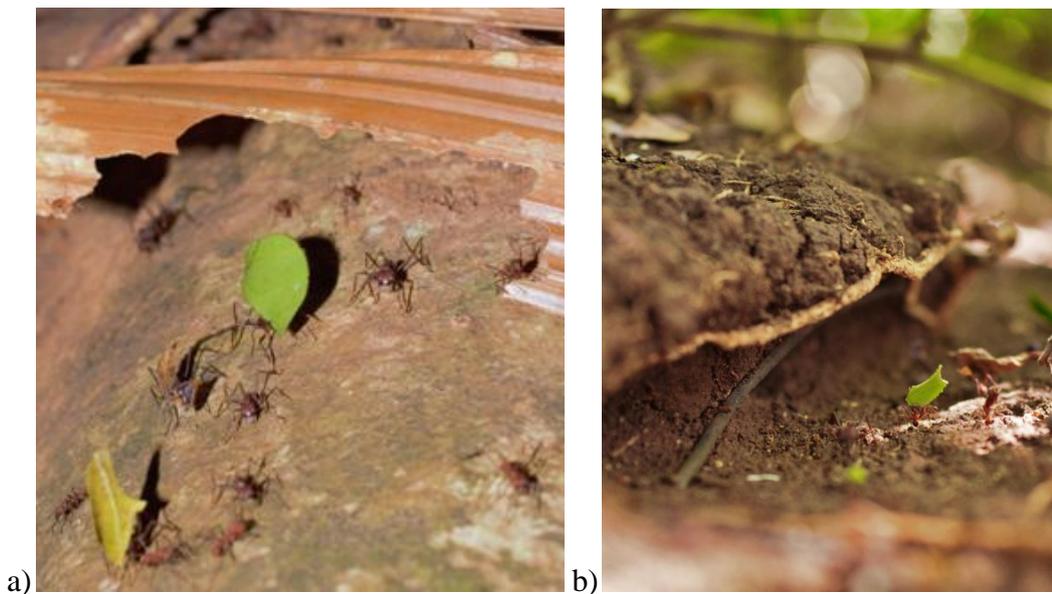
Figure 1. a) *Atta colombica* transport leaf fragments back the nest along a fallen branch. A long leaf crossing the trail has been cut so as to allow the smooth transportation of leaf-fragments by laden ants. b) *Atta colombica* transport leaf fragments along a trail that has been levelled and heightened.

6.3 -Methods

This experiment was performed on Barro Colorado Island and mainland Panama between the 10th of March and the 3rd of April, 2014, between the hours of 8:45 am and 6:30 pm with *Atta colombica*. One large colony with multiple trails was the subject of most replicates (n=24). Additionally, a colony from the mainland was sampled once and a second colony was sampled twice. Individual trails were sampled multiple times but never more than once in a day and never from the same location twice. The intention of the experiment was to impede laden ants while allowing unladen ants free passage. I used an overhead hindrance of a 1 mm diameter metal wire stretched across the trail in different treatments. These treatments either did or did not hinder laden ants depending on the height of the wire, but never hindered unladen ants (fig 2). Poles of 1 mm diameter wire were placed 3 cm apart across the width of the trail to provide ants access from the trail to the overhead wire. The ants attacked the wire apparatus, but were unable to cut or clear it, thus providing a constant stimulus for the duration of the experiment. Once the experimental wire

was in place for each treatment, a five minute delay allowed any disturbance of the ant traffic to pass before data recording. Video recording was taken for half an hour for each replicate and clearing attacks by ants on the apparatus were counted. I defined clearing attacks as occurring when ants opened their mandibles and made contact with any piece of wire or lunged at it. Only one attack was counted per ant, and the overall rate of attack in half an hour provided the response variable of the experiment. Four treatment levels were applied to each trail:

1. The No Obstruction treatment functioned as the control (fig. 2a). The wire was high enough (2 cm) that laden ants could pass underneath without hindrance.

2. The Laden Ant Obstruction was intended to hinder the passage of laden ants without stopping them entirely, while being sufficiently high that non-laden ants would not directly encounter it (fig 2b). The wire was 0.9-1.2 mm above the ground, conforming with the contours of the trail floor as much as possible. The Laden Ant Obstruction achieved the desired effect: ants transited the apparatus on average 13% (see results) slower in the Laden Ant Obstruction treatment than the No Obstruction treatment.

3. The Visual Cue treatment was intended to provide a strong visual stimulus to the ants while not significantly hindering the progress of laden ants. This was achieved by hanging thin white plastic strips (fig. 2c) from the horizontal wire, so that they fell to the same height as the Laden Ant Obstruction. However, in contrast to the solid wire of the Laden Ant Obstruction, laden ants could easily brush through the hanging strips.

4. In order to test whether the presence of laden ants was necessary for the clearance of trail height, the Laden Ant Removal treatment attempted to remove all the incoming laden ants from the trail. The wire barrier was set at 0.9-1.2mm cm above the trail, i.e., at the same height as in the Laden Ant Obstruction treatment (fig. 2d). Two wire mesh barriers upstream of the experimental apparatus temporarily obstructed both laden and unladen ants, but had a much greater effect on

laden ants, allowing time for any laden escapees to be removed by hand. It was common for laden ants to drop leaf-fragments while being obstructed and often these ants would continue inbound along the trail, leaving their leaf-fragment behind. While the vast majority of the laden ants were prevented from traversing the experiment, some did slip though. These were mostly carrying very small pieces of plant material.

The treatments were applied in random order, except for Laden Ant Removal, which was always applied last. This was done as this treatment involved significant trail disruption and, had it been run before the other replicates, it is likely that it would have significantly affected traffic movement for subsequent replicates. The other treatments had only mild and immediate effects on the traffic flow.

In addition to the number of attacks on the wire barrier, I also recorded the Average Speed During Transit, which was the speed at which ants transited from one side of the apparatus to the other across a fixed distance. This was usually a distance of 10 cm, but on one occasion it was 6 cm. To obtain these data, the videos of 10 replicates were randomly selected and from these transit time was recorded for two randomly selected ants for each of the following class of ant: outbound and unladen, inbound and unladen, and inbound and laden. Laden was not recorded for the removed treatment as there were few to no laden ants on the trail. Flow was obtained by randomly choosing one random minute per treatment and counting all ants that passed an imaginary center line.

6.31 -Statistical Analysis

An ANOVA followed by a post-hoc Tukey's test was used to compare the Average Speed During Transit of unladen ants among the four treatment levels.

I used an ANCOVA to compare the effect of the four treatment levels on the rate of clearing attack of the obstruction, with Flow and Trail Width as covariates. Homogeneity of slopes among all four treatments was checked and found to be consistent with the assumptions of ANCOVA.

Flow was defined as the number of ants that passed a set point on the trail in one minute and was considered a relevant covariate because it is expected that each ant would have a chance to attack the apparatus and so more ants may equal a larger absolute number of attacks. Trail Width captures characteristics of usage not captured by flow, such as whether the trail was a main or peripheral trail. The Average Speed During Transit for different treatment groups was compared using ANOVA for laden and unladen ants. A Tukey-Kramer multiple comparison test for ANCOVA was calculated for post-hoc analysis of treatment differences in the clearance attack rate (Huitema, 2001). Statistical analyses were performed with R (R Development Core Team, 2012) and Systat 10.
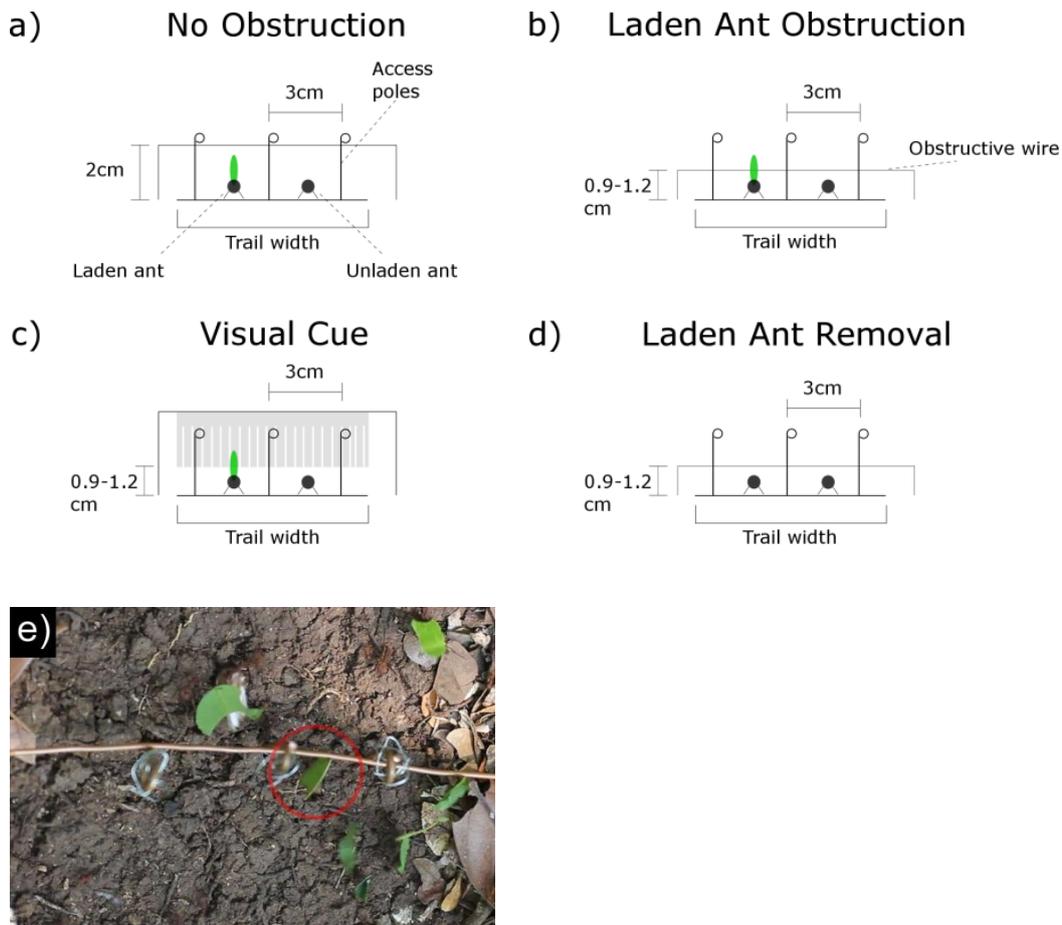
Figure 2. a) The cross wire is high enough to allow laden ants pass underneath without hindrance. b) The cross wire is low enough that laden ants are hindered by its presence, but unladen ants are not. c) Light plastic strips hang down to the same height as Laden Ant Obstruction, but laden ants are able to pass through without hindrance. d) Laden ants are removed from the trail. e) Laden Ant Obstruction seen from above. The red circle shows a laden ant being hindered by the obstructive wire.

6.4 -Results

Average Speed During Transit was 13.8% slower for the Laden Ant Obstruction treatment (2.68 cm/s) than the average of the remaining treatments (3.11 cm/s). There was a significant effect of treatment on Average Speed During Transit for laden ants for No Obstruction, Laden Ant Obstruction, and Visual Cue ($F_{2,55} = 6.734$, $p = 0.002$). A Tukey's test showed that Laden Ant Obstruction was significantly slower than No Obstruction, and Visual cue, but that the last two

categories were not significantly different from each other. There was no significant difference among treatments in the Average Speed During Transit for unladen ants ($F_{3,152} = 1.068$, $p = 0.364$).

The clearing attack rate was significantly related to both traffic flow ($F_{1,97} = 7.24$, $p = 0.0084$) and trail width ($F_{1,97} = 60.36$, $p < 0.001$), and the attack rate was significantly different among treatments ($F_{3,97} = 9.53$, $p < 0.001$). The overall ANCOVA model had an adjusted R^2 of 0.51. Post-hoc analysis showed a significantly higher rate of clearing attacks in the Laden Ant Obstruction treatment over all other treatments ($p < 0.05$) and no significant difference among the remaining treatments (fig. 3).
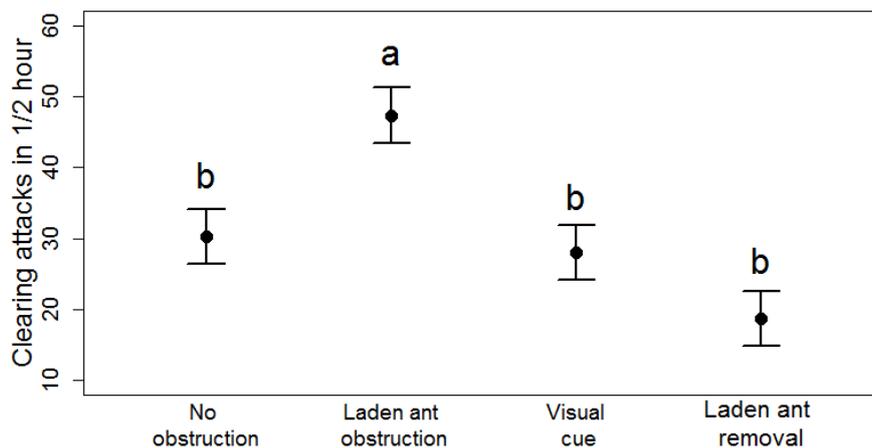
Figure 3. Adjusted mean and standard error of clearing attacks over half an hour for all treatments with post-hoc Tukey-Kramer test.

6.5 -Discussion

The results show that clearing attacks occurred at a significantly higher rate when laden ants were obstructed than when they were in the No Obstruction and Visual Cue treatments, or when they were absent in the Laden Ant Removed treatment (fig. 3). Two conclusions can be drawn from this result. Firstly, unladen ants are not simply responding to anything on or above the trail at a fixed attack rate regardless of whether laden ants are obstructed. This is shown by the significantly

higher rate of clearing attacks for the Laden Ant Obstruction treatment compared to the No Obstruction treatment (fig. 3). Secondly, ants are not stimulated to clear trail height purely by the visual cues of an obstruction at a low height. If they were, then the Visual Cue apparatus would have been attacked at the same rate as the Laden Ant Obstruction apparatus, but it was not (fig. 3). This final result leaves open the possibility that tunnel height and trail height are created via the same mechanism, since the trail height investment does not appear to require visual stimulus, which would not be available in deep, lightless tunnels. Finally, the presence of laden ants may be necessary to stimulate the clearing of overhead obstructions. The results hint at this because the experimental obstruction was at the same height as in both the Laden Ant Obstruction and Laden Ant Removal treatments, yet Laden Ant Removal had a lower rate of clearing attacks (fig. 3). The difference was not significant, however, so at present the question is unresolved. It would be economically efficient, of course, for unladen workers to refrain from height clearance if there were no laden ants using a trail.

What could be the stimulus that triggers the clearing of trail height in leaf-cutting ants? It may be that when laden ants are delayed by some obstacle, the increase in congestion also slows the transit speed of unladen ants. The reduction in their own speed could then stimulate some unladen ants to switch from traversing the trail to clearing any immediate obstacles and possibly to search for obstacles further afield. However, I found no significant difference in Average Speed During Transit for unladen ants for any of the treatments. Therefore, this explanation does not appear likely.

Alternatively, unladen ants could be sensitive to encounter rates, which have been shown to be important in the regulation of other ant colony functions in previous work (Czaczkes, Grüter, & Ratnieks, 2013; Deneubourg et al., 2002; Depickère et al., 2004, 2008; Gordon et al., 1993; Jeanson et al., 2004). It is possible that unladen workers might increase their clearing attacks when they

experience a high encounter rate with either laden or unladen nest-mates. Under this scenario, the hindering of laden ants causes their speed to reduce and their density to increase, thus increasing the rate at which unladen workers encounter them as they move along in the trail traffic. Indeed, Dussutour, Deneubourg, et al. (2009) found that the rate of contact between outbound workers and inbound laden ants was twice as high in the presence of a height obstruction. Therefore it could be that increases in rates of contact lead to increases in the rates of clearing. Furthermore, increased rates of contact can be caused by other factors, such as increased trail flow. In these cases it is possible that there would be an increased rate of clearing on the trail margins in order to increase width.

Finally, there may be some form of direct communication from laden ants to unladen ants indicating their obstruction. For example, the laden ants could release a pheromone when they are obstructed that alerts unladen ants to the problem and triggers an increase in their clearing rate. Direct communication through antennation or other tactile contact may also transfer such information. Further studies will be required to test these hypotheses.

I suggest that the system of clearing trail height is likely to be the same as that for tunnels. There is a similarity of physical characteristics between trail overhangs and tunnels (fig. 1), especially foraging tunnels, which can be both high and wide (Moreira, Forti, Boaretto, et al., 2004). It may be that the trail is an extension of tunnel digging behaviour laid out over two dimensions instead of three.

Ant trails function as a form of communication that facilitates foraging. Information is normally thought to flow downstream or upstream along foraging trails (Czaczkes, Grüter, Jones, & Ratnieks, 2011; Farji-Brener et al., 2010; Robson & Traniello, 1995; Wilson, 1962), but trail obstructions are local problems that require a localized signal and response for their efficient resolution. I have shown that information of overhead obstruction is acquired locally by unladen

Atta colombica workers, even though they do not experience it as an obstruction. Their response depends on a functional impairment experienced by nest-mates. A graded response that matched the degree of impairment would allow a colony to manage efficiently its investment in transport infrastructure, matching the investment to needs for colony-level goals rather than to individual experience. This modulation of function is part of how leaf-cutting ant trails reach the level of sophistication that they do.

Investigations into information asymmetry have long been part of economics (Akerlof, 1970; Spence, 1973; Stiglitz & Weiss, 1981). Here we see a self-organised system using local information to resolve a mismatch between a group with information but no capacity to solve a problem (laden ants) and another group with capacity but no information (unladen ants). The resolution of this imbalance helps to create a smoothly functioning system despite difficult terrain. While the mechanism that resolves this difficulty is yet to be determined, the common properties between ant and human economic systems indicates that there may be underlying general principles common to both.

Acknowledgements

6.6 -References

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7.0 -Thesis Conclusion

This thesis has shown that the presence of leaf-laden ants is necessary for trail height clearance and put forward the hypothesis the resolution of the asymmetry between information rich but capability poor laden ants, and information poor but capability rich unladen ants is regulated by encounter rates (chapter 6). It has shown that ants can regulate their digging rate in response to tunnel space (chapter 3), and provided evidence against a digging pheromone being involved in this process (chapter 4). Subsequently, automated tracking resolved behavioural variables that led to the creation of a conceptual model for digging regulation without the use of a pheromone (chapter 5). Finally, it has pointed the way forward for new pathways of investigation into nest construction organisation.

A point that appeared multiple times in the thesis is the importance of encounter rates in nest construction organisation, as has been shown for other forms of organisation in other species (Bouchebti et al., 2015; Gordon, 1999; Gordon & Mehdiabadi, 1999; Greene & Gordon, 2007; Jeanne, 1986; Pratt, 2005). Encounter rates have emerged as a leading contender for explaining both regulation of investment into tunnel length and trail height. However, the hypothesised mechanisms behind each are slightly different. In the regulation of nest size, the tendency of ants to maintain proximity to other ants regulates individuals to stay within a particular context, and the behaviour is then determined by that context (chapter 5). In trail height regulation ants are also hypothesised to be monitoring their encounter rate, but in this case the hypothesis suggests that once ants detect an anomalous encounter rate they change their behaviour, and either go looking for an obstruction to clear or are more likely to clear the next obstruction that they encounter (chapter 6).

The regulation of nest size was hypothesised to be involve multiple factors (chapter 5). The role that arousal plays is likely to interact with the tendency for ants to maintain proximity to one another. These would both occur within the context of the nest size and shape to produce regulation.

For the regulation of tunnel length, some ants would be in the middle of a tunnel and be more likely to stay there in order to maintain their proximity to other ants. They would then perform context appropriate behaviours such as excavating from that area of the tunnel. Any group at the tunnel face would similarly maintain their proximity to one another and also perform context appropriate behaviour, in this case excavation at the tunnel face. As the tunnel expands, ants are more likely to be in middle-of-the-tunnel groups than tunnel-face groups and therefore less excavation occurs in places that would increase space. This understanding can be expanded to other nest shapes without significant modification. The tendency to maintain proximity may also be part of forming functional subgroups, such as are found in soil excavation and foraging (Hubbell et al., 1980; Pielström & Roces, 2013; Röschard & Roces, 2003).

Trail height may be regulated by encounter rates. In chapter 6 it was hypothesised that the behaviour of unladen ants is modified upon experiencing a high rate of laden ants encounters. This would then cause unladen ants to display a modified searching behaviour and a lower threshold to attack items on or near the trail. It would be interesting to test this hypothesis by observing the encounter rates of ants that attack the apparatus and determining whether they are significantly different from ants that do not attack the apparatus, and how their movement might be different prior to the attack. It remains possible that laden ants release a pheromone when they are obstructed that triggers unladen ants to clear the trail, and so this possibility should also be investigated.

These two examples may demonstrate how an elegant core behavioural mechanism can solve different problems with little modification. It is likely that this process of evolution modifying established behavioural mechanisms to solve different social coordination tasks is common throughout social insect behaviour. For example, it may be that the mechanism of ants maintaining proximity to one another is the same as the mechanism found by Römer and Roces (2015), where ants maintain proximity to fungus and brood in the creation and regulation of nest chambers. It

would be interesting to perform phylogenetic analysis on common regulatory behaviours such as these and see which systems are derived and which are basal, and what effect this may have on the rate of speciation.

This thesis suggests that digging pheromones are not the mechanism that regulates nest space construction. As digging pheromones are an important part of leading models of digging regulation (Buhl et al., 2005; Franks & Deneubourg, 1997) and are supported by experimental data (Chen & Zhang, 2013; Pielström & Roces, 2013), it is a surprise to find a lack of evidence in direct tests (chapter 4, fig 2b). It is my hope that my work inspires further research into how movement and response thresholds or other mechanisms regulate nest excavation.

It was also important to test whether ants test space directly as part of developing an understanding of the regulation processes. I was unable to show that ants respond to space removal in an 'open feedback loop' system (chapter 3, fig. 6a), likely due to methodological issues. Ants are very sensitive, and the process of removing space may be too disturbing to do without confounding the results.

It might be that social insects would be surprised that humans ever achieved mass social living, poorly adapted as we might appear to be. This is not stated to diminish the accomplishments of humans, which are epoch-making in scale (Williams et al., 2015), but to draw attention to the sophistication of social insect societies and how much we may yet learn from them. Many fields stand to benefit from a greater understanding of the many processes of self-organisation. As computers have become smaller and more powerful, robotics have also moved to smaller scales and increasing sophistication. However, with the end of Moore's Law (Courtland, 2013; Harald, Veira, & Weig, 2013; Vardi, 2014), it seems that the processing capacity of small robots will continue to be limited by their size. Robots are already following a process of bio-mimicry for their physical characteristics (Li et al., 2012; Pappalardo, 2011; Sang-Ho & Mita, 2002) and it makes intuitive

sense that their software might also be bio-inspired. Just as with the investigation of theoretical questions, ants make a particularly good inspiration for the development of new systems due to the simple rules that they follow to highly successful outcomes. The application of rules to unsupervised clusters of robots have the potential to revolutionise many fields of construction and manufacturing. Imagine a group of bio-inspired robots being dropped on Mars and being left to construct habitation before the arrival of humans (Engwirda, 2006; Serugendo et al., 2006). In the meantime, leaf-cutting ants remain an inspiration of organisation and a reminder that humans were not the first to achieve mass infrastructure and logistics.

While the organisation of social insect societies is an important field of biology, it can also be seen as a subset of a much larger question: "How can interacting units organise themselves to productive purposes without requiring outside guidance and input." In order to answer this question, model systems are important. These systems should be complex enough to allow the exploration of important questions, but experimental manipulation should be simple enough for studies to be economically and practically feasible. Furthermore, results should be both important in themselves and generalisable to other biological systems. Ants are ideal for this kind of work, being as they are a self-organising system that is manipulable with forceps and which can profoundly affect the ecology and economy of the world. Fungus growing ants have the additional advantage of being required to care for and maintain an organism other than themselves, with attendant behavioural complexities. Finally, leaf-cutting ants have reached the pinnacle of social organisation and have many features that remain to be investigated, the understanding of which can greatly assist the wider field of social organisation studies.

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"And real talk, like, seeing these ants and studying them and respecting them, it's like, man, they're in their own community too. They're trying to survive. They love. They fight. They telling themselves something. We can't understand, but one day we will."

— Brandon McCartney