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Organisation of Foraging in Ants

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Submitted for the degree of Doctor of Philosophy

Submission data: September 2012

University of Sussex

Declaration

I had a large role in the planning, data collection, and discussion of the results in chapter 4. The data analysis and writing of the manuscript was carried out by Dr. Christoph Grüter. I had a large role in the planning, data collection, and discussion of the results in chapter 9. The data analysis and writing of the manuscript was carried out by Dr. Christoph Grüter, and the agent based modelling was carried out by Dr Roger Schürch. The modelling in chapter 11 was carried out by Dr Pierre Nouvellet, and appendix E, which outlines the model, was mostly written by him.

I certify that, with the above qualifications, the work carried out in this thesis is entirely my own, and that any help provided by other individuals with data collection and analysis is fully acknowledged. In addition, I certify that this thesis has not been, and will not be, submitted in whole or in part to another university for the award of any other degree.

I hereby declare that this thesis has not beenm, and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature:

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UNIVERSITY OF SUSSEX
TOMER JOSEPH CZACZKES, DOCTOR OF PHILOSOPHY
ORGANISATION OF FORAGING IN ANTS

Summary

In social insects, foraging is often cooperative, and so requires considerable organisation. In most ants, organisation is a bottom-up process where decisions taken by individuals result in emergent colony level patterns. Individuals base their decisions on their internal state, their past experience, and their environment. By depositing trail pheromones, for example, ants can alter the environment, and thus affect the behaviour of their nestmates. The development of emergent patterns depends on both how individuals affect the environment, and how they react to changes in the environment.

Chapters 4 – 9 investigate the role of trail pheromones and route memory in the ant *Lasius niger*. Route memories can form rapidly and be followed accurately, and when route memories and trail pheromones contradict each other, ants overwhelmingly follow route memories (chapter 4). Route memories and trail pheromones can also interact synergistically, allowing ants to forage faster without sacrificing accuracy (chapter 5). Home range markings also interact with other information sources to affect ant behaviour (chapter 6). Trail pheromones assist experienced ants when facing complex, difficult-to-learn routes (chapter 7). When facing complicated routes, ants deposit more pheromone to assist in navigation and learning (chapter 7). Deposition of trail pheromones is suppressed by ants leaving a marked path (chapter 5), strong pheromone trails (chapter 7) and trail crowding (chapter 8). Colony level 'decisions' can be driven by factors other than trail pheromones, such as overcrowding at a food source (chapter 9). Chapter 10 reviews the many roles of trail pheromones in ants.

Chapters 11 – 14 focus on the organisation of cooperative food retrieval. *Pheidole oxyops* workers arrange themselves non-randomly around items to increase transport speeds (chapter 11). Groups of ants will rotate food items to reduce drag (chapter 12). Chapters 13 and 14 encompass the ecology of cooperative transport, and how it has shaped trail pheromone recruitment in *P. oxyops* and *Paratrechina longicornis*. Lastly, chapter 15 provide a comprehensive review of cooperative transport in ants and elsewhere.

Publications arising from this thesis

Grüter C, **Czaczkes TJ**, Ratnieks FLW. 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. Behav. Ecol. Sociobiol. 64:141–148. (Chapter 4)

Czaczkes TJ, Nouvellet P, Ratnieks FLW. 2011. Cooperative food transport in the Neotropical ant, *Pheidole oxyops*. Insect. Soc. 58:153–161. (Chapter 11)

Czaczkes TJ, Ratnieks FLW. 2011. Simple rules result in the adaptive turning of food items to reduce drag during cooperative food transport in the ant *Pheidole oxyops*. Insect. Soc. 58:91–96. (Chapter 12)

Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW. 2011. Synergy between social and private information increases foraging efficiency in ants. Biol. Lett. 7:521–524. (Chapter 5)

Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW. 2012. Uncovering the complexity of ant foraging trails. Commun. Integr. Biol. 5:78–80. (Chapter 6)

Czaczkes TJ, Ratnieks FLW. 2012. Pheromone trails in the Brazilian ant *Pheidole oxyops*: extreme properties and dual recruitment action. Behav. Ecol. Sociobiol. 66:1149–1156. (Chapter 13)

Czaczkes TJ, Ratnieks FLW. 2013. Cooperative Transport in Ants (Hymenoptera: Formicidae) and elsewhere. Myrmecol. News. (Chapter 15)

Grüter C, Schürch Roger, **Czaczkes TJ**, Taylor K, Durance T, Jones SM, Ratnieks FLW. In press. Negative feedback enables fast and flexible collective decision-making in ants. PLoS ONE. 6(9): e44501. DOI: 10.1371/journal.pone.0044501 (Chapter 9)

Czaczkes TJ, Grüter C, Ratnieks FLW. Accepted. Ant Foraging on Complex Trails: Route Learning and the Role of Trail Pheromones in *Lasius niger*. J. Exp. Biol. DOI: 10.1242/jeb.076570 (Chapter 7)

In the course of my doctoral research, I have contributed to studies led by my colleagues in the Laboratory of Apiculturea and Social Insects, which has resulted in co-authorship on the following paper, which was not included in the thesis:

Jones, S., van Zweden, J., Grüter, C., Menezes, C., Alves, D., Nunes-Silva, P., **Czaczkes, T.J.**, Imperatriz-Fonseca, V. & Ratnieks, F.L.W. 2012. The role of wax and resin in the nestmate recognition system of a stingless bee, *Tetragonisca angustula*. Behav. Ecol. Sociobiol., 66, 1–12

Chapters 8 and 14 are in the process of revision and resubmission.

Acknowledgements

I have been helped over the course of my PhD studies by many, many people in many ways. Whilst I cannot mention all the people with whom I have had fruitful discussions and been helped in other ways, specific acknowledgements must go to the following people.

- Firstly I must thank my supervisor, Prof. Francis Ratnieks. Francis was an ideal supervisor for me: always full of ideas and curiosity, and possessing a real knack for picking up on interesting patterns. His door is always open, and he always provided fantastic feedback. Critically, he gave me free rein to explore my own ideas and trusted me to get on with my work.
- Many, many thanks to Dr Christoph Grüter. Christoph has provided guidance and advice in all aspects of my work, from experiment planning and data collection to statistics and writing. He has a wide and insightful view of behavioural ecology, and any manuscript he comments on is dramatically improved. He has been an absolute pleasure to work with. He whistles very well, but refused to sing during data collection.
- Many thanks to all the other members of LASI. In no particular order, thanks to Dr Margaret Couvillion for bringing joy and life to the lab - her help with writing grammar and style are also much appreciated! Thanks to Gianluigi Bigio for many interesting conversations, both scientific and not. Thanks to Fiona Riddell Pearce for many laughs and fun times. Thanks to Sam Jones for supporting myrmecology in LASI, and stoically putting up with my occasionally bossiness. Thanks to Dr Jelle van Zweden for demonstrating that one can combine fun with being a mature, successful scientist.
- Thanks to Dr. Jonathan Green, Rosie Foster, Lynne Robinson, Mike Clease and the entire JMS crew, for being wonderful and friendly, for many rounds of badders, and for being a link to the world outside LASI. Dr Greens' thesis was both inspirational and highly intimidating during my own write-up.
- Thanks to Prof. Tom Collett for many constructive comments about my manuscripts. He asks fantastic questions, finds unnoticed patterns, and makes great suggestions to take the work to the next level. These suggestions usually involved a lot more work.
- Many thanks to Dr. Paulo Nogueira-Neto for allowing us to work and stay at Fazenta Aretuzina, and making us feel so welcome.
- A belated, but much deserved, thanks to Dr Terry Newsome, my A-level biology teacher, who was a great inspiration to me by expecting more than just answers to the questions in the textbook. I suspect I would not have studied biology further if it were not for him.

- Thanks to the BBSRC for funding me for four years (grant reference number BB / D526888/1). I can honestly say I couldn't do it without them, or an equivalent funding body. Also thanks to the Northwest European section of the IUSSI, and ASAB, for funding my travels to various conferences.
- And lastly, I would like to thank Dr. Katja Rex, for encouraging me to persevere in the search for the ideal PhD position, for reading and improving every part of this thesis, for her unceasing love and support, and for agreeing to marry me.

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Part 1 – General Introduction and Methods

Chapter 1: General Introduction

Ants are eusocial - they exhibit reproductive division of labour, with one or a few individuals dominating reproduction, and the rest, usually their offspring, are termed workers and perform other roles. The workers, instead of producing their own offspring, maximise their inclusive fitness (Hamilton 1964) by assisting their mother in producing siblings. This is analogous to somatic cells ensuring the genes they carry are maintained in the population not by directly reproducing, but by assisting the gametes to be in a position to reproduce (Wheeler 1911, 1926). Many authors argue that a eusocial colony comprised of thousands or millions of workers can be regarded as one super-organism (Wheeler 1911, 1926; Lumsden 1982; Hölldobler & Wilson 2009). Thus, much like organs or cells in a multicellular organism, members of a eusocial colony share the same goals and cooperate in fulfilling these goals. This requires a high degree of organisation, and in the following chapters I will investigate several aspects of this organisation.

One of the most essential tasks for all organisms is the acquisition of food, and this is true for colonies of social insects as well. The foraging behaviour of social insects has been under examination for over two thousand years (Aristotle, circa 343 BC), and has been systematically studied for almost 200 years (Bonnet 1779; Lubbock 1884; Forel 1921). Indeed, the self organisation and foraging of ants is famously praised in the bible:

"Take thee to the ant, thou sluggard; consider her ways, and be wise:
Which having no guide, overseer, or ruler,
Provideth her meat in the summer, and gathereth her food in the harvest."
Proverbs 6:6-8

This thesis is composed of two main topics: the use of route memories and trail pheromones in the organisation of foraging, and the organisation and behavioural ecology of cooperative transport. As chapter 10 is a review of trail organisation and chapter 15 is a review of cooperative transport, the general introduction and discussion will be kept short to minimise redundancy.

Organisation of recruitment – the classical view

The study of foraging in social insects has focussed to a great extent on recruitment of workers to a food source (Carroll & Janzen 1973). Honey bees recruit foragers to productive feeding patches via the famous waggle dance (von Frisch 1967). Ants, on the other hand, have several modes of recruitment, the most simple (and presumably most primitive) of which is tandem running (or tandem carrying), where a recruiting ant leads (or carries) another ant to the food source (Guénard & Silverman 2011). The emission of chemical way-markers by the recruiter for the recruit to follow is thought to have derived from this (Hölldobler & Wilson 1990). More advanced yet is group recruitment, in which the recruiter leads a group of recruits to the target. Finally, in mass recruitment – the most derived form of recruitment – the recruiter is no longer needed to guide recruits to the food source. A line of trail pheromone deposited by the recruiter leads nestmates to the food source.

Several general rules, according to which mass-recruiting ants use trail pheromones, were described in the 1960's. Wilson (1962) demonstrated that the number of workers leaving the nest can be controlled simply by the amount of trail pheromone released by the foragers. The number of recruited foragers can thus be regulated: at a newly discovered food source, worker numbers increase logistically as workers return to the nest depositing trail pheromone, inducing more workers to exit the nest, feed, and deposit pheromone themselves. Eventually, as the food source becomes overexploited, and workers can no longer feed (or fill their crop, Mailleux et al., 2000), they return without depositing trail pheromone, so the number of workers leaving the nest does not increase. The pheromone eventually decays, and the number of ants leaving the colony begins to fall. Hangartner (1969a) found that more pheromone is deposited when the colony is starved, or when the food quality is high (also shown in Jaffe & Howse 1979; Crawford & Rissing 1983; Breed et al. 1987; Beckers et al. 1993). Hangartner (1969b) also demonstrated that at a choice between two pheromone trails, ants will follow the trails in direct proportion to their relative strengths. Combined, these findings predicted that ants would lay stronger pheromone trails to higher quality food sources, and recruits would then follow these paths preferentially, causing the colony as a whole to concentrate on the higher quality food source (Beckers et al. 1990). This simple auto-catalytic system allows the amplification of small differences in trail strength, resulting in a rapid 'choice' made at the colony level. Similar self amplification processes are used during colony emigration in both ants (Mallon et al. 2001; Visscher 2007) and bees (Seeley & Morse 1978; Seeley 1995). One of the main advantages of such a decision-making mechanism is that it does

not require any one individual to directly compare available options, nor does it require top-down control. It is thus robust, flexible, and requires little individual processing power. It is these basic features that appeal to computer scientists and roboticists, resulting in meta-heuristics such as Ant Colony Optimisation (ACO)(Dorigo & Di Caro 1999; Dorigo & Stützle 2004) and the study of swarm intelligence (Kube & Bonabeau 2000).

As a result of the non-linear recruitment of ant workers to a food source, small random differences in initial recruitment strength will lead to one trail 'out-competing' the other if an ant colony simultaneously discovers two food sources of identical quality (Sumpter & Beekman 2003). This effect is known as symmetry breaking, and has been demonstrated repeatedly in laboratory studies and models (Beckers et al. 1990; Sumpter & Beekman 2003)(although one can often observe colonies foraging at multiple locations in the wild – see chapter 9). A corollary to this is that once foraging is well under way along a strong pheromone trail, newly discovered food sources cannot be effectively recruited to, even if they are of higher quality. This has also been demonstrated in laboratory experiments and models (Beckers et al. 1990; Sumpter & Beekman 2003) (and see chapter 9).

Thus, even with a relatively simple model of foraging organisation in ant colonies, surprising sophistcation, and emergent behaviours and properties, arise. However, as in all matters biological, the reality is never that simple.

Complexity in trail pheromone organisation

Even as early as the 1970s, it became apparent that the organisation of foraging in ants was much more complex than described above. Trail pheromones were found to be formed of multiple components, which caused different effects (Traniello 1977; Cammaerts 1984; VanderMeer et al. 1990; Robinson et al. 2005; Jackson et al. 2006; Witte et al. 2007a; Dussutour et al. 2009a). The rules by which ants deposit trail pheromone were also discovered to be highly complex and contingent on many factors apart from resource quality and hunger levels, such as food quantity, the presence of brood, the type of food discovered, and many other factors (Breed et al. 1987; Beckers et al. 1992a; Aron et al. 1993; Biseau & Pasteels 1994; Detrain & Deneubourg 1997; Robson & Traniello 1998; Portha et al. 2002). In the following chapters, I present further complexity in the rules used by ants to modulate recruitment, including modulation due to trail usage (chapter 8), trail pheromone concentration (chapter 7), the presence of home range markings (chapters 5 and 6) and the interaction of these factors

with past experience (chapters 4 and 5). The multiple components of trail pheromones and the complex rules employed by ants in using pheromone trails will be reviewed in chapter 10.

Route memory and the combined use of route memory and pheromone trails

For a scout to recruit, it generally must first successfully return to the nest. The methods by which ants make their way home are varied, and one species may use several methods of orientation. Homing, path integration, route learning and navigation in social insects have been heavily studied for many years, and the existing body of knowledge is too vast for the scope of this introduction (for reviews see Collett & Collett 2002; Collett et al. 2003). It is important to stress, however, that route memory in ants can develop very rapidly and can be extremely accurate, reaching over 90% accuracy at a single trail choice after short periods of training in *Formica rufa* (Rosengren & Fortelius 1986). Similarly, high accuracy at a single bifurcation was reported in *F. lugubris (*95% (Fourcassie & Beugnon 1988)), *Lasius flavus* (97% Jones *et al* (in prep)), and as part of this thesis in *L. niger* (95% (Grüter et al. 2011) (see chapter 4)). Thus, an experienced ant might have two sources of information it could follow to reach the feeding location: its own route memory (a private information source) and the pheromone trail (a social information source). Indeed, potentially they could also follow home range markings laid down passively by nestmates (Cammaerts & Cammaerts 2000). These information sources are not necessarily redundant, and ants do not have to choose one over the other. On the contrary, these information sources can be used synergistically, with trail pheromones acting as a reassurance to ants relying on route memory, allowing them to walk faster and straighter, and also causing experienced ants which stray from the trail to reduce trail pheromone deposition, thus preventing other ants from being led astray (Czaczkes et al. 2011a) (chapter 5). Further demonstration of a complementary interaction of route memories and trail pheromones is given in chapter 7, where I show that trail pheromones and route memory can act additively – especially on complex routes where forming a route memory is difficult – by both increasing navigational accuracy and by promoting route learning.

However, with the use of two information sources, the possibility of conflict emerges. This may be quite common during natural foraging, as colonies may be foraging on multiple food sources simultaneously and so have multiple bifurcating pheromone trails (Rosengren & Fortelius 1986). Honey bees and several species of ants (e.g. *L. niger, Paraponera clavata and F. lugubris)* follow private information over social information (Grüter et al. 2008; Harrison et al. 1989; Fourcassie & Beugnon 1988; Aron et al. 1993), but at least one ant species,

Iridomyrmex humilis, follows social information preferentially (Aron et al. 1993). Of course, both information sources may differ in strength, and thus so may the certainty of followers using them. However, with the exception of Aron et al. (1993), the interaction between the strength of the two information sources was never taken into account in previous studies. Thus, chapter 4 systematically explores this interaction, finding that followers rely on their route memory, even when pheromone trails are strong and the memory is based on only one previous visit to the food source.

Information gathered by social insects is not necessarily explicitly communicated by nestmates. A distinction should be made between signals, where information is deliberately transmitted, and cues, which are acquired from sources not primarily intended for communication (Seeley 1989; Detrain et al. 1999; Jarau & Hrncir 2009). Such cues can be used to regulate foraging effort. For example, in honey bees, foragers attempting to unload nectar will take the delay between arrival and unloading as a cue, informing them of how necessary further foraging and recruitment is (Seeley 1995). If the load is unloaded rapidly, further foraging is necessary. A long delay in unloading suggests that all unloaders are either busy or unmotivated to unload further nectar, indicating that further foraging is not needed. Wasps use similar cues to regulate foraging for pulp and water during nest construction (Jeanne 1999). Contact rate with nestmates is another easily assessed cue that can inform foragers about the density of nestmates or the relative density of non-nestmates, and does not require the ability to count (Hölldobler 1981b; Gordon et al. 1993; Gordon & Mehdiabadi 1999). By observing what contacted nestmates are doing, density cues can cause individuals to switch behavioural roles. For example, harvester ants that encounter nestmates performing midden work are more likely to perform midden work themselves (Gordon & Mehdiabadi 1999). Such cues can be useful for regulating foraging, as reported in chapter 8. *L. niger* assesses encounter rates with nestmates on a foraging trail and reduce the amount of trail pheromone deposition when sensing high encounter rates. This constitutes a negative feedback component in the organisation of foraging, which may limit the maximum trail strength a trail can reach, without compromising the initial rate of pheromone build-up. This may play a role in preventing trails from becoming over-strong, and thus allow colonies to maintain foraging flexibility, and to some degree prevent getting trapped in a sub-optimal foraging situation. A similar negative feedback component was uncovered in chapter 7, although in this case it is the presence of high levels of trail pheromone, which reduce further pheromone deposition.

Cooperative transport

The vast majority of research on the organisation of foraging focuses on recruitment and navigation. This ignores the critical step of retrieving the food to the nest. Ants will often encounter a food source too large to be retrieved by a single worker. Most ants can retrieve liquid food by taking it into their crop, and thus retrieval of large quantities does not pose a great difficulty, as many individuals can act independently. Large items of solid food present more of a challenge. One option available to ants is to dissect the item *in situ*, allowing ants to carry back portions individually. This does not require a great degree of organisation, although tough items may need to be dismembered by workers of a larger caste (personal observation on *Pheidole oxyops*). However, dissection of large items is time-consuming, leaving the food item exposed to theft by other animals , and workers to predation or desiccation (Hölldobler et al. 1978; Traniello 1983, 1987; Traniello & Beshers 1991; Cerdá et al. 1998a). Thus, many ants have developed the ability to cooperatively retrieve large food items. This allows ants to avoid competitors and dangers, but is in itself no trivial task. With poor organisation deadlocks can occur (Sudd 1965), causing such delay in retrieval that dissection may be faster (Moffett 1992). Nonetheless, many ant species perform remarkably effective cooperative transport, transporting items hundreds or thousands of times the weight of a single individual at respectable speeds (Moffett 1988). Although this behaviour is highly conspicuous and charismatic, it is surprisingly poorly studied. A handful of studies in the 1960s (Sudd 1965; Chauvin 1968) examine the organisation of workers around a food item, and decades later some studies examine how workers decide whether or not to recruit to an item (Traniello 1983; Detrain & Deneubourg 1997; Robson & Traniello 1998, 2002). I address this gap by investigating the cooperative transport behaviour of the Neotropical ant *Pheidole oxyops*, examining the rules it uses to arrange itself around an item during carriage (chapter 11). Furthermore, I describe the ability of this ant to reorient food items so as to reduce drag during carriage (chapter 12).

Cooperative transport is not merely a charismatic and interesting behaviour, but seems to inspire many roboticists working in the field of swarm robotics (e.g. Eustace et al. 1993; Bay 1995; Kube & Bonabeau 2000; Groβ & Dorigo 2008; Fink et al. 2009; Berman et al. 2011). However, with the exception of Berman *et al.* (2011), such work tends to ignore the biological data on this behaviour. This may be due in part to the lack of comprehensive reviews of this topic. Chapter 15 provides a review of cooperative transport in ants and elsewhere, and

attempts to synthesise and organise what is known about this behaviour, and encourage interdisciplinary collaborations between biologists and roboticists.

Recruitment organisation and cooperative transport

Although recruitment organisation and cooperative transport seem to be two separate themes, cooperative transport usually follows successful recruitment. In some ants, trail following accuracy is low. For example, chapter 4 (Grüter et al. 2011) shows that in *Lasius niger* as few as 62% of the ants chose the pheromone marked branch at a trail bifurcation. Similarly, *Monomorium pharaonis* achieve an accuracy of 70% on paper substrate (Jeanson et al. 2003). Both species rely on making multiple return trips to the food source. Cooperative transport is usually employed to rapidly remove a food item before competitors do (Traniello 1983; Robson & Traniello 1998). In such a situation colonies cannot afford to make multiple return trips to the food source. Thus, I predicted that in *Pheidole oxyops*, in which 78% of retrieved food is retrieved via cooperative transport (Czaczkes et al. 2011b) (chapter 11), trail following will be more accurate. So as to achieve higher accuracy and allow rapid following of the trail, I expected the *P. oxyops* pheromone trails to be more volatile, causing a stronger presence in the head-space above the trail. Moreover, high volatility will result in the pheromone trail persisting for only a short amount of time - an adaptive trait when a food item can only be collected once, and continual recruitment is not necessary. Thus, pheromone trails of *P. oxyops* should decay much more rapidly than those of *L. niger* or *M. pharaonis*. In chapter 13 (Czaczkes & Ratnieks 2012), both of these predictions were confirmed for *P. oxyops*, and chapter 14 shows that the invasive ant *Paratrechina longicornis*, which also relies heavily on cooperative transport, possesses an almost identically balanced short-term recruitment pheromone. Furthermore chapter 13 presents an additional role for pheromone trails in *P. oxyops*: Pheromone trails not only lead ants from the nest to the food source, but also intercept workers in the environment and channel them towards the food source. This allows workers to find a food item faster, and extends the foraging range of the colony. Local recruitment is also performed by *P. longicornis* (chapter 14), especially to live prey, using volatile chemicals emitted into the air. It is also used to form an 'escort' during the cooperative transport of live prey. Thus, chapters 13 and 14 link together the two main parts of the thesis, demonstrating that the specialised ecology of cooperative gathering large food items requires equally specialised pheromone trail organisation.

Chapter 2: Study species and general methods

Lasius niger

Figure 2.1 *Lasius niger* workers foraging on honeydew from aphids

Lasius niger is perhaps the most conspicuous ant in Britain (Pontin 2005) and very common throughout Europe. Workers are 4-5mm long and dark brown or black. *L. niger* is an ideal study organisms for several reasons. The workers are very robust to disturbance and large enough that individuals can be marked with paint dots on the abdomen. This is easiest to achieve while the ant is drinking from a syrup feeder. Colonies can contain over 10,000 workers, so colony fragments can be used to study colony-level behaviours. Whilst individual and colony level behaviour may differ between mature colonies and small colony fragments, fragments containing several hundred workers or more forage and recruit effectively, and have been used to study foraging and colony organisation in many previous studies (e.g. Beckers et al. 1989; Mailleux et al. 2000; Dussutour et al. 2005; Evison et al. 2008). Queenless colony fragments settle quickly in artificial nest boxes and forage and care for brood normally. Colony fragments can be collected by placing concrete slabs in a habitat suitable to *Lasius niger*, such as meadow edges. After a few weeks a colony is likely to move in under the slabs, which warm up rapidly and so promote brood development. Once a colony moves in, colony fragments can be collected periodically by lifting the slab and using a portable vacuum cleaner to suck up workers and brood, which are then transferred into a foraging box. Ants collected from under the same slab were assumed to be from the same colony, so whilst multiple collections could be made from the same slab, the resultant fragments were not used as independent fragments within any one experiment. We used plastic foraging boxes with a lid measuring 30x30x15cm. The bottom 2cm of the box were covered in a layer of gypsum and the sides coated with Fluon to prevent the ants escaping. Colonies were also provided nest-boxes made of gypsum or wood, in which they rapidly settled. Colonies were provided water *ad-libidum*, and were fed three times per week on Bhatkar mix – a mixture of agar, honey, and vitamins (Bhatkar & Whitcomb 1970). Once to three times per week this diet was supplemented with either mealworms (*Tenobrio molitor*) or fruit-flies (*Drosophila melanogastar*).

Lasius niger use mass recruitment via trail pheromones to lead nestmates to food sources and to lead nestmates to new nest sites during emigration (pers. obs.). The pheromone deposition behaviour of *L. niger* is very characteristic, involving a 0.2 second pause in walking in which the ant curls its gaster downwards and dots the substrate with the tip of the gaster. This behaviour is easily observed by eye or in standard video-recordings, allowing quantification of pheromone deposition. It must be noted that this quantification is approximate, as it is possible, and indeed likely, that ants vary the amount of pheromone deposited with each deposition, and that larger ants deposit more pheromone.

Pheidole oxyops

Figure 2.2 *Pheidole oxyops* minor workers dragging a cockroach

Pheidole oxyops is a ground nesting Neotropical ant. Like most species of *Pheidole*, they are dimorphic, with smaller, 4-5mm long, workers ("minors") and larger, 5-6mm long, workers (" majors") with disproportionately large heads. The number of workers in a colony is unknown, but by the size and depth of the nest cavities (mean depth 2.23m, range 1-5m, mean number of chambers 8, range 4-14, each chamber diameter 12.5cm, height 3cm, (Forti et al. 2007)) and by the number of workers seen leaving the nest, I estimate most colonies to contain at least 1000 individuals. I studied *P. oxyops* because they conspicuously perform well-coordinated cooperative transport, retrieving 78% of their food that way (Chapter 11 and Czaczkes et al. 2011b). Cooperative transport is almost exclusively performed by minors. The largest item I observed being retrieved, by a group of approx. 30 individuals, was a mantid approximatly 50mm long. A scouting ant that finds a food item decided whether to initiate recruitment in a similar manner to other ant species that perform cooperative transport (Hölldobler et al. 1978; Schatz et al. 1997; Robson & Traniello 1998; Daly-Schweitzer et al. 2007; Cerdá et al. 2009; Amor et al. 2010): if the scout cannot move the item, or can only move the item very slowly, it releases the item and returns to the nest, depositing a pheromone trail. On reaching the nest entrance, recruits begin pouring out of the nest, follow the pheromone trail to the food item, and transport the item back to the nest. A video of this process can be found as an online supplementary file to Czaczkes & Ratnieks (2012).

Early experimentation showed that these ants are very partial to a particular Brazilian cheese, described to me as “Mozarella”. This allowed the production of standardised food items with a regular size, shape and mass. By presenting colonies with a piece of cheese affixed to a large weight of modelling clay, surges of recruits could easily be directed to a desired location, and cooperative transport of standardised items could be initiated.

General laboratory methods for working with *Lasius niger*

Colonies to be tested were deprived of food for several days prior to testing, to ensure high motivation to forage. Mazes on which ants can be tested can be cut out of thin sheets of stiff plastic. The plastic maze was balanced on supports surrounded by a water moat, preventing ants from leaving the apparatus. The maze was overlaid with standard printer paper. A small acetate sheet with a drop of sucrose solution could be placed anywhere on the apparatus to form a feeder. Colonies were attached to the apparatus using a bridge, which could be raised or lowered to allow or prevent access. By removing the paper overlays on which ants deposited pheromones, I could manipulate the amount of pheromone on a trail. By following individually-marked ants, the amount of experience they have with the apparatus can be tracked.

Trail choice behaviour was mostly examined using a T maze. Ants crossing a certain point on the left or right branch of the T maze were considered as having made a decision for that direction. T mazes were used in preference to Y mazes, as Y mazes do not necessarily force the ant to make a decision, whereas T mazes do (see figure 2.3). The behaviour of ants was either scored by eye during the experiment, or video recorded and analysed frame-by-frame.

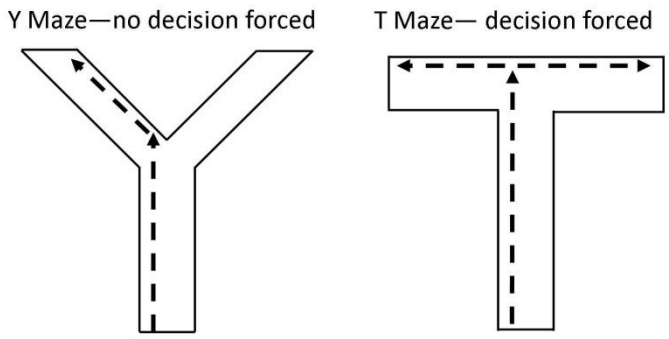

Figure 2.3 – comparing Y and T mazes. In a Y maze, ants walking straight on one side of the stem are passively led in that direction, and are not forced to make an active decision. In a T maze, such an ant is always forced to make an active decision.

General field methods for working with *Pheidole oxyops*

As described above, waves of *Pheidole oxyops* workers could be directed to a particular location by presenting a scout with an immovable bait. Once the scout left the bait to recruit nestmates, the bait could be replaced by a test item. Experiments were usually conducted on a ceramic tile covered in graph paper, providing a standard surface, and facilitating accurate measurement of walking speeds. When necessary, the tile could be raised on fluon-coated posts, allowing access only via a removable cardboard bridge. This allowed carrier group size to be limited. T mazes similar to those used in the lab were adapted for field use. Video recordings were analysed for transport speed of the item, the behaviour of individuals, and the number of individuals involved in the transport event.

Statistical analysis

Since much of the data collected during the course of this thesis came from multiple individuals of multiple colonies, most data were analysis using Generalised Linear Mixed-effect Models (GLMMs). All GLMMs were carried in R (R Development Core Team 2009), using the LME4 package (Bates et al. 2007).

Data were analysed according to Zuur et al. (2009). The exponential family was chosen depending on the data type, and by graphing the data. Normally distributed data were modelled using a Gaussian distribution. Binomial data (i.e presence/absence or binary choice data) were modelled using a binomial distribution, and count data using a Poisson distribution. Count data were often zero inflated, and therefore analysed in two steps: first as presence/absence binomial data, and subsequently a subset of only present data (thus excluding all zeros) were analysed using a Poisson distribution.

Analysis followed the following steps: First, I explored the random effect structure of the data, modelling them either with a varying intercept or a varying intercept and varying slope. The slope of a random effect can vary over continuous variable in the model, such as visit number. Where necessary, models contained either “Colony” or “Ant” as a random effect, or both. Random effect structures were evaluated by comparing their Akaike Information Criterion (AIC) index, with the lowest AIC index being chosen.

Once the random effect structure was chosen , I searched for the ideal model structure. Beginning with a saturated model (which attempts to explain the variation in the independent variable using all the possible explanatory variables and their interactions), I removed the least

significant of the highest-level interactions and re-ran the model until all the highest-level interactions were significant ($P \leq 0.05$ post-correction for multiple testing – usually using the Benjamini-Hochberg method (Benjamini & Hochberg 1995)). For example, the saturated model might be

Pheromone Depositions = treatment * travel direction * visit (+ random effects)

And might produce the results:

treatment * travel direction * visit - P > 0.05
treatment * travel direction - P < 0.05 ***
treatment * visit - P < 0.05 ***
travel direction * visit - P > 0.05
(non-interaction terms not shown)

The highest interaction term is non-significant, and so is removed from the model, and the new model-rerun. Once no more terms in the model are non-significant, the model was chosen as the final fixed-effect structure. If a three-way interaction was found to be significant, this was analysed by sub-setting: for example, if the interaction treatment * travel direction * visit was significant, I would split the data by the various treatments, and test interaction of travel direction * visit in each subset. I would then subset the data according to travel direction, and test the interaction treatment * visit in each subset. Continuous variables (such as visit number) could not be subsetted.

When only one colony was tested, or if there were no significant random effects, simpler General Linear Models or standard non-parametric tests were used.

Chapter 3: How the thesis developed

I joined the Laboratory of Apiculture and Social Insects (LASI) in September 2008 just as the lab had moved from Sheffield to Sussex. Prof. Ratnieks suggested that I join Dr Grüters' project, exploring information conflicts between private and social information in ants. Dr. Grüter had explored similar questions in honey bees previously, and was well versed in the literature and working with social insects. However, neither of us had done lab work on ants before, so we developed most of the key techniques and skills for our future experiments during the course of this project. The results of this experiment were strong and surprising: ants preferred their own private information to social information regardless of the relative strengths of the information source. Moreover, they learned to take the correct choice at a bifurcation very rapidly. Lastly, and also very surprisingly, the results indicated that the ants were not very good at following pheromone trails. These results were published in Grüter et al. (2011) and form the first experimental chapter of this thesis (chapter 4). During data collection, we observed several odd behaviours: ants would suddenly stop depositing pheromone when walking over an area where the pheromone trail had been removed. Likewise, ants returning to the nest from one arm of the T maze seemed to deposit more pheromone just after making a turn. Based on this, I planned experiments to explore the first of these observations. Since experienced ants followed their own memory in preference to trail pheromones, but nonetheless continued to deposit pheromone trails, I hypothesized that pheromone trails have roles apart from guiding ants to the food source. Indeed, additional roles for pheromone trails became somewhat of a repeating theme in my experiments. The experiment was very successful and make up chapters 5 and 6 of this thesis (Czaczkes et al. 2011a). Concurrently, Dr. Grüter and I were collecting data to explore how effective ants are at differentiating between pheromone trails of varying relative and absolute concentrations, but were unable to collect sufficient data to produce meaningful results.

During the second year of my studies, Dr. Grüter and I began taking on the supervision of two undergraduate students investigating the distribution of ants between limited rate feeders. We demonstrated that ants could switch from a poor food source (limited rate feeder) to a good food source (less-limited or unlimited rate feeder). These results were published in (Grüter et al. 2012), and make up chapter 9 of this thesis.

Considering alternative roles for trail pheromones, we realized that the rapid learning we found in *L. niger* might be due to experiments being carried out using a single bifurcation. A

more complex route might be more difficult to learn. Trail pheromones may be important for guiding experienced ants back to a food source which lies at the end of a complex route. With the help of a new set of undergraduate students, we tested this hypothesis, and demonstrated that indeed ants found alternating routes difficult to learn, and trail pheromones were helpful for navigation and promoted route learning. To our surprise, ants which made mistakes when searching for the food deposited more pheromone on their return journey, resulting in more pheromone being deposited on the difficult-to-learn routes. These results are reported in chapter 7 and Czaczkes et al. (2012a).

The results from chapters 4, 7 and 9 convinced me that route memories play a much more important role in ant foraging than previously assumed. I considered modelling ant foraging using route memories and trail pheromones, and contacted Dr Thomas Nowotny from the University of Sussex computer science department, who kindly wrote a basic simulation program. It became apparent that a key piece of data was missing: the speed with which ants change their memory after not finding food at a former food source. I conducted a pilot study on the subject with interesting results (see appendix A), but unfortunately I had to abandon this line of enquiry due to time constraints. I later took up the problem again, this time employing a pair of MSc students at the Ludwig-Maximilian University in Munich, and we replicated the results and gathered some data on pheromone deposition behaviour in this system. However, more data is needed before these results are ready for publication.

During data collection for an experiment which involved the collection of trail pheromone, we were continually frustrated that highly active colonies refused to deposit enough pheromone for our experiments. However, data from the limited-rate feeder experiment showed that this was not due to crowding at the food source, so I suspected that it might be due to crowding on the trail. An experiment where crowding on the trail was varied, using either real ants or dummy ants – glass beads coated in nestmate cuticular hydrocarbons – showed that higher traffic on a trail indeed reduced pheromone deposition on the trail. These results are reported in chapter 8.

Parallel to the lab work, every year Prof. Ratnieks takes a group from the lab to Brazil. In the first year I went out with a rather vague objective: Prof. Ratnieks had seem some ants dragging prey items in groups, and thought this would be worth studying. By offering the ants little pieces of shaped cheese, which they had a great fondness for, and recorded how they transported it, I uncovered a series of behavioural rules which the ants used to arrange themselves around a food item . These results are reported in chapter 11 and Czaczkes *et al.*

(2011b). I also discovered how the ants decided whether or not to recruit nestmates to a food item, but this mechanism had been described in multiple species by several authors.

The following year I carried out a study on task partitioning and division of labour during fruit cutting in *Acromyrmex* leaf cutter ants, as a follow-up to a series of papers carried out by past LASI members (Evison & Ratnieks 2007; Helanterä & Ratnieks 2008). We collected interesting data about task partitioning and morphological adaptation in leaf cutter ants, which I chose not to include in this thesis. I also carried out an experiment on *Pheidole oxyops* and demonstrated that groups of ants can rotate large prey items to reduce drag during retrieval, as reported in chapter 12 and Czaczkes & Ratnieks (2011).

Before my third visit to Brazil I was very puzzled by our finding that *Lasius niger* were so poor at following pheromone trails, and one plausible explanation for this was that making such 'mistakes' was in fact adaptive: If this was the case, ants should follow trails more accurately when ants are trying to navigate to a specific point, (e.g. a nest site) rather than to a distributed patch of feeding locations, (e.g. aphid colonies on a tree). Unfortunately, although I could make ant colonies deposit pheromones to a new nest site in the lab, the ants did not seem to follow the pheromone trail at all, possibly due to a lack of physical recruitment displays. Another situation in which ants must navigate to a specific point is during recruitment to a large prey item. As the Brazilian ants, *P. oxyops*, rely heavily on such food items, I expected this species to follow trails more accurately than *L. niger*. Thus, on my third visit to Brazil, I tested this and found that not only were the pheromone trails followed with great accuracy, but were also very short-lived. This raised questions about the foraging ecology of *P. oxyops*, and in a further series of experiments I demonstrated that *P. oxyops* forages at a greater range from their nest than expected, and that this was at least in part due to the trail pheromone acting to intercept foragers for the environment, and channel them to the food item. These results are reported in chapter 13 and published in Czaczkes & Ratnieks (2012).

Our final visit to Brazil was spent on the Riberaõ Preto campus of the University of Saõ Paulo, kindly hosted by Prof. Nacimento. I knew the campus was overrun by giant *Atta* colonies, and so planned a further experiment on fruit cutting by leaf cutting ants. The idea was to investigate whether larger ants – which are used preferentially to cut fruit – are actively recruited when fruit is found, or whether they are simply more likely to begin cutting fruit. However, the ants stubbornly refused to cut any of the many types of fruit I offered them. My attention therefore turned to the Longhorn Crazy ant *Paratrechina longicornis*, which was very common around the campus buildings, and also performed cooperative transport. I spent the rest of my visit investigating *Pa. longicornis*' recruitment, trail following, and foraging

behaviour, as presented in chapter 14. We have just received reviewer comments on this manuscript, in which a follow-up experiment looking at the adaptive significance of the curious “escorting” we observed was requested. I am in the process of coordinating this experiment with Ayrton Vollet, a PhD student in Riberaõ Preto.

As the end of my third year approached, I had collected so much data that, although I had been analysing and writing continuously, I had to stop data collection and focus on writing. For personal reasons I moved to Munich for the last year of my PhD, where I was kindly taken in by Prof. Witte and his group, an ant group working on broadly similar topics to those covered in LASI. While I still returned to Sussex regularly, I also supervised two masters students working on my abandoned memory-switching experiment. At the same time I contacted Prof. Heinze, who leads an ant research group in Regensburg, and we are currently waiting to hear back from a couple of grant proposals, in the hope that I can continue studying ants for many years to come.

Part 2 – Research chapters

Chapter 4: Decision-making in ant foragers (*Lasius niger*) facing conflicting private and social information

Christoph Grüter, Tomer J. Czaczkes & Francis L.W. Ratnieks

Abstract

Foragers of many ant species use pheromone trails to guide nest-mates to food sources. During foraging, individual workers can also learn the route to a food source. Foragers of the mass-recruiting ant *Lasius niger* use both pheromone trails and memory to locate a food source. As a result, an experienced forager can have a conflict between social information (trail pheromones) and private information (route memory) at trail bifurcations. We tested decision-making of *L. niger* foragers facing such an informational conflict in situations where both the strength of the pheromone trail and the number of previous visits to the food source varied. Foragers quickly learned the branch at a T-bifurcation that leads to a food source, with 74.6% choosing correctly after 1 previous visit and 95.3% after 3 visits. Pheromone trails had a weaker effect on choice behaviour of naïve ants, with only 61.6% and 70.2% choosing the branch that had been marked by 1 or 20 foragers versus an unmarked branch. When there was a conflict between private and social information, memory overrides pheromone after just one previous visit to a food source. Most ants, 82-100%, chose the branch where they collected food during previous foraging trips, with the proportion depending on the number of previous trips (1 v. 3) but not on the strength of the pheromone trail (1 v. 20). In addition, the presence of a pheromone trail at one branch in a bifurcation had no effect on the time it took an experienced ant to choose the correct branch (the branch without pheromone). These results suggest that navigational memory dominates foraging decisions in experienced *L. niger* foragers.

Introduction

Social insect foragers exploiting renewable or large food sources often direct nest-mates to the location of the food source (von Frisch 1967; Hölldobler & Wilson 1990; Jarau & Hrncir 2009). Many ants (Hölldobler & Wilson 1990), termites (Wilson 1972) and stingless bees (Lindauer & Kerr 1960; Barth et al. 2008; Jarau 2009) use pheromone trails to do this. The use of trail pheromones in ants is remarkably diverse and sophisticated, involving multicomponent pheromone blends (Hölldobler 1995) and different pheromones deposited at different locations on the trail having different functions (Robinson et al. 2005; Jackson & Ratnieks 2006; Ratnieks 2008). During foraging, workers also acquire navigational memories when travelling between food sources and the nest (reviewed in von Frisch 1923; Collett & Collett 2002; Collett et al. 2003). The ability of ant and bee foragers to learn the route leading to one or several food sources allows foragers to make repeated visits, a phenomenon called site fidelity, is well known ("Ortstreue") (Ribbands 1949; Hölldobler 1976a; Traniello 1977; Rosengren & Fortelius 1986; Quinet & Pasteels 1996). Ants can remember food locations for weeks or even months (*Lasius fuliginosus*, Quinet & Pasteels 1996; *Formica spp.*, (Rosengren & Fortelius 1986; Salo & Rosengren 2001)).

Experienced foragers leaving the nest can thus make use of both social information (pheromone trails) and private information (navigational memory) to locate a food source. As described in Rosengren and Fortelius (1986), experienced ant foragers will face situations in which the two types of information are in conflict, such as at a trail bifurcation with trail pheromone on both branches. In wood ants, for example, trail pheromone guides foragers lacking knowledge on food source locations, whereas private navigational information is more important for experienced foragers who know a specific food location (Rosengren & Fortelius 1986; Salo & Rosengren 2001). Several studies have investigated the relative importance of social chemical information versus private navigational information by creating conflicts between the two information sources (*Linepithema humile* =*Iridomyrmex humilis*, *L. niger*, (Aron et al. 1993); *Pogonomyrmex sp*., (Hölldobler 1976a); *Formica sp.*, (Rosengren & Fortelius 1986); *Paraponera clavata*, (Harrison et al. 1989). These studies show that foragers of some species rely more on the chemical trail and others more on memory.

However, with the exception of Aron et al. (Aron et al. 1993), experiments have not systematically investigated the effect of factors such as the strength of the pheromone trail, the number of previous visits to a food source, and the quality of the food or the distance to the food source. It is, therefore, not clear how flexibly foragers can choose among different

information sources. In vertebrates, the study of information use strategies (e.g. private vs. social) and the factors affecting decision-making is an active and rapidly expanding field of research (reviewed in Laland 2004; Kendal et al. 2005; Seppänen et al. 2007). Social insects and particularly ants are ideal model systems for investigating decision-making strategies in foraging, as they can be interrogated both at the individual and colony level. One area of special significance concerns the prioritization of alternative information sources as foragers have access to multiple, and often conflicting, information sources (Hölldobler 1999; Grüter et al. 2008). For example, does an ant prioritize social information when its private information is subject to a high degree of uncertainty, as will occur if the ant has made only a few previous visits to a particular feeding location? Or does an ant prioritize social information when the reliability or strength of the social information is high, as will occur if a trail or branch is well marked with pheromone? Various strategies are possible and different ant species might use different strategies depending on the type of food that is collected, the temporal and spatial distribution of food, colony size, worker size or predation pressure (Carroll & Janzen 1973; Beckers et al. 1989; Aron et al. 1993; see Laland; 2004 for a discussion of different information use strategies in vertebrates).

Foragers of the common garden ant *Lasius niger* use both pheromone trails and private navigational information acquired during previous foraging trips to locate food sources (Beckers et al. 1990, 1993; Beckers 1992; Aron et al. 1993; Evison et al. 2008). A preliminary study on *L. niger* showed that a substantial proportion of foragers uses private navigational information even if it conflicts with the information provided by a pheromone trail (Aron et al. 1993). Aron et al. (1993) is an important study because, to our knowledge, it is the only study in social insects in which the quality of both types of information were experimentally manipulated. However, the sample size of this study was too small to draw solid conclusions about the effect of these manipulations on decision-making in experienced *L. niger* foragers. Furthermore, their findings raise another question—whether informational conflict affects decision-making speed. In this study, we investigated the extent to which the number of previous foraging trips (variation in private information) and the strength of the pheromone trail (variation in social information) affect decision-making in *L. niger* at a trail bifurcation, when acting as sole or conflicting information source. We then investigated whether the presence of an informational conflict (private information vs. social information) at a trail bifurcation confuses ants and affects the decision-making process. Our results show that even very limited experience with a trail override chemical information, and that the time it takes an experienced ant to make a decision is not affected by the presence of a pheromone trail.

Methods

Study species

We studied *L. niger* collected on the University of Sussex campus. Experimental colonies were housed in plastic foraging boxes (40×30×20 cm high) containing a wooden nest box (15×15×2 cm high). The bottom of each plastic box was covered with a layer of plaster of Paris. The colonies were queenless with 700–1,500 workers and small amounts of brood. Queenless colonies forage, make trails and are frequently used in foraging experiments (e.g. Dussutour et al. 2005; Evison et al. 2008). We fed the colonies 3 times per week with a food mixture made from honey, raw egg and agar (see Hölldobler & Wilson 1990, p. 632) and once per week with dead mealworms, *Tenebrio molitor*. Colonies were given water *ad libitum*.

Experimental set-up

Colonies were deprived of the food mixture for 4-6 days prior to testing to ensure that foragers were motivated to forage and recruit nestmates to a sucrose syrup feeder. The foraging box was connected to a T-shaped foraging trail via a paper (white photo copier paper) bridge. The stem of the trail was 15cm long and each branch was 11cm long (Figure 4.1).

Experiment 1: Memory and trail choice

We studied 6 colonies. A 1.8 molar sugar solution (50% w/w) was offered at the end of one of the T branches (X or Y; Figure 4.1). This high quality food source guaranteed that ants were motivated to collect the offered solution. Foraging ants were marked individually with acrylic colour dots on the abdomen when they were feeding for the first time. To investigate the effect of private information, we tested their choice at the bifurcation after 0, 1, 2, 3 and 4 training visits. The room in which the experiment was carried out contained many visual cues that could serve as landmarks.

When a marked forager returned from the nest we put a fresh piece of paper (without any pheromone) on the branches of the T-shaped trail (Figure 4.1b). In order to exclude any location information transferred during physical contacts between outgoing and incoming ants before testing, we put a small piece of paper in the middle of the trail where two ants might meet to create two corridors, one for each ant. Hence, the focal foragers could use only their own memory, without any additional social information from either trail pheromones or direct

contact in choosing which branch to take. We considered an ant as having chosen a particular branch after she first crossed either the left or right decision line (Figure 4.1b).

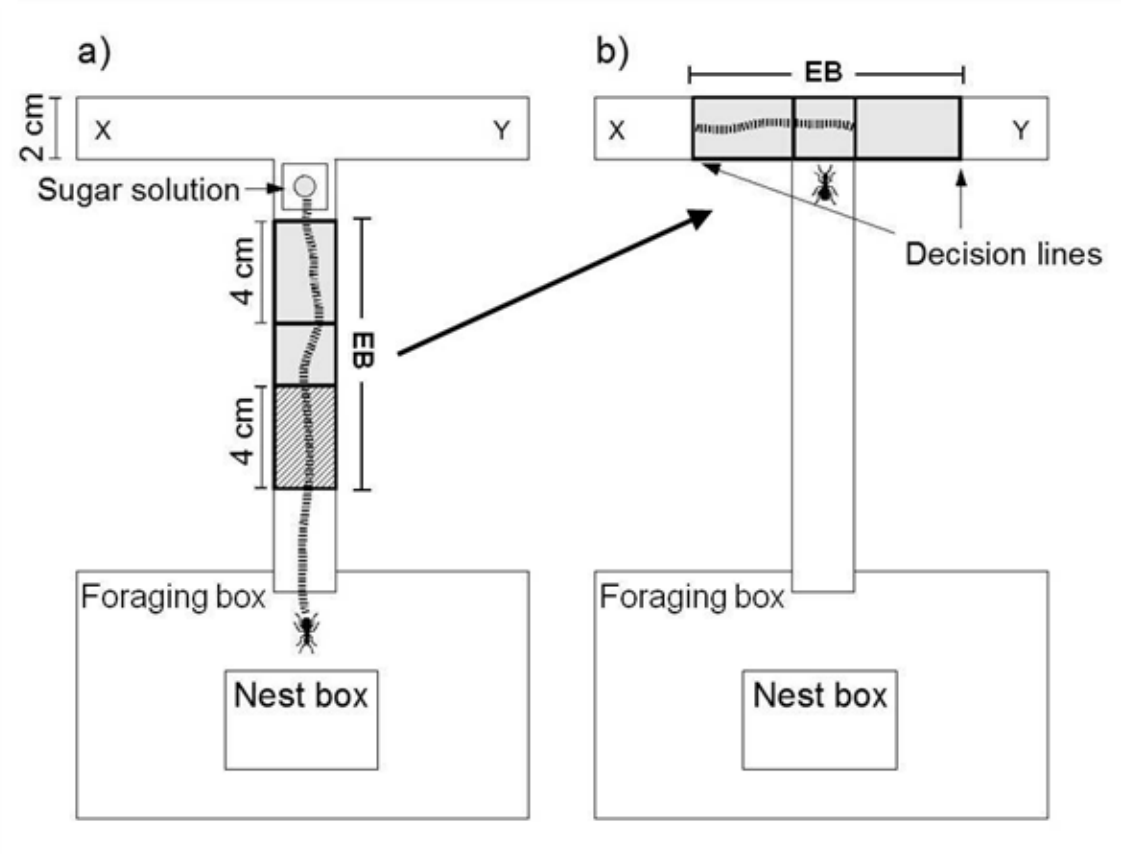

Figure 4.1 - Schematic of the experimental setup used to investigate the effect of private information (memory from previous foraging trips to a feeder on the same side branch) and social information (pheromone trail) on branch choice by outgoing *Lasius niger* forager ants at a trail bifurcation: a trail marking phase. Foragers feed at a drop of 1.8 M sucrose solution and deposit trail pheromone on the experimental branch (EB) when returning to the nest. One part of the EB was masked with another piece of paper. b Testing phase. After a trail of a certain strength had been formed, we moved the food source either to X or Y on one of the branches. Ants collecting sugar solution were marked, and the number of visits recorded. To determine an ant's decision, the piece of paper that masked the hatched part was removed and the EB (grey) was transferred to the junction. An ant was considered to have made a decision when she crossed one of the decision lines. This procedure was used for experiments 2–5.

Experiment 2: Pheromones and trail branch choice

We used 8 colonies to investigate the effect of pheromone trail strength on branch choice. To establish a pheromone trail of a particular strength, we placed a 1.8 M sucrose solution near the bifurcation (see Figure 4.1a). The trail between the food source and the nest was covered with a piece of paper (experimental branch, EB); 10×2 cm; Figure 4.1a). One part of the EB was masked with another piece of paper (4×2 cm; Figure 4.1a). This guaranteed that only the unmasked part of the EB was marked with any trail pheromone being deposited.

The EB was removed after the trail had been established. The time allowed for trail formation was up to 30 minutes but was usually <15 minutes. When trail formation was complete we

removed the food source and all the ants on the trail. The EB was then transferred to the end of the T-shaped trail (Figure 4.1b). The paper that masked one part of the EB was also removed, thereby making two new arms, only one of which was marked with pheromone (Figure 4.1b). For the next 20 minutes we recorded the decisions of foragers leaving the nest and searching for food. Hence, the maximum time of the experiment was 50 minutes. Previous research has shown that trail pheromone persists this long in *L. niger* (Beckers et al. 1993; Evison et al. 2008).

We tested the attractiveness of a branch marked by 1, 5 or 20 ant passages compared to an unmarked branch. A passage is defined as one ant performing at least one pheromone deposition while walking along the EB either towards or away from the nest. A forager *L. niger* does not lay a continuous pheromone trail. Rather, the worker interrupts its walk for a fraction of a second and curves its abdomen vertically to the ground to deposit trail pheromone in a highly stereotyped manner (see Beckers et al. 1992a for more details). One such behaviour was considered a pheromone deposition. We counted both passages and depositions.

Experiment 3: Conflicting information

Seven colonies were used to investigate the relative importance of strong or weak memory versus strong or weak pheromone trail on forager decisions at trail bifurcations. We tested 4 different situations: (i) strong experience (3 visits) versus a strong pheromone trail (≥20 passages or ≥40 depositions), (ii) strong experience versus a weak pheromone trail (1 passage), (iii) weak experience (1 visit) versus a strong pheromone trail and (iv) weak experience versus a weak pheromone trail. Each colony was tested in all four combinations. Pheromone trails and experience treatments were established as described above. We tested a total of 176 ants, 79 encountered food on the left branch (X; Figure 4.1) and 97 on the right (Y; Figure 4.1).

Experiment 4: Control for intra-nest communication

We prevented direct contacts between foragers returning to the nest and focal foragers that were about to be tested as described above. However, it is possible (but has not been shown) that active foragers might also exchange location information inside the nest as in honey bees (*Apis mellifera*; von Frisch 1967). If this occurred, it would confound the results of the conflicting information experiment (previous section). To eliminate this possibility, we did an experiment that repeated the weak experience versus strong trail combination. We worked with only one forager at a time so that no other ant in the colony had information about the

food location. Six different colonies were used and food was offered once on each side for each colony (total 21 foragers, 1-3 foragers per colony and side).

Experiment 5: Time taken to make a decision

Ants walking on the EB (Figure 4.1b) often spent a considerable amount of time walking back and forth, before finally crossing one of the decision lines. To investigate this further, we measured the time ants spent on the EB before crossing a decision line. Measurements were made on ants that had visited the food source once before testing (X or Y; Figure 4.1) and then encountered a strong pheromone trail in the opposing direction (combination iii). The time of walking on the EB until crossing the decision line near the food source was recorded by video and later analysed.

Statistical analysis

We mainly used generalized linear mixed-effect models (GLMM) with a binomial response variable in R 2.9 (R Development Core Team 2009). R fitted the models with the lmer function (Bates et al. 2007). We included colony as a random effect to control for the non-independence of data points from the same colony (Zuur et al. 2009; Bolker et al. 2009). For model selection we used the protocol proposed by Zuur et al. (2009). We first explored the optimal structure of the random components (comparing random intercept models with random intercept and slope models). We then explored the significance of the fixed effects. For most hypotheses we had only one fixed effect, either number of visits (0-4) or trail strength (1, 5 or 20 passages). For the conflicting information experiments we included both pheromone strength (strong, weak) and memory strength (strong, weak) as fixed effects. Wald-tests were used to test the significance of the fixed effects (Zuur et al. 2009; Bolker et al. 2009).

Results

Experiment 1: Memory and trail choice

Figure 4.2a shows that ants rapidly learned the branch leading to food. Even a single visit led to a considerable bias towards the side that had food on the previous trip (74.6%; Figure 4.2a). There was a highly significant difference between naïve ants, (0 previous visits), 52%, and those with just 1 previous visit, 74.6% (Wald Z test: $Z = 3.06$, $P = 0.002$). Most foragers, 95.3%, had learned the location of the food source after 3 trips (Figure 4.2a). The

difference between 1 visit and 3 previous visits is also significant (Z = 2.46, P = 0.014). As shown in Figure 4.2a, there was no further increase from 3 to 4 previous visits.

Experiment 2: Trail strength and trail choice

Ants made an average of 3.09 (N = 122, range 1-8) depositions per passage on the exposed part of the EB. Pheromone that had been laid by just one ant led to a significant increase in the probability of the marked trail being chosen, from 50% for random choice to 61.6% (χ^2 = 6.73, df = 1, P = 0.009). However, increasing the strength of the pheromone trail had relatively little additional effect (Figure 4.2b). A trail with pheromone from 20 ant passages resulted in 70.2% correct choices, some 8.6% higher than a trail of 1 passage. However, this difference was not significant (Z = 1.61, P = 0.107, N = 333).

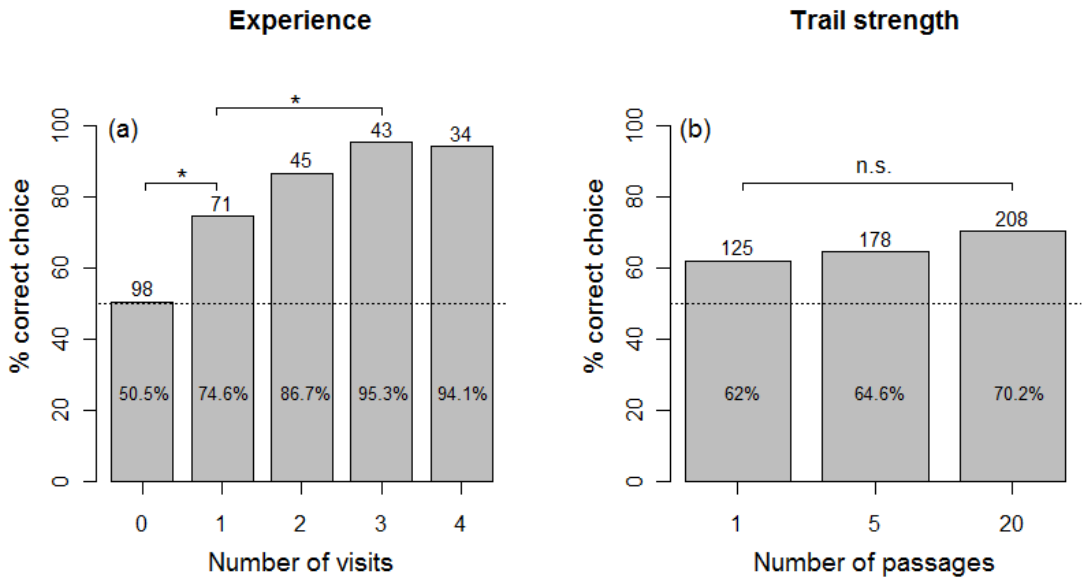

Figure 4.2 - Experiments 1 and 2. The effect of previous foraging experience and trail pheromone strength at a particular branch on trail choice at a T bifurcation. (a) Proportion of ants choosing the branch with syrup after 0-4 previous visits. (b) Proportion of naïve ants choosing the branch with syrup when marked with pheromone by 1, 5 or 20 forager ants (ant passages). The hatched line shows the random expectation, 50%. Numbers above the bars indicate the number of ants tested. Asterisks indicate that bars are statistically different.

Experiment 3: Conflicting information

In all 4 combinations ants relied more strongly on memory than on the pheromone trail (Figure 4.3). After one visit, 84.4% chose an unmarked branch on the side that they had found food on their previous trip versus a branch marked with trail pheromone (situation iii and iv; Figure 4.3). This was actually 10.2% higher than when the alternative branch had zero

pheromone (74.6% Figure 3.2a, 1 visit). However, this difference was not statistically significant (Z = -1.49, P = 0.14). A GLMM analysis investigating all 4 combinations showed no effect of pheromone strength (strong vs. weak) but a significant role of the number of visits (pheromone strength: Z = 0.39, P = 0.69; 1 versus 3 visits: Z = 2.51, P = 0.012; the interaction between both terms was not significant and removed for the final model: Z = 0.004, P = 0.99).

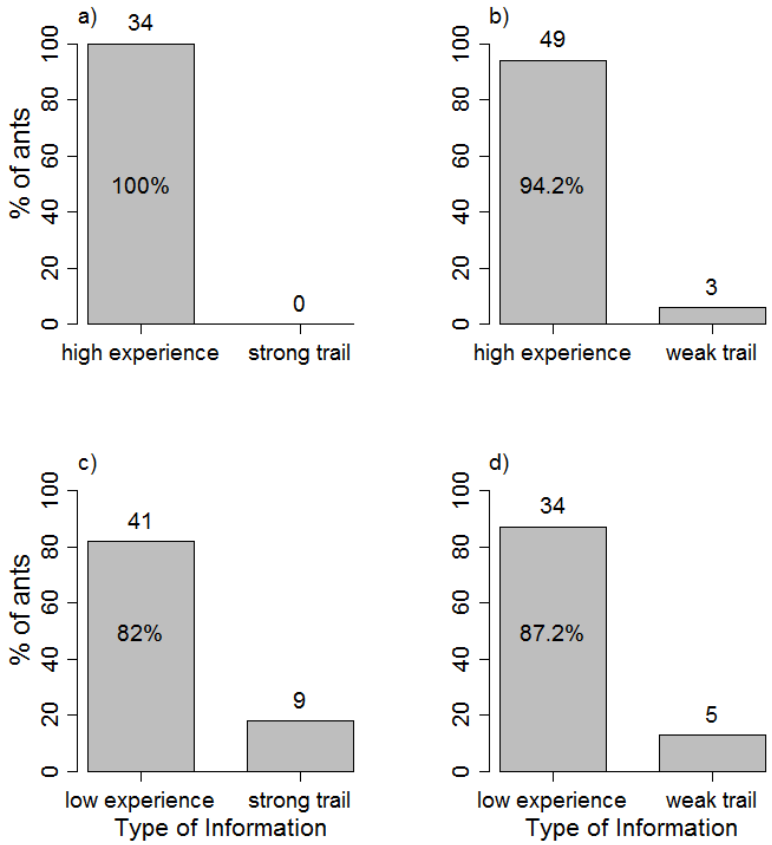

Figure 4.3 - Experiment 3. Branch choice at a trail bifurcation in four combinations of conflicting private and social information. The left bars show the proportions of ants choosing the branch where they had fed previously. The right bars show the proportions choosing the other branch, which was marked with pheromone. (low experience = 1 previous visit; high experience = 3 previous visits, strong trail ≥ 20 ant passages or ≥40 depositions, weak trail = 1 passage).

Experiment 4: Control for intra-nest communication

When testing ants individually in combination (iii) of Experiment 3 (weak experience versus strong pheromone) private information overrides a strong pheromone signal. 90.5% of all ants chose the branch where they found food previously (19 out of 21 ants; χ^2 = 13.76, df = 1, P < 0.001 when compared to the random expectation of equal choice). This confirms that experienced ants rely on private information. This value of 90.5% is again higher than when testing memory alone (74.6%, experiment 1). However, the difference was not statistically significant (Z = -1.48, P = 0.14). It should be noted that the conditions in experiment 1 were slightly different. Experiment 1 was performed some weeks earlier and there were more ants on the trail, hence more disturbances by other ants and the experimenters manipulating the trail. This may have affected the behaviour of ants.

Experiment 5: Time taken to make a decision

The time needed to take a correct decision (cross the decision line near the food source) did not depend on whether there was a conflict between private information and a strong pheromone trail (situation iii; median = 5.9 seconds [1st and 3rd Quartile: 3.54s, 13.2s]) or no pheromone at all (5.7 seconds [4.4s, 13.5s]; Z = -0.6, P = 0.55; Fig. 4.4).

Discussion

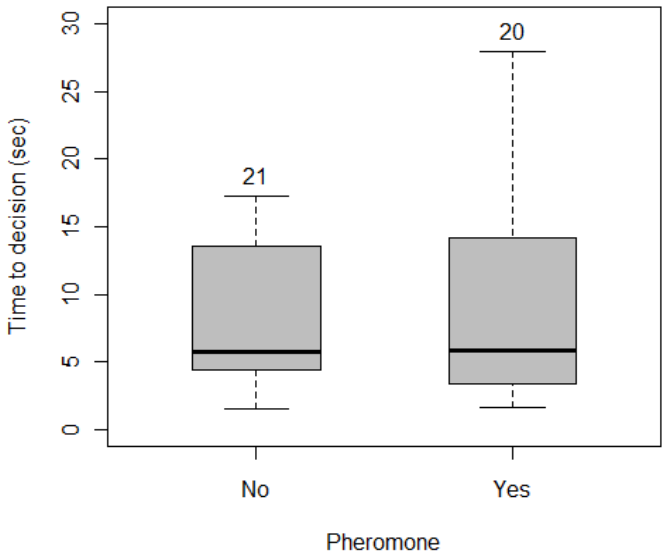

Figure 4.4 - Experiment 5. The time taken by ants between stepping onto the piece of paper on the branches of the T bifurcation (grey part in Fig. 1b) and crossing the decision line. All ants had a low level of experience (1 previous visit to the food source). The grey piece of paper was either unmarked (no pheromone) or strongly marked with trail pheromone (≥ 20 passages or ≥ 40 depositions) was on the opposite branch to the ant's previous experience of feeding side. The box plots show medians, quartiles, 5th and 95th percentiles, and number of ants tested.

Our results show that when an informational conflict occurs, private navigational information (memory) overrides social information (trail pheromone). The effect of memory is very strong, and even a single previous visit has a large effect. Thus, 84% of ants chose a branch unmarked with trail pheromone in the direction that they had previously collected food just one time versus an alternative branch marked with pheromone (combinations iii and iv; Figure 4.3c,d). This increased to 96.5% for ants that had made three previous trips to a feeder consistently on one branch (combinations i and ii; Fig. 4.3a,b). We found no effect of pheromone strength on trail choice. This contrasts to the Argentine ant *Linepithema humile* where the proportion of experienced foragers choosing a marked branch at a trail bifurcation depends on the strength of the pheromone trail in situations of conflicting private and social information (Aron et al. 1993).

When memory and the pheromone trail were tested separately on branch choice we found that memory had a much stronger effect than trail pheromone (Figure 4.2). Even though a small amount of pheromone, the amount deposited by one ant, did have a significant effect on branch choice (62% of correct choices versus the random expectation of 50%), increasing trail strength had a relatively small effect on branch choice (70.2% of correct choices with 20 passages). Hence, a branch that was marked by many more foragers did not have a significantly greater proportion of ants choosing it versus an unmarked branch than a branch marked by only one ant. Aron et al. (1993) found a much stronger effect of the chemical trail (> 95% correct choice; see their figure 1) but did not provide information about trail strengths.

Our results are similar to what has been found in Pharaoh's ants (*Monomorium pharaonis*) where a strong pheromone trail leads to a preference of 70 or 78% for the marked branch (Jeanson et al. 2003) depending on substrate. It has been argued that "wrong" choices at a T-bifurcations can be beneficial if they lead to the discovery of new or better food sources (Deneubourg et al. 1983; Detrain & Deneubourg 2008). The same idea, however, would apply to mistakes made by ants relying on memory. Hence, the "adaptive error" argument does not explain why ants relying on pheromone information make more mistakes than ants relying on memory.

Why did our ants preferentially use private information versus pheromone information? *L. niger* often forages on relatively long-lasting food sources like honeydew producing aphid clusters (Aron et al. 1993). Thus, foraging location may be stable over several days and, therefore, predictable. The foragers we studied learned fast, choosing the correct branch 95% of the time after only 3 previous visits to the food source. Hence, private navigational information seems to be more reliable than pheromone information. Furthermore, ants following a trail often keep their antennae close to the ground, which is likely to reduce walking speed. *L. niger* foragers familiar with a route increased their travelling velocity by about 50% after 5 visits (Mailleux et al. 2005).

These results are not in agreement with the conclusion of Aron et al. (1993) that chemical communication dominates foraging in *L. niger*. This difference may be due to the conditions under which foraging experiments with *L. niger* are often performed. Usually, colonies are given access to one or a few *ad libitum* food sources after several days of starvation and recruitment is studied during a short (< 1.5 hours) initial phase immediately after the food is located (Beckers et al. 1990, 1992a, 1993; Aron et al. 1993; Mailleux et al. 2005; this study). This is different from the natural situation, discussed above, where individual

ants may collect food for days or even weeks at the same food locations. Hence, while a colony is exploiting various carbohydrate food sources simultaneously, the majority of individual foragers may show attachment to particular locations and use route memories to relocate these. This also seems to be the case in other ant species including *L. fuliginosus* (Quinet & Pasteels 1996), *Formica* wood ants (Salo & Rosengren 2001) and also honey bees (Ribbands 1949; Grüter et al. 2008; Grüter & Farina 2009). Trail pheromone probably marks the entire active foraging system and may have a major role in helping naïve ants to locate profitable food sources. In addition, trail pheromone could help experienced foragers to travel faster along trails, or to mark out the colony's territory as occurs in the closely related species *L. neoniger* (Traniello 1980). On the other hand, home range markings in *L. niger* (Devigne & Detrain 2002) and trail pheromones in *Lasius fuliginosus* (Hangartner 1967) do not seem to be colony specific. More research testing the roles of the pheromone trail for experienced foragers is needed.

During Experiment 3 we had noticed that ants sometimes made U-turns directly after the bifurcation or they spent some time on the T before crossing the decision line, so we specifically tested whether a conflict between the pheromone trail and private navigational information somehow confused the ants and made the decision-making process slower. However, we found no difference in the time taken by individual ants to make a decision when faced conflicting private and social information versus ants only having private information. This is further evidence that ants with navigational information seem to be little affected by the presence of pheromones at trail bifurcations in our experimental set-up.

Our results raise the question of the role of trail pheromones in *L. niger*, and in particular whether they may play a more important role under different circumstances. One possibility is that increasing trail complexity affects decision-making strategies, such as if the route to a food source is difficult to learn because it has many bifurcations. This would correspond to a *copy when uncertain strategy* (Laland 2004). Similarly, if a food source is not particularly good or is far away, ants might be more affected by a strong pheromone trail (corresponding to the *copy when dissatisfied strategy* (Laland 2004). Research on factors affecting the adaptive use of social and private information has revealed considerable behavioural plasticity in vertebrates (reviewed in Laland 2004; Kendal et al. 2005). Social insects are ideal models to test the circumstances that favour the use of social or private information (see e.g. Leadbeater & Chittka 2007, 2009; Grüter et al. 2008). Our results also highlight the need to combine laboratory experiments with field studies. We can only understand the behaviour of foragers in the light of information on foraging ecology, including

the spatiotemporal availability of food sources, the proportion of foragers with field experience, their constancy to natural food sources and the role of competition with other ant colonies of the same or other species.

Acknowledgements

We thank Thomas Durance and Lucy Taylor for help with data collection. C.G. was supported by a postdoctoral fellowship from the Swiss National Science Foundation (SNSF grant PBBEP3-123648). T. C. was supported by a PhD studentship from the BBSRC.

Chapter 5: Synergy between social and private information increases foraging efficiency in ants

Tomer J. Czaczkes, Christoph Grüter, Sam M. Jones & Francis L.W. Ratnieks

Abstract

Insect societies integrate many information sources to organise collective activities such as foraging. Many ants use trail pheromones to guide foragers to food sources, but foragers can also use memories to find familiar locations of stable food sources. Route memories are often more accurate than trail pheromones in guiding ants, and are often followed in preference to trail pheromones when the two conflict. Why then does the system expend effort in producing and acquiring seemingly redundant and low quality information, such as trail pheromones, when route memory is available? Here we show that in the ant *Lasius niger* trail pheromones and route memory act synergistically during foraging, increasing walking speed and straightness by 25% and 30%, respectively, and maintaining trail pheromone deposition, but only when used together. Our results demonstrate a previously undescribed major role of trail pheromones: to complement memory by allowing higher confidence in route memory. This highlights the importance of multiple interacting information sources in the efficient running of complex adaptive systems.

Introduction

The integration of multiple information sources is necessary for the functioning of adaptive biological systems at cell, organ, organism and society levels (Camazine et al. 2003; King 2009; Bro-Jørgensen 2010; Cahill et al. 2010) and also in technological systems (Tanenbaum 2003). The successful coordination of the many individuals in a colony of social insects typically involves the gathering and transfer of information from several sources (Robinson et al. 2005; Leadbeater & Chittka 2007; Ratnieks 2008). Workers may gain information either by interacting with their environment (private information) or by interacting with their nestmates (social information)(Kendal et al. 2005; Dall et al. 2005).

During foraging workers commonly transfer information in order to enhance the ability of the colony to forage efficiently in a constantly changing environment. Honey bees, for example, use the waggle dance to inform nestmates about the location of food patches in the environment (von Frisch 1967) whereas many ants use trail pheromones. Pheromone trails, however, do not provide perfect information and naïve foragers frequently make mistakes when using trail pheromones at trail bifurcations (Deneubourg et al. 1983; Harrison et al. 1989; Jeanson et al. 2003; Grüter et al. 2011). Grüter et al (2011) found that in *Lasius niger* only 70% of foragers chose a trail with high levels of trail pheromone at a bifurcation. Route memories, on the other hand, may be acquired rapidly leading to 95% correct choices by *L. niger* workers at a bifurcation after only 3 previous visits to a food source on one branch (Grüter et al. 2011).

In situations where these information types conflict, many species follow route memories in preference to trail pheromones (Lubbock 1884; Rosengren & Fortelius 1986; Beugnon & Fourcassie 1988; Harrison et al. 1989; Grüter et al. 2011), as do honey bees when social and private information conflicts (Grüter et al. 2008). Aphid tending ants may return to stable food sources for days, weeks or months (Rosengren & Fortelius 1986; Quinet & Pasteels 1996; Salo & Rosengren 2001). Why then do ants continue laying pheromones on a trail past the initial recruitment phase when they have more reliable route memories? One possibility is that pheromones are only used by naïve ants. However, this does not explain why pheromone deposition continues past the initial recruitment. Could pheromones interact with route memory to play a yet unknown function for the vast majority of foragers which are travelling along familiar routes? We propose an alternative hypothesis that trail pheromones may complement route memory. Foraging ants may be using the trail pheromone as a reassurance marker, like a white line on a road; allowing ants to increase travel speed without sacrificing trail following accuracy. Ants could travel faster and straighter, safe in the knowlage that if

they do stray from the path the lack of pheromone trails will alert them to this, and allow them to take remedial action. Foraging efficiency could thus be increased on familiar trails. We therefore tested whether the presence of trail pheromone causes an increase in walking speed and path straightness, and a decrease in the rate of U-turning, in both experienced and naïve ants.

Materials and methods

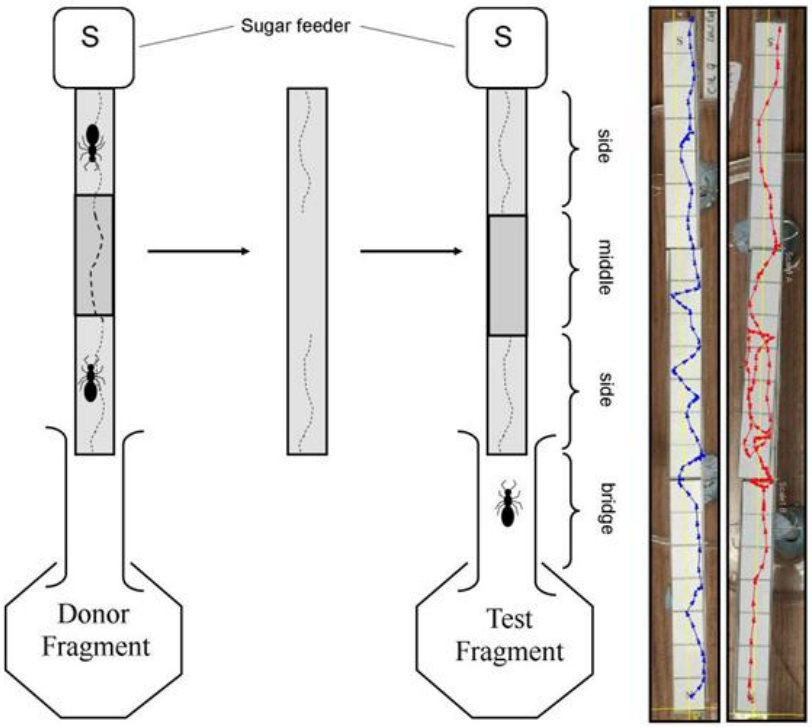

Figure 5.1 - Apparatus used to create a walkway marked by trail pheromone. An ant colony is split into two equal fragments. The donor fragment is allowed access to a 21cm long walkway covered in printer paper with a 1 molar sucrose feeder at one end. The middle 7cm section has an additional paper overlay. Once either 1 or 20 ants had deposited pheromone at least once on the middle section, the 7cm overlay is discarded and the 21cm walkway cleared of ants. This was then used to replace the walkway in the test fragment colony. Simultaneously, the test ants are also visiting a sucrose feeder at the end of a 21cm walkway. Ants are marked individually at the feeder and allowed to make 1 or 3 return visits. The trail pheromone marked walkway from the donor colony is then used to replace the original walkway. A fresh 7cm paper overlay is placed over the middle section to mask any possible marks, and discarded and replaced every time an ant walks over it. On the right are 2 representative paths of an experienced ant returning to the feeder. The blue path (left) is of an ant with one previous visit to the feeder. The red path (right) is an ant with three previous visits to the feeder. Both paths are outbound walks on a heavily pheromone marked walkway.

4 queenless colonies of *Lasius niger* were divided into 2 fragments each. Workers from one fragment – the donor fragment – laid a pheromone trail on paper substrate on which workers from the other fragment – the test fragment – were studied. To obtain the pheromone trail, donor fragment ants were allowed access to a 21cm x 1cm walkway overlaid with office printer paper leading to a 1M sucrose syrup feeder (see Figure 5.1). The middle of the walkway was overlaid with a 7cm long strip of paper on which pheromone laying behaviour could be observed. 7cm was used as it was the longest stretch in which pheromone depositions could reliably be observed and counted. Pheromone laying behaviour in *L. niger* is very characteristic, with the ant pausing momentarily and moving the body backwards while touching the tip of the gaster to the substrate (Beckers 1992). We used pheromone deposition on the middle section as a proxy of pheromone levels on the side sections as it proved impossible to reliably observe two sections simultaneously. We observed workers collecting syrup at the feeder and returning to the nest until a) 1 ant or b) 20 ants had performed at least one pheromone deposition on the 7cm overlay. This gave us weak and strong pheromone trail sections, which were used as treatments for the test fragment colony.

The last ant to deposit pheromone was allowed to leave the walkway before the walkway was disconnected and all other ants removed.

Simultaneously with the above procedure on the donor colony, ants from the test colony were allowed access to a separate 21cm walkway overlaid with fresh printer paper. Up to 8 ants were allowed to reach the feeder, after which additional ants were prevented from accessing the walkway. Each ant was individually marked on the abdomen with a dot of acrylic paint while ingesting syrup. These marked ants were then allowed either to make one (low memory) or three (high memory) return journeys to the feeder before being tested on their next visit to the feeder. If an ant had attained the required number of visits before the pheromone trail was ready, it was gently brushed from the bridge back into the nest box, or the bridge lifted up so that it could not climb on, until we were ready to test the ants. Ants rarely had to wait more than 3 minutes before testing. All experiments were performed in a room with both natural and artificial light, bright walls and ceilings and many different available landmarks.

The test fragment ants were presented with a walkway covered by trail pheromone from the donor ants in which the overlay in the middle 7cm section had been replaced by a fresh overlay, resulting in three distinct 7cm sections: the nest-side and the feeder-side sections both marked with trail pheromone with an unmarked middle section. The behaviour of the marked ants was recorded as they walked to the feeder and back. Every time an ant walked over the middle section the overlay was replaced with clean paper ensuring that it was always totally unmarked. In addition to the marked ants, up to 5 naïve test ants (no memory) were also allowed to walk onto the apparatus via the bridge.

To test for possible effects of home range markings (Devigne et al. 2004), we ran an experiment presenting naïve and low memory test ants with a walkway covered only by home range markings but without trail pheromone. To obtain substrate covered in home range markings, several 21x1cm paper strips were placed within the nest box of the test colony for 20 hours prior to the control experiment, allowing unfed ants to walk over them thereby marking them with home range markings but without laying trails to specific feeding locations. As above, ants were individually marked at the feeder. On their second outward journey the original walkway was overlaid with a 21x1cm home-range-marked paper strip with the middle 7cm section overlaid with a fresh strip of paper to give 2 side sections bearing home range markings (but no trail pheromone) and a middle section which was free of all markings. Whenever an ant laid pheromone onto the 21cm overlay the overlay was discarded and

replaced with a new piece marked only with home range markings. After any ant walked over the middle 7cm section overlay it was replaced by a fresh overlay.

In this way we could designate each ant as being subject to one of four general information combinations: both trail pheromone and route memory, only trail pheromones, only route memory, or no information other than home range markings, which were present in all treatments. Each combination with memory or pheromone can further be divided into high or low memory and strong or weak pheromone. In all cases home range markings were present.

In all experiments, behaviour was recorded using a high definition video camera pointing vertically down on the walkway. A mirror parallel to the walkway and tilted at 45 degrees also allowed a side view so that pheromone laying behaviour could be observed. Videos were analysed frame-by-frame using the programmes Virtualdub (Lee 1998) and Videopoint (Luetzelschwab et al. 1997). The numbers of pheromone depositions and U-turns performed by each ant on each sector were recorded. U-turns were defined as a turn of 180° followed by walking in the opposite direction for at least 1cm. The position of an ant's thorax was marked every 0.1 second yielding a series of coordinates. From these positions change in direction in degrees per 0.1 second could be calculated. In addition, we calculated the ant's average speed per 0.1 second on each 7cm section. Speed values were corrected by allowing 0.2 seconds for every pheromone deposition, during which time an ant is stationary.

Statistical analysis

We analysed the data using a generalized linear mixed-effect models (GLMM) (Bates et al. 2007) in the statistical analysis software R2.9 (R Development Core Team 2009). Models were fitted using either the lmer or lm function (Bates et al. 2007). We included 'colony' and 'ant' as random effects to control for the non-independence of data points from these sources (Zuur et al. 2009; Bolker et al. 2009). Data from 'nest-side' and 'sugar-side' sections were tested for differences using GLMMs. If no significant differences were found, data were pooled into a 'side section' group. Where significant differences were found, comparisons between side sections and the middle section were carried out separately.

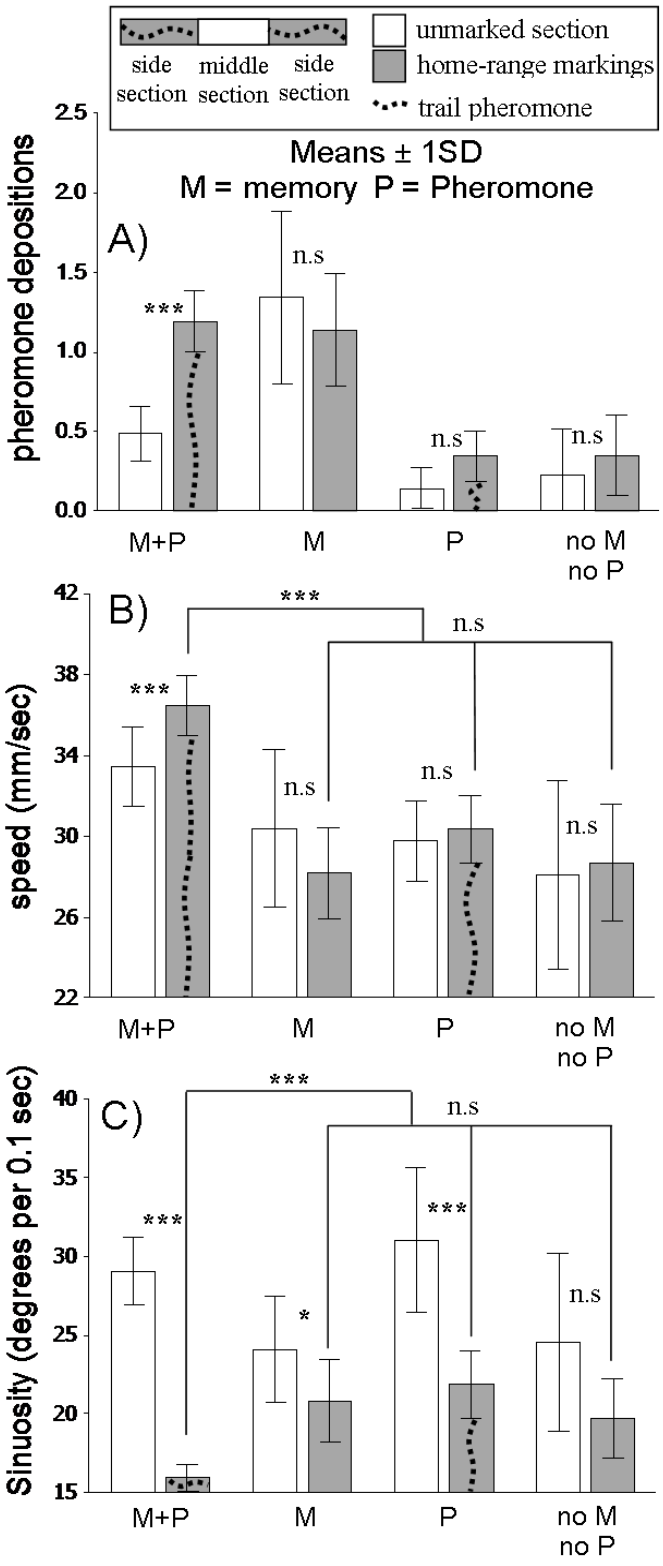

Figure 5.2 - Comparing behaviours on middle and side trail sections. Side trail sections always have home range markings plus trail pheromones where indicated. Middle sections are always unmarked. Pheromone laying reduced when experienced ants step off a pheromone-marked section (A). Walking speed increased and sinuosity decreased when experienced ants walk on substrate marked with pheromone (B & C). M = route memory P = side sections marked with trail pheromone. Annotations refer to significance level (GLMM, *** = p < 0.001, ** = P < 0.01, * = P ≤ 0.05, n.s = P > 0.05). Thick connecting lines present statistical comparisons. Whiskers on bars represent 2 standard deviations.

Results

We found that experienced ants walked 30% faster and 30% straighter on walkways marked by trail pheromone versus unmarked walkways, 20% faster and 37% straighter than naïve ants walking on pheromone-marked walkways, and 29% faster and 24% straighter than naïve ants walking on unmarked walkways (GLMM, speed; all comparison t > 4. 00, P < 0.001, sinuosity; all comparisons t > 2.88, P < 0.003)(see table 5.1 and figure 5.2b and c). This shows that there is a synergistic interaction between the two sources of information, as no increase in walking speed or straightness occurred when only one of the information sources was present. Ants with route memory that step off a pheromone marked path also reduce their trail pheromone deposition rate significantly, by 59% (see table 5.1 and figure 5.2a). This explains why previous authors (Breton & Fourcassie 2004) report no effect of trail pheromone on path sinuosity and speed in *Lasius niger*: only in combination with route memory do these effects occur. Stepping off a

path marked with trail pheromones causes an increase in U-turning rates regardless of the presence of route memory (GLMM, Z > 2.78 P < 0.01, see table 5.1).

The strength of the trail pheromone (1 ant passage or 20 ant passages) had no effect on trail pheromone deposition rates (GLMM, t=0.035, P > 0.5), U-turning rates (GLMM, t = 1.24, P > 0.5), walking speed (GLMM, t=1.39, P > 0.05) or path sinuosity (GLMM, t=0.101, p > 0.5). This suggests the presence of trail pheromone releases an all-or-nothing effect in foraging ants, and agrees with previous research *on L. niger* in which more heavily marked trails did not lead to greater accuracy in trail choice at a T junction (Grüter et al. 2011).

	Trail pheromone deposition rate	U-turning rate	Walking speed	Path sinuosity
Only home range markings	**No** difference z =0.758 p = 0.45	**fewer U-**turns on the sides z = 2.778 p = *0.005*	**No** difference t = 0.237 p > 0.05	**No** difference t = 1.77 p > 0.05
pheromone & home range markings	**No** difference z = 1.756 p = 0.08	**fewer U-**turns on the side z =5.312 p < *0.001*	**No** difference t = 0.543 p > 0.05	**straighter** path on the sides t =5.83 p < 0.001
Memory & home range markings	**No** difference z= 0.924 p = 0.36	**No** difference z =1.087 p = 0.28	**No** difference t =1.449 p > 0.05	**straighter** path on the sides t = 2.39 p <0.05
Pheromone, memory & home range markings	**more** laying on the sides t =4.869 p < 0.001	**fewer U-**turns on the side z = 6.503 p < 0.001	**faster** walking on the sides t = 3.111 p < 0.005	**straighter** path on the sides t = 15.24 p < 0.001

Table 5.1 - Is there a difference between behaviour on the middle section lacking in trail pheromone and home range markings and the side sections, which may be marked with either home range markings and trail pheromones or only home range markings? Tests are GLMMs (see methods section for details).

Experience level, home-range markings, and direction of travel also affected ant behaviour. Ants that made three visits to the feeder performed fewer U-turns (GLMM, z=2.837, P < 0.01) and walked faster (GLMM, t=4.89, P < 0.01) than ants that made only one visit to the feeder, an effect which is present even when controlling for pheromone level effects and direction of travel. We also found significant interactions between direction of travel and position on the walkway on U-turning rates: While the vast majority of U-turns on a pheromone-marked trail occur in the middle sector lacking in pheromone, significantly more U-turns occur on the first sector the ant enters (GLMM, z = 2.837, P <0.01). Thus, ants leaving the nest are more likely to U-turn on the nest side sector than the sector near the feeder, and vice-versa for ants leaving the feeder. Seen another way, we can consider the ants on the far sector from their origin to have committed to their direction of travel. Likewise for walking

speed on trails marked only by home-range markings, ants walked faster on the sector furthest from their origin (GLMM, t=2.898, P <0.001), implying a commitment to their direction of travel. Our study also demonstrates a role of home range markings for foragers. Home range markings are small amounts of cuticular hydrocarbons laid down passively as ants walk on substrate, and may inform ants of how heavily a location is frequented by their sisters or other ants. Ants on paths marked only by home range markings deposit less trail pheromone on their outward journey, and more trail pheromone on their return journey (GLMM, z= 3.984, P <0.001)(see also (Devigne et al. 2004; Devigne & Detrain 2006). On unmarked paths ants show an intermediate deposition rate on both outgoing and returning journeys (GLMM, z = 0.696, p = 0.486, see figure 6.1 in chapter 6).

Discussion

The presence of trail pheromones seems to "reassure" experienced foragers that they have not strayed from the trail. This allows a reduced investment in error checking, leading to increased speed until a lack of trail pheromone indicates that they have strayed. In real terms, ants can walk faster and straighter, relying on the lack of pheromones to inform them that they have strayed from the trail. The trade-off between speed and accuracy is a common problem for many animals (Chittka et al. 2009). Here, with the presence of trail pheromone information reassuring the foragers, the need to make this trade-off can be lessened by allowing ants to increase foraging speed without sacrificing accuracy. The reduction in pheromone deposition shown by experienced foragers when they step off a marked path will also have the effect of maintaining path integrity, avoiding erroneous informational cascades (Bikhchandani et al. 1992) by ensuring that ants that do make an error will not compound this error by marking false paths with trail pheromone. But why require a route memory for the cessation of pheromone deposition when suddenly leaving a pheromone marked path? We suggest this cessation does not occur on the first return trip from the feeder so as to allow the formation of new continuous trails by ants on their first return journey whilst maintaining trail cohesiveness of established trails.

A distinction should perhaps be made between stepping off the trail and being off the trail. These may be responded to differently by the ants. In this experiment, there did not seem to be any difference in the behaviour of ants soon after they stepped onto an unmarked section from a marked section (see figure 5.1), although no formal analysis was conducted. Thus, we could perhaps consider the behavioural changes reported here to be in response to

'stepping off the trail'. However, if the non-marked sections were longer we would perhaps see a change in behaviour further down the unmarked path – a response to 'being off the trail'. This question is amenable to future study.

We suggest that outgoing ants on heavily home range marked paths reduced trail pheromone deposition as they had some evidence (heavy home-range marking) that the food source may be overexploited. On their return journey, the ants knew that the food source was not overexploited, and had evidence (heavy home range markings) that the route is safe, and so a good food source to exploit and recruit foragers to. Home range markings also reduce U-turning rates, an effect which is additive with the reduction of U-turning rates caused by trail pheromones.

It is often assumed that social insect foragers have to decide between social information and memory (Kendal et al. 2005; Leadbeater & Chittka 2007). Our results show that the combination of these seemingly mutually exclusive information sources leads to the emergence of adaptive properties in the colony's foraging system, and lead to further questions concerning interactions between information sources in insect societies and other complex adaptive systems.

Acknowledgements

We thank Thomas Collett for comments on a previous version of this manuscript. TC was supported by a PhD studentship from BBSRC. GC was funded by a postdoctoral fellowship from the Swiss National Science Foundation (SNSF grant No: PA00P3 129134). SJ was funded by a Sussex University GTP studentship.

Chapter 6: Uncovering the complexity of ant foraging trails

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(Written as an addendum to Czaczkes, T.J., Grüter, C., Jones, S.M. & Ratnieks, F.L.W. Synergy between social and private information increases foraging efficiency in ants. Biology Letters 2011)

Abstract

The common garden ant *Lasius niger* use both trail pheromones and memory of past visits to navigate to and from food sources. In a recent paper we demonstrated a synergistic effect between route memory and trail pheromones: the presence of trail pheromones results in experienced ants walking straighter and faster. We also found that experienced ants leaving a pheromone trail deposit less pheromone. Here we focus on another finding of the experiment: the presence of cuticular hydrocarbons (CHCs), which are used as home range markers by ants, also affects pheromone deposition behaviour. When walking on a trail on which CHCs are present but trail pheromones are not, experienced foragers deposit less pheromone on the outward journey than on the return journey. The regulatory mechanisms ants use during foraging and recruitment behaviour is subtle and complex, affected by multiple interacting factors such as route memory, travel direction and the presence trail pheromone and home-range markings.

The foraging behaviour of ants, with its interplay between the individuals and the group, plays an important role in the study of self organisation and the emergent behaviour of complex systems (Camazine et al. 2003; Dorigo & Stützle 2004), and has inspired the well known metaheuristic Ant Colony Optimization (ACO) (Dorigo & Stützle 2004; Dorigo & Di Caro 1999; Mullen et al. 2009). However, in ACO foraging ants are usually considered to utilize a rather simple set of behavioural rules (Dorigo & Di Caro 1999; Beckers et al. 1990; Ratnieks 2008) often limited to simply "If you find food, return to the nest laying trail pheromone" and "preferentially follow trails with more pheromone" (Hangartner 1969b; Beckers et al. 1990; Dorigo & Di Caro 1999). Subsequent study of ant foraging has uncovered further foraging rules and properties of the pheromone trail network (Ratnieks 2008). For example, Pharaoh's ant deposit two types of attractive trail pheromone: a short-lived pheromone that decays within 20 minutes and a longer lasting pheromone that acts as an external long term memory, allowing colonies to re-use trails laid one or two days previously (Robinson et al. 2008b). They also deposit repellent pheromones on branches leading to depleted food sources (Robinson et al. 2005). In the ant *Lasius niger*, rules such as "Deposit more pheromone when food quality is higher" (Beckers et al. 1993), "Deposit more pheromone if the colony is starving" (Mailleux 2006) and "Deposit more pheromone the closer you get to the food source" (Beckers et al. 1992a) have been uncovered. However, foraging ants do not rely solely trail pheromones. *L. niger* and other ants can form accurate route memories after just a few visits to a food source (Rosengren & Fortelius 1986; Fourcassie & Beugnon 1988; Grüter et al. 2011), and these route memories are followed in preference to trail pheromones when in the two conflict (Lubbock 1884; Fourcassie & Beugnon 1988; Harrison et al. 1989; Grüter et al. 2011).

In a recent paper (Czaczkes et al. 2011a), we allowed *L. niger* foragers which had already made several trips to a feeder to walk along a walkway with alternating segments marked and unmarked by naturally-laid trail pheromone. We found that the two information sources, route memory and trail pheromone, interact. Experienced ants use the presence of trail pheromone as what we termed 'reassurance' that they are on the correct path. Reassured, the ants walk faster and straighter. If, by chance, they do make an error and step off the path, they reduce speed, walk more sinuously, and perform more U-turns. We suggested that this might help them to get back on the right path. Furthermore, we showed that ants with a route memory greatly reduce the amount of pheromone they deposit, which we quantified by counting the number of times they dot the tip of their abdomen on the substrate, when they step off the marked path. This represents another rule used by ants for

modifying pheromone deposition: “Reduce pheromone deposition if you step off a pheromone trail and have been to the food source before”. Presumably, this reduces the likelihood that nestmate ants will be diverted down the wrong path, so maintaining trail integrity and prevents an error cascade.

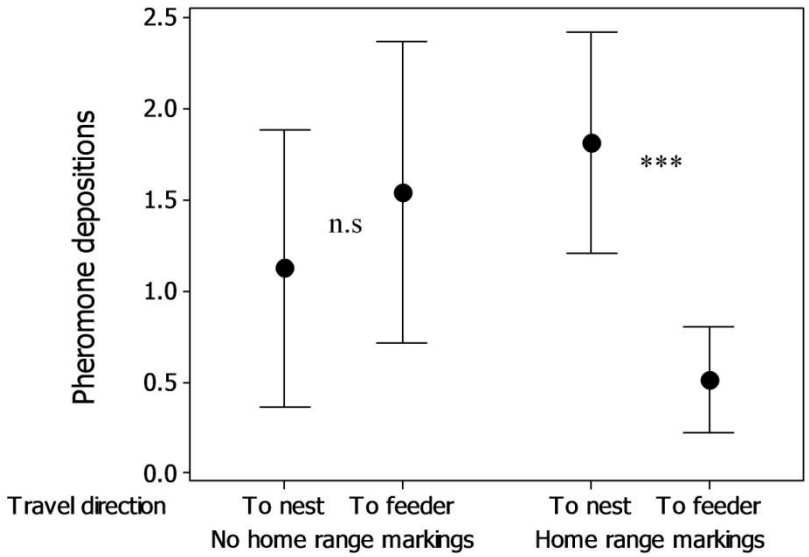

Figure 6.1 - Number of pheromone depositions by experienced ants either travelling towards the feeder or returning to the nest source on a 7cm trail section either marked or unmarked with home range markings (see chapter 4). When home range markings are present, outgoing ants deposit significantly less pheromone than returning ants (Generalised Linear Mixed-Effects Model, z= 3.984, P <0.001). When home range markings are absent, pheromone deposition rates are not different between outwards and return journeys (z = 0.696, p = 0.486) (data from chapter 5).

However, the complexity found in this experiment extended further than the interaction between trail pheromones and memory; the ants also changed their behaviour in the presence of home range markings. Home range markings in *L. niger* consists of cuticular hydrocarbons (CHCs) secreted from tarsal glands on the feet (Yamaoka & Akino 1994; Lenoir et al. 2009) and are passively deposited on surfaces that ants walks over (Lenoir et al. 2009). They are non-volatile, long lasting, and unlike trail pheromones, which lead to specific locations, CHCs are considered to be home range markings. Due to heavier ant traffic closer to the nest, a CHC gradient forms from the nest entrance outwards, defining the areas frequently visited by the colony’s foragers (Devigne & Detrain 2006). Ants can sense CHCs on a surface and on other ants.

The presence of CHCs on the substrate increases aggression levels (Devigne & Detrain 2002) and reduces food discovery time (Devigne et al. 2004) and walking sinuosity (Devigne et al. 2004) in *L. niger* and has also been shown to increase pheromone deposition on the first return to the nest (Devigne et al. 2004; Devigne & Detrain 2006). However, by observing ants making repeated trips to a feeder, we found that this was only half the story. When walking on a substrate with home range markings but without trail pheromone, experienced ants lay less pheromone on outward journeys to a food source, and deposit more pheromone on the return

journey (see figure 6.1). When home range markings are not present, deposition on both the outwards and return journey is of intermediate intensity. In other words, the ants seem to have a further rule modifying pheromone deposition intensity: “If returning to a feeder on a home range marked path, deposit less pheromone”.

Sensing that a trail is heavily marked by CHCs on an outward journey but unmarked by trail pheromones may indicate that the food source has been heavily exploited, and may now be depleted. In that case it would make little sense to increase recruitment of foragers on the outwards journey, as the food source may be depleted. However, on the return journey, when the ant knows there is food at the end of the trail, the colony would benefit from further recruitment to this location. Indeed, a high level of CHCs suggests that this food source was visited frequently in the past, so is not only productive but also (if no alarm pheromone is present) safe. Whilst these explicit arguments are most likely not considered consciously by the ants, the behavioural rules with which ants are equipped suggest a complex and subtle tuning of recruitment behaviour, based on multiple information sources.

A picture is emerging of great complexity in the rules affecting foraging and recruitment in *L. niger*. Individual ants are equipped with many rules governing their behaviour, and alter their behaviour depending on multiple factors including, but no doubt not limited to, trail pheromone presence, home range marking presence, travel direction and experience level, and the interactions between these information sources. This mirrors work uncovering similar sophistication in the communication of honey bees, which have at least four mechanical signals and two pheromones which affect foraging (Seeley 1998; Pankiw 2004; Thom et al. 2007), and foraging in Pharaohs ants, which have multiple trail pheromones and can even extract information from the geometry of the trail system (Jackson et al. 2004; Robinson et al. 2005, 2008b). Multiple signals and information sources seem to be the rule in natural complex systems such as ant foraging, and we predict that by studying individual foragers over multiple foraging trips more such rules might emerge. Progress is being made in understanding the intricate rule sets ants use when foraging, but we are still far from a complete understanding of the system. Uncovering new behavioural rules may inspire development of next generation ACO logic systems (Ratnieks 2008). After all, if so much can be built on basic behavioural rules uncovered over half a century ago, the application of current and future findings may provide a great step forward.

Chapter 7: Ant Foraging on Complex Trails: Route Learning and the Role of Trail Pheromones in *Lasius niger*

Tomer J. Czaczkes , Christoph Grüter, Laura Ellis, Elizabeth Wood & Francis L. W. Ratnieks

Abstract

Ants are central place foragers and use multiple information sources to navigate between the nest and feeding sites. Individual ants rapidly learn a route, and often prioritize memory over pheromone trails when tested on a simple trail with a single bifurcation. However, in nature ants often forage at locations which are reached via more complex routes with multiple trail bifurcations. Such routes may be more difficult to learn so that ants benefit from additional information. We hypothesized that trail pheromones play a more significant role in ant foraging on complex routes, either by assisting in navigation, or route learning, or both. We studied *Lasius niger* workers foraging on a doubly-bifurcating trail with 4 endpoints. Route learning was slower and errors greater on alternating (e.g. left-right) versus repeating routes (e.g. left-left) - error rates 32% and 3%, respectively. However, errors on alternating routes decreased by 30% when trail pheromone was present. Trail pheromones also aid route learning, leading to reduced errors in subsequent journeys without pheromone. If an experienced forager makes an error when returning to a food source, it reacts by increasing pheromone deposition on the return journey. In addition, high levels of trail pheromone suppress further pheromone deposition. This negative feedback mechanism may act to conserve pheromone or to regulate recruitment. Taken together, these results demonstrate further complexity and sophistication in the foraging system of ant colonies, especially in the role of trail pheromones and their relationship with learning and private information in relation to the challenges of foraging in a complex environment.

Introduction

Central place foragers find their way back to their nest or roost by a variety of mechanisms, including by keeping track of their location (path integration) (Collett & Collett 2002; Collett et al. 2003), by depositing a pheromone trail which can be retraced (Cook 1971), or by navigation (Wallraff 2010). Many central place foragers are social and also communicate information that assists other individuals in locating the nest or a feeding site. Examples include primates (Dittus 1984), Lepidoptera larvae (Fitzgerald & Peterson 1983), and eusocial insects such as bees and ants (von Frisch 1967; Hölldobler & Wilson 1990).

For the first few visits to a food source, foraging bees and ants keep track of their location using path integration, allowing them to return directly to the nest from the position they find themselves in (Collett & Collett 2002). As foragers gain more experience they begin to use information gathered during orientation flights (Bees and wasps) (Lehrer 1991; Zeil 1993) or U-turns (ants) (Judd & Collett 1998; Nicholson et al. 1999) to guide themselves back to the nest by matching stored images, such as of the view around their nest, with their current view. Eventually, this is superseded by a series of snapshot images acquired en-route to the goal, with each image eliciting a specific behaviour which brings the individual to the next image and the beginning of the next segment (Collett & Cartwright 1983; Judd & Collett 1998; Graham & Collett 2006). Such route memories can be very accurate in both honey bees (Menzel et al. 2011) and ants (% correct choices at a single bifurcation >90% *Formica rufa* (Rosengren & Fortelius 1986), 95% *Formica lugubris* (Fourcassie & Beugnon 1988), 95% *Lasius niger* (Grüter et al. 2011), 97% *Lasius flavus* (Jones et al, in prep).

The location of resources is often communicated to nestmates, by waggle-dances in honey bees *Apis mellifera* (von Frisch 1967) or by trail pheromones in many ants and some bees and termites (Lindauer & Kerr 1958; Wilson 1972; Nieh 2004). One benefit of such communication is that the information communicated can guide naïve nestmates to a resource such as a feeding site. However, for experienced individuals communication may result in a conflict between an individuals' private information in the form of memory and the social information supplied by nestmates, such as trail pheromone. When a conflict arises, it seems that private information is often prioritized (Grüter et al. 2011; Harrison et al. 1989; Fourcassie & Beugnon 1988; Grüter et al. 2008) (but see *Linepithema humile*; Aron et al. 1993). Even in naïve individuals without private information, social information does not eliminate errors. As

few as 32% of honey bees that attend a waggle dance find the advertised feeder (Mautz 1971) and *L. niger* ants chose the branch at a T-bifurcation marked with trail pheromones only 62% or 70% of the time when it had been marked by 1 or 20 nestmates, respectively (Grüter et al. 2011). In the Pharaoh's ant *Monomorium pharaonis* only 70% of foragers chose the branch at a bifurcation marked by hundreds workers (Jeanson et al. 2003) and only 9 to 65% of *Tetramorium impurum* ant foragers succeed in following a 10cm long pheromone trail (Verhaeghe 1982).

Apart from providing naïve worker insects with information on the location of a food source, what other roles does social information have in foraging? The waggle-dance can reactivate experienced honey bee foragers to foraging (von Frisch 1967) resulting in revisiting of a location that had ceased being rewarding (Grüter & Ratnieks 2011). Trail pheromones allow experienced ant foragers to reach a food source more rapidly by walking faster and straighter (Czaczkes et al. 2011a). It has also been suggested that pheromone trails may assist ants in acquiring route memories (Collett & Collett 2002). Similarly, pheromone trails might be of importance to experienced ants if the route to the food source is hard to learn. Whilst route learning in ants has often been reported to be both rapid and accurate (Rosengren & Fortelius 1986; Fourcassie & Beugnon 1988; Grüter et al. 2011), most studies were conducted on simple trails with a single bifurcation. Trails with multiple choice points may provide a greater challenge, as more information must be stored, and route learning at one bifurcation may interfere with route learning at another.

This study investigated foraging by *Lasius niger* ants in a doubly bifurcating maze leading from the nest to 4 end points, one of which had a food source. We predicted that this more difficult and realistic challenge would lead to greater errors than found with a single bifurcation. In addition, this design allowed us to investigate the possibility that routes requiring alternating choices (e.g. left at the first T-bifurcation and right at the second) would be more difficult to learn than routes requiring repeating choices (e.g. left at both bifurcations) and whether the presence of trail pheromones affected learning and errors. We found that ants do indeed make more errors on alternating routes, but that trail pheromones can assist experienced ants to relocate a feeder, both by decreasing errors a T-bifurcations and by facilitating route memory formation.

Methods

Study species

We studied 8 *Lasius niger* colonies collected on the University of Sussex campus. Colonies were housed in plastic foraging boxes (40×30×20 cm high). The bottom of each box was covered with a layer of Plaster of Paris. Each foraging box contained a circular plaster nest box (14cm diameter, 2 cm high). The colonies were queenless with 500-1,500 workers and small amounts of brood. Queenless colonies forage, make pheromone trails and care for brood, and are frequently used in foraging experiments (Devigne & Detrain 2002; Evison et al. 2008). Colonies were fed three times per week with Bhaktar diet, a mixture of egg, agar, honey and vitamins (Bhatkar & Whitcomb 1970) supplemented once per week with dead mealworms, *Tenebrio molitor*. Colonies were deprived of food for four days prior to a trial in order to achieve uniform and high motivation for foraging. Water was provided *ad libitum*.

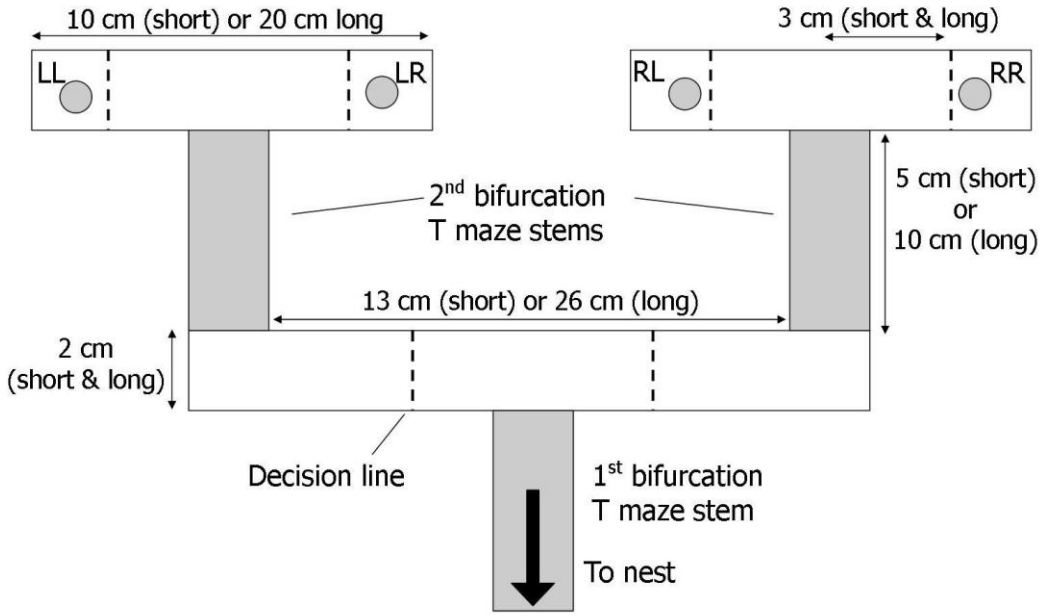

Figure 7.1 - Double-bifurcation maze: Pheromone depositions were recorded on the T-maze stems (shaded grey). Ants were considered to have chosen left or right at a bifurcation when they crossed the relevant decision line (dashed lines). A syrup feeder (1M sucrose) was placed at one of the four end points of the maze. Each section of the maze was covered in paper, which could be replaced to remove any pheromone on the maze surface. In the long maze (experiment 1) the heads and stems of the T-maze were doubled in length, but the distance of the decision line from the centre of the T-maze stem, and the width of the maze, remained constant. LL: left-left, ants reaching this feeder had to turn left on both bifurcations, etc.

Experiment 1 a) comparing repeating routes vs. alternating routes; and (b) effect of route length

We investigated route learning and pheromone deposition behaviour using an experimental maze (Figure 7.1) that bifurcated twice on the way from the nest to give four end points. To start an experimental trial the maze was connected to the colony's foraging box using a paper bridge. Once four ants had found the feeder, the remaining ants were removed from the maze and further ants prevented from entering. Each feeding ant was individually marked with an acrylic paint dot on her abdomen, and allowed to make 7 more trips to and from the feeder. For each ant, we recorded the decision it took on every outward trip at each bifurcation, and the number of pheromone depositing behaviours it made on each stem section of the maze (see figure 7.1). Decision lines 3 cm to the left and right of each bifurcation were used to define the choices, left or right, made by each individual foraging ant when walking away from the nest. Choice accuracy was scored independently for both bifurcations: if an ant should have taken a left-right route to reach the feeder, but instead took a right-right route, it would be scored as having made an incorrect decision at the first T-bifurcation and a correct decision at the second bifurcation. The maze was covered by pieces of white printer paper. A drop of 1 molar sucrose solution was placed at one end point. To reach this feeder, an ant had to make a choice at each trail bifurcation, either left-left or right-right for a repeating route or left-right or right-left for an alternating route.

Pheromone deposition is a characteristic and easily observed behaviour in *L. niger* in which the ant makes a brief, c. 0.2s, pause to touch the tip of the abdomen to the substrate. Every time an ant deposited pheromone, the paper overlay covering that maze section was replaced by a fresh piece, thereby removing pheromone information. Thus, ants had to rely solely on their own route memory. Each ant was tested at only one feeder location, but different individuals from each test colony were tested at all four locations. Tested ants were removed from their colony after being studied in order to prevent the same ant being studied twice.

The entire experiment was repeated using a maze of the same overall shape but with all length dimensions doubled to explore the effect of route length on route learning and pheromone deposition behaviour. The decision lines remained at the same distance (3 cm) from the centre of each bifurcation. Longer routes may provide foragers with more opportunity to learn the route, as the image of their surrounding is stable for longer between turns. Similarly, longer routes would give foragers more time between the first and second bifurcation to notice any errors they have made on the first T-bifurcation, and so correct that

error before reaching the second bifurcation. Furthermore, on a larger maze the visual difference between different end-points of the maze will be greater, and consequently errors should be easier for the ants to detect. In addition, on longer mazes errors would be more costly in terms of time and energy wasted so that ants may thus invest more in error checking.

Experiment 2 – effects of trail pheromone

This experiment was carried out to test the effect of trail pheromone on route learning and pheromone deposition. It used the same short-length double-T maze as in Experiment 1. Ants were tested only on alternating routes (right-left or left-right) because this combination proved the most difficult to learn, thus giving a better opportunity to detect any effect of trail pheromones on reducing errors. In half the trials the trail pheromone was removed as above, and in the other half the pheromone was allowed to accumulate. To control for any disruption due to changing the paper overlays in the pheromone removal treatment, we sham removed (removed and replaced) overlays in the trials in which pheromone was allowed to build up. At the end of a trial, an additional 12 naïve ants were allowed onto the maze and their decisions recorded to determine the effectiveness of pheromone without route memory in locating the food source. Each colony was tested twice with pheromone removal (feeder positions right-left and left-right), and twice with pheromone accumulation. When comparing naïve and experienced ants, only the last visit of each experienced ant was used, to ensure that both groups experienced a similar, high, level of pheromone.

Experiment 3 – trail pheromone as an aid to route learning

We investigated whether pheromone trails improve the formation of a route memory on a difficult-to-learn, alternating, route. The experimental protocol had one change from Experiment 2: trail pheromone was either removed after each visit or allowed to accumulate (as in experiment 2) or allowed to accumulate for the first 6 visits but removed on the final return to the feeder. Thus, ants in the final treatment had the benefit of an accumulating pheromone trail on visits 1-6, but no pheromone on visit 7, their final visit. These ants ("Visit 7 memory-test") could be compared with a) their own behaviour on visit 6, in which pheromone was present ("Visit 6 memory-test"), b) naïve ants on a pheromone trail ("Naïve"), c) experienced ants on their 7^{th} visit where pheromone trails were allowed to build up over all visits ("Always-pheromone visit 7"), or d) ants on the 7^{th} visit that had never had a pheromone trail, and thus had to rely on memory alone ("Never-pheromone visit 7"). The final outward journey from the start of the maze to an end point was timed.

Statistical analysis

Data were analysed using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) using R2.9.2 (R Development Core Team 2009). Models were fitted using the lmer function (Bates et al. 2007). Model selection followed Zuur *et al.* (2009): A model with all pertinent variables and all interactions was constructed. Random effect structure was explored by comparing different structures using the Akaike Information Criterion. Random effects that might need to be included were colony, trial and individual ant. By including these as appropriate we controlled for non-independence of data points. Non-significant terms were then removed from the model, beginning with the least significant, until an 'ideal model' – containing only significant terms - was reached. Decision data were analysed using a binomial distribution, and data on pheromone deposition behaviour were analysed using a Poisson distribution, after visual verification of the distribution structure. Interactions were explored by subsetting. For example, if we found an interaction between treatment and visit number the data would be split into the various treatments, and the effect of visit number would be analysed separately in each subset. All P values presented are corrected for multiple testing using the Benjamini-Hochberg method (Benjamini & Hochberg 1995).

Results

Trail choice accuracy

Experiment 1: The effect of a) repeating vs. alternating routes and b) short vs. long mazes on trail choice accuracy

We provided a feeder at the end of a repeating (left-left or right-right) or alternating route (left-right or right-left). The maze was either short or long (doubled lengths). The model included the terms 'maze length (short or long)', 'bifurcation (first or second)', 'route type' (alternating or repeating routes), and 'visit number' (1-7) as explanatory variables. We found an interaction between bifurcation and route type ($Z = -2.802$, $P = 0.00817$; figure 7.2): on alternating route, the error probability was greater at the first T-bifurcation ($Z = 10.658$, $P < 0.00001$), whilst on repeating routes there was no difference in the error probability between the two bifurcations ($Z = 0.981$, $P = 0.327$). There was also a significant interaction between route type and visit number ($Z = 5.542$, $P < 0.00001$): On repeating routes ants made fewer

errors in later visits (Z = 6.233, P < 0.00001). On alternating routes ants also made fewer errors on later visits (Z = 4.595, 4.595, P <0.00001), but the effect was much weaker than on repeating routes (see figure 7.2). Ants also made more errors on short mazes versus long mazes (Z = -2.808, P = 0.00817). Over the whole data set, ants made 97% correct choices per bifurcation on repeating routes versus 68% on alternating routes, 76% correct choices at the first T-bifurcation versus 89% at the second, and 81% correct choices on the short maze versus 84% on the long maze.

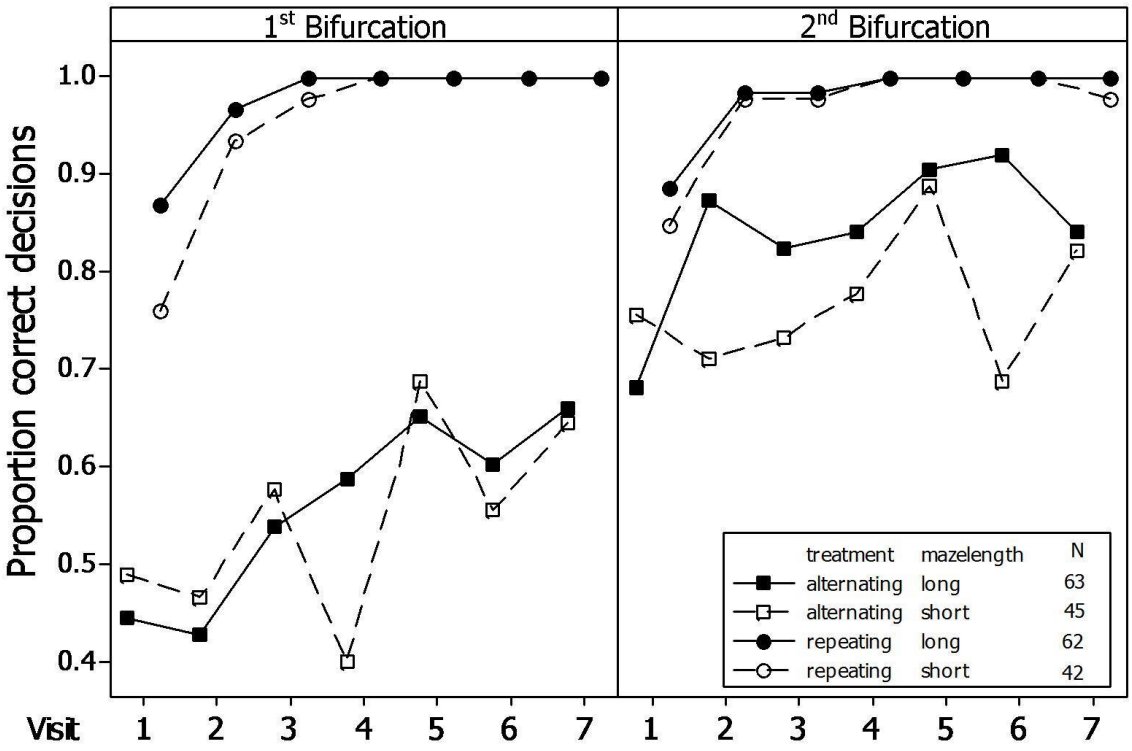

Figure 7.2 - Experiment 1. Route choice accuracy on a doubly bifurcating maze. Proportions of ants choosing the correct branch at each of the two bifurcations, over seven consecutive re-visits to the food source. The initial, "naïve" visit, visit 0, is not shown. The food source was either at the end of a repeating route (left-left or right-right, circles) or an alternating route (left-right or right-left, squares). The maze may be short (open shapes, dashed line) or long (double length; closed shapes, unbroken line). Ants made more errors on alternating versus repeating mazes, on the first versus the second bifurcation on alternating but not repeating mazes, and on short versus long mazes.

Experiment 2: Effects of trail pheromone presence on trail choice accuracy

Trail pheromone was either removed after deposition, as in experiment 1, or allowed to remain on the trail. Only the short maze was used, and the feeders were always at the end of alternating routes (LR or RL). The statistical model included the terms 'bifurcation', 'treatment (pheromone present or removed), and 'visit number' as explanatory variables. Figure 7.3A shows that more errors were made when pheromone was removed (56% vs. 73% correct

choices averaged over all visits, GLMM, Z = 6.756, P < 0.00001). We also found an interaction between bifurcation and visit number (Z = 3.044, P = 0.00291): whilst errors reduced in later trips, the reduction was less on the first versus second bifurcation (55% correct choices on first T-bifurcation vs. 74% correct choices on second bifurcation, averaged over all visits, see figure 7.3A).

When we compared the error rates of ants in both treatments on their last visit with naïve ants we found an interaction between treatment and bifurcation (Z = -2.871, P = 0.00614): On the first T-bifurcation experienced ants with no pheromone information were less accurate (46% correct choices) than both experienced ants with pheromone information (84% correct choices, Z = -2.871, Z = 0.00614) and naïve ants with pheromone information (71% correct choices, Z = -4.814, P < 0.00001)(Figure 7.3B). Naïve ants did not make significantly more errors than experienced ants with trail pheromones (Z = 1.847, P = 0.0648) (Figure 7.3B). On the second bifurcation, however, experienced ants with no pheromone information and naïve ants with pheromones were equally accurate (78% vs. 75% correct choices, Z = 0.315, P = 0.753), but ants with experience and pheromone information made fewer errors than the other two groups (97% correct choices, Z = -2.780, P = 0.00543, vs. naïve Z = -3.004, P = 0.00399) (Figure 7.3B). Thus, on the first T-bifurcation pheromone seems more helpful than route memory, and on the second bifurcation trail pheromones and route memory have a synergistic effect on trail choice accuracy.

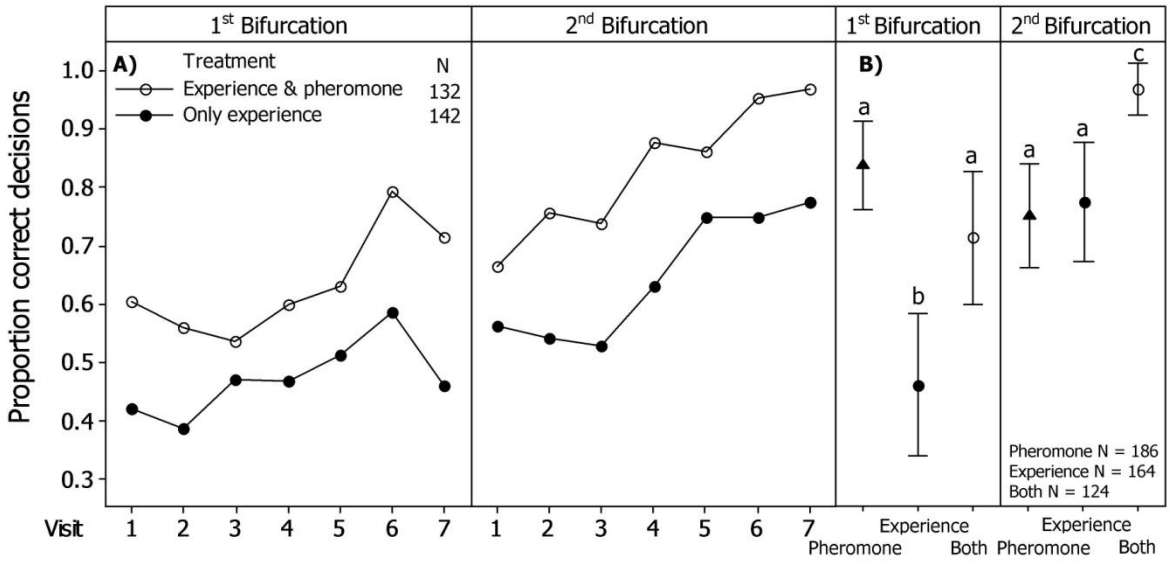

Figure 7.3 - Experiment 2. Route choice accuracy with or without trail pheromone: A) Proportion of ants choosing the correct branch at each of two bifurcations, over seven re-visits to the food source and B) on the final visit. A. Pheromone is either removed from the trail after each visit (filled circles) or allowed to build up (open circles). B. Triangles represent naïve ants with trail pheromone information. Bars in B represent 95% C.I for the mean, different letters are significantly different at 95%. On the second bifurcation, naïve ants and experienced ants without access to trail pheromone information are equally accurate, whilst experienced ants with access to trail pheromone information are more accurate. Only the alternating direction treatment was used in this experiment.

Experiment 3: Trail pheromone as an aid to route learning

In order to ascertain whether the presence of pheromone assists in route learning we allowed pheromone to accumulate on the trail, and removed it either i) after each visit, ii) only on the ant's final visit, or iii) never. The model included the terms 'bifurcation' and 'treatment' as explanatory variables. Treatment has five levels: experience + pheromone (trip 6), experience, no pheromone (trip 7), only pheromone (naïve), pheromone always removed, and experience + pheromone (trip 7). We found significant interactions between some of the treatment comparisons and bifurcation, and in other treatment comparisons we found no effect of bifurcation. A summary of all treatment comparisons at both the first and second bifurcations is given in appendix B, and summarised in figure 7.4. Our key findings are, first, that ants which have had access to trail pheromone for the first 6 visits, and then had the pheromone removed on the 7^{th} visit, made fewer errors on both bifurcations than ants on the 7^{th} visit that never had access to trail pheromone information (73% vs. 62% correct choices Z = -2.322, P = 0.0326). Second, ants which have had access to trail pheromone for the first 6 visits, and then had the pheromone removed on their last visit were less accurate on their last than on their penultimate visit on the second bifurcation (93% vs. 75% correct choices, Z = -2.476, P = 0.0275) but not on the first T-bifurcation (83% vs 71% correct choices Z = 1.563, P = 0.148). These ants also made more errors than ants which always had access to trail pheromones on the second bifurcation (76% vs. 97% correct choices, Z = -2.937, P = 0.00674), but error rates were equal on the first T-bifurcation (71% vs. 71% correct choices, Z = -0.052, P = 0.9586) (interaction term Z = -2.584, P = 0.01956). Thus, trail pheromone aids both the learning of a complex route and also the navigation of a complex route by an experienced ant revisiting a location for the 7^{th} time.

We also found that experience and trail pheromone act synergistically, allowing ants to reach the feeder faster. The time taken for experienced ants on a trail without pheromone and naïve ants on a trail with pheromone to reach the end of the maze was not significantly different (18.3 ± 2.2 SD sec vs. 17.8 ± 1 sec, Z = 0.211, P = 0.833), but experienced ants with trail pheromone are c. 34% faster (13.7 ±1.5 sec) than both experienced ants without trail pheromone (Z = 5.804, Z < 0.00001) and naïve ants with trail pheromone (Z = 2.047 P = 0.0407).

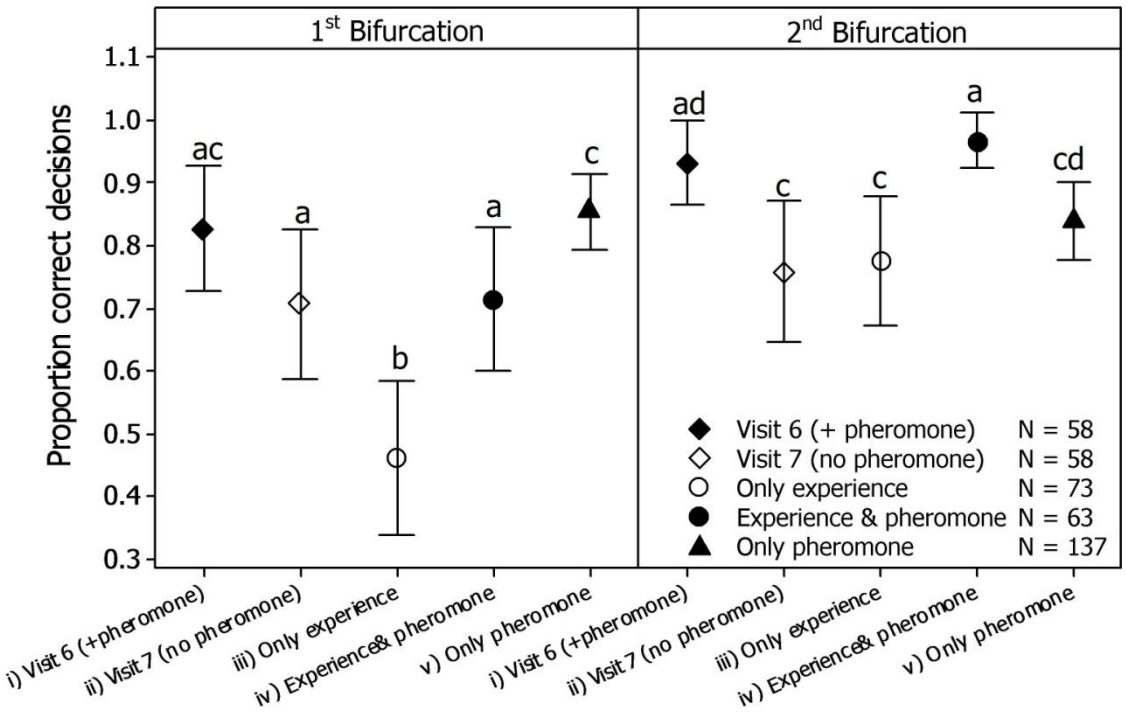

Figure 7.4 – Does the presence of trail pheromone assist in route learning? Proportions of correct choices at the first and second bifurcations for i) ants on their 6th visit , trail pheromone present (closed diamond), ii) the same ants on the 7th visit, trail pheromone absent (open diamond), iii) ants on their 7th visit, trail pheromones absent throughout all visits (open circles), iv) ants on their 7th visit where trail pheromones were not removed (closed circles), and v) naïve ants that are walking on a maze where trail pheromone was not removed (closed triangles). Bars represent 95% C.I for the mean. On the first bifurcation, where most errors occur, fewer errors are made in II than III, indicating that trail pheromone can assist in route learning, and that the effects remain even once pheromone has been removed.

Pheromone laying behaviour

Experiment 1: The effect of repeating vs. alternating routes, short vs. long mazes and navigation accuracy on trail pheromone laying behaviour

The model included the terms 'bifurcation', 'route type' (alternating or repeating), 'travel direction', 'correct last visit?' and 'maze-length' as explanatory variables. Travel direction is a very important determinant of pheromone deposition behaviour, and interacts with many other factors. We found an interaction between travel direction and treatment ($Z = -4.198$ $P = 0.000153$): ants deposited more pheromone on an alternating versus repeating route when returning to the nest ($Z = -5.311$, $P < 0.00001$), but there was no difference between route types when going to the feeder ($Z = -2.109$, $P = 0.134$; figure 7.5A). Thus, ants deposit more pheromone on their homeward journey when faced with a route that is difficult to learn (2.1 ±0.04 vs. 1.5 ± 0.03 depositions per 5 cm for alternating and repeating routes, respectively, averaged over all visits, maze lengths and travel directions).

There was also an interaction between travel direction and maze length ($Z = 8.442$, $P < 0.00001$): ants deposited more pheromone on shorter mazes when returning to the nest ($Z = 6.982$, $P < 0.00001$), but there was no difference when going to the feeder ($Z = 0.137$ $P = 0.9726$).

There was a three-way interaction between visit number, bifurcation and direction ($Z = -3.91$ $P = 0.000305$): When returning to the nest ants deposited less pheromone on the second bifurcation in later visits ($Z = -4.836$, $P < 0.00001$), but there was no change over visit number on the first T-bifurcation ($Z = 1.125$, $P = 0.266$). When going towards the feeder ants deposited more pheromone in later visits on the second bifurcation ($Z = 2.618$, $P = 0.0401$), but there was no change over visit number on the first T-bifurcation ($Z = -0.814$, $P = 0.519$).

Lastly, there was a three way interaction between bifurcation, direction and whether the ants made a error on their last visit to the feeder ($Z = -3.133$ $P = 0.004170$): When returning to the nest, ants that made an error on the outwards journey of their current visit deposited more pheromone on both the first and second bifurcation, although the effect is strongest on the first T-bifurcation, where more errors are made (1^{st} bifurcation, $Z = 12.388$ $P < 0.00001$, 2^{nd} bifurcation $Z = 9.432$, $P < 0.00001$. 2.2 ± 0.04 vs. 3 ± 0.1 depositions per 5cm, averaged over both bifurcations - see figure 7.5B). When going towards the feeder, however, ants that made an error on their previous visit did not change their pheromone deposition behaviour on the first T-bifurcation ($Z = 1.719$, $P = 0.1713$), and deposited slightly more pheromone on the second bifurcation ($Z = 2.188$, $P = 0.0344$)(1.5 ± 0.08 vs. 1.5 ± 0.03 depositions per 5 cm, averaged over both bifurcations). Thus, it seems that ants monitor how successfully they navigate a route, and if they make an error they lay more pheromone (i.e., provide more information for nestmates or their own subsequent journey) on their return journey (figure 7.5B).

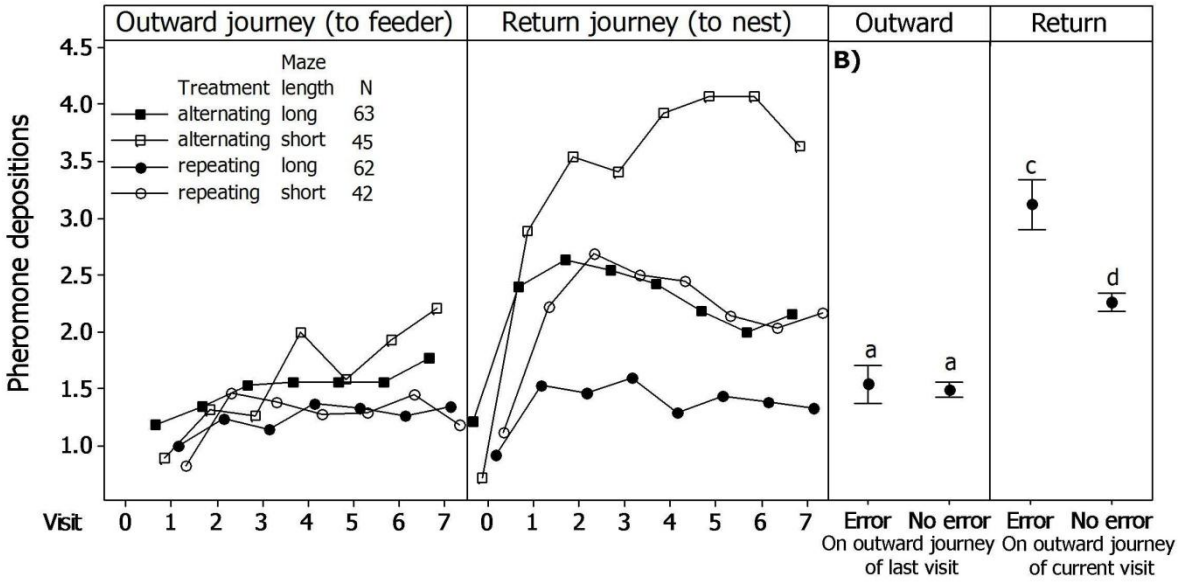

Figure 7.5 - Effects of alternating versus repeating direction, maze length and ant visit number on pheromone deposition: A) Significantly more pheromone is deposited on alternating routes, on shorter mazes and on the return journey. B) Ants that made a mistake in their outward journey to the feeder deposit more pheromone when returning from the feeder once they eventually find it. Symbols signify means, whiskers 95% C.I. Different letters signify statistically significant difference at 95%. Pheromone laying behaviour rates were divided by two on the long maze to allow comparison with the data from the short maze. Error bars for the means in A have been omitted for clarity.

Experiment 2: the effects of trail pheromone presence on pheromone deposition

In this experiment pheromone deposited on the maze was either removed or allowed to build up. The short maze and alternating direction treatment were used. The model included the terms 'bifurcation', 'treatment' (pheromone present or removed), 'travel direction' and 'visit number' as explanatory variables. We found a significant three-way interaction between travel direction, visit number and treatment ($Z = -4.067$ $P = 0.0001$): when pheromone was removed from the maze, ants returning to the nest deposited more pheromone in later visits on both bifurcations ($Z = 2.346$, $P = 0.0190$) (figure 7.6B), with a non-significant trend for more pheromone to be deposited on the 2nd bifurcation ($Z = 1.863$, $P = 0.0625$). When pheromone was allowed to build up, ants increased pheromone deposition on the first visit (when pheromone had not yet been deposited) and decreased pheromone deposition in later visits ($Z = -5.539$, $P < 0.00001$) (figure 7.6B). This pattern held true for both bifurcations, although pheromone deposition was higher on the 2nd bifurcation ($Z = 2.955$, $P = 0.00469$). Ants walking towards the food source did not change their deposition rates in later visits (pheromone allowed, $Z = -0.365$, $P = 0.955$, pheromone removed $Z = 0.649$, $P = 0.774$; Figure 7.6A) on either bifurcation (pheromone allowed, $Z = 0.056$, $P = 0.955$, pheromone removed $Z = -0.1$, $P = 0.92$).

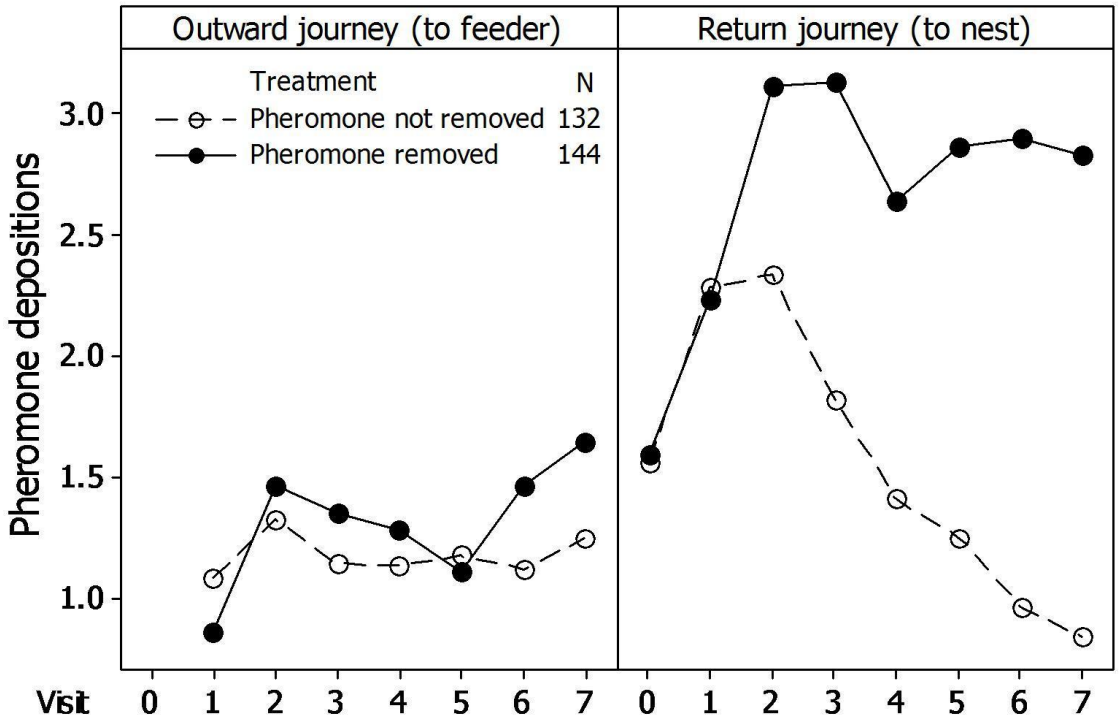

Figure 7.6 - Effect of trail pheromone presence on trail pheromone deposition. Left) Outward journey from nest to feeder. Right) Return journey from feeder to nest. The presence of pheromone on the trail greatly reduces pheromone deposition rates on the return journey but has little effect on the outward journey. Error bars for the means have been omitted for clarity.

Discussion

Route choice

Our results show clearly that a more complex trail with two sets of bifurcations is more difficult for ants to learn than the single bifurcation trail that has been most studied by researchers. *Lasius niger* foragers make over 95% correct choices after three visits to a feeder via a single bifurcation (Grüter et al. 2011), as compared to foragers after three visits to a feeder reached via an alternating route (left-right or right-left), which make 79% correct decisions at the second bifurcation and only 56% correct decisions on the first. Foragers made more errors on alternating routes than on repeating routes (left-left or right-right), but error rates on alternating trails could be reduced by 30% with the provision of trail pheromones. Trail pheromones not only helped guide ants, but also facilitated the formation of route memories.

Ants making return journeys to the feeder were most likely navigating by attempting to match their current view to learned landmark or their visual panorama to their current view, or by walking towards more familiar views (Collett 2009; Baddeley et al. 2012). *As L. niger* cannot learn routes in the dark (Jones et al, in prep), we are confident that ideothetic cues were not being learned in this case, although other ants can learn such cues (Macquart et al. 2008).

It is unclear why more errors were made on alternating routes. One possibility is that the memory of the right direction to turn at one bifurcation interferes with the (different) decision at the other bifurcation. The experiment was conducted in heterogeneous lab space with many large objects which could serve as landmarks for the ants. The view to the left on the maze was very different to the view to the right, providing ample information for landmark learning. Classic models suggest that as ants return to a goal using view-based navigation they attempt to match their current image of the landscape with images acquired close to the goal (Wehner & Räber 1979; Collett & Cartwright 1983; Wehner et al. 1996; Judd & Collett 1998; Graham & Cheng 2009). A more recent model suggests ants take account of all views experienced during training, and attempt to align their path in such a way as to experience the most familiar view (Baddeley et al. 2011, 2012). On repeating routes attempting to match the views acquired at the goal with their current view will lead ants in the correct direction, as on both bifurcations the ants must match their view with the view seen when arriving at the feeder to reach the feeder. However, on alternating routes ants attempting to match their view with the view seen when approaching the feeder will take the wrong turn at the first T-bifurcation, as to make a correct decision at the first T-bifurcation they must head away from the feeder location. Baddeley *et al.*(2011) also demonstrate that alternating routes are more difficult to navigate. Short-term memories from one learning event can also be very unstable and prone to interference from contradictory information arriving too soon after the original learning event (Menzel 1979). Scouting ants also tend to take repeating turns so as to perform an outline-tracing search pattern (Jander 1990), in which ants follow the edge of an area so as to systematically explore the entire circumference of the area. This may apply to ants making foraging trips to a known food source as well. The reason for higher accuracy on the second bifurcation is also unclear. It may be linked to the panorama matching hypothesis mentioned above, or to possible pheromones emitted by feeding nestmates, or to visual orientation to feeding nestmates. Also unclear is why accuracy is greater on long routes. Possibly, longer routes may provide more time and opportunities to learn intermediate route images,

facilitating learning. Visual differences between maze end-points will be greater on longer routes, also facilitating learning and error-checking. Lastly, errors are more costly on longer routes, so ants may invest more in error checking on these routes. These possibilities are not mutually exclusive, and these questions remain open for future studies.

As predicted, the results show that pheromone trails are of value even to experienced foragers. This is in contrast to the results obtained using a trail with a single bifurcation, which by definition is not alternating, where pheromones did not seem to be used by experienced ants (Grüter et al. 2011). Trail pheromone and route memory information are used additively, not redundantly, with pheromone trails increasing accuracy in experienced foragers beyond that achievable with memory alone (figure 7.3). In experiment 2 when considering just the last visit, path choice accuracy of ants guided by memory alone is in fact lower than that of naïve ants guided by pheromones alone. This is likely due to the memory of the correct turn at the 2nd bifurcation interfering with the decision at the 1st T-bifurcation. However, some of this pattern could be attributed to the experienced ants depositing pheromone on their last visit to the feeder, thus making the pheromone trail the naïve ants experience slightly stronger. This slight increase in trail strength could also have affected the results of the naïve ants in experiment 3 to some extent. The order in which ants visited the feeder would also affect to some degree the amount of pheromone experienced on the trail, as later ants would have access to the newly-deposited pheromone from previous ants.

The results also show that in addition to helping in navigation, the presence of trail pheromone also improves learning. These two effects are somewhat separate, and demonstrated by the results of different experiments. In experiment two we can see the benefit of pheromone trails in navigation can be immediate: in the presence of trail pheromones, ants on alternating mazes make significantly more accurate choices even on their first return visit to the feeder (figure 7.3A). That trails pheromone improving route memory formation can be seen in experiment 3: On the first T-bifurcation, where most errors occurred, ants which walked on mazes with trail pheromone for six visits, but then walked on an unmarked route on the seventh, made fewer errors than ants that never had access to trail pheromone information (figure 7.4). The fact that this effect is only present on the first T-bifurcation suggests that the presence of trail pheromones is assisting ants in learning to reduce interference from memories relevant to later parts of the journey. Collett & Collett (2002) suggested that pheromone trails might assist learning, either by constraining ants onto

a narrow route and thus facilitating the formation of intermediate snapshot memories, or by providing a training signal, informing ants that they are on the trail and thus should learn the surrounding landmarks. The role of trail pheromones as a training signal is especially reasonable in the case of a difficult-to-learn route: foragers which are unsure of their location should not invest effort in memorising a route and location. The presence of trail pheromone may thus act to "reassure" ants that they are on the correct path, and thus that the location and route is worth learning. As ants in this experiment were constrained by the maze, our data support the second suggested role of trail pheromones as a training signal. Furthermore, Steck *et al.* (2011) recently demonstrated that desert ants learned the location of their nest much more rapidly when they had access to both visual and olefactory cues than when they had access to only one cue modality. Trail pheromones in our experiment seem to similarly act as a secondary modality used during navigation. However, the two proposed roles of trail pheromones in improving route learning are not mutually exclusive. It is also possible that trail pheromones not only promote route learning, but promote learning in general, for example associative learning of odours with rewards. This possibility is very amenable to experimental investigation.

Pheromone deposition behaviour

The deposition of trail pheromones by ants mirrors their success at navigating a route. Ants deposited more pheromone on routes in which they made more mistakes: the alternating and short routes (figure 7.5A). Moreover, ants walking on a straight route with no bifurcations deposit even less pheromone (mean 0.45 depositions per 5cm, StDev = 0.93, data taken from Czaczkes et al., 2011) than ants walking on repeating routes. Thus, ants deposit more pheromone on more complex or more difficult-to-learn routes. This is due, at least in part, to ants that make a mistake on their outward journey up-regulating pheromone deposition on the subsequent return journey (figure 7.5B). Similarly, when honey bees experience a delay in finding their goal they recommence performing learning flights (Wei et al. 2002) to assist memory formation. In addition, honey bee workers that have difficulty in finding the nest entrance release an attractant pheromone to assist nestmates to find the entrance (Butler et al. 1970). By increasing pheromone deposition after experiencing difficulties in finding the food source, an ant assists both her nestmates and herself on a subsequent visit.

One reason that an ant increases her pheromone deposition rate on the return journey may be that it is only once she has reached her goal that she can evaluate whether she made

an error in getting there. If an ant were to increase pheromone deposition on the outward journey and make a mistake, this could result in more ants being lead into error. However, this reasoning assumes that ants make more errors on their outward than return journey. Although we did not collect data on how accurately ants returned to the nest, there are several reasons why this is likely. Firstly, by definition ants on a return journey have travelled a route once more than when they were making their previous outward journey. Secondly, on return journeys ants can rely on path integration (Collett et al. 2003) to guide them to the nest, without having to rely on possibly confusing and conflicting landmark information. Thirdly, ants may spend less time at the feeder than in the nest, and so their memory of the route on the return journey may be more recent than on their outward journey. Lastly, ants may simply avoid recruiting heavily during their outward journeys as they cannot be sure that the food source is still productive.

Negative feedback also occurs in the *L. niger* recruitment system. Our results (figure 7.6) show that the presence of high levels of trail pheromone suppresses further pheromone deposition. This may have important implications for the organisation of colony-level foraging. Ants which successfully find a food source return to the nest depositing trail pheromone (Beckers et al. 1992a). This elicits more ants to exit the nest (Wilson 1962). Some of these ants will follow the trail successfully, feed, and return, also depositing trail pheromone. This positive feedback quickly results in a strong trail being established. By depositing more pheromone for higher quality food sources, such positive feedback loops can allow ant colonies to concentrate their foraging on one or a few best feeding locations from multiple possible feeders (Beckers et al. 1990; Aron et al. 1993). However, as recruitment in many mass-recruiting ant species is non-linear (Detrain and Deneubourg, 2008; Sumpter and Beekman 2003), foraging trails can rapidly become very strong. If a new feeding location is located once foraging at the first feeding location is well underway a colony may not be able to switch feeding locations, even if the newly discovered feeder is of a higher quality (Beckers et al. 1990; Sumpter & Beekman 2003). This is because the pheromone trail to the first feeder is too strong. The negative feedback system described here, of trail pheromone presence suppressing further pheromone deposition, may counteract the positive feedback system responsible for fixing forager allocation. This may act to protect colonies from becoming too firmly entrenched in the exploitation of any one feeding location, without sacrificing the speed at which a consensus ‘decision’ is made, as the initial rapid build-up of pheromone would not be hindered; only the later increase to extremely high levels. Furthermore, once feeding is well

underway and the pheromone trail is sufficiently strong to guide ants accurately, this reduction of pheromone deposition in response to pheromone presence will result in less metabolically expensive pheromone being used unnecessarily.

Foraging in ants is a complex process involving the use of route memories and pheromone trails, and is both a collective and an individual behaviour. The simplified situations tested in laboratory studies often mask much of this complexity. By introducing a slightly more complex and realistic situation, we detected features of ant foraging and recruitment that would otherwise have remained hidden. For example, *L. niger* appears to have an innate bias towards learning repeating routes, and this may have ecological implications. We predict ants would disproportionately exploit food sources at the end of repeating routes. However, when the bias in route memory formation hinders exploitation of food sources, an increase in trail pheromone deposition can compensate, by helping to guide ants to food sources that require an alternating route, and enhancing route learning. These results are a compelling example of the sophistication of ant foraging, and the interplay and complementarity of different information sources in collective organisation.

Acknowledgements

We would like to thank Tom Collett and two anonymous reviewers for comments on earlier versions of this manuscript. T.J.C. was supported by a PhD studentship from BBSRC. G.C. was funded by a postdoctoral fellowship from the Swiss National Science Foundation (SNSF grant no: PA00P3 129134).

Chapter 8: Negative feedback in ants: crowding results in less trail pheromone deposition

Tomer J. Czaczkes, Christoph Grüter & Francis L.W. Ratnieks

Abstract

Crowding in human transport networks reduces efficiency. Efficiency can be increased by appropriate control mechanisms, which are often imposed externally. Ant colonies also have distribution networks to feeding sites outside the nest and can experience crowding. However, ants do not have external controllers or leaders. Here we report a self-organized negative feedback mechanism, based on local information, which down-regulates the production of recruitment signals in crowded parts of a network by *Lasius niger* ants. We controlled crowding by manipulating trail width and the number of ants on a trail, and observed a 60-fold reduction in the number of ants depositing trail pheromone from least to most crowded conditions. We also simulated crowding by placing glass beads covered in nestmate cuticular hydrocarbons on the trail. After 10 bead encounters over 20cm, forager ants were 55% less likely to deposit pheromone. The mechanism of negative feedback reported here is unusual in that it acts by down-regulating the production of a positive-feedback signal, rather than by direct inhibition or the production of an inhibitory signal.

Introduction

Both human and insect societies face the challenge of coordinating many individuals. Top down hierarchical control is evident in human organisations such as government, business corporations, and the military. However, many modern challenges, such as dynamic task allocation in factories and routing of data and goods, can be too complex for any one controller to manage or even to have a global view of events (Papadimitriou 2003). Insect societies face similar challenges and have evolved bottom-up self-organized mechanisms to regulate collective behaviours.

Collective behaviours in social insects, including foraging and nest-site selection, are mediated in part by positive feedback loops, in which one effect (e.g. foragers returning from a food source depositing a pheromone trail) up-regulates another effect (e.g. more workers leave the nest, follow the trail, and feed) which in turn up-regulates the first effect. Successful individuals, such as a scout who has found a new nest or feeding site, recruit nestmates by making an appropriate signal such as a waggle dance or by laying pheromone (Seeley 1995; Jarau & Hrncir 2009). The number of recruits and their distribution among sites is modulated according to resource quality (Beckers et al. 1990, 1992b; Seeley 1995; Portha et al. 2002; Franks et al. 2003; Breton & Fourcassie 2004) and other factors (Traniello & Beshers 1991; Mailleux et al. 2005; Mailleux 2006) via an interplay of positive and negative feedback processes. Negative feedback may be passive, such as by the decay of pheromone trails in ants or reduced waggle dancing in honey bees (Wilson 1972), or active in the form of a deliberate signal. Examples of inhibitory signals include the stop signal used by honey bees to reduce recruitment to dangerous foraging location (Nieh 1993, 2010) or competing alternative nest sites (Seeley et al. 2011), and the 'no entry' trail pheromone used by Pharaoh's ants to deter foragers from taking the wrong branch at a trail bifurcation (Robinson et al. 2005).

Overcrowding in a trail network leads to a decrease in traffic flow with subsequent loss of efficiency (Burd & Aranwela 2003; Dussutour et al. 2005a). Colonies of *Lasius niger* ants can adjust their foraging in parts of a trail system in response to crowding. For example, longer routes are used more when shorter routes are overcrowded (Dussutour et al. 2004, 2006). This adjustment is mediated at least in part by ants being "pushed" onto the longer route (Dussutour et al. 2006). In this way, direct environmental constraints, in this case crowding, can lead to the emergence of improved network use without any explicit adjustment of the

information shared between individual foragers (Dussutour et al. 2005a; Czaczkes & Ratnieks 2011). However, responses to crowding are not only passive. In this study we test and support the hypothesis that *L. niger* foragers actively respond to crowding by depositing less trail pheromone. As such, crowding causes negative feedback by down-regulating the production of a positive feedback signal—trail pheromone.

Methods

Study species

Colonies of the black garden ant, *Lasius niger*, were collected on the University of Sussex campus and housed in plastic foraging boxes (40×30×20cm). The bottom of each box was covered with plaster of Paris and contained a circular plaster nest (14cm diameter, 2 cm high). Colonies were queenless with 500-1000 workers and small amounts of brood. Colonies were fed three times per week with Bhatkar jelly (Bhatkar & Whitcomb 1970) and deprived of food for four days prior to each trial to give high and consistent motivation to forage and recruit to experimental sucrose syrup feeders. Water was provided *ad libitum*.

Part 1– Effect of trail crowding

This experiment was designed to investigate the effect of trail crowding on pheromone deposition rates by foraging ants walking between the nest and feeder. Five colonies were used. A hungry colony was allowed access to a 20cm long walkway covered with printer paper leading to a 1M sucrose syrup feeder at the end. The walkway was either 0.5cm (narrow) or 2cm (wide) in width. Walking *L. niger* workers are about 2.5mm wide across their antennae, so ants passing on the narrow trail almost invariably contact each other. Ants were either allowed freely onto the bridge (many ants) or restricted (few ants: only the first 7-9 ants to reach the feeder allowed to continue foraging; additional ants were excluded by using a drawbridge). In all trials, the first 7-9 ants to reach the feeder were individually marked with a dot of acrylic paint. Foraging was then allowed to proceed for 30 minutes from the time the first ant found the feeder. The walkway was videoed from above using a high definition camera (Sony HDR-XR520). A mirror angled at 45° was placed beside the trail, allowing the video to capture views of walking ants both from above and side. The side view allowed pheromone depositions to be clearly detected.

Individually-marked ants were followed throughout a trial, recording both pheromone depositions on each trip to or from the feeder and head-on contacts with other ants. In

addition, pheromone deposition behaviours made by all ants, and the number of head-on contacts between ants, were counted on the 4cm section of trail nearest to the feeder so as to measure the overall state of the trail. Pheromone deposition in *L. niger* is a highly stereotyped behaviour in which the ant pauses for circa 0.2 seconds and presses the tip of her abdomen firmly on the substrate. This behaviour is easily observed and counted (Beckers et al. 1992b). Five colonies were tested, and each colony was tested in all four treatment combinations (wide path/many ants, wide path/few ants, narrow path/many ants, and narrow path/few ants). The paper overlay on the walkway was replaced and the plastic walkway backing cleaned with ethanol after every trial.

Part 2 – Simulating crowding with glass beads

To further investigate how ants perceive crowding and to control for possible auto-correlation between the number of ants on the trail and the amount of trail pheromones on the trail (more ants on the trail results in more pheromone on the trail, and separating these effect statistically may not be possible) we ran an experiment with glass beads (artificial ants) coated in nestmate cuticular hydrocarbons (CHCs). Glass beads coated in CHCs have been used successfully to mimic both nestmate (Greene & Gordon 2003; Ozaki et al. 2005; Greene & Gordon 2007a; Akino et al. 2004) and non-nestmate ants (Wagner et al. 2000; Akino et al. 2004; Ozaki et al. 2005; Martin et al. 2008). To prepare the beads we collected ten workers from the test colony, chilled them for two minutes at -20°C, and then placed them in a glass vial with 500µl pentane for ten minutes to dissolve the CHCs. 2.5µl drops of solution were then dripped over ten black glass beads (diameter 2mm), allowing the pentane to evaporate and deposit the CHCs on the beads. CHCs were extracted and beads were prepared immediately prior to use. The 10 beads were then placed at 2cm intervals on the 20cm long walkway, and a single marked ant was allowed to make two return trips to the feeder. The walkway was 0.5 cm wide to ensure that marked ants contacted the beads. Trail pheromone deposition rates were recorded for each journey. The paper overlay on the walkway was replaced and the plastic walkway cleaned with ethanol after every test. 4 ants from each of 10 colonies were tested.

Given that *L. niger* are black in colour, we also tested the hypothesis that the colour of the glass beads, black versus clear, affects the perception of foragers. Four ants from each colony were also tested with the following treatments: clear glass beads coated with CHCs, uncoated (blank) black beads, uncoated (blank) clear beads, and no beads. The same beads

were used for each of the four ants tested per colony per treatment. Treatment orders were pseudo–randomised.

Statistical analyses

Data were analysed using generalised linear mixed models (GLMM) in the statistical package R 2.9.2 (R Development Core Team 2009). Models were fitted using the lmer function (Bates et al. 2007). Model selection followed Zuur et al (2009). We first constructed a saturated model, including all predictor variables we had an *a priori* reason for testing, and all interactions between them. Only three way interactions or lower were modelled. Random effect structures were explored and competing models compared using Akaike Information Criterion (AIC). Random effects included were colony (in all analyses) and ant (where individual ants' behaviour was followed over multiple visits). We removed non-significant effects and interactions, then explored the significance of fixed effects, and removed non-significant effects and interactions. Interaction effects were explored by making subsets. For example, if a significant interaction was found between trail width and collision rates, the data would be split into wide and narrow trail treatments, and the effect of collision rates explored in both subsets. Binomial data, such as whether ants deposited pheromone or not, were analysed using a binomial distribution, and count data, such as number of pheromone depositions per ant, were modelled on a Poisson distribution. All P-values presented are adjusted using the Benjamini-Hochberg (Benjamini & Hochberg 1995) correction to account for multiple testing.

Results

Part 1 – Effect of crowding

The density of ants on the trail (many or few), trail width (wide or narrow), visit number (e.g., first, second, n^{th} visit to the feeder for an individually-marked ant), and the number of head-on encounters were used as predictor variables.

Individual ants

As shown in figure 8.1, less trail pheromone was deposited on both narrower and more crowded trails. There was a significant interaction between trail width and ant density ($P = 0.0232$, $Z = -2.55$, figure 8.1). When many ants were present on a trail, focal ants deposited less pheromone on narrow than wide trails ($P = 0.00017$, $Z = -3.928$). When the number of ants on

the trail was low there was no effect of trail width on pheromone deposition (P = 0.507, Z = -0.664). Focal ants deposited less pheromone on later visits (both trail treatments P < 0.0001, Z > 8) and this trend was more pronounced when many ants were allowed onto the trail (interaction: P < 0.0001, Z = -14.483, see appendix C part 1). This can be seen in figure 8.1 by the steeper decline in the curve in the many-ants treatments. This finding is mirrored in the trail width treatment, with ants on narrow trails initially making less pheromone depositions than ants on wide trails (Z = -5.139, P < 0.0001) and ants on narrow trails and high crowding depositing no pheromone after making a few visits (figure 8.1). In addition, we found that on narrow trails more collisions resulted in focal ants making fewer pheromone deposition behaviours (P = 0.00017, Z = -3.931). There was no significant relationship between collision rates and pheromone deposition on wide trails (P = 0.507, Z = -0.664, interaction between collisions and trail width: P=0.000145, Z = 3.888). The changes in total trail pheromone depositions were driven primarily by a reduction in the probability of ants depositing trail pheromone. Reduction in the number of pheromone-laying behaviours by those ants that did lay pheromone played a smaller role (see see appendix C part 1).

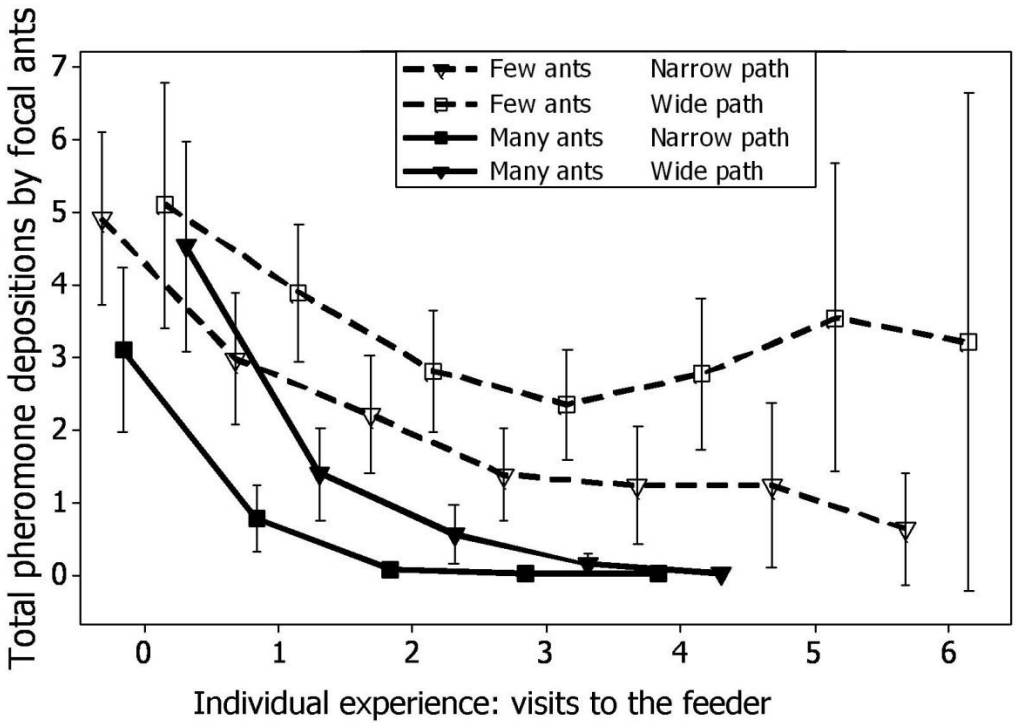

Figure 8.1 - Effect of trail width (narrow, 5mm, versus wide, 20mm), crowding (few versus many ants), and individual experience, in terms of number of previous visits to the feeder, on pheromone laying behaviour by individual foraging ants. Whiskers = 95% confidence intervals for the mean.

All ants

Total pheromone depositions on the first four cm of the trail gave a similar picture to the data obtained by observing individual ants (above). Less pheromone in total was deposited on narrow than on wide trails at high ant numbers ($P < 0.0001$, $Z = -8.584$) but not at low ant numbers ($P = 0.894$, $Z = -0.189$, interaction between trail width and ant: $P = 0.00159$, $Z= -3.255$, see see appendix C part 2 for figures and details). Unexpectedly, we found that when many ants were on the trail there was a positive correlation between collision number and total pheromone depositions ($P = 0.000171$, $Z =-3.931$), although we did not find this at low density ($P = 0.507$, $Z = -0.664$, interaction between collision rate and ant number treatment: $P < 0.0001$, $Z = -4.429$). This resulted in more pheromone depositions with higher ant density on the wide trail but not on the narrow trail (see figures in appendix C part 2). However, if we consider pheromone depositions *per ant* (appendix C part 2), we find fewer depositions per ant when many ants are on the trail ($P < 0.00191$, $Z =-3.189$) and when the trail is narrow ($P = 0.0233$, $Z = -2.267$), mirroring the data from individual ants. We also find that more collisions result in less depositions per ant ($P = 0.00142$, $Z = -3.386$). These effects are mainly driven by a reduction in the proportion of ants depositing pheromone, not by a reduction in the number of pheromone depositions per depositing ant (see appendix C part 2).

Part 2 – Crowding with glass beads

There were four bead treatments: black beads with CHC, clear beads with CHC, black beads without CHC, clear beads without CHC, and one control (no beads). As figure 8.2 shows there were significant differences in pheromone deposition between treatments (figure 8.2, see also appendix C part 3). Foraging visit number was not a significant predictor of deposition probability ($P = 0.3355$, $Z = 1.081$). Treatments were also compared in terms of pheromone depositions per journey by all ants (excluding the first journey to the food, when ants never deposit pheromone), in terms of whether ants deposited pheromone or not, and in terms of the number of depositions by depositing ants (see appendix C part 3 Tables 1A-C).

The presence of black CHC+ beads reduced the average pheromone depositions of ants compared to all other treatments (figure 8.2, appendix C part 3). The presence of clear CHC+ beads also caused a reduction in the average number of pheromone depositions compared to the blank bead and control treatments (figure 8.2, see appendix C part 3). To determine whether the reduction in total pheromone deposited was driven by ants making fewer pheromone depositions, or by ants choosing not to deposit pheromone at all, we also analysed the effect of the treatments in terms of proportion of ants depositing pheromone,

and number of pheromone depositions per depositing ant (see appendix C part 3). Both a reduction in depositions per ant, and the proportion of ants depositing pheromone, play a role in the reduction of total pheromone deposition in CHC+ trials.

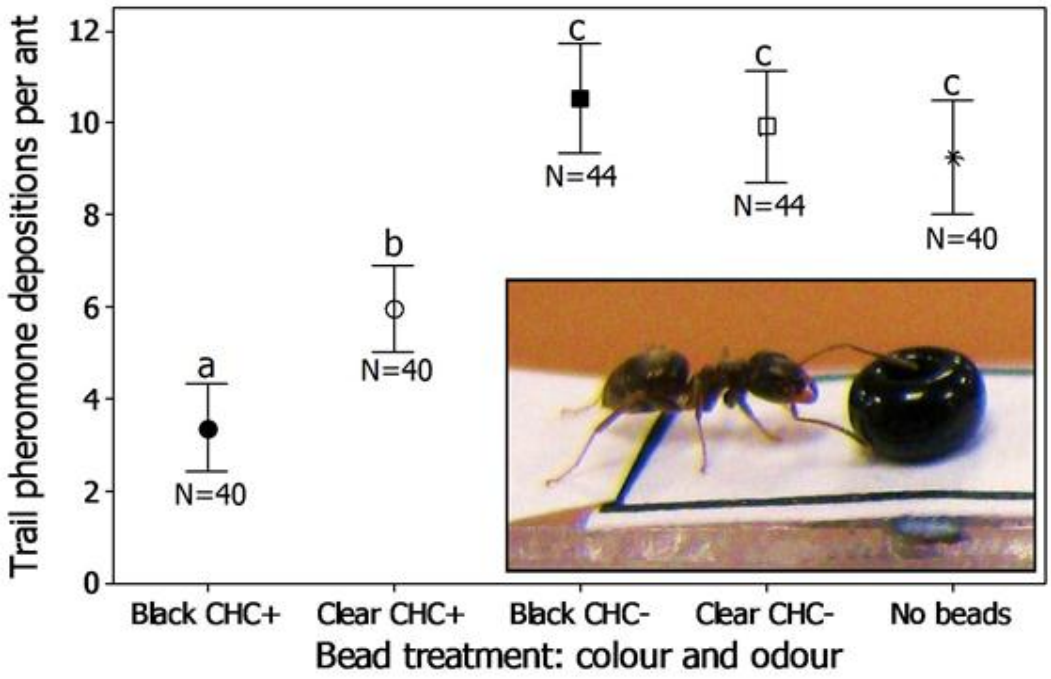

Figure 8.2 - Effects of bead treatments on the number of pheromone depositions performed. Beads could either be black or clear, and either covered by CHCs (CHC+) or not (CHC-), or could be absent altogether, resulting in four treatments: black CHC+, black CHC-, Clear CHC+, clear CHC-, and a control (no beads). Dots represent means, whiskers 95% C.I. Treatments headed by the same letter are not significantly different. N = number of ants observed. Insert: an ant walking off the bridge (left) encountering and antennating a black bead coated in CHCs.

Discussion

Our results show clearly that crowding on trails reduces the amount of pheromone deposited by each foraging ant. Ants on crowded trails are both less likely to deposit pheromone at all, and perform fewer deposition behaviours when they do deposit pheromone at least once. Foragers assess crowding on trails at least in part by noting the number of times they collide with nestmates. An object is assessed as a nestmate primarily by the presence of cuticular hydrocarbons, but also by its colour. Black beads, which are the same colour as the ants, have a stronger effect than clear beads.

Although crowding reduces the amount of pheromone laid per ant, it is noteworthy that the total amount of pheromone deposited on the wide trail is nevertheless higher when

many ants are allowed onto a trail (see appendix C part 2 figure A). This is reasonable, as the colony was hungry and had access to only one food source which was of high quality (1M sucrose). However, the extent of the positive feedback signal, in terms of pheromone deposition to that food source, was reduced. In other words, crowding in the *Lasius niger* foraging system down-regulates positive feedback. This dampening of positive feedback can indeed bring recruitment to a halt, as can be seen in figure 8.1 for visit three onwards at the highest crowding treatment. Our interpretation is that foraging ants have determined that the level of foragers on the trail is sufficient, and no more foragers should be recruited. Recruitment to crowded trails is thus lower than what it would have been without this negative feedback. Reduction in the number of foraging ants on part of the trail system can also be caused via other negative influences, such as pheromone decay (Wilson 1962; Jaffe & Howse 1979), feeder abandonment, and cessation of foraging due to satiation (Grüter et al. 2012, and see chapter 9).

The modulation of positive feedback detected in this study probably plays several roles, including adjusting and limiting the number of workers recruited to a food source in relation to the capacity of the trail to handle traffic and preventing heavily-used trails from becoming so strongly marked with trail pheromone that other trails cannot develop (Beckers et al. 1990). In addition, by possessing an active response to trail crowding, a colony can react to crowding on straight sections of trail in addition to at trail bifurcations. This could not occur if ants relied solely on U-turning due to the passive effects of overcrowding (Dussutour et al. 2004).

Our results also show that *L. niger* use contact rates to estimate nestmate abundance, as previously reported in other ant species for both nestmates and non-nestmates (Hölldobler 1981b; Gordon et al. 1993). Contact rate is a simple cue to use as the information is gathered at little or no cost (Detrain & Deneubourg 2009) and does not require actual counting (Gordon 1999). However, contact rates are not the only possible source of information for determining ant density or levels of trail use. Ants monitor trail pheromone levels in order to follow trails, and can thereby incidentally collect information on trail use by nestmates. Thus, high levels of trail pheromone on a trail can also cause ants to reduce further pheromone deposition (Czaczkes et al. 2012a, see chapter 7). Ants can also estimate the number of ants that have visited an area by sensing levels of home-range markings, which are cuticular hydrocarbons (CHCs) deposited passively as ants walk over a substrate (Detrain & Deneubourg 2009), and

regulate trail deposition accordingly (Devigne et al. 2004; Czaczkes et al. 2011a). By modulating trail deposition in reference to long-term, indirect cues (home-range markings), medium-term indirect cues (trail pheromones) and short-term direct cues (encounter rates) ants can respond to changes in colony needs and the environment over a wide range of time scales.

The modulation of positive feedback described here is a further example of a bottom-up self-organised mechanism in the regulation of collective foraging behaviour in ants. A picture is emerging of complex organisation in social insect foraging, with a combination of positive and negative feedback loops acting to adjust the numbers of workers directed to specific locations as appropriate to both environmental and colony conditions. Social insect colonies use both passive processes and active responses to information integrated from multiple internal and external sources to organise themselves in an adaptive manner.

Acknowledgements

T.C. was supported by a PhD studentship from the BBSRC. G.C. was funded by a postdoctoral fellowship from the Swiss National Science Foundation [SNSF grant no: PA00P3 129134].

Chapter 9: Negative feedback enables fast and flexible collective decision-making in ants

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Abstract

Positive feedback plays a major role in the emergence of many collective animal behaviours. In many ants pheromone trails recruit and direct nestmate foragers to food sources. The strong positive feedback caused by trail pheromones allows fast collective responses but can compromise flexibility. Previous laboratory experiments have shown that when the environment changes, colonies are often unable to reallocate their foragers to a more rewarding food source. Here we show both experimentally, using colonies of *Lasius niger*, and with an agent-based simulation model, that negative feedback caused by crowding at feeding sites allows ant colonies to maintain foraging flexibility even with strong recruitment to food sources. In a constant environment, negative feedback prevents the frequently reported bias towards one feeder (symmetry breaking) and leads to equal distribution of foragers. In a changing environment, negative feedback allows a colony to quickly reallocate the majority of its foragers to a superior food patch that becomes available when foraging at an inferior patch is already well underway. The model confirms these experimental findings and shows that the ability of colonies to switch to a superior food source does not require the decay of trail pheromones. Our results help to resolve inconsistencies between collective foraging patterns seen in laboratory studies and observations in the wild, and show that the simultaneous action of negative and positive feedback is important for efficient foraging in mass-recruiting insect colonies.

Introduction

Positive feedback is the basis for the emergence of many different types of collective behaviours in a wide range of organisms from bacteria to mammals, including group migration, aggregation, nest-site choice, nest construction, and collective foraging (Bonabeau et al. 1997; Camazine et al. 2003; Amé et al. 2006; Buhl et al. 2006; Lukeman et al. 2010; Zhang et al. 2010; Seeley 2010; Sumpter 2010). One consequence of strong positive feedback is that groups may focus on or choose only a sub-set of the available options (Beckers et al. 1990; Traniello & Robson 1995; Sumpter & Beekman 2003; Camazine et al. 2003; Schmidt et al. 2006; Halloy et al. 2007; Jeanson & Deneubourg 2009; Sumpter 2010). For example, rather than exploiting two identical resources equally, groups of spiders (Jeanson et al. 2004), cockroaches (Amé et al. 2006) or ants (Beckers et al. 1990; Sumpter & Beekman 2003) often predominantly use just one site. This "symmetry breaking" can be a consequence of the amplification of small, sometimes random, differences in the amount of socially-transmitted information, for example ant trail pheromones, favouring one of the options (Sumpter & Beekman 2003; Sumpter 2006; Detrain & Deneubourg 2008; Couzin 2009). Species of ants and stingless bees with mass-recruitment of foragers via trail pheromone often show limited ability to switch to a better food source because of the strong non-linear response of recruits to the pheromone (Beckers et al. 1990; Traniello & Robson 1995; Camazine et al. 2003; Schmidt et al. 2006, but see Dussutour et al. 2009a). For example, in a classic experiment Beckers et al. (1990) found that *Lasius niger* ant colonies were unable to switch from a low quality feeder to a high quality feeder that became available later. Inflexibility in the reallocation of foragers to newly appearing food sources is surprising because it could lead to reduced colony foraging efficiency, especially in natural environments where changes in food source profitablity and location are inevitable. On the other hand, it has been suggested that focusing on one or a few food sources is advantageous because it helps a colony to defend these against competitors or predators (Camazine et al. 2003; Detrain & Deneubourg 2008; Sumpter 2010).

Information about the dynamics of forager allocation in natural environments in mass-recruiting ants such as *Lasius niger* is scarce. However, one study suggests that while competition does affect the number of foragers at natural food sources, the allocation of ant foragers in nature seems to differ from that observed in laboratory studies (Dreisig 1988). In particular, under natural conditions colonies do seem to be able to allocate foragers according to food source profitability in ways that suggest collective flexibility rather than the strong symmetry breaking and near-irreversible collective decisions seen in laboratory studies (Dreisig

1988). Dreisig (1988) found that in several ant species the presence of more workers at natural food sources decreases the rate of energy gain per individual, suggesting that workers inhibit each other's energy intake. This suggests that reduced individual gains due to crowding may cause negative feedback. Negative feedback such as from crowding can counterbalance positive feedback (Bonabeau et al. 1997; O'Toole et al. 1999; Robinson et al. 2005, 2008a; Detrain & Deneubourg 2008; Couzin 2009; Nieh 2010; Sumpter 2010; Seeley et al. 2011) and crowding has been shown to lead to an ideal free distribution of cockroaches underneath shelters (Halloy et al. 2007) and more equal traffic flow in foraging *L. niger* ants (Dussutour et al. 2004) with access to two parallel pathways on the main trail to the nest.

Although effects of crowding on the foraging behaviour of individual ants have been shown, and the potential for crowding to affect the collective exploitation of food sources has been recognised (Wilson 1962; Verhaeghe & Deneubourg 1983; Mailleux et al. 2003a; Detrain & Deneubourg 2008), the critical experiments have not yet been performed to show that crowding can prevent "symmetry breaking" in foraging ants. In addition, the hypothesis that negative feedback allows flexibility in a changing environment remains untested. Our study addresses these hypotheses in two ways. First, we used laboratory colonies of *L. niger* to investigate the effect of crowding at food sources on forager allocation under both stable and changing food source distributions. Second, we built an agent-based simulation model to test the role of crowding in the same situations. A stable foraging environment was set up by simultaneously offering each colony two identical food sources. The changing environment was set up by initially providing just one food source, with a second, better food source being provided 15 minutes later by which time this first source was already being exploited. In both experiments, in order to vary the strength of any negative feedback due to crowding, we varied the number of feeding holes, thereby mimicking food patches of different sizes. We hypothesized that in the stable situation an increase in the strength of the negative feedback due to crowding would lead to a more even distribution of foragers. In the changing environment we hypothesized that negative feedback caused by crowding at the first food source would allow colonies to reallocate foragers to the second, better, food source.

Methods

Study species

We studied six colonies of *Lasius niger* collected on the University of Sussex campus. Like many ants, *L. niger* collect carbohydrates in the form of honeydew secreted by aphids and

nectar from flowers (Oliver et al. 2008). Experimental colonies were housed in plastic foraging boxes (40×30×20 cm high) containing a circular plaster nest box (15cm diameter, 2cm high). The colonies were queenless and had 2400-4700 workers (individually counted at the beginning of the experiments). Queenless colonies forage, make trails and are frequently used in foraging experiments (Dussutour et al. 2004; Evison et al. 2008; Czaczkes et al. 2011a; Grüter et al. 2011). Colonies were fed 3 times per week with a mixture of honey, raw egg and agar and given water *ad libitum*. Colonies were deprived of food for 4 days prior to a feeding trial to ensure that the ants were motivated to forage and recruit nestmates to a sucrose syrup feeder. Pheromone deposition in *L. niger* is a very characteristic behaviour and is easily observed. To deposit pheromone on the substrate, a forager interrupts her walk for a fraction of a second and curves the abdomen to touch the substrate with the tip (Beckers et al. 1992a). Only successful foragers deposit pheromone in *L. niger* (Beckers et al. 1992a but see Mailleux 2006).

Experimental setup

Ants were given access to a T-shaped trail system with an 18 x 2cm stem and two 10 x 2cm branches. The end of each branch widened into a circular platform 8.8cm in diameter to accommodate a feeder. The entire apparatus was covered in standard printer paper that was replaced after each trial. This was to ensure that the foraging substrate for each trial was unmarked by ant pheromones or other secretions. A 1M sucrose feeder was placed on each circular platform. The distance between the two feeders was approx. 30cm (branch + platform). Each feeder consisted of a sealed petri-dish, 5cm in diameter, with a number (1, 3, 9 or 27) of 1mm diameter holes in the base (appendix D figure S1). The ants stood underneath the feeder to collect syrup. The feeder was raised on four 2cm long disposable wooden legs (appendix D figure S1). The holes were large enough for up to 8 ants to feed simultaneously at any one hole. Sucrose solution was available in unlimited quantity.

Experiment 1: stable environment with two identical food sources

We used four different feeder combinations to create different levels of crowding by using two identical feeders each with 1, 3, 9 or 27 holes. Each of the six colonies was tested in each of the four combinations. Each trial lasted for 120 minutes from the time the first ant started feeding. The number of ants feeding and the number of unoccupied feeding holes on each feeder were counted every 5 minutes. The number of full and empty ants leaving the feeder and the number of pheromone depositions on each branch were counted for 2 minutes

every 15 minutes. Full ants are easily recognised by an observer by the extended and striped (separated abdominal segments) abdomen. To facilitate counting, a 6cm section on each branch was marked on the substrate paper and ants and pheromone depositing behaviours were counted on this section. Two observers collected these data, one per 6cm section. Additionally, the 2 sections were filmed with a high definition video camera (Sony HDR-XR520) to analyse whether empty ants leaving a feeder chose the branch leading to the second feeder or the branch leading to the nest.

Experiment 2: changing environments with unequal access to equal-concentration food sources

In this experiment, the second food source was introduced 15 minutes after the discovery of the first food source. The second food source had 3 times as many feeding holes as the first. The feeder combinations were 1 versus 3 holes, 3 versus 9, and 9 versus 27 holes. Each of the six colonies was tested in each of the three combinations. A trial lasted 90 minutes from the time the first ant started feeding. Fifteen minutes later, the second feeder was introduced and was usually discovered within 3 minutes. The number of ants at each feeder and the number of unused feeding holes were counted every 2 minutes for 90 minutes.

The agent based simulation model

We developed a spatially explicit agent-based model of foraging agents using NetLogo 4.1.2 (Wilensky 1999) (the NetLogo file can be found in the online material of the published article. Please rename the file extension from *.txt to *.nlogo). The model description follows the ODD (Overview, Design concepts, Details) protocol (Grimm et al. 2006, 2010).

Purpose

The purpose of the model was to explore the effects of different crowding thresholds on the allocation of foragers as described in experiments 1 and 2. Additionally, we tested the role of pheromone decay rates on forager allocation in a changing environment. The model is not intended to be an exact and fully parameterized model of *L. niger* foraging. While the modelled situation is based on our experimental set-up, the aim was to build a more generic model that captures the key elements of ant foraging and recruitment to investigate how crowding affects worker allocation in a species with strong positive feedback via pheromone trails and negative feedback via crowding at food sources.

Model entities, state variables and scales

For most simulations, we used 500 agents (see Table 9.1 for parameters), which corresponds approximately to the number of ants that can be expected to forage during a typical experimental trial using colonies with several thousand ants (i.e., not all the ants in a colony forage). Agents could assume any one of 6 different states: idle inside the nest, searching for food, feeding at a food patch, at a food patch but unable to feed due to crowding (dissatisfied), laying a pheromone trail while returning to the nest (recruiter), unloading.

Colony size	500 agents
Leaving rate of idle foragers	(2/1000 * no. agents in the nest)/sec
Crowding threshold	8, 24, 72, 216
Drinking time	60 time steps (60 seconds)
Return-to-nest time	ca. 40 time steps
Unloading time	60 time steps
Time delay between introduction of both food sources	0 time steps (Part C); 900 time steps (Part D)
Amount (*c*) of chemical deposited per patch	60 pheromone units
Pheromone decay rate r	0.4 (corresponds to a decay in c. 2700 time steps or 45 min)
Amount of pheromone at t	$C_i(t) = C_i(t - 1) \times (100 - r)/100$
Pheromone detection threshold per patch	0.05 pheromone units

Table 9.1 - Overview of processes, parameters and default values used in the model.

The simulated agents occupied a specific location at every point in time and were located on a two-dimensional square grid with the shape of a T-maze connected to the nest. The default branch width was 4 squares, the stem width was 5 squares. The default lengths were 24 squares for the stem and 11 for each arm. The nest was located at the base of the T-maze (5 x 4 squares), with one food patch at the end of each branch (4 x 4 squares). Multiple agents could occupy the same square. Simulations were run in discrete time steps (*t*). One time step was made to correspond approximately to one second in the experiment in the following way. It took real ants approximately 40 seconds to walk from a food source to the nest. Hence, for the model we chose branch and stem lengths that required approximately 40 time steps with an agent walking-speed of 1 square per time step (agents did not always walk in a perfectly direct way from nest to feeder). Thus one time step in the model corresponds to one second. Total model running time was 5400 time steps, corresponding to approximately 90 minutes. Because of the stochastic nature of the model, 30 model runs were performed for each combination of parameter values. For each run, the random number generator was uniquely seeded based on the operating system's time and date (Wilensky 1999).

Model process overview and scheduling

Agents that left the nest started to perform a random walk (searching) until a food patch or, at a later stage of the simulation, a pheromone trail was encountered. Agents finding a food patch spent 60 time steps taking on food at the patch if there was no crowding. Successful agents then walked directly to the nest and then took 60 time steps to unload. After unloading, agents could leave the nest and start searching again (random walk) or follow a trail. If food patches were crowded, agents became dissatisfied.

During the simulation, the behavioural states and variables were updated for each agent at every time step. The different states were updated asynchronously in sequence (idle agents -> foraging agents -> feeding agents -> dissatisfied agents -> recruiting agents -> unloading agents). However, the model was robust to changes in the sequence (see appendix D figure S2).

In model 1 (corresponding to experiment 1), two identical food sources were offered simultaneously. Figure 9.2B shows that 1 feeding hole can accommodate a maximum of 8 foragers. Hence, we again used 4 different crowding thresholds, which corresponded to the crowding levels in the experiment: high (8 agents ≈ 1 feeding hole), medium (24 agents ≈ 3 feeding holes), low (72 agents ≈ 9 feeding holes) and very low (216 agents ≈ 27 feeding holes). For simplicity, crowding was modelled as an all-or-nothing state. For example, if 8 agents were already present at a food patch in the high crowding situation, other agents at the feeder location could no longer access the food and became dissatisfied. Apart from crowding, food patches were *ad libitum* as in the experiments.

In model 2 (corresponding to experiment 2), one food source was introduced with a delay of 900 time steps (~ 15 minutes). This second food source permitted 3 times as many agents access to forage before the crowding threshold was reached (8 vs. 24 agents, 24 vs. 72 agents, 72 vs. 216 agents). If the number of agents on a food patch was higher than the crowding threshold for this patch, a newly arrived agent became dissatisfied and performed a random walk.

Model design concepts

The pheromone deposited on the trail system and the proportions of agents at the two food sources, as influenced by both negative and positive feedback, are emergent properties of the model. The concepts of adaptation, objectives and prediction are not important in this model. There is no learning in the model.

Sensing is important in this model: agents leaving the nest were able to detect pheromone left on patches and oriented themselves according to the amount of pheromone. The agents' ability to detect pheromone differences was perfect, and agents always follow the higher pheromone level. This is a simplification, as in reality ants have a limit to their ability to discriminate differences between pheromone trail strengths, and differences in pheromone strengths affect the probability of an ant taking a trail (Hangartner 1969b). Nonetheless, this simplification was sufficient for our purposes. Agents that had fed successfully at either food source, and were walking back to the nest were assumed to know the direction of the nest (implemented by means of a nest odour). Furthermore, agents reaching the food patch were able to perceive whether the number of agents on a food patch equalled the crowding threshold for this patch (implemented by counting the number of feeding agents on the patch.

Stochasticity is used to introduce variability in the number of agents leaving the nest at any time step (Table 9.1) and in their random walks.

Model initialisation

At the beginning of each simulation trial, the nest, the T-maze and the food sources were initialised as described above. The amount of pheromone chemical was set $C_{pheromone} = 0$ for all patches, and the nest scent of patch X was set to C_{nest} = 100 - distance (X_{nest}, X), where X_{nest} is the patch at the centre of the nest. All agents were initiated at the nest centre and their state set to idle, with a probability P_{leave} (Table 9.1) to leave the nest.

Submodels

The move-foraging submodel defined how agents behave after leaving the nest: Agents could follow a pheromone trail by sampling 3 patches in walking direction (0°, 45° left and 45° right) and walk towards the direction of the patch with the most pheromone. Pheromone was detected if the amount exceeded a threshold level of pheromone (Table 9.1). We assumed the pheromone chemical to be volatile, and chose an evaporation rate that led to a decay of the pheromone trail below the perception threshold of the agents that was equivalent to approximately 45 minutes. This was based on the pheromone strengths we measured during test runs and corresponds to experimentally measured values for *L. niger* (Beckers et al. 1993). The decay of pheromone was calculated for each square of the grid at each time step as $C_{i,t} = C_{i,(t-1)} \times (100 - r)/100$; where C_i is the chemical on patch i, r is the evaporation rate in % and t is the time point, leading to an exponential decay (see also table 9.1). If the 3 patches in walking

direction had no pheromone or below-threshold pheromone levels, then the agent moved in a random direction (towards 8 possible patches).

The move-to-nest submodel defined the behaviour of agents after successful foraging. Full agents perceived the strength of the nest-odour (see section Initialisation) on patches and behaved as they did in the case of pheromone. In nature, ants find their nest relying on various methods such as land-mark learning, path-integration and olfaction (Collett et al. 2003; Steck et al. 2009; Collett 2009). For the purpose of this model, the method of finding the way back to the nest was irrelevant. The quality of food patches was high in that all successful agents deposited a pheromone trail with amount *c* on each patch they cross when walking back to the nest. This is a simplification as in nature not all ants deposit trail pheromones and ants also deposit pheromone when walking from the nest to the food source (Mailleux et al. 2005). However, for the purpose of our model this was irrelevant, because we simply wanted the agents to establish an attractive pheromone trail with a certain decay rate.

Dissatisfied agents performed a random walk without paying attention to the pheromone trail or laying a pheromone trail. For all agent movements described above, agent step size was equivalent to 1 patch length independent of direction. Movements were therefore off-lattice.

Sensitivity analysis

In order to test how strongly our results depended on the values of key parameters we systematically varied the number of agents, pheromone decay rates, side branch lengths, and the length of the stem. The model was robust over a wide range of these parameters. Some deviations (e.g. with larger T mazes) are given in the Results section of the paper.

Statistical analysis

We used linear mixed-effect models (LME) and the statistical package R 2.9 (R Development Core Team 2009) to analyse the experimental data. R fitted the models with the lme-function of the nlme-package (Zuur et al. 2009). In experiment 1, the response variables in the different models were (i) the relative difference between the two branches, (ii) the number of empty ants leaving the nest and (iii) the ratio between empty and full ants leaving the nest. In experiment 2, the response variable was the proportion of ants foraging at the second feeder. We included colony and trial as hierarchically nested random effects to control for the non-independence of data points from the same colony and the same trial (Zuur et al. 2009). If necessary, we transformed the response variable with a square-root transformation

to achieve a normal distribution. For model selection we used the protocol proposed by Zuur et al. (2009). We first explored the optimal structure of the random components (comparing random intercept models with random intercept and slope models). We then explored the significance of the fixed effects. Our fixed effects were the number of holes and time of measurement. Time of measurement was included because previous studies showed temporal changes in forager allocation in similar experiments (e.g. Beckers et al. 1990) The interaction between the two fixed-effects was removed for the final model if it was not significant ($p > 0.05$). The final model always included both fixed-effects. If we tested datasets multiple times, we adjusted the significance levels using the sequential Bonferroni method (Sokal & Rohlf 1995).

Results

Experiment 1: stable environment with two identical food sources

When a trial began, the feeders were discovered within a few minutes and a rapid build up of foragers was observed. When both feeders had 1 feeding hole (1:1) both had very similar numbers of ants (Figure 9.1A). Conversely, when both had 9 (9:9) or 27 (27:27) holes, foraging activity was strongly biased towards one feeder (Figure 9.1C,D). An intermediate pattern is found when both feeders had 3 (3:3) holes (Figure 9.1B). When feeders had 9 (9:9) or 27 (27:27) holes, the feeder that had more foragers after 5 minutes was usually (11 of 12 trials, which is significantly different from the 50:50 random expectation: $\chi^2 = 8.33$, df = 1, $p = 0.004$) the feeder that was exploited more, on average, during the entire 120 minute trial. The relative difference in the number of ants foraging at the two feeders differed significantly between treatments (LME, random intercept and random slope [for "time"] model: t-value = 4.58, $p = 0.0003$; Intraclass Correlation Coefficient (ICC): colony = 0.45; trial = 0.12; Figures 9.1 and 9.2A; see table 9.2 for pair-wise comparisons). Overall, the differences between the two feeders tended to decrease over time (t-value = -1.86, $p = 0.064$). When analysing each treatment separately, we found that the proportion of ants feeding at the feeder that had more ants after 5 min was not different from 0.5 when both feeders had 1 hole (0.51 ± 0.04 [mean ± SD], one-sample t-test: t-value = 1.37, df = 22, $p = 0.18$). If feeders had more holes, the proportion of ants feeding at this feeder was significantly higher than 0.5 (3:3 holes, 0.59 ± 0.04, t-value = 12.7, df = 22, $p < 0.0001$; 9:9 holes, 0.62 ± 0.05, t-value = 11.3, df = 22, $p < 0.0001$; 27:27 holes, 0.63 ± 0.06, t-value = 10.6, df = 22, $p < 0.0001$.

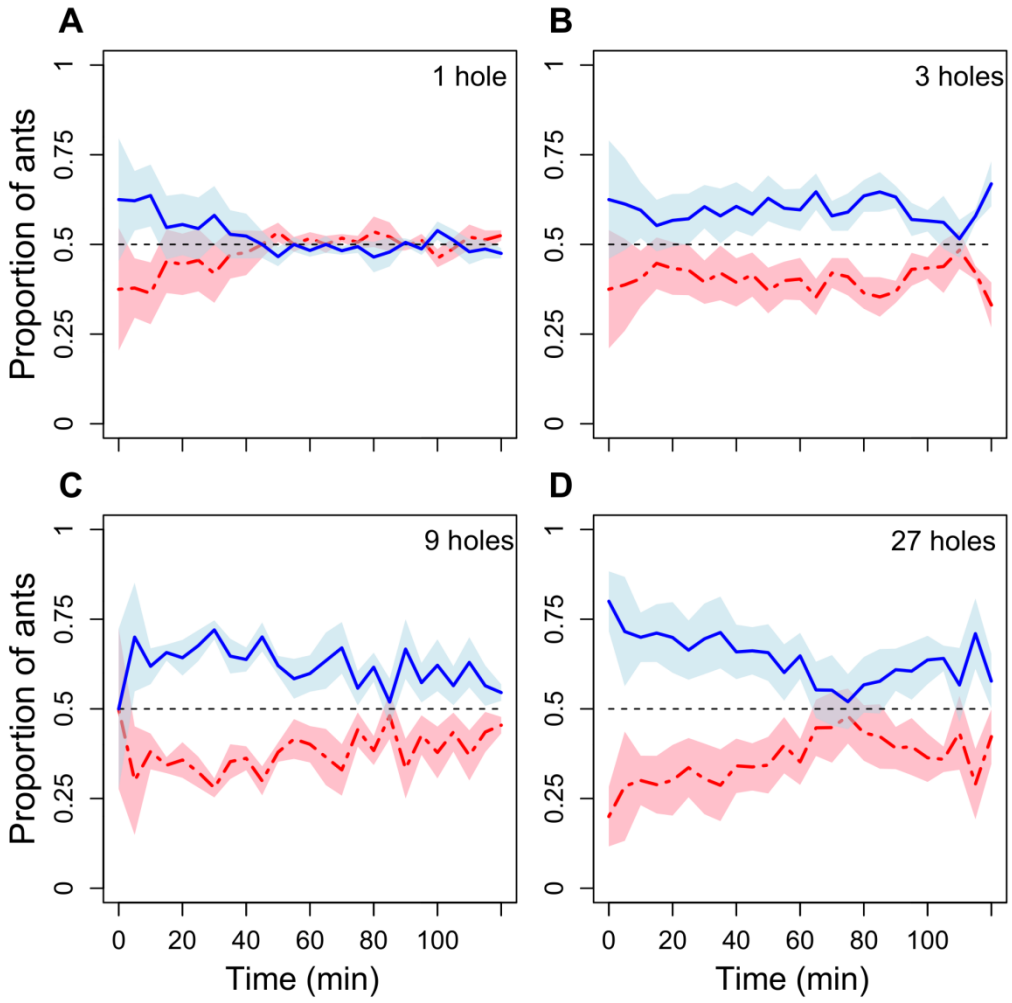

Figure 9.1 - Experiment 1. Proportions of ants visiting two identical 1 molar sucrose feeders each with 1, 3, 9 or 27 feeding holes. The blue line represents the feeder that had more ants after 5 minutes, the red line the other feeder. The dashed black line indicates an equal distribution of ants at both feeders. Data represent the mean of 6 test colonies, 1 trial per colony for each number of holes. The shaded areas (light blue and pink) represent the standard errors (SE) of the mean.

Figure 9.2B shows that crowding, quantified as the number of syrup-drinking ants per feeding hole, was negatively correlated with the number of holes. The fact that feeder use was also more similar at 3 (3:3) holes versus 27 (27:27) holes (Figure. 9.2A) shows that also moderate levels of crowding (Fig. 9.2B) cause enough negative feedback to have some balancing effect. The number of feeding holes affected the number of unsuccessful (empty) ants leaving a food source (first 60 min of experiment: LME, random intercept model: t-value = -3.16, p = 0.0056; ICC: colony = 0.21; trial = 0.50). More empty ants left the feeder if it had only 1 feeding hole (pair-wise comparisons shown in table 9.2). The number of full ants leaving a feeder is shown in Fig. 9.2C. As a consequence, the resulting ratio between empty and full ants leaving the feeders was also affected by the number of feeding holes (Figure 9.2D). All

ratios differed significantly between treatments except 9 versus 27 holes (Table 9.2). Our videos also showed that a substantial proportion of empty ants leaving the feeder under high crowding conditions (1:1) walked towards the second feeder, instead of walking towards the nest (Figure 9.2F). The probability of full ants to walk to the second feeder instead of returning to the nest was much lower (Figure 9.2F, LME, random intercept model: t-value = -6.8, $p < 0.0001$; ICC for colony = 0.12). Overall, the proportion of ants walking back to the nest increased with time (t-value = -3.1, $p = 0.003$), probably due to satiation (Figure 9.2F). The same model also showed an interaction between “time” and “state” (full or empty) (t-value = 2.91, $p = 0.005$), indicating that the difference between full and empty ants in their propensity to walk back to the nest decreased over time.

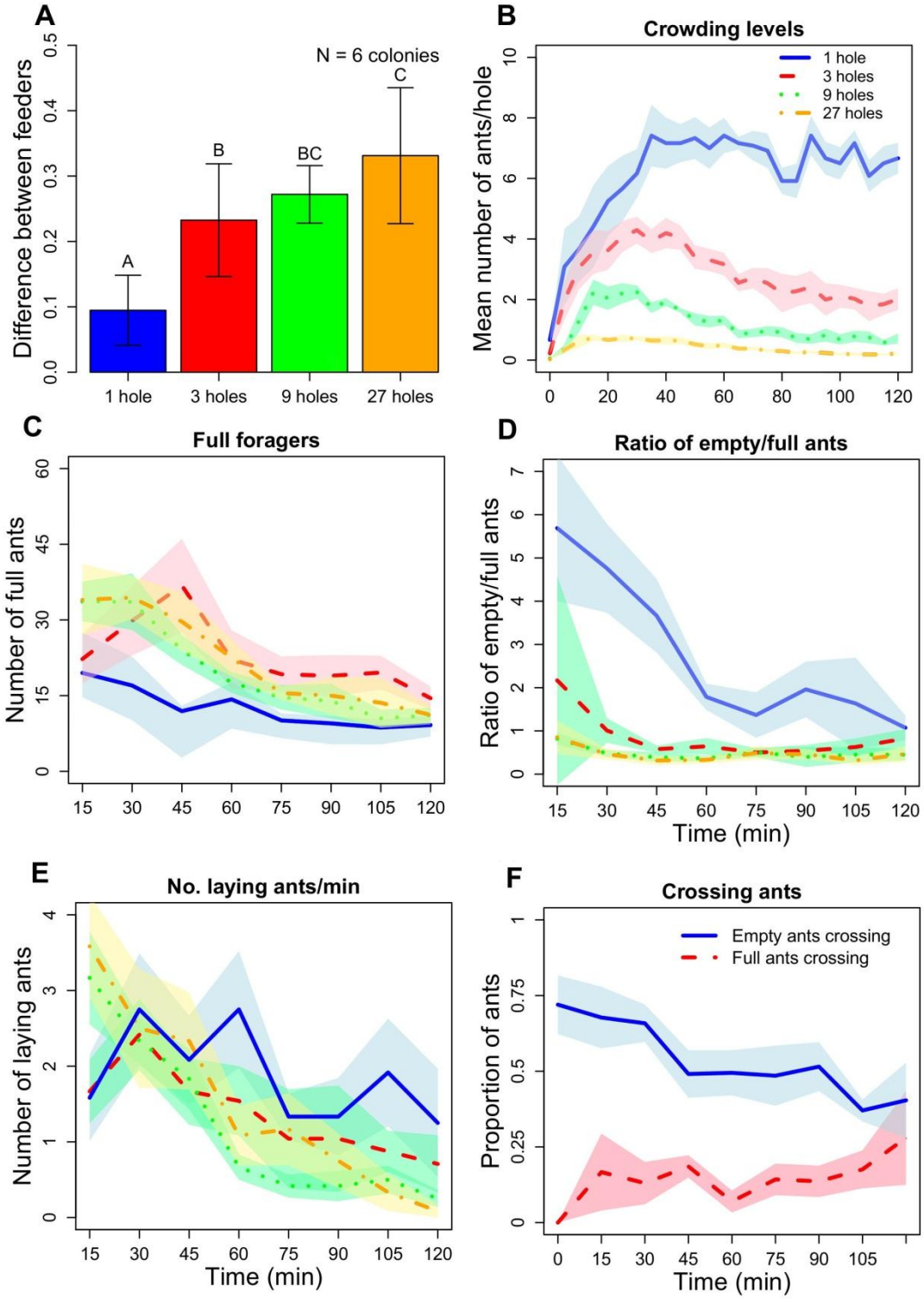

Figure 9.2 - Experiment 1. (A) Mean average difference in the proportions of ants feeding at the two feeders during the whole of the 120 minute trial. Bars show the mean and standard error for the 6 test colonies, one trial each per treatment. The letters above the bars indicate statistically significant differences (linear mixed-effect models: $P < 0.05$; see results for details). (B) Mean number of ants per hole during the whole of the trial. (C) The mean number of successful, i.e. full ants leaving a feeder (averaged for the two feeders) counted over 2 minutes. (D) The mean ratio of empty to full ants returning to the nest for the 6 colonies, measured every 15 min. (E) The mean number of ants laying trail pheromone on either branch. Ants were counted during 2 min every 15 min. (F) The mean proportion of empty (blue line) and full (red line) ants leaving a feeder under high crowding conditions (both feeders had 1 hole) and walking towards the other feeder instead of back to the nest. As can be seen the proportion of empty ants walking towards the other feeder was considerable higher than the proportion of full ants. The shaded areas represent the SE of the mean.

Exp 1 - Difference between identical feeders	t-value	p-value
1 vs. 3 holes	2.95	< 0.0099*
1 vs. 9 holes	4.96	< 0.0002*
1 vs. 27 holes	6.34	< 0.0001*
3 vs. 9 holes	2.01	0.063
3 vs. 27 holes	3.39	< 0.004*
9 vs. 27 holes	1.39	0.19
Empty ants leaving feeder		
1 vs. 3 holes	-2.56	0.01*
1 vs. 9 holes	-4.38	< 0.0001*
1 vs. 27 holes	-4.72	< 0.0001*
3 vs. 9 holes	-1.81	0.071
3 vs. 27 holes	-2.16	0.031
9 vs. 27 holes	-0.35	0.73
Effect of time	-9.85	< 0.0001
Ratio empty/full ants		
1 vs. 3 holes	-6.6	<0.0001*
1 vs. 9 holes	-9.2	< 0.0001*
1 vs. 27 holes	-10.83	< 0.0001*
3 vs. 9 holes	-2.66	0.018*
3 vs. 27 holes	-4.28	<0.0007*
9 vs. 27 holes	-1.61	0.13
Effect of time	-4.97	<0.0001
Exp 2 – difference between non-identical feeders		
1/3 holes vs. 3/9 holes	-2.06	<0.066
1/3 holes vs. 9/27 holes	-4.38	0.0014*
3/9 holes vs. 9/27 holes	-2.32	<0.043

Table 9.2 - Effects of the number of feeding holes on the difference in the proportions of ants visiting two identical (Exp 1) or two different (Exp 2) feeders, the ratio between ants returning full or empty from the feeder. *Significant after sequential Bonferroni correction. The raw data for the tests presented in this table is provided in appendix D.

Experiment 2: changing environments with unequal access to equal-concentration food sources

This experiment investigated how crowding affects the allocation of foragers to a second feeder made available 15 minutes after the first, but with 3 times as many feeding holes. Colonies with high levels of crowding at the first feeder (1:3) quickly, within an average of 10 minutes, reallocated the majority of foragers to the new 3-hole feeder (seen by the crossing of lines in figure 9.3A). Conversely, colonies continued to allocate foragers mainly to the first feeder when the first feeder had generous feeding access, 9 holes (9:27; figure 9.3C). A situation with moderate crowding (3:9) leads to an intermediate pattern. The proportion of foragers visiting the second feeder during the last 50 minutes of a trial differed significantly between the treatments (LME, random intercept model: number of holes: t-value = -4.12, p = 0.00171; effect of time: t-value = 3.38, p = 0.0008; ICC: colony < 0.001; trial = 0.44; pair-wise comparisons are shown in table 9.2). The significant effect of time shows that, overall, the proportion of ants feeding at the second feeder increased during the last 50 minutes of the experiment.

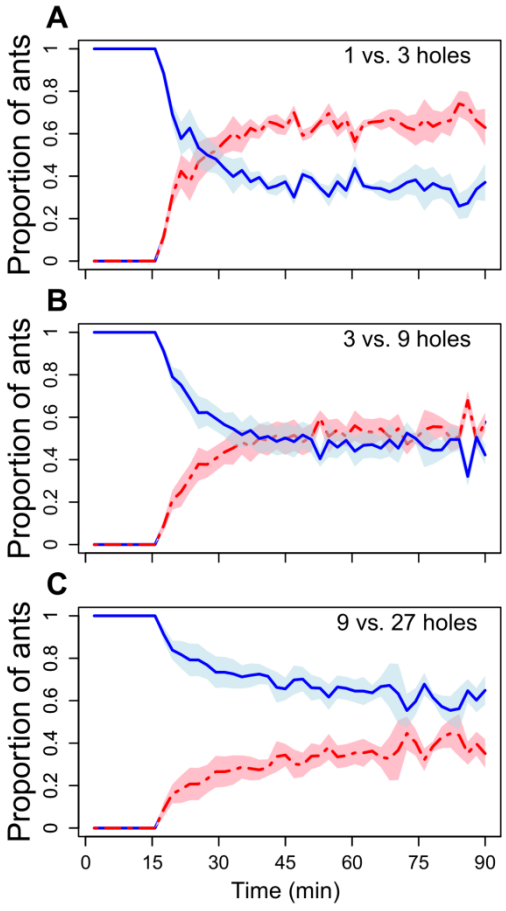

Figure 9.3 - Experiment 2. The mean proportion of ants at the two feeders, in which the second feeder (red line) had three times as many feeding holes but was made available 15 minutes after ants starting collecting syrup at the first feeder (blue line). As can be seen, the lines cross for the 1 versus 3 hole situation, but not for 9 versus 27. The shaded areas represent the SE of the mean.

Agent-based models 1 and 2

Figure 9.4 shows how crowding affects the proportion of agents exploiting the food patch that had more agents after 600 time steps (corresponding to 10 minutes). There is a clear effect of the number of agents that can simultaneously forage at a patch on the degree of symmetry breaking. While strong crowding, in which a low number of agents can simultaneously forage at a given patch, leads to a more equal distribution of agents at both food patches (figure 9.4A), low crowding leads to strong symmetry breaking.

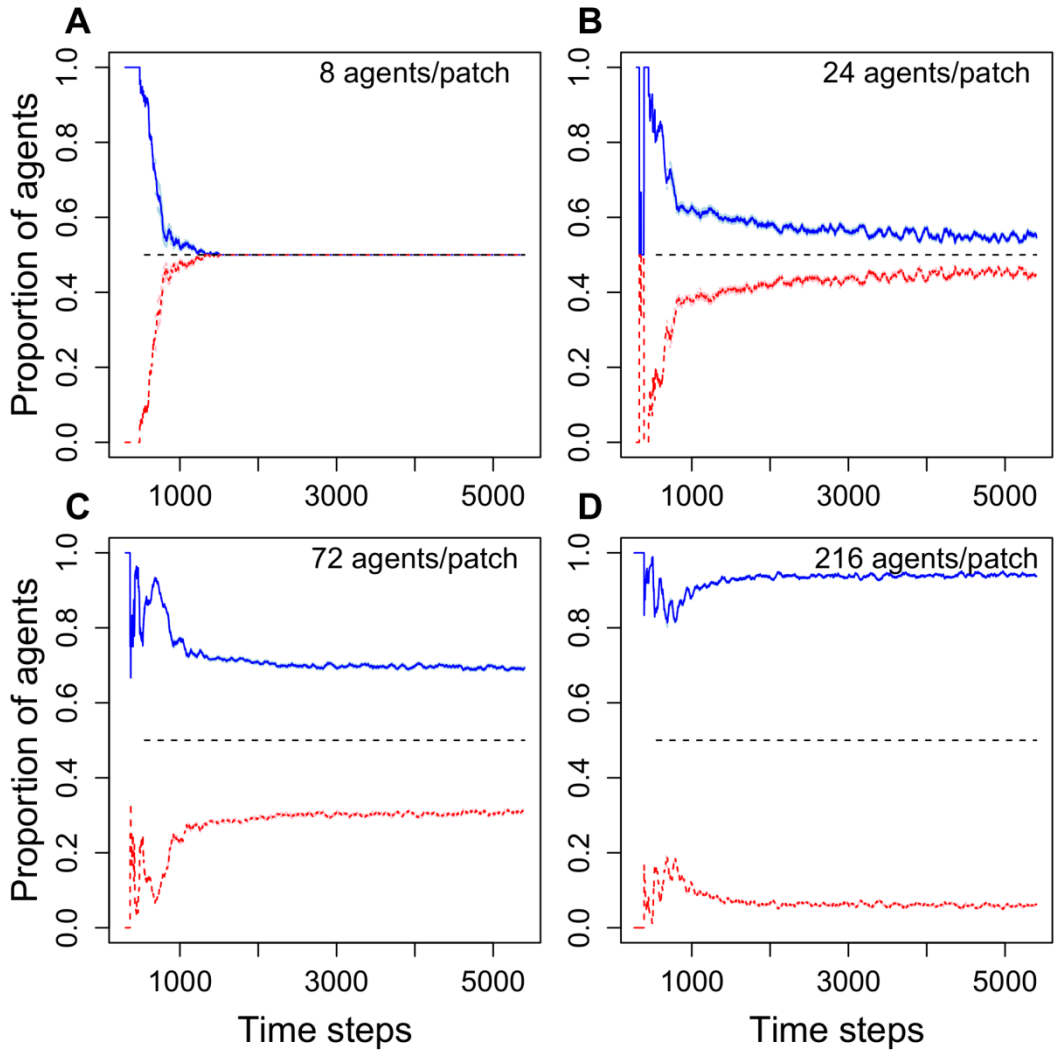

Figure 9.4 - Model 1. Proportions of agents visiting two identical food patches each with space for 8, 24, 72 or 216 foraging agents. The blue line represents the patch that had more agents after 600 time steps, the red line the other. The dashed black line indicates an equal distribution of agents at both feeders. Data averaged from 30 simulations in each situation. The standard deviation (StDev) is shown in light blue and pink, but is too small to be seen by eye.

As in experiment 2, if a superior food patch is made available after a delay, high levels of crowding lead to rapid reallocation of foragers to the superior new patch (figure 9.5A), but without crowding agents do not reallocate (figure 9.5C). Intermediate levels of crowding lead to an intermediate pattern (figure 9.5B). As the switch to the superior patch is more rapid under high crowding conditions this suggests that flexibility does not require pheromone decay. Indeed, the food patch that is introduced with a time delay received more foragers even before its branch had more trail pheromone (figure 9.6A). On average, more agents were present at the second feeder after 1115 time steps, while the amount of pheromone present was only greater after 1374 time steps (averages of 30 simulations). However, pheromone decay rate does affect the time taken to switch: the faster the decay, the faster colonies reallocate agents to the second food patch (linear regression with log[decay rate]: t-value: -13.26, $p < 0.001$, $R^2 = 0.95$, figure 9.6B). However, even with zero pheromone decay on the branch leading to the first food patch, colonies can still switch to the more profitable second patch (figure 9.6B).

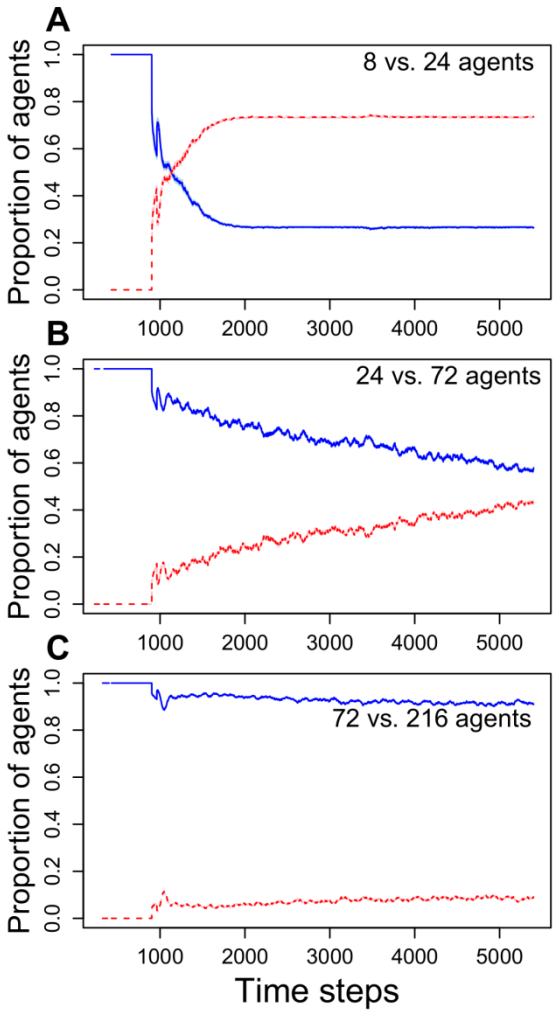

Figure 9.5- Model 2. Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 900 times steps after agents started foraging at the first food patch (blue line). Data averaged from 30 simulations in each situation. The StDev is shown in light blue and pink, but is again too small to be seen.

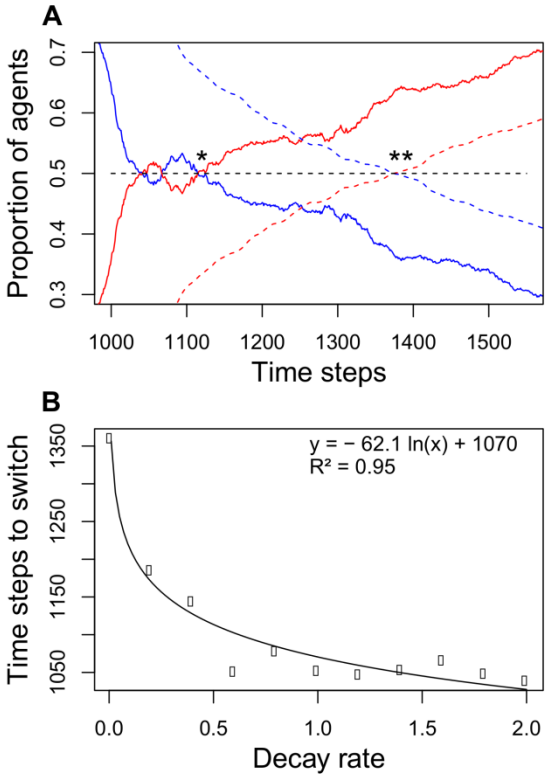

Figure 9.6 - (A) Switch point of agents (solid lines, *) and pheromone trail strength (dashed lines, **). The solid lines show the proportion of ants foraging at the first (blue) and second patch (red). The second patch was available after a 900 time step (15 minutes) delay, but allowed 3 times more agents to collect food simultaneously (8 vs. 24 agents). The dashed lines show the relative amounts of pheromone on the branches leading to the first (blue) and second patch (red). The pheromone switch happened some time after the switch in the number of foraging ants (average of 30 simulations). (B) Relationship between the pheromone decay rate and the time until more agents foraged at the second food patch. Note that the switch happens even with a pheromone decay rate of zero. A decay rate of 2.0 corresponds to a pheromone decay below the perception threshold of the agents in less than 10 minutes.

Sensitivity analysis

We tested whether the results of our model are sensitive to changes in the values of some key parameters to determine the robustness of our main findings. We found that colonies as small as 150 foraging agents were able switch to the second food patch at the strongest crowding threshold (8 vs. 24 agents that were allowed to forage simultaneously at a patch, corresponding to 1 vs. 3 feeding holes). Below this colony size, allowing 8 agents to forage simultaneously at the first patch no longer leads to a sufficient number of agents becoming dissatisfied due to crowding to cause a switch (appendix D figure S3). On the other hand, with the lowest crowding conditions (72 vs. 216 agents/patch, corresponding to 9 vs. 27 feeding holes) only colonies with more than c. 2000 foraging agents switched to the second food source (appendix D figure S4). The effect of decay rate was relatively small as shown in figure 9.6B. Increasing the length of the stem of the trail system to a distance corresponding to approximately 2 metres did not affect the ability to quickly switch to the second food patch with high crowding conditions (8 vs. 24 agents/patch) (appendix D figure S5). However, increasing the distance between the food patches, equivalent to longer arm length in a T-maze, had a stronger effect on the time it took for the switch to take place (appendix D figure S6). At a distance corresponding to about 2 metres between the two food patches, colonies

with 500 agents no longer switched (8 vs. 24 agents; appendix D figure S6C). We also tested if the probability of dissatisfied agents to walk to the nest instead of to the second food source affects how long it takes to switch to the second food source. The simulations show that the time to switch increases if the probability to walk to the nest increases (appendix D figure S7).

Discussion

Our results show that crowding results in negative feedback and enables colonies to allocate foragers more evenly between two feeders in a stable environment and to reallocate more foragers to a superior feeder in a changing environment. Our agent based simulation model confirms the role of crowding as a mechanism enabling this group-level flexibility.

Our experimental results indicate that the ability of a colony to switch to a superior food source is unlikely to depend strongly on pheromone trail decay to the first food source, which has been suggested as a potential mechanism (Dreisig 1988). Colonies switched on average after only 10 minutes, which is faster than expected if it were due to pheromone decay given that trail pheromones in *L. niger* persist for at least 40-60 minutes (Beckers et al. 1993; Evison et al. 2008). In addition, even under high crowding conditions ants continued to deposit pheromone when walking to and from a feeder (figure 9.2E). Our simulation supports this conclusion as colonies switched to a superior food source even if the pheromone trail to the first food source had not decayed. Indeed, there is a period during which the branch leading to the first food patch still has more pheromone even though the majority of agents are already foraging at the second food patch. Hence, the relative strength of the pheromone trails on both branches under crowded conditions is a consequence rather than a cause of the switch to the second food source. However, the model also shows that colonies can reallocate foragers more quickly if the pheromone decays faster (figure 9.6B). In our experiments, the two feeders were identical in terms of the sucrose concentration (1M), which is another important determinant of food source quality. We anticipate that the switch would have happened even faster if the second feeder would have had a higher sucrose concentration, as was the case in previous studies (e.g. Beckers et al. 1990). This is because higher sucrose concentration increases the intensity of pheromone depositions (Beckers et al. 1993).

Recent theoretical work suggests that stochasticity in the decision-making process or the use of two different types of pheromones could potentially lead to flexibility in collective decision-making in the ant *Pheidole megacephala* (Deneubourg et al. 1983; Dussutour et al. 2009a, 2009b). However, the underlying mechanisms for flexibility in *P. megacephala* require

further investigation. Our results demonstrate a simple mechanism in addition to stochasticity in *L. niger*. In crowded situations, many ants are unable to gain sufficient access to the food source, resulting in reduced food source profitability as experienced by individuals. As a consequence, many unsuccessful foragers leave the feeding site (figure 9.2D). A large proportion of these ants did not return to the nest but found the branch leading to the alternative feeder (figure 9.2F), thereby increasing the probability of using this feeder. Overall, unsuccessful foragers were approx. 3.2 times more likely to walk towards the alternative feeder than successful ants (figure 9.2F). The importance of this simple mechanism is supported by the results of the agent models. Here, dissatisfied agents (agents unable to collect food due to crowding) did not deliberately leave the crowded food source to search for another food source but found it via a random walk.

We suggest that in nature these three mechanisms (pheromone decay, stochasticity and searching by unsuccessful foragers) could potentially all result in colony-level flexibility, but would act on different time scales and might be more or less important depending on factors such as the geometry of the trail network and the distances between the food sources. For example, in the simulation model the probability that dissatisfied agents will discover the second food source, and, therefore, the ability of colonies to reallocate foragers, depended on the distance between the two sources (see sensitivity analysis). If the two food sources are far apart, dissatisfied agents performing a random walk are less likely to find the second source. On the other hand, the distance of the two food sources from the nest did not affect the ability of colonies to reallocate foragers quickly. Also the angle of the bifurcations have the potential to affect collective flexibility because bifurcation angle has been shown to affect branch choice and the U-turn probability of foragers of other ant species (Jackson et al. 2004; Gerbier et al. 2008; Helanterä et al. 2011). We simulated this by varying the probability of ants to walk back to the nest vs. to the second feeder (appendix D figure S7) and found that this probability indeed affects the speed of switching to the second food source. Depending on species, other feedback signals may also be used, such as pheromonal stop-signals deposited on unprofitable branches in Pharaoh's ants (Robinson et al. 2005, 2008a).

Our results help unify understanding of the distribution of an ant colony's foragers under both laboratory conditions with unrestricted access to food (Beckers et al. 1990) and natural conditions with more restricted availability (Dreisig 1988). In nature, foragers of many ant species depend heavily on honeydew produced by aphids or other Homoptera for their carbohydrate supply (Dreisig 1988; Völkl et al. 1999; Oliver et al. 2008). The amount of aphid honeydew produced per patch depends on species and number (Dreisig 1988; Völkl et al.

1999). The key determinant of aphid patch profitability seems to be the accessible amount rather than the quality of the produced honeydew (Völkl et al. 1999) and ants have been shown to distribute themselves among various patches according to the amount of honeydew produced by each aphid patch (Dreisig 1988). Hence, as in our experiment with high crowding, forager allocation among aphid patches depends on patch profitability rather than the sequence of food patch discovery. This ability to allocate foragers dynamically according to the profitability of food sources is also found in the honey bee, *Apis mellifera*. As in ants, successful honey bee foragers recruit nestmates to profitable food sources, but unlike ants they use the waggle dance (von Frisch 1967; Seeley 1995). The waggle dance is also a positive feedback mechanism, but the relationship between signal and response is more linear than is the case in ant trail pheromones (see Fig. 5.28 in Seeley 1995). As a consequence, honey bee colonies can exploit two identical food sources without symmetry breaking and are able to allocate more foragers to a superior food source that appears later without crowding (Seeley 1995; Detrain & Deneubourg 2008).

In summary, our results show that when strong and non-linear positive feedback occurs, negative feedback can prevent ant colonies becoming trapped in suboptimal collective states. This mirrors the balancing effects of negative feedback in other complex systems. In engineering, James Watt's steam regulator is a classic example and in human physiology a failure in negative feedback in the regulation of blood sugar level causes diabetes. We predict that negative feedbacks will be found to occur widely in other complex biological systems that have strong positive feedback mechanisms, to prevent the system becoming trapped in suboptimal states.

Acknowledgements

We thank Audrey Dussutour, Elva Robinson, Thomas Seeley and three anonymous referees for comments on the manuscript.

Chapter 10: Ant trail pheromones: new roles for an old system

T.J Czaczkes' & F.L.W. Ratnieks

Abstract

When considering ant trail pheromones, most biologists think of a chemical deposited from a food source to the nest, which directs nestmates from the nest to the food source. But this simple view of trail pheromones is just part of the picture. Here, we review the wide array of roles to which trail pheromones are put. Many of these roles are regulatory in nature, determining the foraging effort of colonies. Several are unexpected, such as using the trail network geometry to inform workers of foraging direction, or scouts monitoring intersection rates with trails to estimate a nest cavity size. Trail networks are often composed of two or more pheromones with different properties and longevities, allowing efficient exploration and monitoring of an area, and acting as an external memory. A repeated theme in the use of pheromone trails is complementarity with other information sources, be they social, public or private: how trails are deposited, or how they affect behaviour, is highly dependant on what the individual ant senses or knows. These other information sources are rarely redundant, often complementary and sometimes synergistic with trail pheromones. Whilst decision making in social insects has often been described as being based on simple individuals using simple behavioural rules, research over the last four decades has demonstrated surprising complexity and interdependence in the rules governing the use of trail pheromones.

Few people have not seen a trail of ants leading to a feeding site, frequently in their own kitchen or garden. Ant trails may be a common sight but they embody uncommonly important biological principles. They are outstanding examples of cooperation, with individual workers helping nestmates to find foraging locations, thereby enhancing colony foraging and the inclusive fitness of individual workers. In doing this, ant trails are also remarkable examples of adaptive complex systems, and in particular of adaptive organization at the group level with individual sub-units—worker ants—coordinating their activities to a common goal. What are the underlying mechanisms for achieving coordination? For over 200 years (Bonnet 1779), it has been known that foraging ants release scents, or in more modern parlance, pheromones. A worker who has found a good feeding site can, for example, deposit trail pheromone onto the substrate when walking back to the nest. Naïve nestmates can then follow this signal to food. Trail pheromone acts as positive feedback, directing more ants to where the food is. When the food is gone, negative feedback occurs via the evaporation of existing trail pheromone and the non-laying of additional pheromone. This classical view of an ant pheromone trail is a powerful one and was the inspiration for "ant colony optimization" (Dorigo & Di Caro 1999; Dorigo & Stützle 2004), a technique for obtaining high-quality computational solutions to questions that are unsolvable analytically. However, work over the past decades has shown that trail pheromones do more that simply guide ants from the nest to food sources, and also that ant trail systems rely on more than a single pheromone, indeed they rely on more than just pheromones. Here we review the role of trail pheromones in the organisation of ant colonies. Our main message is that trail pheromones are much more than simply wayposts for ants, and are used in a complex manner, often in conjunction with other information sources.

The classic view of trail pheromones – recruitment and worker allocation

The classic role of trail pheromones is rooted in a few simple responses of workers to trail pheromones uncovered in the 1960s and 70s (see figures 10.1 and 10.2). Firstly, in 1962, Wilson showed that trail pheromones laid at the entrance of a nest is sufficient to draw *Solenopsis saevissima* workers out of the nest, and that these workers would then follow a pheromone trail (Wilson 1962). The number of ants leaving the nest was directly related to the amount of trail pheromone presented. Then, in 1967, Hangartner demonstrated that *Lasius fuliginosus* workers could discern between two trails of different strengths at a bifurcation, with the number of ants taking one branch being proportional to the relative strength of the

pheromone trail on that branch. Lastly, as suggested by Wilson (1962) and demonstrated by Hangartner (Hangartner 1969a, 1970), ants deposit more pheromone to higher-quality food sources. These behavioural rules are simple, but their repercussions for trail organisation are far reaching. Wilson's (1962) experiments demonstrated that with the simple rules 'deposit trail pheromone if you have successfully fed" and "leave the nest in proportion to the amount of pheromone sensed', colonies could regulate the level of recruitment to a food source, and stop recruitment to over-exploited or exhausted food sources (see figure 10.1). With the addition of Hangartner's rules 'follow a trail in proportion to the amount of pheromone on it' and 'deposit more pheromone for better food sources' a very elegant system is constructed: colonies can now "choose" between multiple food sources, selecting the better food source due to preferential recruitment to that food source (see figure 10.2), and curbing recruitment when the food source becomes over-exploited. However, the interaction of these rules to allow decentralised decision-making was only realised and demonstrated several decades later by Beckers et al (1990, 1993). They found that when presented with a high quality (1mol) and low quality (0.1mol) food source *Lasius niger* colonies will eventually send most of their foragers to the better food source, and that this is driven by ants depositing more pheromone to the higher quality feeder. *L. niger* colonies, which rely solely on chemical mass recruitment, could become 'trapped' in suboptimal foraging situations if the poor feeder was presented first (but see Grüter et al. 2012 and chapter 9), whilst *Tetramorium caespitum*, which combine group and mass recruitment, could use the group recruiting behaviour to lead workers to the newer, high quality food source, and thus avoid sub-optimal foraging.

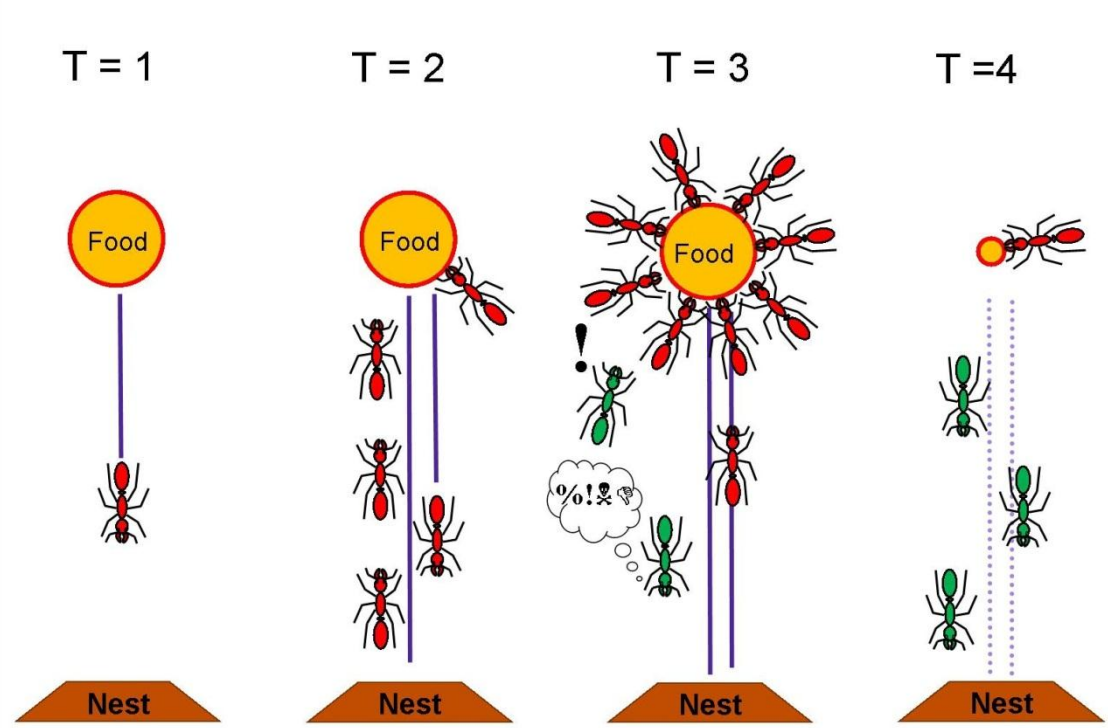

Figure 10.1 - Trail pheromones control the number of ants recruited to a food source.
One of the two 'classic' roles of trail pheromones. T = 1: A scout finds some food, and returns to the nest laying a pheromone trail. T = 2: Ants in the nest smell the pheromone trail and follow it to the food source. T = 3: when the food source is fully exploited, some ants (shown in green) cannot access the food source, and return without laying pheromone. Thus the trail is not strengthened, and the number of ants being sent to the food is regulated. T = 4: when the food source becomes depleted, ants return without feeding, and so do not lay a pheromone trail. The old pheromone trail eventually decays, ending recruitment to the exhausted food source.

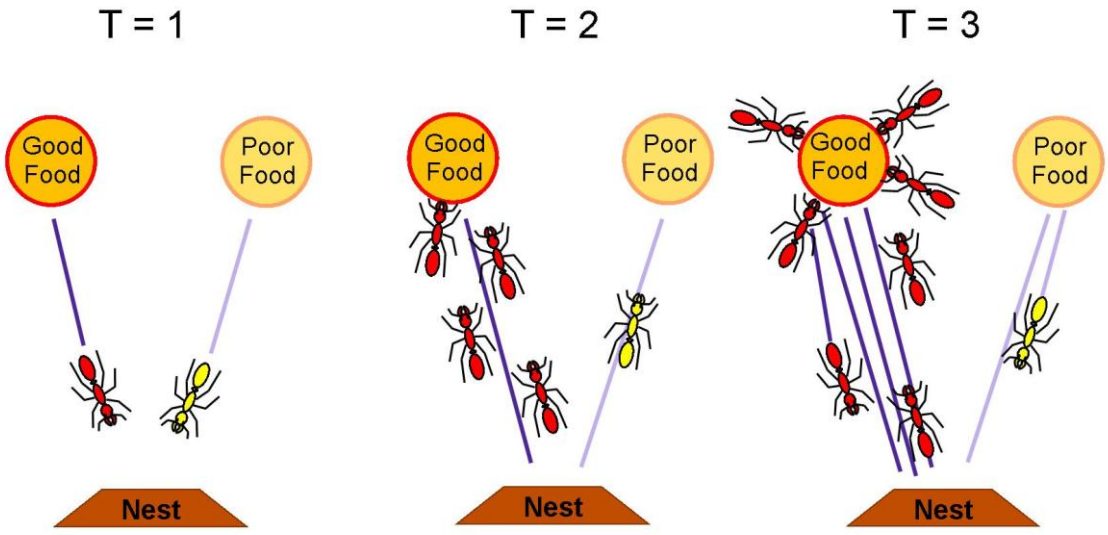

Figure 10.2 - Trail pheromones used to make a colony-level decision between two food sources.
One of the two 'classic' roles of trail pheromones. T = 1: two food sources, one of high quality and one of low quality, are discovered simultaneously by scouting ants. The scouts return to the nest laying a pheromone trail, but the scout that found the better food source lays a stronger trail. T = 2: As the trail to the good food source is stronger, more ants follow that trail, feed, and in T = 3 themselves return laying a pheromone trail. The colony has 'chosen' the good food source without any ant directly comparing both food sources.

The many roles of trail pheromones in the organisation of ant colonies

Whilst this classic model of pheromone trails has lead to valuable insights into collective decision making and bottom-up organisation, and inspired Ant Colony Algorithems and other heuristics, it is overly simplistic. Indeed, the seductive simplicity and elegance of this model can obscure the complexity found in reality. While not wrong, the classic model is far from the whole story. In reality, the role of trail pheromones in colony organisation is many-faceted and complex. One area in which this is becoming increasingly clear is the organisation of trail recruitment.

Trail recruitment can be thought of as consisting of three components: recruiting workers to use a pheromone trail, thereby modulating overall number of ants on the trail, directing recruits along a particular path, thereby giving the recruited ants directional information, and modulating the number of ants taking a particular path, thereby modulating the proportion of traffic on a path. These tasks may all be accomplished by the same trail pheromone, as in the experiments of Wilson and Hangartner mentioned above, but recruitment to the trail can also be completely separate from trail following and path choice.

Modulation of recruitment

Recruitment is modulated according to many factors apart from resource quality, such as resource quantity: *Lasius niger* ants will modulate their recruitment to the productivity of a feeder by being more likely to stop foraging before satiation at slowly-replenishing feeders (Mailleux et al. 2003a). Likewise, ants can modulate recruitment dependent on the size of the food item, with larger food items eliciting the recruitment of more workers (Traniello 1983, T Wenseleers, in prep). If a food source is depleted, the Pharaoh's ant, *Monomorium pharaonis,* will deposit a repellent trail pheromone (Robinson et al. 2005), which prevents ants from taking the path to the depleted food source, thus increasing foraging efficiency (Robinson et al. 2008a). Much as in the honey bee waggle dance (Seeley 1995), trail pheromone recruitment is not just used for recruitment to food sources, but also for recruitment to new nest sites (Hölldobler 1981a; Hölldobler et al. 1996) (also pers. obs of *Lasius niger,* T. Czaczkes). Trail pheromone recruitment also occurs to battle grounds, so as to repel invading ants and defend colony territories (Cammaerts-Tricot 1974; Wilson 1975, 1976; Hölldobler 1976b; Hölldobler & Wilson 1978), or during raids on other colonies (Hölldobler 1981b; Hölldobler et al. 1994, 1996), or to allow colonies to avoid competitors

(Hölldobler 1976a; Franks & Fletcher 1983; Farji-Brener & Sierra 1998), (but see Califano & Chaves-Campos 2011).

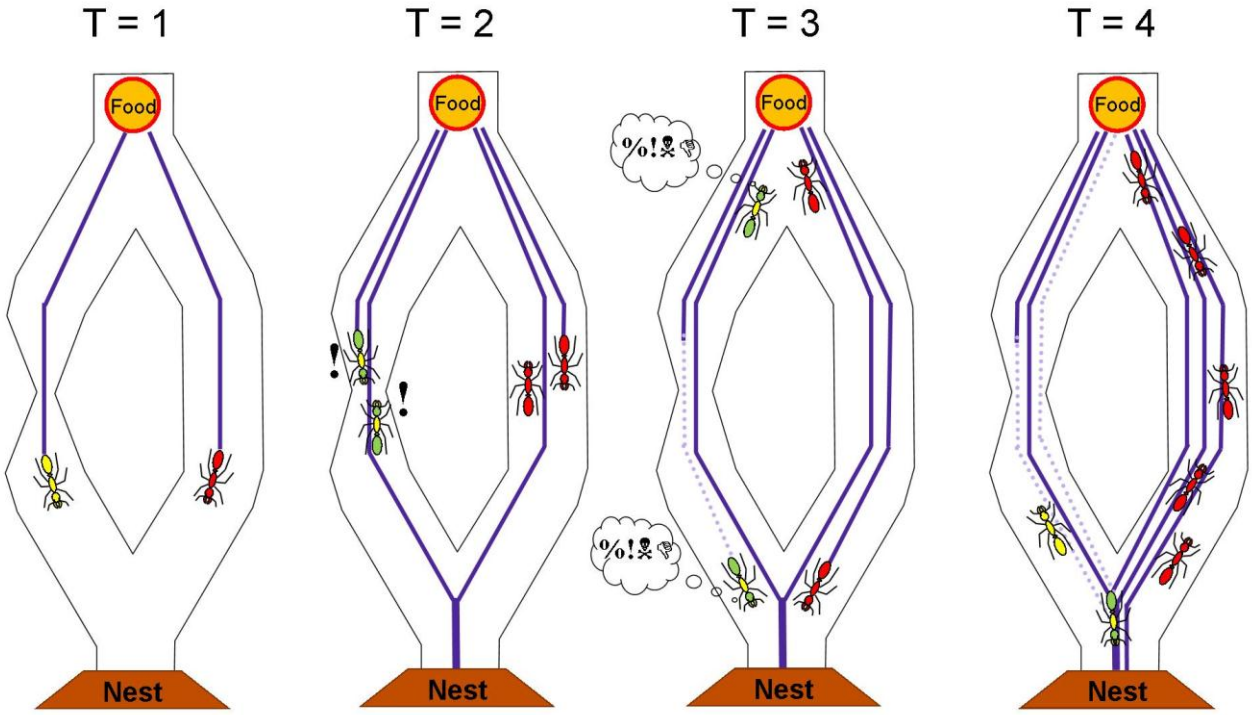

Figure 10.3 - How trail crowding might affect colony level route choice
High contact rates on trails were found to result in a reduction in trail deposition. This may affect foraging in the following manner: T = 1: two ants return from a food source via different routes. The left route contains a bottleneck. T = 2: as recruitment continues, ants at the bottleneck experience crowding. T = 3: ants which experienced crowding deposit less trail pheromone, both on the current journey and subsequent journeys. T = 4: As the pheromone trail on the unconstrained path is stronger, more ants take the unconstrained path. The colony thus 'decides' to allocate workers to each path relative to the crowding level on the path.

Modulation of trail use

In many ants, the trail pheromones cause recruitment, give recruits directional information, and affect path choice, and thus can be used simultaneously to recruit workers, direct recruits, and channel workers differentially along different parts of the trail network. This is the case for *Lasius niger,* and thus *L. niger* can modulate how workers are sent along different routes by varying the amount of pheromone they deposit. Foragers encountering heavy traffic on trails are almost 60 times less likely to deposit trail pheromone than foragers encountering very light trail traffic (see chapter 8). This will have the effect of capping recruitment if only one path to the food source is available (modulation of recruitment), or directing foragers down the least crowded path when there are multiple paths to the food (modulation of trail use – see figure 10.3). In this case, the foragers estimate trail usage according to their rate of contact with nestmates. *L. niger* also responds to the amount of trail

pheromone already on the trail, and reduces pheromone deposition rates if a large amount of pheromone has already been deposited (Beckers et al. 1992a; Czaczkes et al. 2012a, see chapter 7). By reducing recruitment to specific portions of a trail, traffic might be re-routed to alternate routes and crowing on branches reduced, with a subsequent rise in efficiency (Burd & Aranwela 2003; Dussutour et al. 2005a). Even if traffic is not re-routed, such a feedback mechanism will act to reduce the number of foragers on an over-crowded trail.

Directing of recruits and other roles for pheromone trails

Trail pheromones can play an important role in colony organisation in ways other than by differential recruitment and modulation. Directing foragers along a specific path using trail pheromones is clearly critical in sending workers to where work is needed, be that in the context of foraging, house hunting or fighting. However, the directing of foragers has other effects as well: for example it constrains foragers to a particular path. These paths can then be engineered to increase the efficiency of traffic flow by smoothing surfaces and removing obstacles (Rockwood & Hubbell 1987; Howard 2001). Confinement along a set path also supports route learning (see below for details).

The structure of the pheromone trail network itself can encode information: Pharaohs ants use the trail's geometry to provide a polarity to the trail network: as the trail branch at about a 60^0 when leading away from the nest, ants encountering a bifurcation with both paths deviating by 30^0 know they are heading away from the nest, while ants heading towards the nest will encounter one path at 30^0 and another at 120^0 from their current direction. They then can then use this polarity to reorient themselves along a trail (Jackson et al. 2004). *Temnothorax albipenis* workers can use a pheromone trail to assess potential nest sites: workers scouting out a new potential nest site lay pheromone trails inside the new nest site on their first exploration. On returning for a second visit, they use the number of times they cross their own pheromone trail to estimate, with surprising accuracy, the area of the potential nest site (Mallon & Franks 2000; Mugford et al. 2001).

Multi-component trails: Long-lasting and short-lived pheromones

The classic model of pheromone trails involves a single attractive trail pheromone that causes ants both to exit the nest and then to follow it. Indeed, as shown by Wilson (1962) and Beckers *et al.* (1990), this can be enough to achieve adaptive organisation. However, in reality many ant species use multiple pheromones on their trails. Why are such complex, multi-

component trails needed? The use of multiple trail pheromones allow a more adaptable and efficient foraging organisation than a single trail pheromone would allow (Jackson & Ratnieks 2006; Robinson et al. 2008a). The 'no entry' pheromone used by Pharaohs ants has already been mentioned above, but most reported multi-pheromone pheromone trail systems involve using two or more attractive pheromones with different longevities (Maschwitz & Mühlenberg 1975; Maschwitz & Schönegge 1977; Traniello 1982; Witte et al. 2007a; Dussutour et al. 2009a). The long-lasting pheromones may either act as home-range markings and exploration trails– signalling that many conspecifics or colony members have visited the area before (Traniello 1982; Deneubourg et al. 1990; Fourcassié & Deneubourg 1994), or as a form of 'external memory', which may be used by nestmates (Jackson & Ratnieks 2006; Jackson et al. 2006; Dussutour et al. 2009a; Evison et al. 2012) or be individual specific and ignored by nestmates (Breed et al. 1987; Mallon & Franks 2000).

Long-lasting pheromones to be used as home-range markings are deposited by scouts even before they have discovered a food source, and are mostly deposited by outgoing individuals (Hölldobler & Wilson 1977; Traniello 1982; Deneubourg et al. 1990; Fourcassié & Deneubourg 1994). The trail laid by an exploring ant is likely to be followed by other exploring ants. As the ants move further away from their origin, the territory will be more weakly explored, and so the home-range trails will be weaker, resulting in a higher likelihood of ants stopping to follow a trail and begin to explore at random. This results in a fanning out of the exploration trails, and scouts are rapidly led to the outskirts of the explored territory. Thus large areas are efficiently explored without extensive repeat exploration of already marked areas (Deneubourg et al. 1990; Fourcassié & Deneubourg 1994).

Long-lasting pheromone trails may also act as an 'external memory'. As workers move between their nest and a place of interest, such as a feeding location or potential nest site, they may deposit a long-lasting trail pheromone as well as a short-lived trail pheromone that causes recruitment and trail following. The long lasting pheromone may not be sensed by all workers (Jackson et al. 2006), or may not by itself recruit nestmates to follow the trail (Maschwitz & Schönegge 1977), or may only recruit weakly (Witte et al. 2007a). If the resource becomes uninteresting – e.g. the feeder becomes exhausted – the short term recruitment pheromone rapidly decays, and the resource stops being heavily visited. If the resource eventually becomes interesting again – the feeder becomes productive, or a new nest-site is needed – an individual that re-discovers the resource can reactivate the trail by re-depositing short-lived attractive and recruiting pheromone. The presence of the long-lived pheromone allows foraging to resume more rapidly (Jackson et al. 2006; Dussutour et al. 2009a), and

ensures that the resource is re-checked periodically. This is particularly useful in nest-relocation, where the presence of trail pheromones leading to previously reconnoitred nest-sites reduces the perilous period of homelessness (Stroeymeyt et al. 2010; Evison et al. 2012).

Short-lived trail pheromone are not just used to guide ants from the nest to a food site: they are often used in local (or short-range) recruitment as well (Traniello 1983; Hölldobler et al. 1995; Czaczkes & Ratnieks 2012). Local recruitment can increase the foraging range of species and decrease recruitment delay by recruiting workers in the local vicinity, thus not requiring a return to the nest (Czaczkes & Ratnieks 2012). In *Aphenogaster cockerelli* the poison gland pheromone is used both for short-lived recruitment from the nest by drawing a line across the substrate with the sting, and used for local recruitment by releasing poison gland secretions into the air (Hölldobler et al. 1995). A similar situation occurs in *Lasius neoniger*, except that in this case the local recruitment is achieved by dotting poison and hindgut secretions on the ground around a food item, instead of releasing the secretions into the air (Traniello 1983). The ant *Pheidole oxyops* achieves local recruitment by using the short-lived recruitment trail laid from a food item as a net: foragers intersecting the pheromone trail follow it away from the nest and are thus channelled towards the food source (Czaczkes & Ratnieks 2012). By concentrating nestmates into a smaller area, local recruitment can allow ant colonies to overcome the minimum group size requirement of mass recruitment (Beekman et al. 2001). Short-lived pheromones are also commonly used in conjunction with long-lasting pheromones to recruit ants, which then begin following the long-lasting pheromones (Cammaerts-Tricot 1974; Hölldobler et al. 1994).

The interaction of pheromone trails with other signals and information sources

Ants have access to many more information sources than pheromone trails alone. Foraging ants also use their own internal state (Mailleux 2006), their own memories (Rosengren & Fortelius 1986; Aron et al. 1993; Grüter et al. 2011), their interactions with nestmates and other ants (Hölldobler 1976a; Gordon & Mehdiabadi 1999), and cues inadvertently provided by nestmates (Devigne & Detrain 2002, 2006, and chapter 8) to inform their behaviours. It has become apparent that ants can use multiple information sources or signals concurrently to fine-tune their behaviours (see table 10.4).

One example of the use of multiple signals is the use of physical (motor) displays or stridulation to enhance, or even change, the effect of a pheromone trail. Stridulation, and the

subsequent substrate-born vibrations, can increase the attractiveness of a pheromone trail (Hölldobler 1999). In some ant species, such as *Pachycondyla marginata* and *Camponotus socius,* pheromone trails are only followed if accompanied by physical displays (Hölldobler 1971, 1999).The meaning, and thus response, to a pheromone trail in *C. socius* is entirely dependent on the accompanying physical displays of the trail-laying ant: a waggle display indicates recruitment to food, and causes only workers to exit the nest. A jerking display signals emigration and is responded to by workers and males, with workers picking up eggs, brood and other workers before leaving the nest (Hölldobler 1971). Similarly in *Oecophylla longinoda*, pheromone trails from the anal gland combine with different pheromonal factors and motor displays to signal either foraging recruitment, defensive recruitment, or exploration recruitment (Hölldobler & Wilson 1978).

An information source commonly used by social insects is route memory, which can guide workers from their nest to semi-permanent food sources, such as honeydew-secreting Hemiptera colonies (Rosengren & Fortelius 1986; Salo & Rosengren 2001). Indeed, when route memory information and trail pheromones conflict at a bifurcation i.e. a pheromone trails leads in one direction but the ant remembers successfully visiting a feeder in another direction, ants will often, but not always, choose to follow their own route memories (Lubbock 1884; Vilela et al. 1987; Aron et al. 1988, 1993; Fourcassie & Beugnon 1988; Harrison et al. 1989; Grüter et al. 2011). This may suggest a hierarchy of information source use, with one being used until it becomes unavailable, at which point the next information source in line is used (Rosengren & Fortelius 1986; Vilela et al. 1987). This is not the case, however, as can be seen when route memories and pheromone trails do not conflict: When a foraging *L. niger* ant is travelling to a feeder to which she has been before, she can rely on her memory to guide her, but also use the presence of trail pheromone to confirm that she has not made a navigational error. This allows the ant to walk faster and straighter, and perform fewer U-turns. If the ant does step off the trail, and so seems to have made a navigational error, she reacts by slowing down, walking in a more sinuous manner and performing more U-turns, in order to re-find her original path. She also reduces her own pheromone deposition so as to prevent other ants following her in her error (Czaczkes et al. 2011a). Thus, trail pheromones can synergise with and compliment route memory. The trail pheromone acts as a "reassurance" to ants that they are still on the trail, although the ants may be using other information sources to navigate. A similar situation is found in *Atta cephalotes*, where ants turned by 180° will quickly reorient when replaced on a pheromone trail, but will walk about aimlessly if placed on an unmarked surface (Wetterer et al. 1992). The presence of a

pheromone trail can work additively with route memories as well, increasing trail choice accuracy of experienced ants by up to 30% in *L. niger* (Czaczkes et al. 2012a, see chapter 7). Ants can thus use pheromone trails to complement route memory, and do so especially where route memories are inaccurate by depositing more pheromone after making and subsequently correcting a navigational error (Czaczkes et al. 2012a, see chapter 7).*Temnothorax albipenis* ants also use memories and trail pheromones in combination to make note of future potential nest sites. If they have both the memory of their local environment and their trail pheromones, intact colonies can avoid moving to previously visited, low-quality nest sites if their nest is destroyed (Franks et al. 2007). If either memory is disrupted (by re-arranging landmarks) or pheromone trails are removed, the ants do not avoid previously visited low-quality nest sites.

Trail pheromones also affect route memories by improving memory formation. Collett and Collett (2002) suggest two ways in which this might occur: firstly, pheromone trails might constrain ants to follow repeatedly the same path, resulting in ants being repeatedly exposed to exactly the same visual panorama, which can then be used for navigation (Graham & Cheng 2009). Secondly, the presence of trail pheromones might prime ants to memorise routes, as the presence of trail pheromones effectively 'reassures' ants that they are on the right path (Czaczkes et al. 2011a). While no experimental evidence exists for the first hypothesis, Czaczkes et al (2012a) provide some support for the second possibility. They show that *Lasius niger* foragers which have made several trips to a feeder over a trail-pheromone marked path make fewer mistakes than ants with a similar amount of experience at travelling to the feeder, but had the trail pheromones on their path constantly removed.

A common manner in which other information sources affect the use of trail pheromones is by adjusting the amount of trail pheromone deposited. This can be seen in the effect of home-range markings on trail pheromone deposition. Home-range markings are long lasting and non-volatiles, and rather than signal a location, they provide cues[1] as to how often nest-mates or non-nest-mates have visited a location (Devigne & Detrain 2002, 2006). Often, home-range markings take the form of cuticular hydrocarbons laid down passively as ants walk over a substrate (Yamaoka & Akino 1994; Lenoir et al. 2009). The presence of home-range markings causes *L. niger* foragers to increase pheromone deposition when returning to the nest (Devigne et al. 2004; Devigne & Detrain 2006). However, pheromone deposition by

[1] Whether home-range markings are cues or signals is debatable, but it beyond the scope of this chapter.

experienced ants decreases when returning to a food source on a trail marked by home-range markings but unmarked by trail pheromones (Czaczkes et al. 2011a, 2012b). It seems that in this situation, foragers returning to the nest from a food source marked by home-range markings are assured that, since the feeder has been heavily visited in the past, it is safe and worth recruiting to. Conversely, foragers returning to a feeder via a route marked by home-range markings but not by trail pheromones deposit less pheromone, as it is likely the food source has been depleted (Czaczkes et al. 2012b). In this example, two information sources – route memories and home-range markings – interact to affect the deployment of a third: trail pheromones. Having two compounds that decay at different rates, such as home range markings and trail pheromones, allows ants to distinguish areas that have been recently visited by a few ants and areas that have been visited by many ants longer ago, effectively allowing ants to 'smell the past' (Cammaerts 1984; Detrain & Deneubourg 2009). Thus route memory information, home-range markings and trail pheromone presence interact to adjust foragers' trail pheromone deposition behaviour.

Table 14.1 - (table continuation and caption on the next page)

Role for trail pheromones	Description	Other information source	references
Pheromones interacting with other signals			
Modulation of recruitment dependant on trail usage	By sensing the amount of trail pheromone on the trail, ants can decide if more recruitment to a food source is needed. Less pheromone is laid on heavily marked trails.	Pheromone * pheromone	(Beckers et al. 1992a; Czaczkes et al. 2012a)
As an external memory	As workers foraging on long-lasting food sources they may deposit two trail pheromones: a long lasting pheromone and a short-lived one. If the food source becomes unproductive, the short-lived pheromone decays rapidly, greatly reducing the number of ants visiting the depleted food source. The long-lived pheromones ensure that the food source is checked periodically, and allows rapid resumption of foraging. Can also be used during nest relocation.	Long-lasting pheromone * short-lived pheromone	(Jackson et al. 2006; Dussutour et al. 2009a; Stroeymeyt et al. 2010)
Recruitment to different tasks	By coupling a pheromone trail with specific physical displays, some ants can change the meaning of the pheromone trail to signal, for example, either recruitment to food, unexplored territory, or a battle.	Pheromone * physical display	(Hölldobler 1971; Hölldobler & Wilson 1978; Hölldobler et al. 1996)
Pheromones interacting with cues			
Modulation of recruitment dependant on trail usage	By sensing the presence of other ants on the trail ants can decide if more recruitment to a food source is needed.	Pheromone * contact rates	Chapter 8 of this thesis
Modulation of recruitment dependant on trail usage	By sensing the presence of home-range markings on the trail, in combination with the ant's travel direction and past experience, ants can decide if more recruitment to a food source is needed.	pheromone * home-range markings * route memory	(Devigne et al. 2004; Czaczkes et al. 2011a, 2012b)
Pheromones interacting with private information			
Local recruitment: from surrounding area to a food source	Pheromone laid towards the nest. Ants sensing the pheromone follow the trail away from the nest to the food source using private path integration information to assess their relative location to the nest.	Pheromone * memory of nest location	(Hölldobler et al. 1978; Traniello 1983; Czaczkes & Ratnieks 2012)

Modulation of recruitment dependant on colony satiation	Individuals may increase pheromone deposition when starved, or conversely increase the minimum amount of food required to trigger pheromone deposition.	Pheromone * individual hunger level	(Hangartner 1969a; Mailleux 2006)
Modulation of response to recruitment dependant on colony satiation	Workers from starved nests may decrease their response threshhold to recruitment by trail pheromones, and may even deposit trail pheromone themselves once recruited.	Pheromone * individual hunger level	(Mailleux et al. 2011)
Reassurance to route memories	The presence of trail pheromones can allow ants to rely more heavily on route memories, trading off accuracy for speed. If an error is made ants are informed by sensing the lack of trail pheromone.	Memory * pheromone	(Wetterer et al. 1992; Czaczkes et al. 2011a)
Support of memory use	Trail pheromones allow greater accuracy on complex trail systems even for experianced ants. The benefits of route memory and trail pheromones in terms of reduced errors are additive.	Memory + pheromone	(Czaczkes et al. 2012a)
Facilitate memory formation	Either by constraining ants onto a trail, ensuring rapid learning of a single route, or by triggering learning, possibly through a "reassurance" that the ant is on the correct route, hence should attempt to learn its surroundings.	Memory * pheromone	(Collett & Collett 2002; Czaczkes et al. 2012a)
Pheromones interacting with abiotic public information			
Local recruitment: from surrounding area to a food source	Trail pheromone dotted on the ground or emitted into the air. Ants sensing the pheromone follow it upwind to the food source.	Pheromone * Wind direction	(Hölldobler et al. 1978; Traniello 1983; Czaczkes & Ratnieks 2012)
Support navigation in low light levels	Ants deposit more trail pheromone when foraging in low light levels.	Pheromone * light levels	Jones et al, In prep

Table 10.1 - Complementarity and synergy between trail pheromones and other information sources.
Many of the roles of trail pheromones involve an interaction with other information sources, with the other information source either affecting the use or deposition of trail pheromones, by trail pheromones affecting the other information source, or by multiple information sources being integrated to affect behaviour.

A consistent theme in the use of other information sources in combination with trail pheromones is complimentarity (see table 10.1): the other information sources can complement pheromone trail use by adding nuances and extra meaning to the recruitment, or by strengthening recruitment, or by affecting how trail pheromones are laid down. Conversely,

trail pheromones can complement other information sources, such as by improving memory formation, or increasing the reliability of route memories, or by being specifically laid down where route memories seem to be failing to increase navigational accuracy. Complementarity between information sources is likely to be widespread in biology. Examples range from cellular processes, for example in apoptosis, which sometimes needs both intrinsic and extrinsic signals to be triggered (Alberts et al. 2007), to long range navigation navigation, where magnetic field information may be used as a compass for orientation, but this compass is calibrated using celestial cues (Cochran et al. 2004).

Final remarks

For many years it has been fashionable to remark that social insects are individually simple, using a limited set of basic rules which cause adaptive emergent properties at the colony level. Whilst true to some extent, researchers over the past four decades have been gradually building a more comprehensive, if far from complete, picture of the rules social insects use during decision making. These are surprisingly numerous and complex, many requiring the integration of information from multiple sources, which can be social, public or private. Nowhere is this clearer than in an ant colony's trail system. Multiple trail pheromones with different properties are present, and the information from these can be used in concert and with other information sources. The way they are deployed is affected by many factors, both individual and at the colony level, and outside the colony by factors both biotic and abiotic. They may elicit different responses depending on the state of the receiver; caste, hunger levels, and what other information, both public and private, the receiver has access to. While emergent patterns are the rule in a social insect colony, the organisation of social insect colonies is governed by many rules.

Addendum – Trail organisation in Lasius niger: a mini-review

Unlike the study of honey bee recruitment and foraging organisation, the study of recruitment and foraging organisation in ants is thrown into considerable disarray by the multitude of study species used. The different ecological niches filled by different ant species impose differing demands on the organisation strategies of said species (van Oudenhove et al. 2011; Czaczkes & Ratnieks 2012), and thus conclusions drawn from one ant species are not necessarily applicable to another. Different ant species are appropriate model organisms for studying different questions. However, a systematic review of research on one popular model species would allow not only a less confused understanding of the foraging organisation in that ant species, but would also highlight important gaps in our knowledge. Thus, in this section I hope to provide a brief overview of what is currently know about the foraging organisation in *L. niger*, what this thesis has added to the field, and what gaps in the field still need addressing.

The nature of the trail pheromone in Lasius niger

There have been several attempts to characterise the chemical component(s) of the *L. niger* trail pheromone (Bergström & Löfqvist 1970; Hayashi & Komae 1980; Attygalle et al. 1987; Bestmann et al. 1992; Kern & Bestmann 1994). Whilst 3,4-Dihydro-8-hydroxy-3,5,7-trimethylisocoumarine seems to be the key chemical responsible for trail following, it is likely that other chemicals play secondary roles, as they do in the closely related *L. fuligenosus* (Kern et al. 1997). The complete characterisation of the *L. niger* trail pheromone blend is still missing. Pheromone trails formed for up to 15 minutes to a 1 molar sucrose feeder have been estimated to decay to a point where ants can no longer detect them in 47 minutes (Beckers et al. 1993). However, trails formed by 500 ant passages to a 1mol sucrose feeder may last for 20-24 hours (Evison et al. 2008).

Trail pheromone deposition rules in Lasius niger

L.niger deposit trail pheromone as a series of dots, and trail pheromone deposition is measured by counting these dotting events (Beckers et al. 1992a). *L. niger* generally only deposit pheromone after feeding (Beckers et al. 1992a), although in starved colonies 27% of recruits which received food via trophalaxis also deposit pheromone (Mailleux 2006). Foragers only begin depositing pheromone if they can ingest a minimum volume of liquid (Mailleux et al. 2000), a threshold which rises when colonies are starved (Mailleux 2006). Both the probability of ants depositing pheromone, and the intensity (number of depositions per

depositing ant), can be modulated (e.g. Beckers et al. 1992a). For instance, pheromone deposition is higher for higher quality food sources (Beckers et al. 1993), which leads to colonies concentrating their foraging effort on the higher quality food source (Beckers et al. 1990). Fed foragers deposit more pheromone closer to the feeder (Beckers et al. 1992a). The proportion of returning foragers depositing pheromone increases as colonies become larger, until colony sizes of between 75-180 ants, at which point colony size does not have an effect on deposition behaviour (Mailleux et al. 2003b). The presence of brood slightly increases the proportion of ants depositing pheromone to proteinacious food, with a similar non-significant trend for sugary food (Portha et al. 2004). A greater proportion of foragers deposit pheromone to sugary food as compared to proteinacious food (Portha et al. 2004) because as foragers are more likely to deposit pheromone in home-range marked areas, and as the amount of home-range marking decreases with distance from the nest, foragers are more likely to recruit to food sources closer to the nest (Devigne & Detrain 2006). Pheromone deposition by individuals decreases in their later visits by foragers (Beckers et al. 1992a; Mailleux et al. 2005). Pheromone deposition is lower on paths angled at a more oblique angle to the food-nest axis (Beckers et al. 1992b) and on paths marked with trail pheromone (Beckers et al. 1992a).

The response to trail pheromones in Lasius niger

Trail pheromones induce ants to leave the nest (Mailleux et al. 2011) and induce trail following (Lubbock 1884). Although it is widely assumed that *L. niger* workers can detect differences in pheromone concentrations at path bifurcations, and follow the stronger path, this has not to our knowledge been demonstrated experimentally with naturally deposited pheromone trails. The presence of trails pheromones decreases U-turning (Beckers et al. 1992b). Trail pheromones have been claimed not to affect speed or sinuosity in *L. niger* (Breton & Fourcassie 2004), although I report different results when trail pheromones and route memory are combined (chapter 5). Starvation lowers the response threshold of ants in the nest to recruitment by trail pheormones (Mailleux et al. 2011).

Other aspects of foraging organisation in Lasius niger

As in other ants, given multiple *ad libidum* feeders *L. niger* colonies have been shown to usually concentrate their foraging on one feeder, presumably due to amplification of small initial differences in recruitment to the feeders (Beckers et al. 1990). Increased chances of U-turning, and increased travel time (hence lower overall pheromone levels), cause *L. niger* colonies to preferentially use shorter routes to a feeder (Beckers et al. 1992b), where route

memory does not seem to play a role in this behaviour. Route memories do, however, cause foragers to walk faster (Mailleux et al. 2005), but see chapter 5. Colonies can 'select' the least crowded of two routes due to ants attempting to enter a crowded path being 'pushed' onto the less crowded path (Dussutour et al. 2004, 2006). Under crowded conditions at a bottleneck alternating clusters of ants from either direction take turns in crossing the bottleneck, resulting in little or no loss of food intake (Dussutour et al. 2005a). *L. niger*, like other ants, exhibit 'wall following', and preferentially follow and walk next to walls and ridges, which can affect the structure of their foraging trails (Dussutour et al. 2005b).

The contributions of this thesis to our understanding of Lasius niger *foraging*

This thesis describes many new behavioural rules and aspects of behaviour and learning which affect foraging organisation in *L. niger*. These are discussed in depth in the relevant chapters; here we present a synthesis of the major findings in list form.

- Workers learn the direction a feeder on a T-bifurcation very rapidly, achieving 75% accuracy after only one visit, and over 95% accuracy after three (chapter 4).
- Naive workers can follow pheromone trails at a T-bifurcation, but with surprisingly low accuracy (62% and 70% correct choices for a weakly and strongly marked trail, respectively – chapter 4).
- When trail pheromone and route memory conflict, workers overwhelmingly follow route memory, regardless of the strength of the pheromone trail (chapter 4).
- Experienced ants walking on a pheromone trail walk faster and straighter than inexperienced ants on a marked trail, experienced ants on an unmarked trail, and inexperienced ants on an unmarked trail (chapter 5).
- Experienced ants stepping off a pheromone marked route reduce their pheromone deposition; an effect not seen for inexperienced ants (chapter 5).
- When experienced and inexperienced ants step off a pheromone marked path, they perform more U-turns and walk in a more sinuous manner. When stepping off a pheromone marked path experienced (but not inexperienced) ants begin walking slower (chapter 5).
- On home-range marked paths, experienced ants deposit less pheromone when walking towards a food source, and more pheromone when returning. This effect is not seen on paths unmarked by home-range markings (chapter 6).

- Foragers have great difficulties in learning routes which require alternating path decisions (e.g. turning left then later turning right). Most errors occur on the first decision point (chapter 7).
- The presence of pheromone trails increases accuracy on alternating routes, especially at the first decision point (chapter 7).
- Ants deposit more pheromone in difficult-to-learn routes. This is at least in part due to ants which have made an error on their outwards journey depositing more pheromone on their return journey (chapter 7).
- There is a negative relationship between the amount of pheromone on a trail and the amount of pheromone foragers returning to the nest deposited on a trail (chapter 7).
- Crowding on trails causes foragers to reduce the amount of pheromone they deposit (chapter 8).
- Crowding is measured in terms of head-on collisions with nestmate-like objects: the presence of nestmate cuticular hydrocarbons is the main criteria for categorising objects as nest-mates foragers, but object colour also plays a role (chapter 8).
- Crowding at the feeder does not reduce pheromone deposition rates (chapter 9).
- Colonies can switch their foraging effort from highly limited-rate feeders to less limited feeders, even if foraging is well underway to the highly limited feeder. This redistribution of foragers to a more productive feeder can occur even when the pheromone trail to the less productive feeder is stronger than to the less productive feeder (chapter 9).

Future directions in the study of Lasius niger

While there are many interesting questions about the foraging organisation of *L. niger* left unanswered, two major topics call for immediate attention. Firstly, all studies on the modulation of trail pheromone deposition in *L. niger* rely on the counting of individual pheromone depositions. However, it is possible and indeed quite likely that foragers also modulate the amount of pheromone per deposition. Thus, developing the ability to quantify individual pheromone depositions, and using this ability to explore this potential hidden modulation of pheromone deposition, is key to gaining a full understanding of foraging organisation in *L. niger*. Moreover, an accurate quantification of the pheromone deposited on trails will allow the production of realistic artificial trails, which would be a very valuable tool in the study of *L. niger* foraging.

This thesis has highlighted the importance of route memory in *L. niger.* However, our understanding of the role of route memory in the organisation of foraging in *L. niger* is fragmentary and far from complete. Differential memorisation of food sources depending on foraging success, food quality, and other factors may explain many of the patterns of foraging previously ascribed to differential pheromone deposition. Models and behavioural experiments aimed at differentiating between memory effects and pheromone effects could give us important information about the relative importance of these two information sources to *L. niger*. A better understanding of route memory formation and flexibility would also be of great value in realistically simulating colony level organisation.

Lastly, there is very little work linking laboratory studies to studies on unmanipulated wild *L. niger* colonies. As mentioned in chapter 9, there are indeed some mismatches between laboratory results and field observations. A few studies have powerfully confirmed laboratory findings using field observations (e.g. Völkl et al. 1999), and others provide valuable information about natural distribution of *L. niger* workers between aphid colonies (e.g. Dreisig 1988). However, a systematic evaluation of whether results of laboratory studies reflect natural behaviour in the field is urgently needed. This will highlight which laboratory results are of real importance to the foraging and organisation of *L. niger*, and which results are due to artefacts of laboratory research methods, such as starvation, broodlessness of colonies, or small colony sizes.

Chapter 11: Cooperative food transport in the Neotropical ant, *Pheidole oxyops*

T.J Czaczkes, P. Nouvellet & F.L.W. Ratnieks

Abstract

Cooperation in foraging through information sharing is widespread in social insects and has been much studied. By contrast, cooperative transport of food items by groups of workers is less common and has received comparatively little attention. We investigated collective food retrieval in the Neotropical ant *Pheidole oxyops*, a ground-nesting species in which minor workers, mean body weight 0.6mg, collectively transport larger dead insects back to the nest entrance. In total, 29% of items and 78% of total food mass is transported collectively. We examined the configurations of ants carrying single experimental food items (weight 119mg, size 10 x 10 x 1 mm) and found it to be non-random, with twice as many carrying ants at the corners as expected. This arrangement is achieved by preferential joining of corners and leaving of sides by carriers. Corner carrying increased carrying speed by up to 29%. Ants also preferentially carried food items from the front and back, versus the middle.

Introduction

Social insects stand out in the extent and complexity of their cooperative behaviour. Cooperation among workers is involved in many areas of colony life, such as nest building (Franks et al. 1992; Deneubourg & Franks 1995), defence (Frehland et al. 1985; Hölldobler & Wilson 1990), hygienic behaviour (Hart & Ratnieks 2002), and especially foraging (Hölldobler & Wilson 1990; Robson & Traniello 2002). Foraging in ants differs to that of bees and wasps in that ants must forage and retrieve food on foot. This allows ants many unique foraging behaviours, such as cooperative retrieval of food items.

Cooperative transport of single food items by groups of ants is common though not universal, is documented in 40 genera of ants, and has arisen independently many times (Hölldobler & Wilson 1990; Moffett 2010). It may result in "superefficient" groups, in which multiple ants can collectively carry a larger weight than would be manageable if divided amongst the individual workers (Sudd 1965; Franks 1986; Moffett 1988; Deneubourg & Beshers 1991; Robson & Traniello 1998; Franks et al. 1999, 2001). Cooperative transport can also help reduce interspecific interference (Deneubourg & Beshers 1991; Cerdá et al. 1998b)
Several aspects of cooperative transport have been well studied to date, including the mechanism of carrier number recruitment optimisation (Traniello 1983; Franks 1986), the composition of retrieval groups (Franks 1986; Franks et al. 1999), and the ecological significance of group retrieval (Detrain 1990; Deneubourg & Beshers 1991; Schatz et al. 1997; Yamamoto et al. 2009). Much less work has investigated the coordination and arrangement of carriers. Chauvin (1971) notes that *Formica polyctena* are excited by, and more likely to grasp, moving objects and objects surrounded by conspecifics, which leads to cooperative transport. Sudd (1965) showed that small groups (mean c. 3 ants) of *Formica lugubris* did not position themselves at random around a cooperatively transported prey item, and eventually began pulling in the same direction, although achieving coordination could take a pair of ants as long as 10 minutes.

The most detailed studies of cooperative transport in ants have been carried out on army ants (Franks 1986; Franks et al. 1999, 2001; Powell & Franks 2005). Franks (1986) describes the formation of coordinated teams of new world army ants, *Eciton burchelli*. These typically involve one submajor and one or more smaller workers. The larger submajor carries the food item from the front slung underneath its body, with the smaller worker(s) lifting the rear to reduce drag (Powell & Franks 2005). Franks et al (2001) describe similar teams in the old world army ant *Dorylus wilverthi.* These army ant examples represent highly sophisticated

examples of carrying in species with polymorphic workers. Powell & Franks (2005) suggest that the emergence of the specialist carrier caste in *E. burchelli* arose as a direct consequence of the shift to taking large arthropod prey. Franks and colleagues provide a detailed description of the organisation of prey carriers in these highly specialised, polymorphic species. This study aims to be a counterpoint to these studies, presenting data on cooperative transport in a species with monomorphic carriers that regularly work in groups of four or more, which will be relevant to many other cooperative carriers. Several studies on cooperative transport in less polymorphic ant species investigate recruitment behaviour (Detrain & Deneubourg 1997; Cerdá et al. 2009) or group size adjustment (Robson and Traniello 1998), but few examine the arrangement of carriers during transport. A handful of studies do describe the arrangement of carriers (Sudd 1960a, 1965; Moffett 1988), but the general conclusion seems to be that, excluding army ants, ants collectively transporting a large food item tend to work more as solitary foragers, taking no account of the efforts of their sisters. This results in uncoordinated actions such as pulling in opposite directions until, by chance, the ants are pulling in the same direction and transport can resume (Sudd 1965). One study by Moffett (1988) on the swarm raiding ant *Pheidologeton diversus* reports coordinated cooperative transport, but does not provide data on the organisation of the carriers.

In this study we investigate cooperative food retrieval behaviour by minor workers in the Neotropical ant *Pheidole oxyops.* Cooperative transport is important in this species, with 78% of prey retrieved this way (see below). Our results show that minor workers are not distributed at random around an experimental food item, but rather are over-represented at the corners and that this increases retrieval speed. In addition, we investigate the mechanisms governing ant distribution around an experimental food item during retrieval and show that more ants carry by the leading sections than the trailing sections, and more ants carry by the trailing sections than by any of the middle sections.

Methods and results

Site and study organism

Data were collected between January 29 and February 28 2009 and 2010 at Fazenda Aretuzina, near São Simão, São Paulo State, Brazil. Air temperatures ranged from 23-31C. The study species, *Pheidole oxyops*, is dimorphic, with large headed major workers (mean body mass 61.6mg, SD 11.2mg, n = 20 [5 from each of 4 colonies]) and small headed minor workers (body mass 6.0mg, SD 1.7mg, n as above). Majors were never observed to be involved in

cooperative transport. *P. oxyops* nest in the ground and hunt and scavenge for prey which are often carried back by multiple workers.

We chose to study *P.oxyops* as they display group recruitment from the nest and coordinated cooperative transport, which is a major aspect of their foraging behaviour. During 5 hours of observation on 9 colonies (33 minutes per colony) 103 food items were collected, 69% of which were arthropods. 29% of all food items, accounting for 78% of total food mass, were transported cooperatively by minor workers. Average group size was 3.5 individuals, (SE =0.84), with the largest group observed being 30 ants retrieving a mantid approximately 50mm long. Average load mass of individually carried items was 0.0023g (SE=0.0003) and for cooperatively transported items was 0.0874g (SE=0.0711). Heavier items were more likely to be cooperatively transported (Spearmans rank correlation, rho=0.604, DF=131, P<0.001). A worker who is unable to move a food item is able to cause the recruitment of many additional nestmate workers to the item from the nest, as occurs in several other ant species (Hölldobler et al. 1978; Schatz et al. 1997; Robson & Traniello 1998; Daly-Schweitzer et al. 2007; Cerdá et al. 2009; Amor et al. 2010). Foraging occurred during daytime and all data were collected from 0900 to 1800. Data were not collected during and for at least an hour after rain as this disrupted foraging.

To maintain consistency, all data were collected in the shade, either under tree cover or a parasol, and within 0.5m of the nest entrance using test arenas consisting of an A4 (210x297mm) sheet of 2mm graph paper backed by a tile (figure 11.1). The arena was raised 40mm off the ground on polytetrafluoroethylene coated plastic posts with access provided by a cardboard ramp fixed to the side closest to the nest entrance. The standard food item, used in all experiments except experiment 6, was a 10x10x1mm piece of cheese overlaid with 2mm graph paper, weighing in total 119mg. The perimeter, where the ants would grasp the item, was divided into 20 2mm edge sectors, 2 at each corner and 3 along each side (figure 11.1). Our operational definition of a corner was the two sectors meeting at a corner point. This was large enough for two ants to simultaneously grasp a corner, which occasionally occurred. Group recruitment for cooperative transport is initiated when a worker is unable to retrieve a food item due to too-high tractive resistance (see above), thus a wave of ants could be directed onto the test arena by offering an immovable food item at the far end of the arena to trigger recruitment.

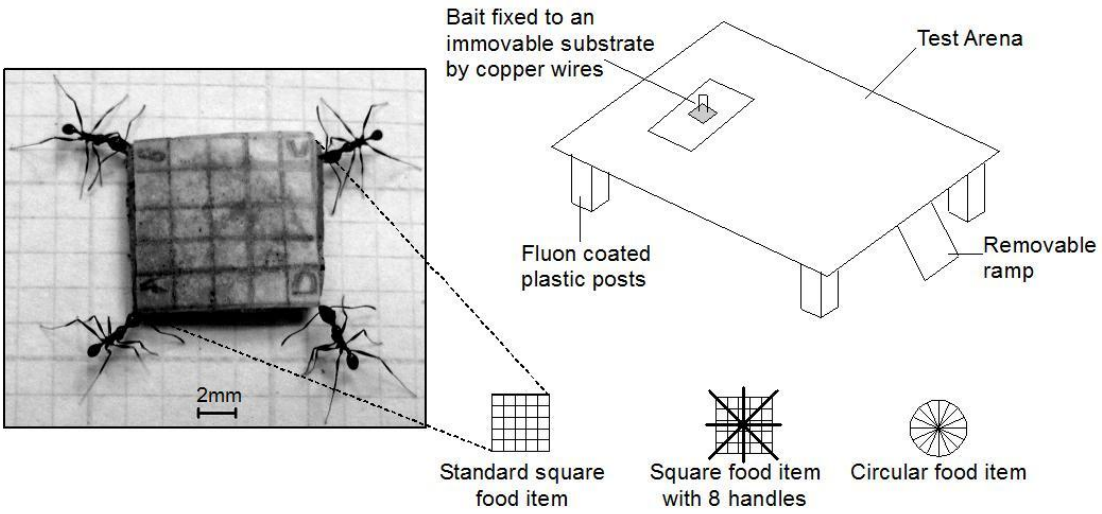

Figure 11.1 - Basic set up of apparatus, and representations of the three food item types used. Photograph shows four ants cooperatively carrying a standard food item by the corners. The food item is a 10X10X1mm piece of mozzarella cheese weighing c.119mg overlaid by a 2mm grid.

Experiment 1. Effect of carrier number on carriage speed

Method - The aim of this experiment was to determine the relationship between the number of carrier ants and the carriage speed of a food item. A colony was presented with a standard food item on the test arena. Recruitment was triggered using a fixed 5x5mm food bait. Forager ants were then presented with a standard food item and allowed to carry it towards the nest. All ants not holding the food item were brushed off the test arena. The test arena was video-recorded from above. Speed was measured from the videos by finding an instance during the carriage when the item was transported in a straight line for at least 3 cm. The distance covered and time taken were used to calculate transport speed. On reaching the nestward side of the test arena, the item was replaced in its original position at the far end of the test arena using a pair of soft forceps. Any ants that fell off the item were removed from the arena, and the item was allowed to be carried towards the nest again. This was repeated until no ants remained, which never took longer than 15 minutes. Whilst resulting in non-independence of data, this allowed large sample sizes, and no group was used more than 3 times. A minimum of 5 repeats with different ants were carried out for each group size. Temperature was measured every trial using a bulb thermometer placed by the test arena. Data were collected from colony A.

Results - A single ant could just drag our standard food item. Carriage speed increased with more ants but levelled off at approximately 5-7 ants (figure 11.2). A quadratic regression

explains significantly more of the variation than a linear regression (Sequential ANOVA, DF=1, F = 12.01, P <0.05) showing that the relationship is non-linear. The best fit quadratic equation was carriage speed (mm per sec) = 0.6764 + 1.325n – 0.06328n^2, (quadratic regression, F=12.18, DF = 88, adjusted R^2 = 67.9%, P<0.001), which fits the data better than the linear regression (F=156.25, DF = 88, adjusted R^2 = 63.8%, p<0.001). Temperature had a significant effect on carriage speed but explained less than 5% of the variation in speed (linear regression, T = 2.28, DF=88, adjusted R^2 =4.6%, P = 0.025).

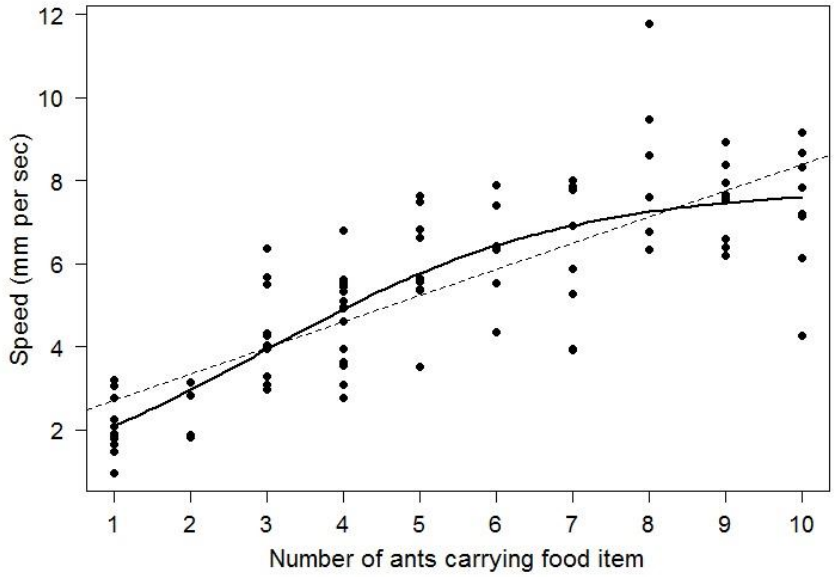

Figure 11.2 - Effect of the number of ants carrying an experimental food item, a 10 x 10 x 1mm piece of mozzarella cheese weighing c. 119mg, on carriage speed. Thick black line: logistic regression (asymptote reached at 7.8 mm/s, #t = 18.962, P < 0.0001. Thin dotted line: linear regression (speed = 2.073 * 0.6301n (S = 1.71, adjusted R2 = 63.8, P<0.001)

Experiment 2. Distribution of carriers

Method - This experiment investigated whether ants distribute themselves randomly around a standard food item on a raised arena. Following the results of experiment 1, we studied groups of 4 ants in this and other experiments as 4 carrier ants could move the food item and yet was below the number of carriers at which the number to speed relationship levelled off (see figure 11.2). As such, we expected that carriage speed with 4 ants would be sensitive to the positions of the carrier ants around the food item. Ants were allowed to carry the item for 2 minutes. Whenever the ants came close to the edge of the test arena they were allowed to walk onto a piece of card and replaced in the centre of the arena. After two minutes in which ants could adjust their distribution around the food item, a photograph was taken to determine the number of corners occupied. Colonies A (N=22), B (N=19) and C (N=26), were studied.

We compared the observed distribution of ants at corners versus middle sectors against the expected distributions based on random choice of middle versus corner sectors (see

appendix E). Given that the food item was a 10mm square, its perimeter is 40mm, and the expected random probability of joining a corner is 0.4, (4x4)/40.

Results - As figure 11.3 shows, the distribution of ants was non-random. An average of 2.8 corners were occupied, compared to 1.5 for the model that best fit the observational data but did not include a preference for corners (see appendix E)**.** Only by adjusting the model so that ants were allowed to have a preference for corners was it possible to obtain an expected distribution that did not differ significantly from observed (G test: G =6, DF = 3 P=0.108). In this model ants cannot share a sector nor a corner (formed of two sectors) and had a preference for joining corner sectors over side sectors ($p_{c=}$ probability of joining one of the corners, estimated by maximum likelihood: p_c =0.196, 95% CI: 0.184, 0.207; see appendix E). As ants have been observed to occasionally share a sector, the likely situation is that the chance of joining a sector drops dramatically to a low but non-zero value when it is already occupied by one ant. However, for the purposes of this study the simpler models suffice to demonstrate corner preference.

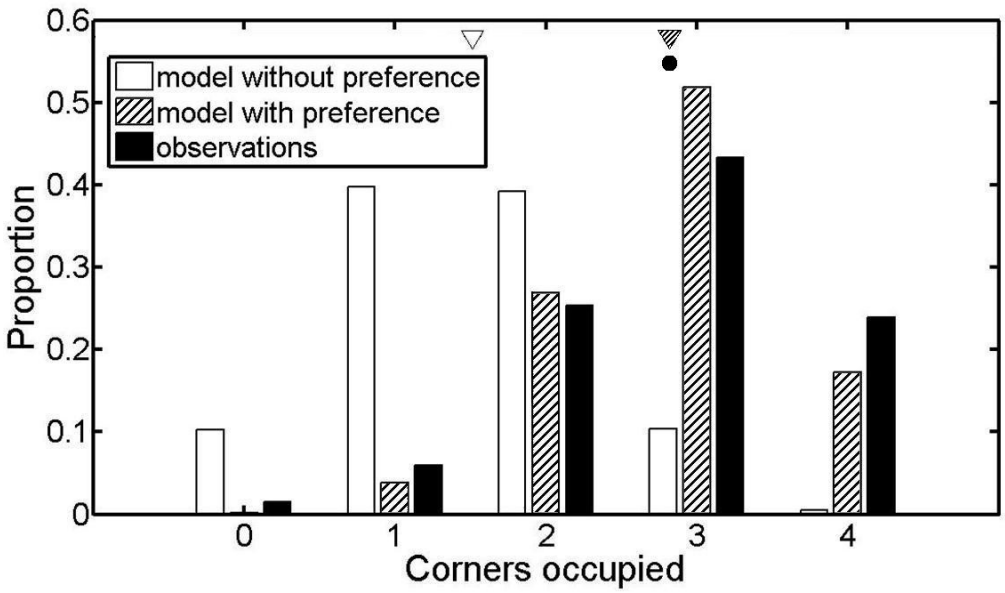

Figure 11.3 - Proportion of corners occupied by a carrying ant. Black bars are observed data, white bars represent the model where ants have no preference for corners and cannot share corner or side sectors. The hatched bars represent the model where ants cannot share corner or side sectors and corner sectors are 5.4 times more likely to be occupied than side sectors (see appendix E). The triangles and circle represent the means of the models and observation respectively.

Experiment 3. Effect of ant distribution on carriage speed

Method - Basic methods followed Experiment 2. The four ants were allowed to walk for up to two minutes, or until they had carried the food item for a minimum of 30mm without

stopping or turning. Trials were video-recorded and a single measurement of carriage speed and the number of corners occupied at that time was taken from each video. Colonies A (N=18), and C (N=20), were studied.

Results - As significant differences were found between the two colonies, colonies were analysed separately. Carriage speed increases significantly with the proportion of corners occupied in colony C (Spearmans Rank Correlation, DF = 18, rho = 0.716, P <0.001), whilst the trend was similar but not significant in colony A (Spearmans Rank Correlation, DF = 16, rho = 0.270, P = 0.185). Figure 11.4 shows pooled results for both colonies, and shows walking speed of 4.5, 5.2, 6.0 and 6.4 mm/s for 1, 2, 3 and 4 occupied corners, respectively. When fewer than four corners are occupied the remaining ants are either carrying at a side sector or co-occupying a corner.

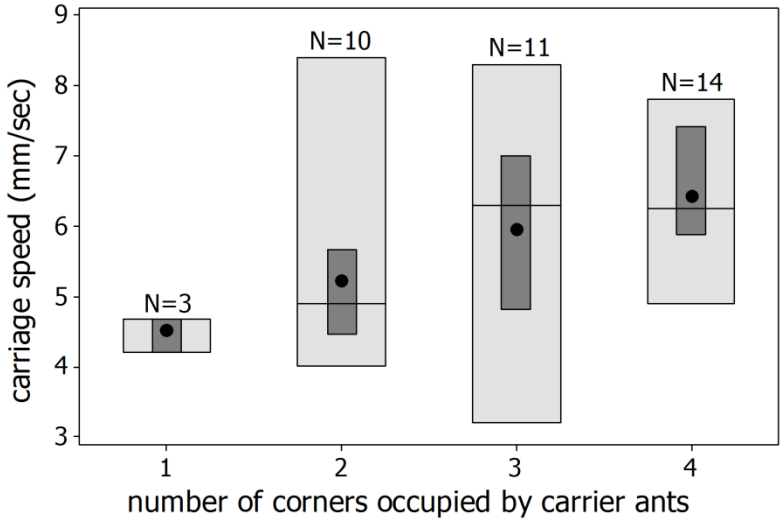

Figure 11.4 - Carriage speeds (mm/s) of 10X10X1mm 119mg food items being carried by four ants depending on how many of the corners of the food item have one or more ants grasping them. Bisecting lines indicate medians, circles indicate means, light boxes indicate interquartile range, and dark boxes indicate 95% confidence interval for the median.

Experiment 4. Mechanism of ant distribution adjustment

Method – Basic methods followed Experiments 2 and 3. A group of 4 carrier ants was video recorded for one minute after the food item first started moving. Instances of joining and leaving at corners and sides were recorded, and rates of joining and leaving calculated. Colonies A (N=12), B (N=11) and C (N=13), were studied. An event was defined as a single act of joining or leaving. For example, if an ant joins onto a side, lets go, and then joins a corner there are 3 events. Ants were considered to have joined when they clearly grasped the food item with their mandibles and remained at a fixed position on the food item for at least 1 second. So as to get a measure of the how long ants grasp the corners and sides, the duration for which each of the four ants grasped the item for the first time was measured. Only the first

grasping event was measured to ensure that no ant was measured twice. Ants that had not released the item by the end of the video were assigned a grasping duration of 60 seconds.

Results – The proportion of joining events on corners and sides was calculated and weighted to reflect the larger proportion of side sectors. The proportion of leaving events on corner and side sectors was weighted by the proportion of joining events for corners and sides. Figure 11.5a shows that ants join the corners at a significantly higher rate than the sides (mean +/- St Dev weighted proportion of joining events 0.81 +/-0.20 versus 0.29 +/-0.14; two sample t-test: t = 9.15, P < 0.001, N=36) and leave the sides at a significantly higher rate than the corners (mean weighted proportion of leaving events 0.63 +/-0.74 versus 1.52 +/-1.25; two sample t-test: t = -4.12 P < 0.001, N=36). Ants grasped the corners of the item significantly longer than the sides (Kruskal-Wallis, N corners =65, N sides=43, Z = 4.05, P<0.001).

Experiment 5. Ant distribution on food items with additional "handles"

Method - By making the sides of a food item as easy to grasp as the corners, this experiment investigated the possibility that corners might be preferred simply because they are easier to grasp than sides. The general method is identical to Experiment 4. Four copper wires were attached to a standard food item to give 8 2mm handles around the perimeter of the food item. Two wires were diagonals, protruding 2mm from the corners, and two were placed perpendicular, protruding from the centres of the sides (figure 11.1). Ants readily grasped these handles when carrying a food item. Colonies A (N=17), B (N = 12) and C (N=17), were studied. Grasping duration was also measured as above.

Results – Again the proportion of joining and leaving events were weighted and compared as above. When the food items were given handles on both corners and sides, ants still joined corners more than sides (mean +/-StDev weighted proportion of joining events 0.74 +/-0.26 versus 0.34 +/-0.17; two sample t-test: t 3.85, P < 0.001, N=35)(figure 11.5b) and left the sides at a higher rate than the corners (mean +/- StDev weighted proportion of leaving events 0.28 +/-0.28 versus 0.48 +/-0.51; two sample t-test: t = -2.1 P < 0.043, N=35). There was a non-significant trend for ants to grasp the corners longer than the sides (Kruskal-Wallis, N corners = 60 N sides = 62, Z=1.72, P = 0.069 adjusted for ties). When comparing ant behaviour around food items with and without handles, there was no significant difference in joining rates of corners or sides (two sample T test, sides; DF = 79 T = 0.139, P =0.169. Corners; DF =

79, T = 0.139, P =0.169) but leaving rates were reduced when handles were provided (two sample T test, sides; DF = 44, T = 4.7, P < 0.001. Corners; DF = 42, T = 2.7, P = 0.01).

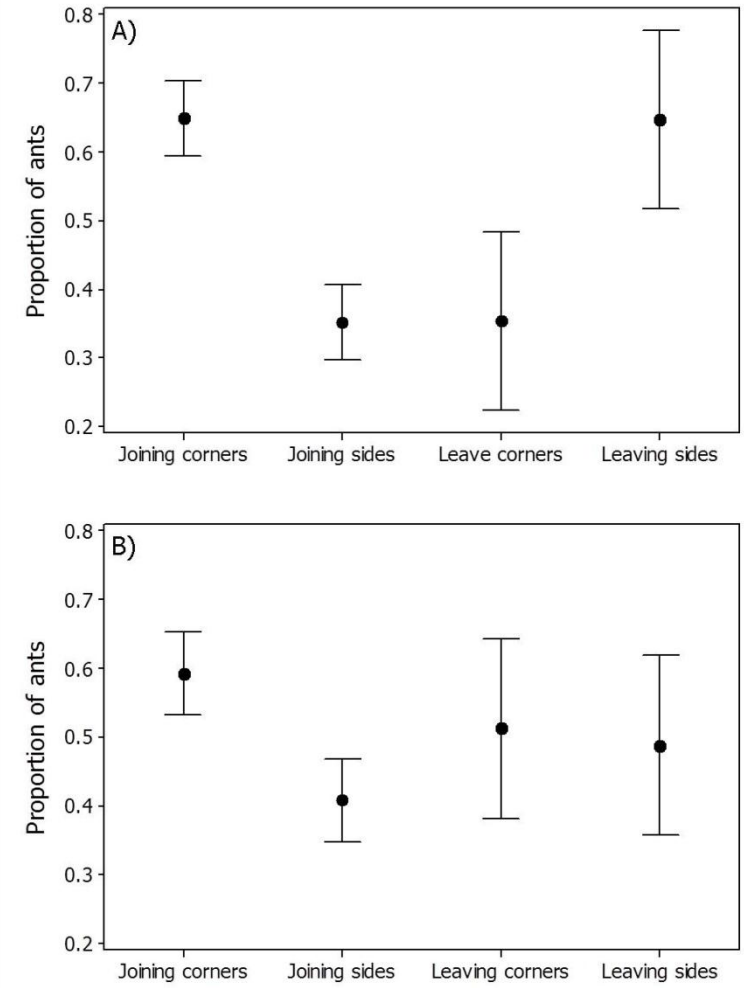

Figure 11.5 - Proportions of ants joining and leaving corners and sides of a 10 x 10 x 1mm food item, without (A) (N= 36) and with (B) (N=46) handles. Circles indicate means, lines indicate 95% confidence intervals for the medians.

Experiment 6. Joining rules for a moving food item

Method - This experiment investigated the order in which ants joined different locations of an already moving food item in relation to the direction of the nest entrance and the movement of the item (i.e., facing the entrance, facing away). To eliminate any preference for corners over sides, a circular food item 10mm in diameter was used. The perimeter was marked into 16 equal sectors. The test arena was lowered to allow access from all sides of the arena. The carriage of the food item was video recorded until it was carried off the arena, a distance of 280mm. Ants were classified as joining the nestward side if they joined one of the 4 sectors facing towards the nest, the back if they joined one of the opposite 4 sectors, and the middle if they joined one of the 8 intermediate sectors. Ants were ranked according to joining order independent of the number of ants already grasping the food item. Colonies A (N=10)

and C (N= 16) were studied. At the end of each video the number of ants carrying by the nestward, middle and back sectors was counted.

Results – The first three ants to join joined the nestward side of a food item at a significantly higher rate than the back or sides (ANOVA, DF = 233, F=22.09, P<0.001: 77%, variance = 18.5). Of the first ants to join a food item 77% joined the nestward side, versus 15% (variance = 13.5) for the far side and 8% (variance=13.5) for the two middle sides combined. As the nestward side became occupied, more ants started joining the middle and back (figure 11.6). Proportionally more ants joined the nestward side or back than the sides, and over the entire carriage period ants joined the nestward side and back equally (two sample T test, nestward vs. middle, T = 4.21, P = 0.002, back vs. middle, T = 4.51, P = 0.001, nestward vs. back, T = 1.08, P=0.299). All ants seemed to carry the item nestwards, with the ants at the back walking forwards, those at the front walking backwards, and those at the middle walking sideways. By the end of the observation period more ants carried from the nestward side than from the back, and more ants carried from the back than the middle (front mean = 2.8, SD 0.80, back mean 2.09, SD = 0.98, middle mean/2 = 1.6, StDev = 0.56. One way ANOVA for difference DF = 104, F=19.14, P <0.001, adjusted R^2 = 25.86%).

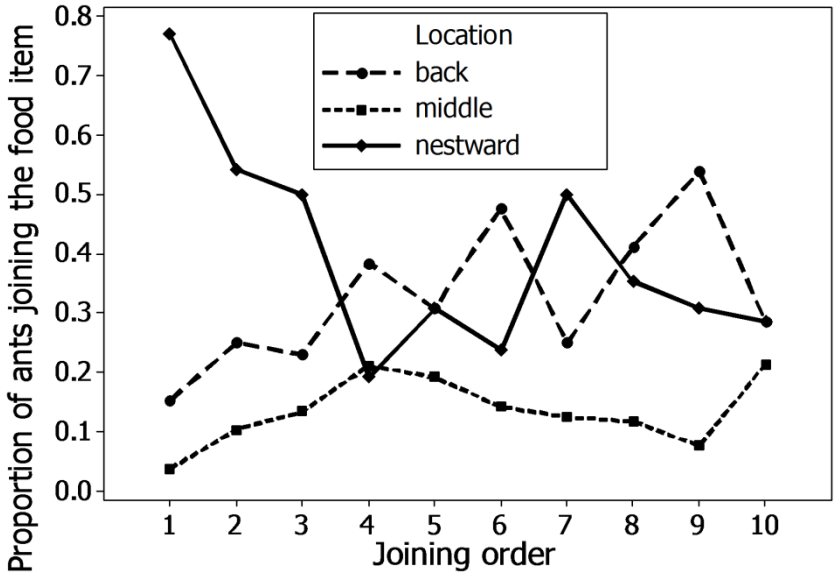

Figure 11.6 - Proportion of ants joining the nestward, middle and back sectors of a mobile circular food item, 10mm diameter, by the order in which the ants join and begin to help transport the item

Statistical analysis

All statistical analyses were carried out using Minitab 14 unless stated otherwise. Specific tests are given in the results sections. All tests were two tailed unless stated otherwise. Data from different colonies were pooled where no significant difference among colonies was found using an ANOVA. Significant colony effects were only found in experiment

3, where colonies were analysed separately. Maximum likelihood estimations were performed on Matlab.

Discussion

Our results show that the organisation of cooperative food transport of large items in *Pheidole oxyops* has features that appear to increase food transport effectiveness. There is a strong preference for carrying by the corners, and this leads to higher carriage speeds. In addition, the position that carrying ants join the food item is also non-random with regard to food item position relative to the nest. A mechanism for adjustment of carrying positions is provided by varying rates of joining and leaving the carried item. These findings demonstrate some sophistication in the organization of cooperative transport.

When only one corner is occupied the mean carriage speed is only 70% of the speed when all four corners are occupied. This gives a possible adaptive explanation for the behaviours observed in experiment 2 and 5, which show that ants preferentially carry food items by the corners. Faster prey retrieval could have several benefits, such as reducing the time the ants are at risk from predation, desiccation and interference from competitors, and the freeing up of workers for other tasks (Feener & Moss 1990; Tanner 2008; Cerdá et al. 2009).

The non-random distribution of ants found in experiment 2 arises in part due to different joining and leaving rates for sides versus corners and in part due to ants remaining at corners longer than sides (experiment 4): ants joined corners at a rate 1.75 times that of sides and left sides at a rate 1.2 times that of corners, and remained at corners 50% longer. Experiment 5 demonstrated that the preference for joining corners cannot simply be explained by corners being easier to grasp: Even when the food items had additional handles at both sides and corners ants joined the corners at 1.4 times the side rate. After weighting the proportion of leaving events by the proportion of joining events, ants were also found to leave the sides at a higher rate than corners. Therefore, there is probably something intrinsic about a corner that is attractive to the ants. The fact that the addition of handles decreased leaving rates of corners and sides whilst not changing joining rates suggests that the reason ants join an area may not be the same reasons it chooses to stay or leave.

This study also raises the mechanistic question of how ants sense a corner. 10 x 10 mm pieces of cheese are notably absent from most ecosystems, and clear 90° corners along with them. What is it about corners that causes a preference? We demonstrated that a corner is

not sensed only by the ease by which it is grasped, by showing that corners are preferred even when the sides are equally easy to grasp (experiment 5). There are several possible explanations for the preference of ants to carry by the corners. Corners may simply be contacted more easily, or carrying by the corner may be more efficient as the food item, and possibly other ants, do not get in the way of the ants' legs so much (Fig. 11.1). Lifting part of a pliable food item by a corner may be easier than lifting by a side, and would result in reduced surface exposure to the substrate and thus reduced friction. Lastly, Czaczkes and Ratnieks (2011) showed that during the turning of food items to reduce drag, most ants causing the turning grasped the item by the corner. Grasping items by a corner provides greater leverage for turning the item. Experiment 3 clearly shows that faster carriage speed is one advantage of carrying by the corners. This may also explain Sudd's (1965) finding that *Formica lugubris* workers tend to space themselves and avoid clustering whe gathered around a food item. Therefore, we suggest that ants simply have a preference for grasping a food item where they will be least crowded, both by their sisters and by parts of the food item itself.

Experiment 6 explored the joining behaviour of ants around a circular food item currently being transported. At first, ants preferentially joined the nestward side. As this became more occupied the back was preferentially joined over the middle (figure 11.6). This is not what one would expect if ants, when finding the front sectors fully occupied, simply moved to the next free space, as this would be the middle. Thus, this tendency to join the back of an item versus the middle probably arises from a direct preference. *P. oxyops* workers appear to walk in a coordinated manner towards the nest, with ants at the front pulling and ants at the back pushing and lifting, similar to behaviour reported by Moffett (1988) but in contrast to the uncoordinated behaviour of *Myrmica rubra* and *Formica lugubris* reported by Sudd (1965). Carrying and walking sideways may be more difficult and thus might be less preferred. Carrying from the sides may also be disfavoured as, if unbalanced by ants on both sides, it could cause imbalance in the carrying force. These results have parallels with teams of army ants retrieving prey (Franks 1986). The larger ant carrying from the front in army ants can be compared to a group of workers in *P. oxyops*. In both cases a large amount of ant-power is directed at carrying from the front, and may be assisted by a smaller amount of ant-power lifting at the back (experiment 5).

Ants must carry a variety of objects, and the method by which they carry is determined by the shape and mass of the object to be carried. Leaf cutter ants, for example, cut pieces of leaves and fruit to be carried resulting in items that can be carried by a single media ant holding the item above its body, although the item size is usually below peak efficiency

(Rudolph & Loudon 1986; Burd 1996). Army ants dissect larger prey items and often carry the resulting items back in cooperative teams of two to four ants, the larger worker holding the food item slung underneath its body and the smaller workers lifting the item from behind (Franks et al. 2001). For ants species that are more susceptible to disruption from other ants dissection is maladaptive, and bulky items are more likely to be retrieved without dissection (Yamamoto et al. 2009), as occurs in our study species, *P. oxyops.*

Cooperative transport by similar individuals of bulky items that cannot be dissected is a common problem for both ants and humans, so the study of such systems may be of importance for both ant behaviour and technological application. Indeed, the study of how similar individuals cooperatively solve complex tasks is finding applications in current and future technology (Woern et al. 2006; Christodoulou 2009). Cooperatively carrying a load by robots may be improved and informed by insights from ant behaviour. Cooperative pushing, pulling and towing have been achieved several times using "swarms" of independent robots (Kube & Bonabeau 2000; Pettinaro et al. 2005; Wang & de Silva 2006; Udomkun & Tangamchit 2008). Real world applications may also require lifting, especially of delicate items or work on irregular terrain, and lessons learned from this and future studies may provide inspiration for solving such lifting problems. The benefits of ant-inspired systems may be of prime importance in robotic applications where direct intervention is impossible or impractical, such as deep sea or extraterrestrial engineering (Huntsberger et al. 2000; Parker & Zhang 2006; Woern et al. 2006). Cooperatively lifting objects is something that humans and their machines need to do regularly. Although at an early stage, insights from cooperative transport by ants, and ant behaviour in general, may have far reaching applications.

Acknowledgments

We thank Dr Paulo Nogueira-Neto for allowing us to work and stay at Fazenta Aretuzina and Dr Jacques Delabie for identification of *P. oxyops*. We thank Thomas Collett, Christoph Grüter, Ellouise Leadbeater, Katja Rex and two anonymous referees for comments on the manuscript. TC was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) doctoral training grant award. PN was funded by Sussex University. FR's travel costs were funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Chapter 12: Simple rules result in the adaptive turning of food items to reduce drag during cooperative food transport in the ant *Pheidole oxyops*

T.J. Czaczkes & F.L.W. Ratnieks

Abstract

Insect workers cooperate to carry out a variety of tasks. One example is cooperative transport of food items by two or more ant workers, which is important in foraging in many species. We predicted that natural selection would result in strategies that improve the performance of this task and tested this in *Pheidole oxyops*, a Brazilian ant in which c. 70% of the biomass of dead insects brought back to the nest is transported cooperatively. We specifically tested the hypothesis that groups would reorient food items to reduce drag, given that legs, wings, and other projections should affect the ease of dragging prey in different orientations. By presenting ants with artificial food items and dead cockroaches, both of which required approximately twice as much force to drag backwards as forwards, and a control item which was equally easy to move in both orientations, we showed that natural groups of 3-20 food-transporting ants usually turned items that were facing backwards (72% and 83% of trials for artificial food items or cockroaches respectively), the orientation requiring greater force, but not items facing forwards (10% and 12% of trials respectively). Turning usually involved a single 'steering' ant. The key role of the 'steering' ant was shown by removing either the current steering ant or a randomly chosen 'non-steering' ant during turning. In 100% of the trials in which the steering ant was removed, turning stopped until another ant took its place. Conversely, turning stopped in only 17% of trials in which a 'non-steering' ant was removed. Turning is an emergent property of the system and may not have been directly selected for. Rather, turning seems to occur through a combination of pre-existing retrieval behaviour and the underlying physics of large loads. Points where the food item catches the ground can act as a fulcrum or pivot around which the item can rotate. Ants furthest from the fulcrum have more leverage and so are more likely to play a key role in turning. A simple rule relevant to individual transport of food items such as "grasp the food item and move towards the nest", when used in the context of cooperative transport, has allowed the ants to solve a seemingly complicated problem requiring coordination.

Introduction

Social insects are renowned for their ability to cooperatively solve complex tasks, such as nest site selection, allocation of workers to tasks, hygienic waste management, nest construction and foraging (Seeley & Morse 1978; Franks et al. 1992; Seeley 1995; Mallon et al. 2001; Hart & Ratnieks 2001; Nicolis & Dussutour 2008). Workers usually coordinate their activities when tackling these complex problems via self organisation (Camazine et al. 2003), where adaptive collective behaviour emerges via the interactions of individual workers with each other and the environment, and without any individual being in charge or having complete knowledge of the state of the system.

Ants forage exclusively on foot. This imposes limitations on foraging distance compared to flying, but also facilitates the use of trail pheromones (Robinson et al. 2005; Morgan 2009), food caching and transfer outside the nest (Ratnieks & Anderson 1999; Anderson et al. 2002), and cooperative transport (Franks 1986; Moffett 1988). Cooperative transport is widespread in ants and has evolved many times (Moffett 1992). It has several benefits, including faster prey retrieval, which reduces competition and theft, and allowing items which are too large for a solitary forager to be retrieved without dissection (Traniello 1987, 1989a; Cerdá et al. 1998b, 2009; Powell & Franks 2005). Rapid retrieval of food items by cooperative transport allows some ant species to gain exclusive use of food sources that would otherwise be dominated by other, more aggressive species (Hölldobler et al. 1978; Traniello 1989a; Detrain 1990).

Cooperative transport may involve help from workers already present nearby (as in army ants (Hölldobler et al. 1978; Franks 1986; Franks et al. 2001) or workers recruited by a forager that has returned to the nest after discovering a food item too large to transport individually (Detrain & Deneubourg 1997; Kube & Bonabeau 2000; Daly-Schweitzer et al. 2007; Czaczkes et al. 2011b). Size is estimated by scouts bu using the tractive resistance of the food item- items that cannot be moved by an individual scout trigger a recruitment event (Detrain & Deneubourg 1997; Robson & Traniello 2002; Amor et al. 2010). Carrier ants assemble around a food item and drag or it away (Robson & Traniello 2002), or in the army ants form a team of 2 to 4 and lift and carry the item (Franks 1986). If the recruited ants are also unable to move the item further waves of recruitment may occur (Daly-Schweitzer et al. 2007). Cooperative transport is used both in retrieving food into the nest and removing large waste items and excavated stones from the nest (pers. obs.).

Cooperatively transported items will often have various protuberances, such as hairs and hooks on seeds or spines and appendages on insects, which make an item easier to drag in some orientations than others. One solution would be to lift the item clear of the substrate, thus eliminating drag (Traniello 1989a). This would be possible for a small item. However, lifting items fully off the ground is often not possible. In such cases the group of ants could retrieve the item more easily if it was in an orientation that reduced drag, and so we hypothesised that ants in which cooperative transport is important these reorientations will occur.

In this study we test this hypothesis on the neotropical ant *Pheidole oxyops* through a series of experiments in which we present foragers with various food items that differ in the amount of drag they present depending on orientation. *P. oxyops* collects the majority of its food using cooperative transport (29% of items and 78% of total food mass, (Czaczkes et al. 2011b), and so is an ideal species for investigating adaptations that improve the efficiency of cooperative transport . We show that *P. oxyops* do turn items during cooperative transport in a way that reduces drag, and that turning is less likely to occur on items that have similar levels of drag when moved in alternative orientations. We also provide data testing a hypothesis regarding the mechanism by which turning is achieved, in which differential drag across the substrate causes the item to pivot around points of high drag. Thus the reorientations are most likely not an active behaviour under selection, but occur due to the physics of the system.

Method

Site and study organism

Data were collected in February 2010 at Fazenda Aretuzina, a farm located near São Simão, São Paulo State, Brazil. The study species, *Pheidole oxyops*, nests in the ground and naturally scavenges and hunts for arthropods which are often carried back by groups of ants (range 2-30) (Czaczkes et al. 2011b). Five field colonies were studied.

Experimental procedure

Experiments were performed using a test arena consisting of an A4 (210x297mm) sheet of grade 100 fine sand paper laid on a tile, placed within 50cm of the nest entrance. The arena was raised 4cm off the ground on plastic posts coated with polytetrafluoroethylene (fluon), a slippery substance that ants cannot climb. Access to the arena was limited to a cardboard ramp on the side facing the nest entrance. Workers could be recruited onto the arena by

offering an immovable food item at the far end of the arena (Czaczkes et al. 2011b), which was then replaced by a test item.

Three test items were used: (A) an artificial prey item weighing 0.33g consisting of a 10x10x1mm piece of cheese attached to a thin 10x10mm plastic sheet with four copper wires, one attached to each corner. Each wire projected forward then bent backwards to form a sled that could easily be pulled forward, but required 1.7 times more force to drag backward (figure 12.1a: 'Directional drag sled' – see below for force measurement method). (B) A similar item but with two double ended runners each attached to 2 corners. Each runner was curved at both ends to produce a sled that was equally easy to pull in either forward or backward directions (figure 12.1b, 'Non-directional drag sled'). (C) A dead cockroach weighing c.0.22g, c. 3cm long. Three dead cockroaches were used, being stored in a freezer between trails. They had backwards pointing spiny legs, making them 2.4 times harder to pull backwards than head-first (figure 12. 1c). Each item was offered to each colony several times facing in either the high drag or low drag orientation (high drag sled: high drag orientation N = 28, low drag orientation N = 26. Low drag sled: one orientation N = 30, the other orientation N = 56. Cockroach: backwards N = 30, head first N = 25). Ants were allowed to carry the item to the end of the arena. We recorded whether the item was turned, categorizing turning into three levels corresponding to turned (> 135°), not turned (< 45°) or partly turned (> 45° and <135°). Trials were video recorded using a Sony Handycam HDR-XR520. Carriage speed of cockroaches was also measured.

Examining the turning mechanism

By close examination of the videos obtained in the previous experiment, we developed a hypothesis for the turning mechanism involving 'steering' ants that play a key role in item turning and 'non-steering' ants, which do not (see results and discussion sections). To investigate the turning mechanism we placed the high-drag sled in its backward (high drag) orientation on the test arena and allowed groups of ants to attempt to move and turn it. Ants were categorised as either 'steering ants' or 'non-steering' ants. Candidate steering ants were defined as the ant(s) whose body orientation was most consistently in line with the turning direction of the item. Non-steering ants - ants that were hypothesized not to be responsible for turning the item - were recognized in three ways. First, an ant whose head moved with the item while its body stayed in place, implying that the food item moved the ant. Second, an ant whose legs and body flailed around, demonstrating it was not gripping the surface well. Third, an ant grasping the item at the pivot point and hence which had no leverage. Once turning

began, either a steering ant (N = 9) or a non-steering ant (N = 24) was removed. All trials were video recorded and examined frame by frame using Virtualdub (Lee 1998). The grasping location of the steering ants was noted, and ants were considered to be grasping the item by a corner if they ant was within 2mm of a corner.

Measuring drag forces

To obtain a relative measurement of the force needed to drag food items forwards and backwards, each item was attached to a 1g weight resting on an electronic balance sensitive to 0.1µg (Sartorius TE64) with fine nylon fishing line via a pulley (see figure 12.1d). The test item was then placed on a piece of the same grade fine sandpaper, which was pulled by hand from under the item at approximately 10mm per second, comparable to the speed of a group of 10 ants dragging an experimental food over fine sandpaper (mean 9.7 mm/sec 0.81 SD). Pulling resulted in a force being transmitted to the weight on the balance, reducing the total down force. Each item was re-tested 7 times and an average of the maximum readings calculated. The directional drag sled caused 1.70 times as much force in the backward (Mean 0.21g SD 0.012) than in the forward orientation (Mean 0.12g SD 0.009), the cockroaches 2.40 times as much (mean 0.33g SD 0.18) than head first (mean 0.14g, SD 0.03), while the non-directional drag sled required only 1.06 times more force to drag backwards (mean 0.17g, SD 0.009) than forwards (mean 0.16g, SD 0.009).

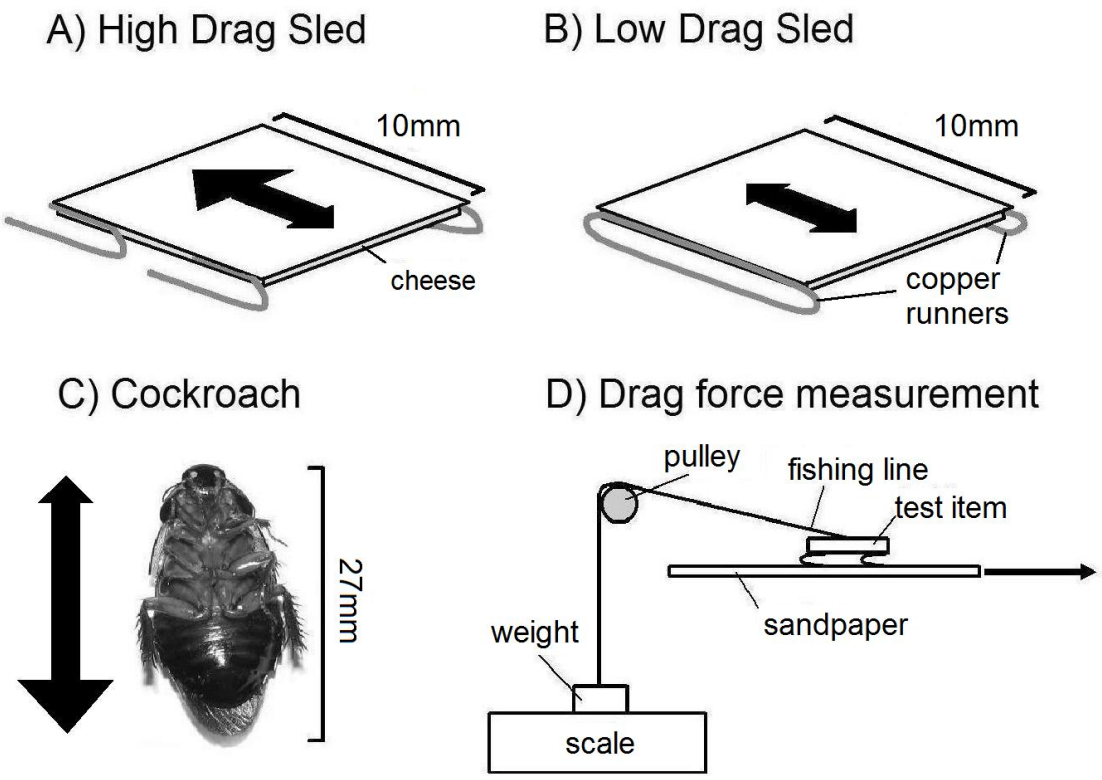

Figure 12.1 - Items used to test cooperative transport and turning: Two artificial prey items consisting of a 10x10mm acetate sheet, to which A) four or B) two runners of copper wire were affixed as shown. A 10x10x1 piece of cheese was attached to the bottom of the acetate sheet, total weight 0.33g A) The directional drag sled required 1.7 times more force to drag backwards (against the arrow) that forwards. B). The non-directional drag sled only required 1.06 times more force to drag one way than another. C) One of three cockroaches, all c.30mm long, weight 0.21-0.24g. Due to backwards pointing spines on the legs, cockroaches require 2.4 times more force to drag backwards than head-first. D) Apparatus used to measure the drag of items. The sandpaper is pulled from under the test item, causing the fishing line to exert an upward force on the balance that can be read from the scale. The purpose of the weight is to ensure that the balance is still subject to a net downward force despite the upward force transmitted by the fishing line.

Statistical analysis

We analysed all data using a generalized linear mixed-effect models (GLMM) in the statistical analysis software R2.9 (R Development Core Team 2009) with a binomial response variable. R fitted the models with the lmer function (Bates et al. 2007). We included colony as a random effect to control for the non-independence of data points from the same colony, as well as cockroach identity for trials involving cockroaches, as three individual cockroaches were used and the data pooled (Zuur et al. 2009; Bolker et al. 2009). We took a conservative approach by modelling random effects with both a random slope and random intercept. As only one colony was tested for the turning mechanism and in the analysis of transport speed of

cockroaches versus orientation, data for these experiments were analysed using a Kruskal-Wallis test in Minitab 14.

Results

Groups of *P. oxyops* workers were significantly more likely to turn the directional drag sled when it faced the nest backwards (high drag) than forwards (72% versus 10% of trials, GLMM, N = 54, Z = 3.618, P < 0.0003) (Figure 12.2A). There was no effect of ant number on probability of item turning when in the high drag orientation (one way ANOVA, DF=16, F=0.4, P = 0.95). Conversely, the non-directional drag sled was equally likely to be turned in both orientations (42% versus 35%, GLMM, N = 48. Z = 0.367, P = 0.713) (Figure 12.2B). The cockroaches, which differed even more in drag than the directional sled, were even more likely to be turned when presented backwards versus forwards (83% versus 12%, GLMM, N=55, Z = 5.974, P <0.0001) (Figure 12.2C). Trials where the item was only part turned were excluded. This allowed the use of a binomial distribution. Part turning only occurred in a minority of trials, 16% with the directional drag sled, 27% with the non-directional drag sled and 25% with a cockroach. Cockroach movement speed was slower on the rare occasions of being dragged backwards, even when accounting for number of carrying ants (mean 5.81 mm/s compared to 8.46 mm/s) (GLM, N backwards = 17, N forwards = 22, F = 16.45, P < 0.001, adj R^2 = 33%). When included in the model, carrier number (4-19 ants, mean 10.9, StDev 3.6) did not have a significant effect on carriage speed (F = 1.6, P = 0.153).

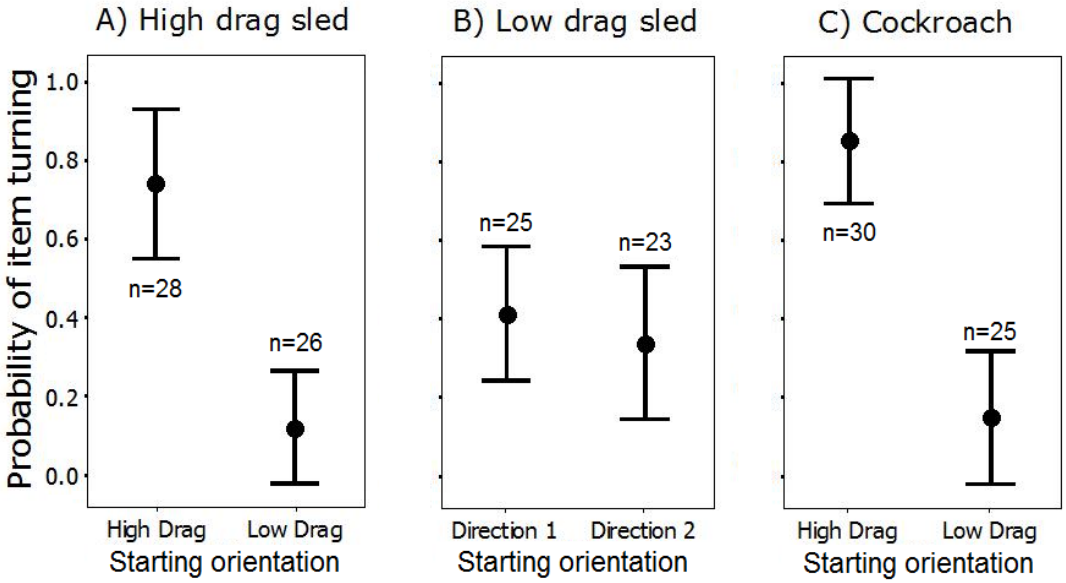

Figure 12.2 - The probability of ants turning each tested item depending on its starting orientation. Dots represent the mean, whiskers indicate the 95% CI . A) Ants turned the directional drag sled significantly more often when it faced in the high drag orientation. B) Ants turned the non-directional drag sled less frequently than the directional drag sled, and there was no difference in the number of turns between the two orientations. C) Cockroaches were significantly more likely to be turned when facing in their high drag orientation.

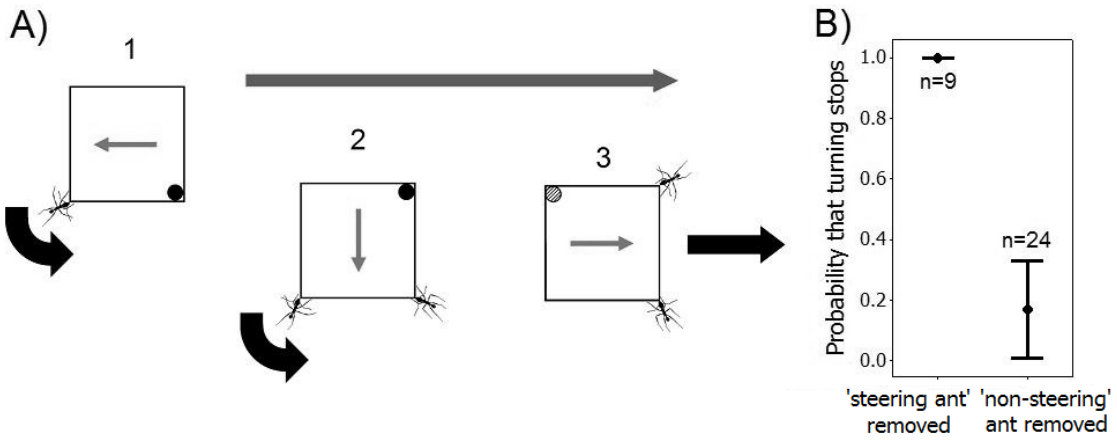

Figure 12.3 - A) The hypothesized mechanism by which a cooperatively transported item is turned. In this example the ant across from the drag point (black circle) drives the rotation. This ant attempts to pull the object in the desired direction (long arrow), but fails due to the object being stuck at the drag point. Thus, the ant's movement causes the object to pivot instead (1). Another ant is now in position to affects another pivot (2). The item is thus oriented in a way that causes the least amount of drag and can be carried onwards (3). B) The removal of a steering ant (see result section) significantly decreases the probability of turn continuation. Dots signify means, whiskers signify 95% CI. Turning stopped significantly more often if a steering ant was removed.

By examining videos of ants turning food items we developed a hypothesis for the turning mechanism: Ants grasp the item and attempt to drag it towards the nest. The item will tend to pivot around a drag point (Figure 12.3A, steps 1 and 2), bringing other ants around the item into a positions where they also begin turning the item by dragging towards the nest (Figure 12.3A step 2). Eventually the item reaches an orientation with a sufficiently low drag to allow it to be moved more easily, or where the point of highest drag is at the back of the item (Figure 12.3A step 3). Ants grasping furthest from the pivot point, such as opposite corners, will have more leverage and will thus more often be the main source of turning. Turning can occur whilst the item is in motion, as drag points will cause parts of the item to move slower than others, allowing pivoting around the point even when the item is in motion. Dragging ants can be categorized into hypothesized 'steering' ants, which cause the item to turn, and 'non-steering' ants, which may assist in moving the item but play little or no part in the turning (see methods section). There is nothing fundamentally different about steering and non-steering ants – it is their position around that item that determines their role. From this hypothesis we predicted that removing the (usually one) 'steering' ant will cause turning to cease while removing a 'non-steering' ant will not. This prediction was tested (see methods). Turning stopped significantly more often if a steering ant was removed than when a non-steering ant was removed (Fishers Exact test, N steering removed = 9, N non-steering removed = 24, DF = 1 Z = 10.95, $P<0.001$) (Figure 12.3B). Of the 9 steering ants successfully identified

and removed, 8 were grasping the item within 2mm of the corner – significantly more than would be expected by chance (proportion of perimeter within 2mm of corner = 0.4. proportion of steering ants grasping by the corner = 0.89. T test T = 4.4, P = 0.002).

Discussion

Our results show that groups of *P. oxyops* reorient food items during cooperative transport in a way that causes the item to require less force to be dragged to the nest. The re-orientation of transported items increases carriage speed and decreases dragging effort, as increased drag causes a reduction in carriage speed (cockroaches were carried 31% faster in the low drag orientation). By being able to remove items to a safe location more quickly and efficiently, cooperatively transporting ants are able to compete successfully against other species that may otherwise effectively dominate food sources (Traniello 1983; Yamamoto et al. 2009).

While load turning by *P. oxyops* is clearly adaptive, the mechanism by which turning occurs appears to result from the combination of the physics of load turning and the tendency of ants to return to the nest when carrying food, rather than specific load-turning behaviours. The turning mechanism can arise by the parsimonious rule “grasp the item and attempt to move towards the destination” combined with the mechanics of the system: Greater drag on part of the item will cause the item to pivot around this point, much as a tethered boat in a river will turn in line with the flow of the river’s current. Of the ants grasping an item, those that are able to move faster or more easily will cause the item to pivot around the point where the item moves slowest or has become caught on the substrate until all ants are moving at the same speed, resulting in no farther turning. Turning can occur even when the item is in motion towards the nest. Coordination is achieved because group members are attempting to move the item in the same direction, towards the nest. Previous research has shown that after cooperative transport is well underway and the item is in a low drag orientation, the ants at the front of the item carry while walking backwards, while ants at the back
carry while walking forwards, as other cooperatively transporting ants do (Sudd 1960a; Moffett 1988).

Our results show that cooperative transport of food items in *P. oxyops* is an effective and sophisticated collective behaviour. A previous study showed that workers distribute themselves non-randomly around food items, and by doing so can increase the retrieval speed (Czaczkes et al. 2011b). This study adds to this by showing that *P. oxyops* also turn food items

to so that they require less force to transport and are thus transported faster. Load transport efficiency is not the only reason for performing rapid cooperative transport: cooperative transport is often used to move a valuable food item to a place of safety, either the nest or under cover (Yamamoto et al. 2009). By reducing transport time ants reduce their exposure to desiccation, predation and parasitism, and free up workers for other tasks faster (Feener & Moss 1990; Tanner 2008; Cerdá et al. 2009). Cooperative transport also allows ants to exploit a much broader size range of food items.

Selection may have acted on *P. oxyops* to prefer grasping items by the corners so as to increase their ability to turn items, but grasping by the corners increases transport rate independently of item turning (Czaczkes et al. 2011b). Thus, our results are also of interest as they demonstrate that a beneficial behaviour may not necessarily exist due to evolutionary adaptation. In the case of load turning, our results indicate that this behaviour may emerge due to the physics of the system combining with pre-existing behaviours that would be adaptive to lone forager ants, rather than novel behaviours that have been directly selected for in the context of cooperative transport and load turning. Indeed, the development of cooperative transport may have been facilitated by the fact that cooperative transporters would benefit from item reorientation from the outset, without the lag of natural selection developing item turning behaviours *de novo.*

Acknowledgements

We thank Dr. Paulo Nogueira-Neto for allowing us to stay and work at Fazenda Aretuzina, Jacques Delabie for identification of the study species, Jonathan Bacon for constructive ideas and Katja Rex, the Sussex Social Insect Journal Club and two anonymous referees for constructive comments on earlier versions of the manuscript.

Chapter 13: Pheromone trails in the Brazilian ant *Pheidole oxyops*: extreme properties and dual recruitment action

Tomer J. Czaczkes & Francis L.W. Ratnieks

Abstract

Communication of feeding locations is widespread in social animals. Many ants use pheromone trails to guide nestmates to food sources, but trail properties and how they are used vary. The ant *Pheidole oxyops* retrieves prey cooperatively using multiple workers. The recruited workers are guided to the prey by a pheromone trail laid by the initial discoverer. In comparison to other ants, this trail has extreme properties. Despite being laid by just one ant, freshly laid trails are followed very accurately (84.4% correct choices at a bifurcation), but decay in only 5-7 minutes. This extreme accuracy and short duration probably reflect adaptations to underlying differences in feeding ecology. In particular, *P. oxyops* needs to rapidly recruit nestmates to a precise location in a competitive environment. Rapid decay combined with a natural walking speed of 1.4 metres per minute should set an upper limit of 4m (an 8m round trip) on recruitment range. However, experimentally placed food items up to 8m from the nest entrance were cooperatively retrieved. This greater range is due to the trail having a dual recruitment role. It not only recruits from the nest but also intercepts ants already outside the nest, causing them to join the trail. 75% of ants joining the trail then followed it towards the food item. Even when direct recruitment from the nest was prevented, this secondary recruitment action resulted in 7 times as many ants locating a food source than by chance discovery, and in items being moved 46% sooner.

Introduction

Animals often forage in a competitive environment. Those possessing adequate weaponry or size may be able to dominate a food source (Hölldobler et al. 1978; Hölldobler 1982). Less aggressive animals may use another niche in time or space (Cerdá et al. 1998a) or employ other strategies such as moving food to a safe location (Smith & Reichman 1984). Many central place foragers, including social insects, must also retrieve food to the nest to feed developing young. Some ants increase the maximum size of the food items they can retrieve by cooperative transport, in which two or more workers collectively move an item (Sudd 1965; Fowler 1984; Moffett 1988; Czaczkes et al. 2011b) thereby making the food safe from competitors (Hölldobler et al. 1978; Traniello 1983, 1987). Ants employ a variety of strategies and techniques to cooperatively transport food items (Sudd 1960a; Moffett 1988; Czaczkes & Ratnieks 2011; Czaczkes et al. 2011b). However, a basic requirement is that sufficient foragers must be recruited to the item to move it. Ideally, recruitment should be accurate and rapid: accuracy to ensure that recruits reach the precise location and rapidity to outpace competitors.

Food gathering locations range along a continuum from point sources to broad areas. In the latter case, recruitment of nestmates can still be effective even if there is some error in the ability to communicate the location, such as in some harvester ants (Greene & Gordon 2007b) or honey bees (Weidenmuller & Seeley 1999) (pp. 272). In some ants trail following accuracy is low. For example, in *Lasius niger*, which often exploits honeydew from aphid colonies (Pontin 1963), as few as 62% of the ants chose the correct branch at a trail bifurcation with only one ant depositing a trail, and only 70% of ants chose the correct branch with 20 depositing ants (Grüter et al. 2011). Pharaoh's ants, *Monomorium pharaonis*, achieve a comparable accuracy of 70% on paper substrate (Jeanson et al. 2003). However, when recruitment is to a single point source, such as to a nest site or a single food item, accuracy is more important (Weidenmuller & Seeley 1999). This study investigates trail following in *Pheidole oxyops*, a neotropical ground-nesting species that retrieves 78% of its food using cooperative transport of large items, mostly dead insects (Czaczkes et al. 2011b). This species provides an excellent opportunity to study adaptations for recruitment where maximum accuracy is expected, because recruits are directed via a pheromone trail laid by one or a few food-discovering ants to a precise location (Fowler 1984; Czaczkes et al. 2011b). This contrasts to the situation in *L. niger* or *M. pharaonis*, were many ants contribute to a single pheromone

trail. Indeed, Beekman et al. (2001) claim that in *M. pharaonis* recruitment to a food source is not possible unless many ants lay the trail pheromone (but see Sudd 1960b).

Pheromone trails should be easier to follow if more of the chemical is present in the headspace around the trail. This will require the chemical to be more volatile, especially if the trail is laid by only one ant. A more volatile trail pheromone will, however, be shorter lived. As *P. oxyops* forages mainly on non-renewable food sources, a long lasting trail pheromone is not necessary and would in fact be harmful. This is because continued recruitment long after the item has either been retrieved or lost to competitors would result in many ants being sent out of the nest needlessly. However, there is a time delay equivalent to one round trip between food discovery and recruits from the nest reaching the item. In the first part of this study we found that the foraging range of a *P. oxyops* colony was double the maximum distance from the nest at which recruitment from the nest should be able to function. This led to the hypothesis that the trail of *P. oxyops* also intercepted nestmates already searching for food near the food item, thereby resulting in the longer than expected colony foraging range.

Methods

Data were collected in February 2011 at Fazenda Aretuzina, a farm located near São Simão, in São Paulo State, Brazil. The study species, *Pheidole oxyops*, nests in the ground and naturally scavenges and hunts for dead and living arthropods, which are often carried back by groups of minor ants (2-30). *P. oxyops* display cooperative transport and recruitment behavior: when an ant attempts to move a food item and fails, it returns to the nest laying a pheromone trail that recruits nestmates to the item (Fowler 1984; Detrain & Deneubourg 1997; Czaczkes et al. 2011b). A burst of recruits can be triggered by providing an immovable food item. In this study we used a piece of mozzarella cheese fixed to a large piece of modelling clay. 11 field colonies were studied in total, labelled A-H and X –Z. A subset of these colonies was used for each individual experiment.

Experiment 1) Determining trail pheromone decay rate and trail following accuracy

Our first experiment determined the properties of the pheromone trails of *P. oxyops* by examining trail following accuracy of recruits and the decay rate of a trail laid by a single minor worker ant that had discovered an immovable food item. A T-maze was formed by placing a plastic platform, 20x220mm, which acted as the arms of the T, 30cm from a nest entrance (see figure 13.1). Each arm was 100mm long, with a 20mm central section between them. The

platform was raised on stilts surrounded by a water moat to prevent ants gaining access except via a cardboard ramp, which formed the stem of the T. The ramp was 100mm long, tapering from 50mm in width at the base to 20mm where it connected to the central section of the platform. The platform was overlaid with clean printer paper, with vertical decision lines marked 50mm from the centre of the overlay. A bait item was placed at the end of one arm. Within a few minutes this would be found by a minor worker ant from the study colony. The discoverer ant would unsuccessfully attempt to move the bait and then return to the nest via the ramp laying trail pheromone. We can be certain that the ant is laying a pheromone trail, as in other experiments ants could be seen to accurately follow the path of recruiting ants (the results of this experiment and experiment 4). On reaching the nest, the discoverer caused a surge of recruits which ran up the ramp and onto the platform. Ants passing a decision line (see figure 13.1) were considered as having chosen either left or right. These ants were then removed from the platform by allowing them to walk onto a piece of paper, and were then shaken off at arms length from the apparatus. Although the ants could in theory return to the apparatus, resulting in pseudo-replication, it seems likely that this disturbance would prevent most ants from reaching the apparatus, or following the trail up onto the platform. We continued monitoring the decisions of ants for up to 7 minutes as new ants left the nest. As the surge of recruits triggered by the first ants can be short lived, lasting sometimes less than two minutes, new surges of recruits must be triggered. To trigger a new recruitment surge, the bait was replaced at the centre of the T-maze when the flow of recruits ceased. This elicited a new surge of recruits without reinforcing the trail pheromone on the branch of the T-maze where the food item had been previously located. Thus, we could observe trail following accuracy up to 7 minutes after the initial recruitment occurred. During some trials no ants crossed a decision line in some of the minutes, hence the varying number of trials per minute in figure 13.2. The number of ants tested per minute, and the number of trials from which they stem, are presented in figure 13.2. Trials were videoed using a Sony HD XR520 camcorder. From the videos, the number of ants making left or right decisions per minute was determined. After every trial the platform was cleaned with ethanol and the paper overlay replaced. All trials were conducted in the shade. Temperature was recorded by placing a bulb thermometer on the ground beside the T maze. Six colonies, B-G, were tested in this experiment. Each colony was tested 2 or 3 times.

Experiment 2) Effect of distance to nest entrance on discovery of food items

The main aim of this experiment was to determine the maximum distance at which a *P. oxyops* colony can locate and retrieve food items. Individually numbered 5x5x1mm cheese pieces were laid out evenly in concentric circles around colony X. The nest entrance was then monitored for four hours, and we noted any food items being retrieved. We chose to stop data collection after four hours as >90% of items were returned within one hour, and no items returned after 2.5 hours. All retrieved food items were removed before they entered the nest, preventing satiation of the colony. Three trials were conducted. In the first trial 4 items were placed 0.5m from the entrance, 8 at 1m, 16 at 2m, 24 at 3m, and 32 at 4m. The second trial used longer distances, with 20 items at 5m and 40 at 6m. In the third trial 92 food items were placed around the nest entrance. Beginning on the outer circle, we laid 30 items at 10 meters, 26 at 8 meters, 18 at 6 meters, 12 at 4 meters and 6 at 2 meters. A larger number of bait items were placed at the more distant locations to maintain a similar item frequency at the various distances, although food item frequency did differ at different distances and in different trials – see appendix F part 1. All items returning to the nest were being cooperatively transported. The study colony was located on the edge of tree cover, at least 12 meters away from the nearest neighbouring *P. oxyops* colony. This colony was chosen as it was active, with many foragers, and isolated, and thus suitable for determining the maximum foraging range for the species. We were unable to study other colonies as their foraging ranges overlapped.

Experiment 3) Ability of the pheromone trail to intercept and direct foragers already outside the nest to the food item

The discoverer ant causes a surge of recruits from the nest along the trail that it has laid. But is the trail also effective at directing foraging ants who are already outside the nest scouting for food to the food source? We tested this by placing a bait item either 2m or 4m from a nest entrance. A single discoverer ant was allowed to find the item and return to the nest laying a pheromone trail. When the ant left the bait, the bait item was removed and video recording of the area surrounding the bait location began. The scout was allowed either to enter the nest, causing a normal recruitment surge (treatment 1: full trail with recruitment from the nest), or was removed 5cm from the nest entrance, resulting in an equivalent pheromone trail across the ground but without causing a recruitment surge (treatment 2: full trail without recruitment from the nest). The number of ants approaching the location of the food item from the direction of the nest was determined from the videos for 6 minutes after the scout had left the bait. A 30cm line perpendicular to the line from the bait to the nest, 150mm from the location of the bait (line A in figure 13.1b), was used as the criterion for an

ant approaching the bait. We also recorded the number of ants crossing a similar line on the pheromone trail 25cm from the nest (line B in figure 13.1b). As a control, before the trial began line A, and a similar line 20cm away (line C), were monitored for two minutes, and the number of ants crossing the lines heading away from the nest were counted. This provided as measure of how many ants arrive at the location of the food by chance. For the 2m distance five colonies (A, G, X, Y, Z,) were studied, with two repeats of the two treatments. At 4m two repeats of each treatment were carried out on 3 colonies (G, X, Y). Only a partial dataset was obtainable for colony A, and colony Z could not be tested at 4m as it was too close to other colonies.

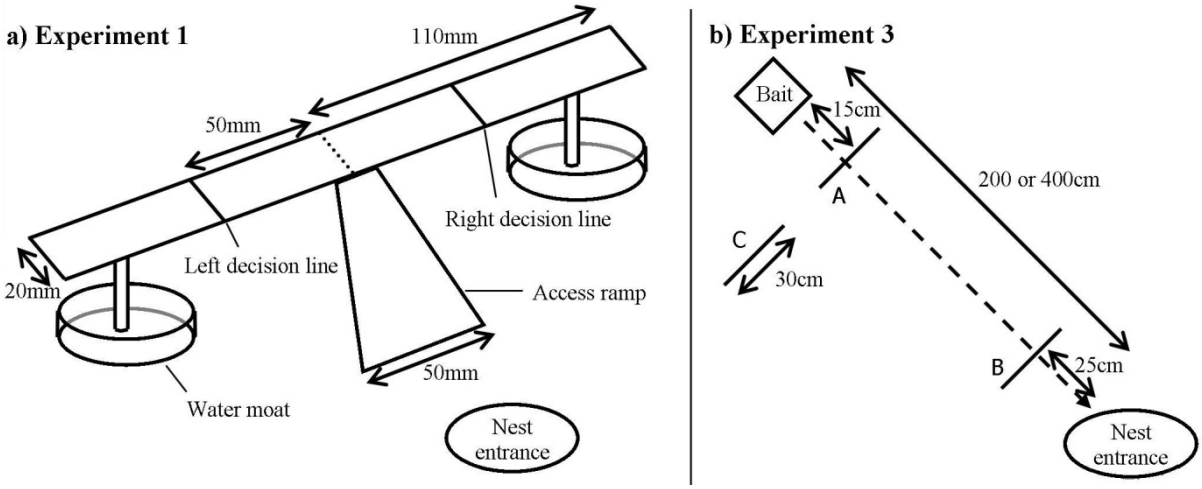

Figure 13.1 - a) T maze apparatus used in experiment 1 (not to scale). An unmovable food item was placed at the end of one arm the T maze. A worker ant would find this and return to the nest, depositing a pheromone trail. Recruits emerge from the nest entrance almost immediately and enter the apparatus via the access ramp. Ants crossing a particular decision line were considered to have chosen either left or right. b) Experiment 3 set up. An ant eventually found the bait and returned to the nest, depositing a pheromone trail. This discoverer ant was either allowed to return to the nest and cause a recruitment surge (treatment 1), or were removed 5cm from the nest entrance (treatment 2). The numbers of ants crossing lines A and B for 6 minutes after the scout left the food item were recorded. Prior to the trial, the number of ants crossing lines A and C had been recorded for 2 minutes.

Experiment 4) Effect of recruitment and the pheromone trail on the movement of food items

This experiment was designed to test the effect of a trail pheromone, with or without recruitment from the nest, on the ability of a colony to assemble sufficient ants to move a food item. A smooth ceramic tile, 15 x 15cm, was sunk with its surface level to the soil surface 2m from the nest entrance, providing a surface with regular friction. A 15 x 15 x 1mm square of cheese was placed in the centre. The nest entrance was either left open (treatment 1: full trail with recruitment from the nest), or temporarily blocked using a 90cm diameter petri dish, thus preventing recruitment (treatment 2: full trail without recruitment from the nest). We waited

until an ant found the food item, attempted to move it, failed, and returned to the nest laying a pheromone trail. We then measured the time from the moment the scout left the food item until the item was first moved by recruited ants and also until it had been moved 5cm from its original position, to represent the successful initiation of the collective retrieval of a large food item. The numbers of ants grasping the food item when it was first moved, and when it had been displaced by 5cm, were also recorded. To get a measure of the rate at which ants find a food item without any form of recruitment, we also ran controls in the absence of a pheromone trail by removing ants as they found the food item by chance. Using data from this last sub-experiment, we constructed a model describing the build-up of ants at a food source without recruitment, using the same methodology as described for constructing GLMM models (see statistical analysis below, and appendix F part 2 for more detail on the modelling of the build-up of ants by chance alone). We then compared the model's results with the time needed to build up sufficient ants to move the item when a pheromone trail (treatment 2), or pheromone trail and recruitment (treatment 1), was present (see results for further details).

Experiment 5) Joining and following a pheromone trail by ants already outside of the nest

To determine the probability that ants already outside the nest will follow a trail that they discover, and the direction they take, a runway (40 X 100cm) of smooth ceramic tiles was placed flush with the ground in front of colony X, leading directly to the nest entrance. The tiles were covered with white printer paper. An immovable food bait was placed at the far end of the runway, and was soon found by an ant which would attempt to move the item, fail, and return in a relatively straight line to the nest along the paper while laying a pheromone trail. The food item was then immediately removed, and the entire runway was video recorded for 7 minutes. 8 repeat trials were performed. When analysing the videos using Virtualdub software (Lee 1998) the path of the returning scout was marked on screen with a line. Other ants were scouting for food in this area, and every ant to cross this line was scored as to whether it followed the pheromone path or not and, if it did, whether it followed the path towards the nest or towards the food item. An ant was considered to follow the pheromone trail when it followed the line for 10cm or more if the ant had initially been walking in a different direction to the pheromone trail (>10 degrees different). If the ant had been walking in a similar direction (<10 degrees different) we required the ant to follow the trail for at least 20cm before scoring it as following the pheromone trail. This was to reduce the chance of including ants that walked in the trail direction by chance alone.

Statistical analysis

We analysed the data using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) and general linear models (GLM) using R2.9 (R Development Core Team 2009). Models were fitted using the lmer function (Bates et al. 2007). Where appropriate, we included 'colony' as a random effect to control for the non-independence of data points from these sources (Zuur et al. 2009; Bolker et al. 2009). Saturated models (containing all measured variables and random effects) were produced and non-significant terms sequentially removed until we arrived at a model containing only significant terms (Zuur et al. 2009). Heterogeneity of variance was controlled for when discovered by adding the appropriate term to models (Zuur et al. 2009). Data were square-root transformed where necessary to achieve normality of error. Sign tests, one sample Z tests and one sample T tests were carried out in Minitab 14.

Results

Experiment 1) Determining trail pheromone decay rate and trail following accuracy

Every ant that discovered the food item tried to move it, failed, returned to the nest, and immediately caused a surge of ants which walked in the direction from which the discovering ant had come. Thus, we are confident that every discoverer ant deposited a pheromone trail. The statistical model contained only one explanatory variable – time after recruitment – and one random effect – colony (with intercept free to vary but a fixed slope). Temperature did not have a significant effect on decay rate (GLMM, $z = -0.664$, $P = 0.507$). A binomial error structure was used. Recruit ants initially chose the branch of the T-maze marked with trail pheromone with high accuracy (84.4% correct, figure 13.2). The age of the pheromone trail was strongly correlated with the proportion of ants taking the correct branch (GLMM, $z = -9.747$, $P < 0.001$, see figure 13.2). Mistakes increased rapidly, and 7 minutes after trail laying there was no difference between the branches (mean 50% correct, figure 13.2). The difference between the branches was non-significant after only 5 minutes (One sample T test, $N = 161$, mean = 0.57.1, $T = 1.83$, $P = 0.07$). Although trail pheromones are normally assumed to decay exponentially (Jeanson et al. 2003; Dussutour et al. 2009a), we found that our data was best explained by a linear distribution (sequential ANOVA, linear $F = 158.54$, $P < 0.0001$, exponential $F = 0.97$, $P = 0.324$). Although a function with an asymptote of 0.5 would be a more realistic biological description, over the timescale in this experiment a linear function is a reasonable, and simpler, approximation.

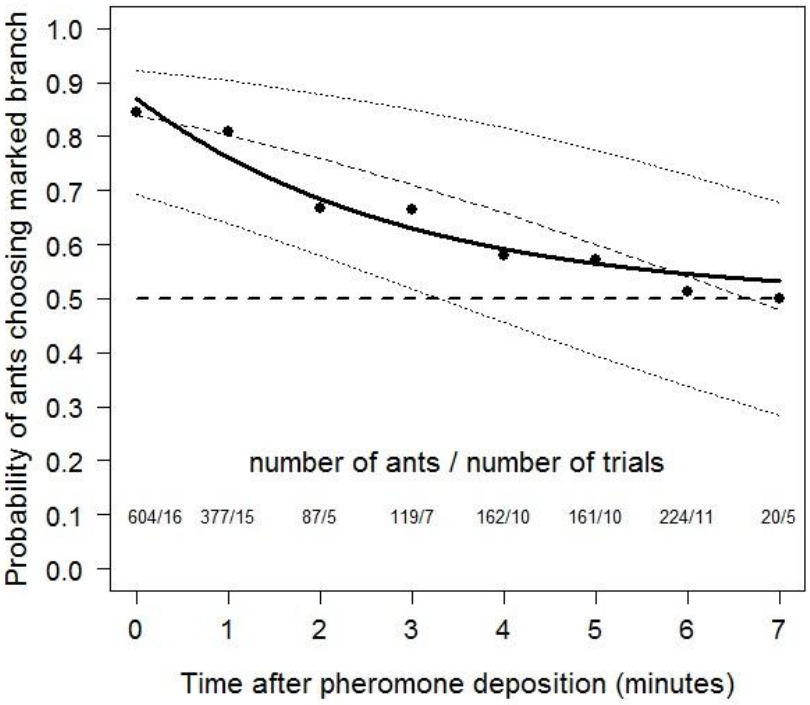

Figure 13.2 - Experiment 1. Decay rate of trails laid by a single discoverer ant. Trail choice accuracy decays rapidly after pheromone deposition. Ants follow a freshly-laid pheromone trail with high accuracy, but within 6-7 minutes branch choice is random, probability 0.5 (dashed line). The thick curved line is the estimated decay rate of the pheromone. The curved dashed line represents the model estimate (GLMM, $z = -9.747$, $P < 0.001$). The dotted lines represent the 95% confidence interval for the estimate, and the dots show the mean for data within one-minute blocks (i.e., 0 minutes = 0 – 59s after the pheromone was laid, 1 minute = 60-119s, etc). Numbers are number of ants tested/number of trials.

Experiment 2) Effect of distance to nest entrance on discovery of food items

Colony X was extremely effective at retrieving nearby food items, collecting 100% within 1m of the nest (figure 13.3). The proportion dropped with distance, decreasing to 45% at 5m and 12% at 8m. At 10m no items were retrieved within four hours. In the first trial some items laid down at 3m were already being returned when observation of the nest entrance started. Thus, the measurement for 3 meters is a slight underestimate. One of the more distant items were later found to have been surrounded by other species of ants, showing that competition occurs and that rapid food-item discovery and retrieval is advantageous in this environment.

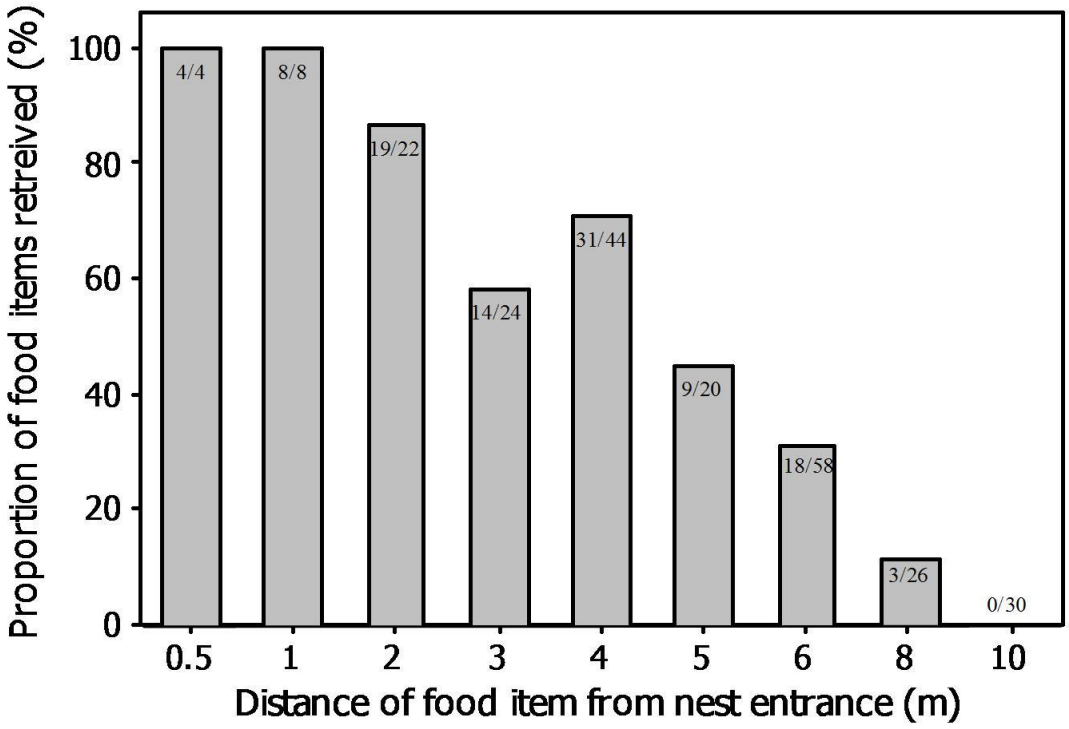

Figure 13.3 - Experiment 2. Proportions of food items placed on the ground that were returned to the nest entrance within four hours as a function of distance from the nest entrance in Colony X. Numbers in bars indicate the number of items retrieved out of the total. All retrieved items were being transported cooperatively.

Experiment 3) Ability of the pheromone trail to intercept and direct foragers already outside the nest to the food item

The full statistical model (comprising distance of the food item, time taken for the discoverer ant to reach the nest, treatment, and the interactions between all of these fixed effects, and colony as a random effect) was pared down to a final model comprising only treatment as a fixed effect and colony as a random effect. A normal error structure was used. Unsurprisingly, more ants arrived at the location of a discovered food item when recruitment from the nest was allowed than when the trail-laying ant that discovered the food item was removed 5cm from the nest entrance (mean n = 26.5 ants from trail + recruitment, [treatment 1] vs. 9.7 , from trail alone [treatment 2], GLMM, DF = 34, t = 3.04, P = 0.0046). However, the trail itself acted as an important recruitment mechanism as only 1.5 ants on average located a food item with no trail leading to it (GLMM, DF = 16, t = 7.86, P < 0.0001, see figure 13.4). Indeed, when the discoverer ant was removed 5cm from the nest entrance more ants passed the point at which the food was found (line A, figure 13.1b) than a point on the trail 25cm from the nest entrance (line B, figure 13.1b), even though this point was closer to the nest and so

would be expected to have more ants passing by on their way to and from the nest entrance (mean ants at 25cm = 3.7, mean ants at full distance = 9.7, GLMM, DF = 16, t = 6.15, P < 0.0001). The distance of the food bait from the nest, 2m or 4m, had no significant effect on the number of ants reaching the food. Therefore, distance was not included as an explanatory variable in the final model. Indeed, of all fixed effects in this experiment treatment was the only significant explanatory variable.

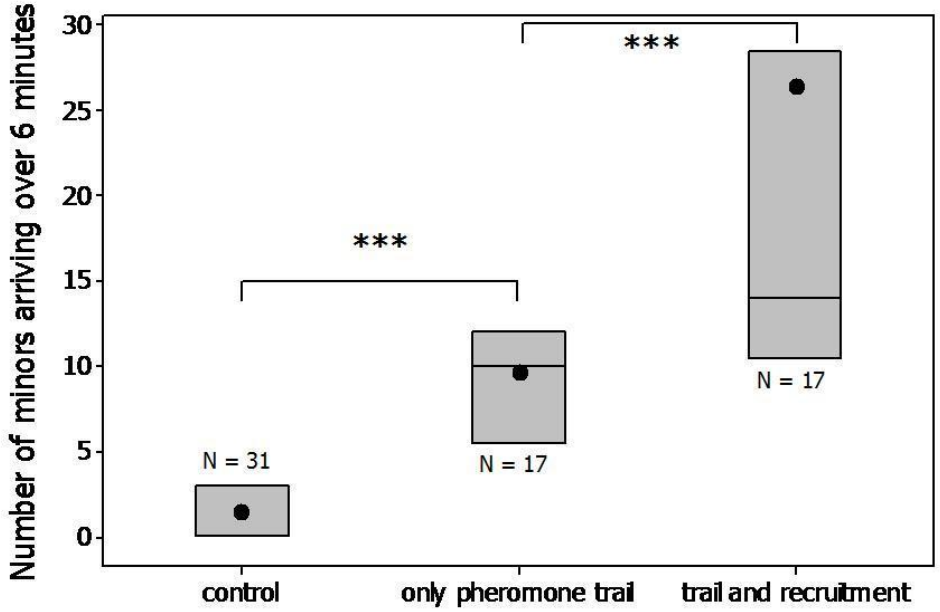

Figure 13.4 - Experiment 3. Number of ants arriving at the location of the food item per 6 minutes, before it was presented (control – a measure of discovery rate alone) and after a forager had found it and returned to the nest. Returning foragers were either prevented from reaching the nest (treatment 2- only pheromone trail: discovery + ants intercepted by the pheromone trail) or allowed to enter the nest and cause a surge of recruitment (treatment 1 - trail and recruitment: discovery + interception + recruitment from nest). Circles represent means, central horizontal lines represent medians (median for control is 0), grey boxes represent inter-quartile range. Three asterisks represent highly significant differences, P < 0.001, between neighbouring treatments. The median in the control bar is 0, and so is not represented.

Experiment 4) Effect of recruitment and pheromone trail on the movement of food items

On average 2.2 (SD 1.96) ants were required initially to move a 15mm^2 food item and an average of 6.55 (SD 2.68) ants were grasping the item when it had been moved 5cm from its original location. These figures were almost identical when scout ants were allowed to reach the nest and recruit or prevented from doing so (mean number of ants at initial move: only pheromone trail, no recruitment from nest =2.11 SD = 0.93 [treatment 2]. Pheromone trail and recruitment from nest = 2.27 SD = 2.57 [treatment 1]. Mean ants at 5cm: treatment 2 = 6.67 SD = 3.32, Treatment 1 = 6.46 SD = 2.16). Using the data on the number of ants that discovered

the food item by chance, we modelled the build-up of ants at a food item without recruitment of any form, resulting in a final model of the time in seconds (s) required to give n ants at a food item: n = -5.71 + 0.0199s. We could use this to interpolate the amount of time necessary for a specific number of ants to find the food item. Thus, it would take 345s for 2.2 ants and 747s for 6.55 ants to find the food item by chance without the help of a pheromone trail (see appendix F part 2 for details). Using these numbers as the expected values (null-hypothesis) we tested whether food items were moved sooner when scouts laid a pheromone trail but were prevented from recruitment at the nest (treatment 2) than by scouts encountering a food item by chance. Items were indeed moved significantly sooner when a pheromone trail was present (One sample Z test, mean time required for food to be moved = 232.1s, SD = 453.7 N = 10, Z = -2.12, P = 0.034), and were also displaced by 5cm sooner (One sample Z test, mean time to move the item 5cm = 468.9s SD = 110.315, N = 10. Z = -5.21, P < 0.001).

Experiment 5) Non-recruited ants joining and following a pheromone trail

The probability that an ant walking in the environment that encounters a pheromone trail will join the trail is strongly negatively correlated with the age of the pheromone trail. For a freshly-laid trail the probability is almost 80%, decreasing to 0% at 7 minutes (see figure 13.5). Of the ants that did join the trail, 75.5% walked towards the food item, which is significantly more than expected by chance (Sign test: N = 46, H_0 = 0.5, P = 0.0016).

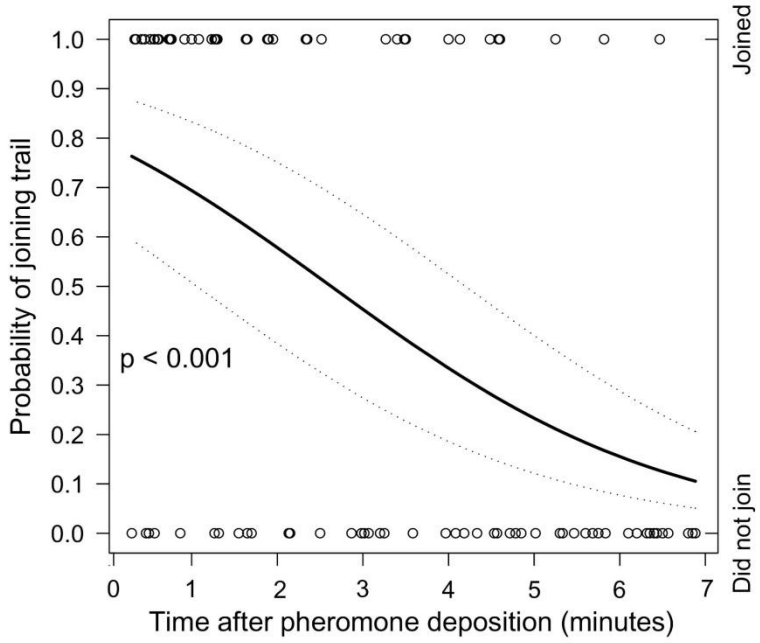

Figure 13.5 - Experiment 4. The probability that an ant walking outside the nest that intercepts a pheromone trail will follow the trail, as a function trail age. The thick black line represents the modelled probability of joining (left Y axis) based on the data. The white circles represent individual ants which either joined or did not join the pheromone trail (right Y axis).

Discussion

The results support our prediction that the pheromone trail laid by a single *P. oxyops* worker recruiting to a single large food item can be followed with considerable accuracy (85% correct choices at a T-bifurcation). In contrast, in *Lasius niger* a trail laid by a single forager is followed through a T-bifurcation with probability of only 62% (Grüter et al. 2011). Even when the trail is laid by 20 ants the proportion choosing the correct branch increases only to 70%. Similar results were obtained for the Pharaoh's ant, *Monomorium pharaonis*, in which only 70-80% chose a branch that had been marked by hundreds of nestmates (Jackson et al. 2006). In both these species there is unlikely to be as great a need for precise communication of location as in *P. oxyops*, as *L. niger* recruit mainly to clustered, long-lasting food sources (Pontin 1963), and neither *L. niger* nor *M. pharaonis* perform cooperative transport, so both can rely on many ants making return trips to the food patch to strengthen the pheromone trail.

The decay rate of the *P. oxyops* trail pheromone also supported our predictions. The trail no longer provided useful information 5 to 7 minutes after it was laid. There was close agreement in the results of our two different bioassays, one on trail choice at a T-bifurcation (experiment 1, figure 13.3) and one on trail joining (experiment 5, figure 13.5). In contrast, pheromone trails of *L. niger* are usually laid by many individuals and are still effective up to 20 hours later (Evison et al. 2008). This difference matches the different feeding ecology of the two species. Whilst the aphid patches which *L. niger* primarily recruit to replenish over time, and can persist for months (Salo & Rosengren 2001), the single large food items which are the main food source of *P. oxyops* do not replenish and must be exploited immediately. Evaporation should lead to a trade-off between trail following accuracy and pheromone decay rate. *P. oxyops* makes this trade-off firmly on the side of high accuracy and short duration. Indeed, trail following accuracy is particularly high when we consider that the trail is laid by a single ant, as compared to tens or hundreds in other species. As individual *P. oxyops* foragers rarely have to reinforce pheromone trails multiple times, one might expect them to use a larger proportion of their pheromone store compared to mass-recruiting species. The behavior of the recruits may also be adjusted to allow for fast and accurate trail following. One might also expect that ants relying on single discoverers will tend to deposit fairly continuous trails, whilst ants recruiting to long lasting food sources might conserve pheromone by depositing a series of dots, as *L. niger* does. These are open questions for future investigation.

The rapid decay rate of *P. oxyops* pheromone trails sets a maximum recruitment distance. In our study (experiment 3) returning scouts took on average 33s and 45s to return from a bait two and four meters away, respectively, walking on natural substrate. This gives a mean walking speed of 1.36 ±0.22 SD meters per minute. Given a 6 minute life span of the pheromone trail, the maximum recruitment range of *P. oxyops* should be 6*1.36 / 2 = 4.08 meters. However, we found that a colony can retrieve food items using cooperative transport from as far away as 8m (figure 13.3). This discrepancy can be explained by the results of experiments 3 and 5 that show that the trail intercepts ants already out of the nest and directs most of these (75.5%) towards the food item. Even without recruitment from the nest (treatment 2), 7 times as many ants reach a food item with a trail pheromone leading to it than would be expected by chance discovery (experiment 3, figure 13.4), and thus allow food retrieval to begin 47% sooner that it would if ants relied on chance discovery alone (experiment 4).

Local recruitment of nestmates has also been reported in the ant *Aphaenogaster cockerelli* (Hölldobler et al. 1978), where workers finding a food item emitted a pheromone which attracted nearby ants. The recruitment effect of the pheromone trail in *P. oxyops* has a similar effect but works via a different mechanism. Whilst the local recruitment signal of *A. cockerelli* is from a point-source using diffusion and air movement, in *P. oxyops* it is in the form of an interception line from the food item to the nest. Effectiveness is increased as the interception line is directed towards the nest, an area where nestmates are likely to occur. 75% of nestmates walking in the environment who crossed the pheromone trail walked towards the food source, demonstrating that *P. oxyops* foragers have additional information, presumably in the form of personal information on the direction in which the nest entrance lies (perhaps by learning local landmarks or by using path integration (Collett & Collett 2002)), that increase the effectiveness of interception. Because pheromone trails recruit ants already outside the nest, the foraging range of a *P. oxyops* colony is increased and the response time is decreased compared to a situation in which the trail only serves to direct recruits from the nest.

The pheromone trails of ants have traditionally been seen as way markers from a resource to the nest, and particularly as a means of directing foragers at trail bifurcations. However, recent research indicates that ant trail systems are more sophisticated than this, including new roles for ant trail pheromones such as activation signals for old trails (Robinson

et al. 2008b), allowing reactivation of long lasting trail pheromones, and as a reassurance to route memory, allowing higher movement speeds (Czaczkes et al. 2011a). Here, we have demonstrated that pheromone trails also have a dual recruitment effect, directing ants from the nest itself to a food item, and also intercepting and directing foragers already in the environment to the food item. This second mechanism allows a colony *of P. oxyops* to forage at greater distances and to start moving large food items more quickly than would be possible if the trail only recruited nestmates from the nest itself. We have also demonstrated that a pheromone trail has the potential to be very effective in its traditionally assigned role, with a trail laid by a single ant being capable of guiding 85% of recruits down the correct branch at a bifurcation.

Acknowledgements

We thank Dr. Paulo Nogueira-Neto for allowing us to stay and work at Fazenda Aretuzina, Dr B. Czaczkes for help with data management and Drs Katja Rex and Margaret Couvillon for comments on the manuscript. T.C was funded by a Biotechnology and Biological Sciences Research Council doctoral studentship.

Chapter 14: Novel escorting behaviour and convergent evolution of recruitment mechanisms in an invasive ant

Tomer J. Czaczkes & Francis L.W. Ratnieks

Abstract

The Longhorn Crazy ant, *Paratrechina longicornis*, is a pest ant species with worldwide distribution. It tends honeydew-producing hemiptera to obtain carbohydrates and is also an effective predator and scavenger. Previous research has shown that during foraging *P. longicornis* uses at least three pheromones with varying properties, which are used in recruitment to point sources and trail following. Our results show that as well as gathering honeydew, this species uses its specialised recruitment pheromones to effectively exploit large food items. 88% of the mass of externally-carried food was retrieved cooperatively by two or more workers. Recruitment to large items is via a pheromone trail laid by the discovering ant. This trail is initially followed with few errors by naïve recruits (82% correct choices at a T-bifurcation) but decays very rapidly (within 6 minutes). We also show that a food-discovering ant can recruit nearby nestmates to a large food item without returning to the nest. These properties of the recruitment system are strikingly similar to two unrelated ant species which also specialise in cooperative retrieval of large food items, suggesting convergent evolution. Lastly, we describe a novel "escort" behavior. In escorting, additional workers accompany a transported item but do not assist in carrying it. Both local recruitment and escorting are much more pronounced when handling live prey, suggesting a role in preventing live prey escaping.

Introduction

The longhorn crazy ant, *Paratrechina longicornis*, is perhaps the most widely-distributed invasive ant in the world. Native to S.E. Asia, it is now found in all continents except Antarctica (Wetterer 2008). It can be a major indoor pest (Harris et al. 2005), and in an invaded habitat can reduce biodiversity by displacing native ants and other invertebrates (Hölldobler & Wilson 1990) (pg 433).

Why is *P. longicornis* so successful as an invader? It demonstrates several traits common to invasive ant species, such as polygyny, polydomy and intranidal mating (Holway et al. 2002; Debout et al. 2007; Pearcy et al. 2011). *P. longicornis* also tends phloem-feeding hemiptera, which provide large quantities of carbohydrates. However, many ant species do this, and unlike many invasive ants *P. longicornis* often does not aggressively displace other ant species from food sources (Levins et al. 1973; Banks & Williams 1989; Lester et al. 2004). *P. longicornis* is, however, a very effective hunter and scavenger (Kenne et al. 2005) and can rapidly recruit workers to large prey items (Trager 1984; Kenne et al. 2005). Thus, it seems that *P. longicornis* may out compete other ants by exploiting food sources more rapidly (Fellers 1987). *P. longicornis* can avoid interference competition (Banks & Williams 1989) from other species by using coordinated cooperative transport to bring large food items to the safety of the nest (Trager 1984; Kenne et al. 2005).

Cooperative transport, in which items are moved by two or more individuals, is common in ants and humans but rare in other animals (Czaczkes & Ratnieks In press, see chapter 15). In ants, one adaptation to cooperative transport is a specialised type of recruitment, in which an ant that has discovered a large food item, such as a dead insect, lays a short-lived but accurately followed pheromone trail that nestmates can follow to the item to help in transport. The trail needs to be accurately followed in order to guide nestmates to the specific location of the item, and strong, so that a functional trail can be laid by a single discoverer (Czaczkes & Ratnieks 2012). However, the trail need not be long lived as there is no need for ongoing recruitment to the food location, as it is not a renewing food source such as a patch of aphids secreting honeydew. Trail pheromones with these properties occurs in the ants *Aphaenogaster albisetosus* and *Pheidole oxyops*, which both specialise in rapid recruitment to large food items followed by cooperative transport back to the nest (Hölldobler et al. 1978; Czaczkes & Ratnieks 2012). In addition, species with cooperative transport of large food items

can display local recruitment (i.e., recruitment of nearby nestmate foragers outside the nest) either by the discoverer emitting a point-source attractant (Hölldobler et al. 1978) or by the recruitment trail laid by the discoverer having a dual effect, intercepting workers outside the nest in addition to recruiting ants from the nest itself where the trail ends. This allows recruitment and retrieval to begin more rapidly and to occur to sites more distant from the nest (Czaczkes & Ratnieks 2012).

The complexity of *P. longicornis's* chemical communication (Witte et al. 2007a) suggests that it has convergently evolved a specialised recruitment behavior similar to that of *Ph. oxyops* and *Aphaenogaster albisetosus*. As *P. longicornis* is a very capable hunter as well as scavenger (Kenne et al. 2005), we also predicted specialised adaptations to hunting live prey. A high accuracy, short term recruitment system would allow *P. longicornis* to compete effectively for protein sources, and would function alongside recruitment to long-lasting food sources. In this study we aimed to test these predictions by comparing the recruitment and foraging of *P. longicornis* to that of ants specialised in recruiting to large, ephemeral food items. We find that *P. longicornis* not only possess such a specialised recruitment system including both high-accuracy, short term recruitment and local recruitment, but also demonstrates a unique escorting behavior of live prey items.

Methods

Study site and organism

Data were collected in March 2012 on the campus of the University of São Paulo, Riberão Preto, Brazil. *P. longicornis* forage extensively on honeydew (Wetterer et al. 1999) (and see results) but also hunt and scavenge arthropod prey very effectivly (Kenne et al. 2005). Large items are retrieved by coordinated encircling cooperative transport (Czaczkes & Ratnieks In press). Wild, unmanipulated colonies were studied. Workers with an empty abdomen weighed on average 0.39mg (St-Dev = 0.045) (see results). As bait we used freshly caught termites (*Syntermes spp.;* mean weight 5.98mg, St-Dev 1.3).

Experiment 1 – The importance of cooperative versus individual transport in foraging

We surveyed the entrances of three *P. longicornis* colonies for four hours in total, collecting any items (excluding brood) being carried towards the nest. Items were weighed on

a microbalance sensitive to 0.1mg (Sartorius TE64) within 30 minutes of collection. The number of ants transporting each item was also recorded.

To ascertain the importance of liquid retrieval in *P. longicornis* we observed active trails of 5 colonies for 10 minutes each, counting the number of ants returning with empty or distended abdomens. Where foraging trails could be traced, they were found to lead into trees, presumably hosting colonies of honeydew producing hemiptera. As a comparison, we also collected data from four colonies of *Pheidole oxyops* in the same location. *P. oxyops* is a native Neotropical species which also specialises in cooperative retrieval of large food items (Czaczkes et al. 2011b). We have never observed *P. oxyops* foraging above ground level. To get a measure of the amount of liquid being retrieved by each *P. longicornis* worker, we weighed 37 ants with non-distended abdomens and 16 with distended abdomens using a balance sensitive to 0.01mg (Mettler Toledo UMT 2 balance).

Experiment 2) Trail choice at a T-bifurcation

This experiment quantified the properties of a *P. longicornis* recruitment trail laid by a single discovering worker. We replicated the methods of Czaczkes & Ratnieks (2012) to determine the longevity of trails made by one discovering ant to an immoveable food item, and the accuracy with which recruits follow this trail. Recruitment to large food items by *P. longicornis* is similar to other cooperatively-transporting species (Detrain & Deneubourg 1997; Robson & Traniello 1998; Czaczkes et al. 2011b). When an ant finds an item it attempts to drag it back to the nest. If the item is too big for the discoverer to move, it returns to the nest depositing a pheromone trail. On entering the nest a surge of recruits leaves the nest and follows the pheromone trail to the food item.

To initiate trail laying, a freshly-freeze-killed termite was tethered to a piece of modelling clay and placed at the end of one arm of a T-maze. The T-maze head was a plastic platform, 20x220mm, raised on stilts each placed in a container of water to prevent access via the stilts (figure 14.1a). The stem of the T-maze was a 100mm long ramp, tapering from 50mm in width at the base to 20mm where it connected to the central section of the platform. The platform was overlaid with clean printer paper, with perpendicular decision lines marked 50mm from the centre of the overlay. The apparatus was placed within 30cm of a *P. longicornis* nest entrance. The bait was typically discovered within c. 2 minutes by a single *P. longicornis* worker, which would then attempt to move the item, fail, and return to the nest depositing a pheromone trail.

Trail laying in *P. longicornis*, as in many other formicine ants which lack stings, is a stereotyped behavior involving brief pauses to lay pheromone, in which the abdomen is bent downwards and dotted on the substrate, although *P. longicornis* can also lay continuous pheromone trails by dragging the abdomen along the substrate (V. Witte, pers. comm.). The recruits would run up the ramp and onto the platform. Those passing one of two decision lines on the head of the T (see figure 14.1a) were considered to have chosen either left or right, and then gently removed by brushing them from the platform with a piece of paper.

We continued monitoring choices for up to 7 minutes as new recruits left the nest. However, as the surge of recruits triggered by the discoverer normally lasts only 1-2 minutes, additional recruitments also had to be triggered. To do this the bait was replaced at the centre of the T-maze when the flow of recruits ceased. The bait would soon be found by an ant which would try to move it, fail, and return to the nest depositing a recruitment trail and causing a new surge of recruits. In this way, we were able to send new recruits to the stem of the T without reinforcing the trail pheromone on one of the two branches at the head of the T-maze which was laid only by the initial discoverer. We monitored trail choice for 7 minutes.

Trials were videoed using a Sony HD XR520 camcorder. From the videos, the number of ants choosing left or right was determined and grouped into one-minute intervals. After every trial the platform was cleaned with ethanol and the paper overlay replaced. All trials were conducted in the shade. Five colonies were tested. Colonies of P. *longicornis* are highly mobile and frequently relocate their nest. Due to frequent nest relocation not all colonies could be tested an equal number of times. Three colonies were tested 6 times, one 3 times, and one twice (n = 23 trials in total).

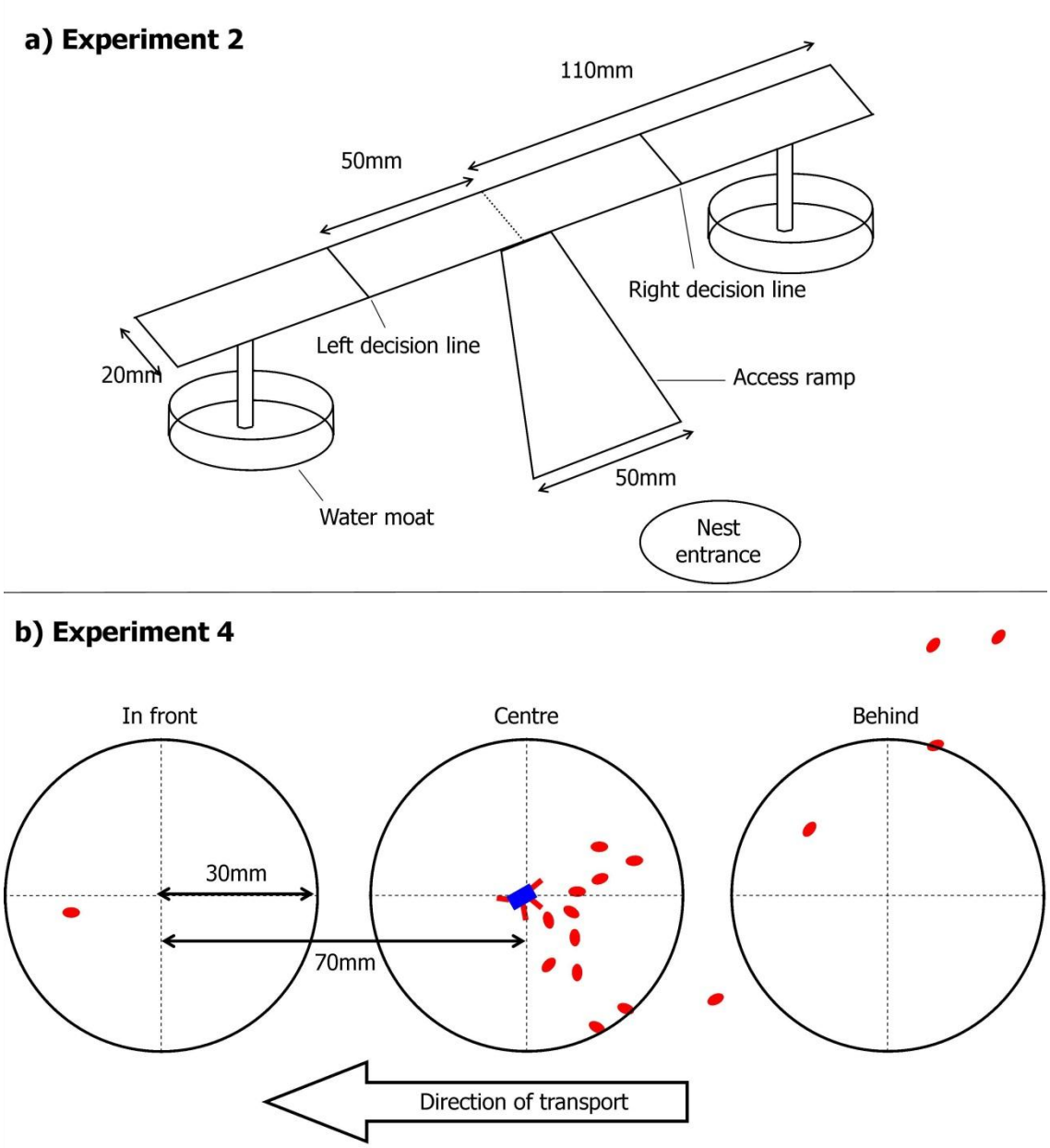

Figure 14.1 - a) T-maze apparatus used in experiment 2 (not to scale). A freeze-killed termite was placed at the end of one arm the T. A *P. longicornis* worker would find this and return to the nest, depositing a pheromone trail. Recruits emerge from the nest entrance almost immediately and enter the apparatus via the access ramp. Ants crossing a particular decision line were considered to have chosen either left or right. b) Diagram based on video recordings representing a termite (blue rectangle) being cooperatively transported by four *P. longicornis* workers (red lines) from right to left. Other P. longicornis workers in the area are marked with red ovals. There are 11 workers in a 3cm radius around the termite, two workers in a 3cm radius circle 7cm behind the item, and one worker in a 3cm radius circle 7cm in front of the item.

Experiment 3) Local recruitment to live and dead food items

To determine whether *P. longicornis* emits volatile recruitment pheromones known from its Dufour gland (Witte et al. 2007b) when encountering large prey items, and whether the prey item being alive or dead affects this, we presented *P. longicornis* colonies with a termite that was tethered to the substrate circa 30cm from a nest entrance. The substrate was white printer paper that had been taped down on all sides to keep the edges flush with the concrete ground surface. The prey item was either alive or freshly freeze-killed. As soon as an ant discovered the item a circular plastic barrier, 1.5cm radius and 1cm high and coated in fluon, was placed around the bait plus ant to prevent the ant from returning to and recruiting workers from the nest. The area around the bait was videoed for 2 minutes before the bait was presented and then for two minutes after the bait was discovered. From the videos, the number of ants entering a 10cm radius around the bait location was determined for both periods. At least 20 minutes were allowed between trials to allow the colony's behavior to return to normal. 6 colonies were tested, each once with a dead termite and once with a live termite.

Experiment 4 – escorting behavior during cooperative transport

While studying cooperative transport in *P. longicornis* we sometimes saw a conspicuous 'escort' of ants following the carried item without assisting in carrying. In addition, it seemed that this escort was only deployed when live prey items were being transported. To test whether *P. longicornis* specifically escort cooperatively-transported food items, and whether this is affected by the item being alive or dead, we presented termites to active *P. longicornis* trails c. 2m from the nest entrance. Live termites were gently held with soft forceps until a *P. longicornis* worker grasped it. The section of the trail where the termite was presented was videoed for two minutes before the termite was presented. The termite was then followed and the area around it videoed as it was transported to the nest. After the termite had been retrieved the original trail section was again videoed for a further two minutes. During transport we counted the number of ants within 3cm radius of the prey item every 30 seconds, and also in a similar 3cm radius area centred 7cm behind and 7cm in front of the item, relative to the direction of transport (see figure 14.1b). Ants actively carrying the termite were counted separately. Before and after transport similar counts were made in a 3cm radius area centred on the trail segment where the termite was originally presented. Each colony was tested three times with live prey and three times with dead prey. 6 colonies were tested in total.

Statistical analyses

We analysed the data using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) and general linear models (GLM) using R2.15 (R Development Core Team 2009). Models were fitted using either the glm or the lmer function (Bates et al. 2007). When colonies are tested multiple times, or multiple measurements are taken per trial, we tested whether 'colony' or 'trial' or both should be included as a random effect to control for the non-independence of data points from these sources (Zuur et al. 2009; Bolker et al. 2009). Saturated models (containing all measured variables and random effects) were produced and non-significant ($P > 0.05$) terms sequentially removed until at a model containing only significant terms was arrived at (Zuur et al. 2009). Binomial data (experiment 2) was modelled using a binomial distribution family. Normally distributed data (experiment 3) was modelled using a Gaussian distribution family. Poisson distributed data (experiment 4) was modelled using a Poisson distribution family. All P values presented were corrected for multiple testing using a Benjamini-Hochberg correction (Benjamini & Hochberg 1995).

Results

Experiment 1 – The importance of cooperative versus individual transport in foraging

We collected and weighed 76 items being transported to the nest. Of these, 18 (25%) were being cooperatively transported versus 54 being transported by single ants. However, the cooperatively-transported items accounted for 87.9% of the total mass (193mg of a total of 219mg), being on average 21 times heavier.

While monitoring ants returning to the nest via active trails we counted 1246 ants, of which 488 (39.2%) had a visibly distended abdomen. By comparison, of 284 *Pheidole oxyops* minor workers returning to 4 nests (excluding those returning from the refuse pile) only 5 (1.8%) had a visibly distended abdomen.

We weighed 37 *P. longicornis* with an apparently empty abdomen and 16 with a visibly distended abdomen. "Empty" ants weighed on average 0.39 mg (StDev= 0.045) and "full" ants weighed on average 0.86 mg (StDev = 0.13). Thus, on average a "full" ant carried 0.49 mg of liquid, c. 120% of its body weight. Thus, per minute a colony retrieves on average 4.78 mg of liquid and 0.9 mg of solid food.

Experiment 2) Trail choice at a T-bifurcation

In every trial ants which discovered the bait returned to the nest depositing a pheromone trail, and caused a surge of recruits to leave the nest and follow the trail. As figure 14.2 shows, trail choice is initially very accurate, with 82% correct choices in the first minute. But this rapidly drops, with the modelled decay curve intersecting random choice (50%) in under 6 minutes. We found that the minute following trail laying in which a recruit was observed making a choice was a strong predictor of whether it chose the correct branch (GLM, DF = 842, Z = -8.403, P < 0.0001, see figure 14.2). These results are almost identical to data collected using the same protocol on *Pheidole oxyops* (Czaczkes & Ratnieks 2012). Indeed, when data from both species are combined in a single statistical analysis, ant species is not a significant factor (GLMM, Z = 0.725, P = 0.468). In addition, the trail pheromone decay characteristics of both species are very similar to those of *Aphaenogaster albisetosus* reported by Hölldobler et al (1978) (see figure 14.2), but statistical comparison between the two data types is not possible because Hölldobler *et al.* used a different bioassay to the ours.

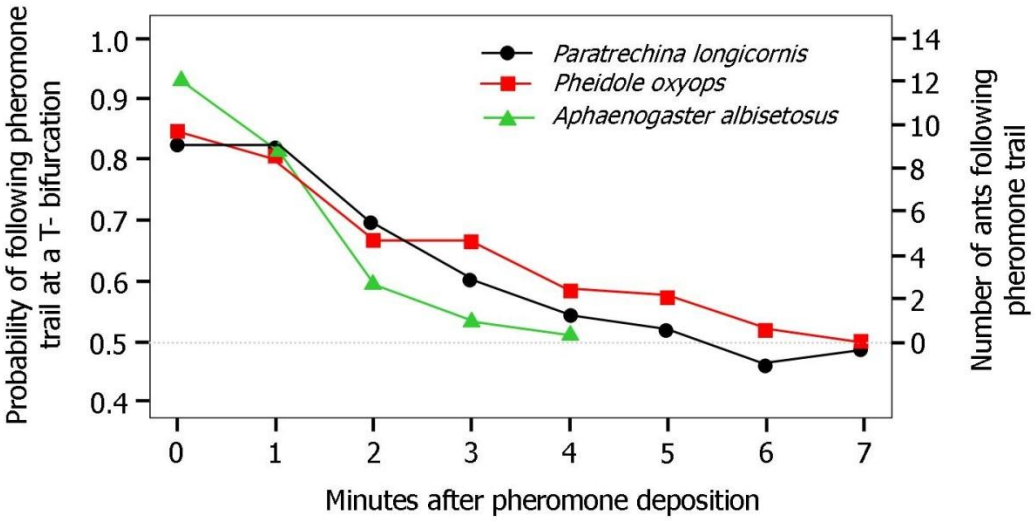

Figure 14.2 - Trail pheromone decay rates in three ant species. In all three a single discoverer ant recruits nestmates to a large food item by laying a pheromone trail to assist in cooperative transport. The data from *Paratrechina longicornis* (black circles) and *Pheidole oxyops* (red squares) refer to the proportions of ants making a correct decision at a T-bifurcation on which a single discoverer ant had deposited a pheromone trail, and were gathered using identical protocols using a T-maze. The data from *Aphaenogaster albisetosus* (green triangles) refer to the number of ants following a pheromone trail out of the nest. The trail was made using extract from a single poison gland and had been aged a varying number of minutes before being presented (right axis). Data on *A. albisetosus* are from Hölldobler, Stanton and Markl (1978). Data on *Ph. oxyops* are from Czaczkes & Ratnieks (2012). The horizontal dashed line marks 0.5 probability (H_0) for trail choice and 0 ants for trail following. That is, random trail choice due to the trail having no behavioural effect.

Experiment 3 – Local recruitment to live and dead food items

As expected, before the termite prey item was presented whether it was live or dead had no significant effect on the number of ants entering the observed area in which it would later be presented (LME, Z = -0.069, P = 0.945). After the prey item was presented, however, many more ants entered the area around a live versus dead item (GLM, Z = 3.077, P = 0.00419, see figure 14.3. Interaction term Z = 2.923, P = 0.0139). Similarly, as expected there was no significant difference in the number of ants entering the area before and after a dead termite was presented (GLM, Z = 1.005, P = 0.315), but significantly more ants entered the area after a live termite versus dead item was presented (Z = 3.696, P = 0.0004, see figure 14.3).

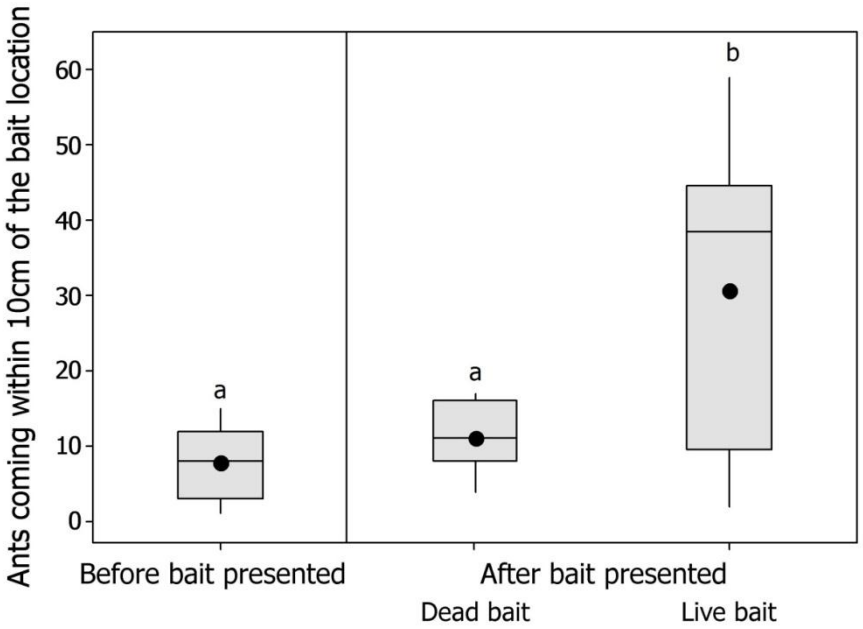

Figure 14.3 - Local recruitment to live and dead baits versus control (before bait presented). A live or dead termite is placed by a colony entrance, and the first ant to find the bait is confined with the bait, preventing it from returning to the nest and recruiting workers by means of its pheromone trail. The number of ants entering a 10cm radius around the location of the bait is counted for two minutes before and after the bait was found by the discovering ant. Dots signify means, horizontal lines signify medians, boxes signify interquartile ranges and whiskers signify the general extent of the data. Groups with the same letter above are not significantly different.

Experiment 4 – escorting behavior during cooperative transport

We found a significant interaction between whether the termite bait was alive or dead and whether the observed area was centred on, in front of, or behind the termite as it was being transported to the nest, on the number of ants in these three specified 3cm radius areas (GLMM, Z = -5.053, P < 0.0001):

As figure 14.4B shows, when a dead termite was presented, slightly but significantly more ants (excluding those grasping the termite) were found around the item than either 7cm in front (GLMM, Z = 6.83, P < 0.0001) or behind (GLMM, Z = 8.986, P < 0.0001) the item. There was no difference between the number of ants found in front the item and the number of ants behind the item (GLMM, Z = -0.445, P = 0.656. see figure 14.4B). However, when a live termite was presented many more ants (excluding those grasping the termite) were found in the area surrounding the termite than in front (GLMM, Z = -16.43, P < 0.0001) or behind (GLMM, Z = -12.49, P < 0.0001) the item (see figure 14.4A). In addition, more ants were found behind the item than in front of it (GLMM, Z = 3.673, P = 0.0002, see figure 14.4A) and more were found than in the equivalent locations with a dead termite.

When comparing the 3cm radius area surrounding the transported item with the same area before the item was presented and after transportation had ended, we found significant interactions between whether the termite was alive or dead and whether the measurement was taken before the item was presented, during transportation or after transportation ended (GLMM, Z = 4.529, P < 0.0001).

As figure 14.4B shows, when a dead termite was presented, more ants were found in the observed area during transportation than either before (GLMM, Z = -5.33, P < 0.0001) or after (GLMM, Z = -6.635, P < 0.0001). There was no difference in the number of ants before and after transportation (GLMM, Z = -0.591, P = 0.555). An identical but stronger pattern was found when live termites were presented: More ants were found in the observed area during transportation than either before (GLMM, Z = -11.94, P < 0.0001) or after (GLMM, Z = -10.18, P < 0.0001)(see figure 14.4A). There was no difference in the number of ants in the area before and after transportation (GLMM, Z = -1.335, P = 0.273, see figure 14.4A).

Lastly, there was no significant difference between treatments in which a live or dead termite was presented either before presentation (GLMM, Z = 0.74. P = 0.46) or after transport had ended (GLMM, Z = 1.468, P = 0.178), whilst there were significantly more ants near a live than dead termite during transport (GLMM, Z = 5.736, P < 0.0001, see figure 14.4A).

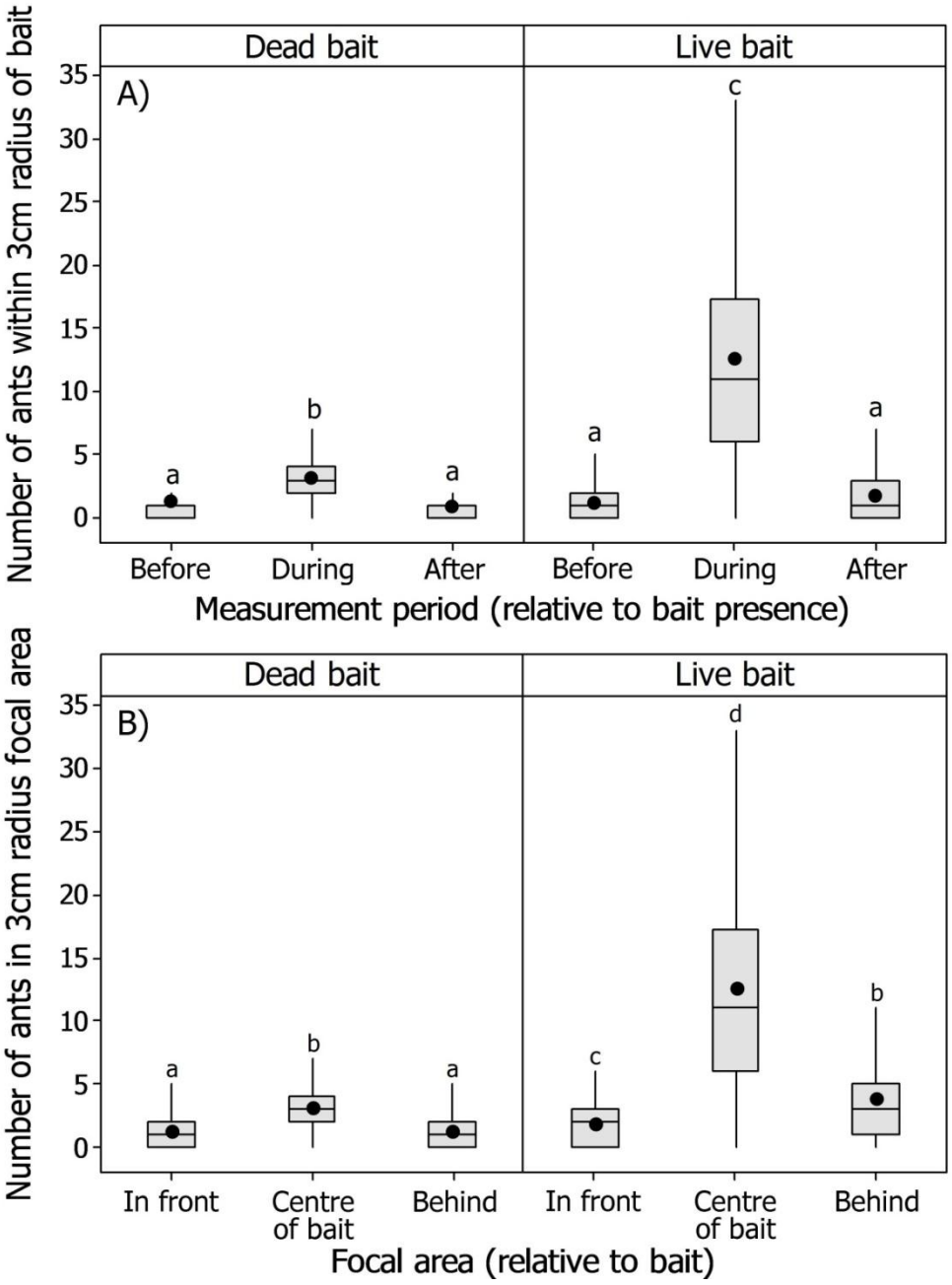

Figure 14.4 - A) Numbers of ants in 3cm radius areas around a transported bait item, and in equal areas 7cm in front of or behind the item. This number does not include ants carrying the bait. The bait item was either a live or a dead termite. B) Number of ants within a 3 cm radius around a transported bait item before, during and after transport of the item in time. This number does not include ants carrying the bait. The bait item was either a live or a dead termite. In both figures dots signify means, horizontal lines signify medians, and boxes signify interquartile ranges. Groups within each figure with the same letter above them are not significantly different.

Discussion

Our data show a striking similarity between the short-term recruitment mechanism of *Paratrechina longicornis* and that of both *Pheidole oxyops* and *Aphaenogaster albisetosus* (figure 14.2). In all three species an ant that discovers a food item that it cannot move always returns to the nest laying a pheromone trail which always results in an immediate surge of recruits that then follow the trail towards the item (Hölldobler et al. 1978; Czaczkes & Ratnieks 2012). Much as in *Ph. oxyops,* trail following in *P. longicornis* is initially very accurate with c. 85% of recruited ants choosing the correct branch at a T-bifurcation. But within c. 5-7 minutes of trail laying, choice between left and right at the T-bifurcation is not different from random indicating that the pheromone laid by the discoverer has dissipated to a level where it no longer has any behavioural effect. Decay of a trail laid by a single discoverer ant to a food item was measured using a different bioassay, ants leaving the nest and following a trail rather than trail choice, in *A. cockerelli* (Hölldobler et al. 1978). In this species the trail also rapidly ceased to have behavioural effects on recruits, with no ants leaving the nest in response to a 6-minute-old trail (Hölldobler et al. 1978).

As these three ant species are not closely related, being members of 3 different genera in 2 sub-families (*Paratrechina* is in the Formicinae, *Aphaenogaster* and *Pheidole* in the Myrmicinae), the results of this study when combined with results of the other two studies suggest a possible convergent evolution of recruitment patterns and trail pheromone properties as a result of a similar organizational and ecological challenge: accurate recruitment to a point source via a trail laid by a single ant, but with no need for the trail to persist for more than a few minutes. Indeed, short persistence and ease of following of a trail laid by a single ant are both presumably due to the use of volatile chemicals, as in the case of alarm pheromones (Hölldobler & Wilson 1990). In contrast ants relying primarily on semi-permanent food sources may have pheromones which even when deposited by a single ant are longer lasting, but more difficult to follow. For example, even with the number of pheromone depositions taken into account, the trail pheromone of *Lasius niger*, an ant that relies heavily on collecting honeydew from aphid patches, were estimated to have a lifetime of 47 minutes (Beckers et al. 1993). However, on an identical maze to that used in experiment 2, a trail laid by a single ant was only followed by 62% of naïve ants (Grüter et al. 2011).

Our results also show clearly that a *P. longicornis* forager emits a recruitment signal, presumably a volatile pheromone and possibly the same pheromone used to lay a short-lived

trail, that recruits nearby nestmates to a food item it had discovered. Similar local or short-range recruitment was described in *Aphaenogaster albisetosus* (Hölldobler et al. 1978) and *Lasius neoniger* (Traniello 1989b). *Ph. oxyops* was shown to also perform local recruitment of nearby ants by means of the pheromone trails laid to the nest by the ant that discovers a large food item also intercepting workers outside the nest who cross the trail, and then directing them towards the food source (Czaczkes & Ratnieks 2012). Indeed, any mass-recruiting species in which trail pheromones alone are sufficient to draw foragers from the environment will thus by definition display local recruitment via interception by pheromone trails. It is possible that all four species use both types of recruitment. Our results can be attributed to recruitment, as *P. longicornis* do not seem to sense prey via volatile odours, relying instead on physical antennal contact (Kenne et al. 2005). This may explain their unusually long and widely-splayed antenna (Kenne et al. 2005).

Our data also clearly show that worker *P. longicornis* provide an escort of non-carrying ants to a food item being cooperatively transported, particularly live prey. As described in experiment 4, this seems to be related to local recruitment behavior, with escorting and local recruitment being elicited more to live prey. Escorting behavior is also absent from cooperatively transported brood items (pers. obs.). This strongly suggests that local recruitment and the presence of the escort around live prey items are adaptations to handing live prey items, which may escape, rather than for defence against predators or competitors. If the latter were true, we would predict escorts to occur for both live and dead prey and also brood. This is in contrast to local recruitment of *A. cockerelli*, which occurs strongly to dead food items (Hölldobler et al. 1978). However, it must be noted that as termites are social insects, where one termite is found more are likely to be found. Thus, the “escorting” behaviour may be a specialised response to preying on termites. As the effect is by far stronger with live termites, this behaviour may even conceivably be released by the termite alarm pheromone. Furthermore, whilst the specificity of this response to live prey suggests a role as an escort for preventing escape, the critical experiments demonstrating this have not been performed. Both these experiments, and experiments demonstrating “escorting” of non-social insect prey, are important future experiments.

P. longicornis has a complex chemical communication system involving at least three orientation pheromones, derived from three separate glandular sources (Witte et al. 2007a). In laboratory experiments using extracts from specific glands, Witte et al (2007a) demonstrated

that hindgut extract elicited strong, long-lasting trail following and weak point-source attraction, poison gland extract showed intermediate trail following, persistence and point-source attraction, and Dufour gland extract (mainly undecane and tridecane (Witte et al. 2007b)) elicited low trail following and persistence but high point source attraction (see table 14.1). Both local recruitment and the escorting behavior are likely mediated by a pheromone emitted from the Dufour gland, which elicits a strong point-source attraction (table 14.1 and Witte et al. 2007a, 2007b). However, it is also possible that these behaviors are elicited by, or modulated by, stridulation, which occurs in *A. cockerelli* and *Atta* leaf cutter ants, although in *A. cockereli* stridulation is not perceived over any appreciable distance (Markl & Holldöbler 1978; Roces & Hölldobler 1996).

	Trail following	**Persistence of trail following**	**Point-source attraction (relative to Dufour gland)**	**Other effects**
Hindgut	90%	Up to 24 hours	61%	
Poison gland	80%	10 – 60 minutes	76%	
Dufour gland	75%	Up to 10 minutes	100%	Increased linear velocity (excitement)

Table 14.1 - the effect of three orientation-eliciting glandular extracts on the behavior of *Paratrechina longicornis*. Adapted from Witte et al (2007a).

The glandular source of the recruitment trail examined in this study is more puzzling. Witte et al (2007a) demonstrate that trail following is elicited most strongly to hindgut extract, followed by poison gland extract, with Dufour gland extract eliciting the weakest trail following response (table 14.1 and Witte et al. 2007a). However, hindgut and poison gland extract demonstrate efficacy for up to 24 hours and 60 minutes respectively, whilst the bioassay performed in experiment 2 (Figure 14.2) demonstrate an efficacy of under 10 minutes, much more in line with the results Witte et al. describe for Dufour gland extract, which are reported not to elicit strong trail following. There are several possible explanations for this inconsistency. Firstly, Witte et al. used very strong artificial trails, probably more than an order or magnitude stronger than those laid by a single discover ant as used in this study. Low levels of poison gland or hindgut pheromone may have a much shorter period of efficacy. Alternatively, different motivational states of the trail-following ants may explain the inconsistency between the two studies. Trail following in Witte et al. was tested only for ants returning to the nest from a carbohydrate food source, whilst in our experiment tested ants recruited towards a large proteinacious food source. Different trail pheromones may be laid depending on the type of food being recruited to (Cammaerts-Tricot 1974; Cammaerts &

Cammaerts 1980), and ants in different motivational states can follow different trail pheromones preferentially (Witte 2001). Thus, either the longer lasting orientation pheromones described by Witte et al (2007a) may have already decayed to such low levels in our experiment that they were not followed, or they were not deposited at all, or the recruited ants were not motivated to follow those pheromones. Future studies can resolve this question by chemical analysis of the trails laid by discoverer ants.

P. longicornis possess a dual recruitment system. Firstly, workers demonstrate similar specialised adaptations to foraging for large food items to *Ph. oxyops* and *A. albisetosus*. Indeed, *P. longicornis* demonstrates an escort behavior for live prey items which has not been previously described. *P. longicornis* also displays recruitment consisting of long-lasting trail pheromones specialised for exploiting semi-permanent carbohydrate sources (Witte et al. 2007a). Unlike *Ph. oxyops*, which appears to rely almost entirely on retrieving large food items, *P. longicornis* is very effective at exploiting liquid long-lasting carbohydrate sources, with almost 40% of worker returning to the nest with a filled crop. *P. longicornis* is thus also likely to possess a well developed route memory, as demonstrated by other hemiptera-tending ants (Salo & Rosengren 2001; Grüter et al. 2011).

Our results show that *P. longicornis* has evolved a specialised system of recruiting nestmates to large food items, which are cooperatively transported and form the major part of the non-liquid food brought back to the nest. This recruitment system works alongside long-lasting mass recruitment to long-lasting food sources. But to what extent this flexible recruitment system contributes to the crazy ants' success as an invader, and how important it is relative to other common characteristics of invasive ants, such as unicoloniality, polygyny, and flexible nesting habits (Holway et al. 2002) are still open questions.

Acknowledgments

We would like to thank Prof. Fabio Nacimento for inviting us to work with him in Brazil, Dr Benjamin Czaczkes for help with data management, Prof. Marlene Sofia Arcifa for the use of her microbalance, and Prof. Volker Witte for comments on a previous version of the manuscript. T.C was funded by a BBSRC studentship. F.R was supported by a travel grant from the São Paulo Research Foundation (FAPESP).

Chapter 15: Cooperative transport in ants and elsewhere

Tomer J. Czaczkes & Francis L.W. Ratnieks

Abstract

Cooperative transport, defined as multiple individuals simultaneously moving an object, has arisen many times in ants, but is otherwise extremely rare in animals. Here we review the surprisingly sparse literature available on cooperative transport. Cooperative transport abilities in ants are a continuum, but three general syndromes are described: uncoordinated transport, in which transport is slow, poorly coordinated and characterised by frequent and long deadlocks; encircling coordinated transport, in which transport is fast, well coordinated, and with few deadlocks; and forward-facing coordinated transport, carried out exclusively by army ants, in which one worker, usually of larger size, straddles an item at the front while one or more smaller workers help to lift at the back. In the two coordinated syndromes, the groups of ants involved constitute teams, and specialised recruitment to large items and adjustment of carrier number to match item size may occur. Some features of cooperative transport are specific adaptations, whilst others are already present in the behaviour of ants carrying items alone. One major benefit of cooperative transport appears to be that it allows a colony to utilize large food items in an environment with aggressive or dominant competitors by quickly removing the item to the nest rather than having to cut it up or consume it on the spot. In addition, compared to individual transport, cooperative transport may have other benefits such as increased transport speed or efficiency.

The study of cooperative transport also includes computer simulations and robots. These provide biologists with new perspectives and also formalise questions for further study. Likewise, lessons learned from cooperative transport in ants can inform computer scientists and roboticists.

Over the last hundred years the range of abilities considered to be uniquely human has diminished. For example, a sense of fairness or an aversion to inequality has been demonstrated in both monkeys (Brosnan & de Waal 2003) and dogs (Range et al. 2009). Tool use, another attribute once considered uniquely human, is now known in many taxa (e.g. chimpanzees (Goodall 1964), crows (Hunt 1996), fish (Paśko 2010), octopuses (Finn et al. 2009), and even insects such as ants (Banschbach et al. 2006) and solitary wasps (Brockmann 1985). However, one behaviour that is almost exclusively confined to humans is cooperative transport. Unlike tool use, our closest relatives the great apes rarely seem to do this. Apart from humans, the only animals that regularly perform large scale cooperative transport are ants. Cooperative transport can be defined as two or more individuals simultaneously moving an item from one location to another. Although cooperative transport is a widely known behaviour of ants, and often features in cartoons and the popular image of ants, it is surprisingly understudied and what information exists has never been comprehensively reviewed in the published literature, although Moffett (1987) surveys cooperative transport in his PhD thesis and later in Moffett (2010). Here we address this deficiency by collating what is known about cooperative transport in ants. In doing this we also discuss whether specific adaptations are used or required for cooperative transport, the ecology of cooperative transport, and also introduce a simple terminology for the different syndromes of cooperative transport observed. Lastly, we examine cooperative transport outside humans and ants including research on cooperative transport in other animals, robots and via computer simulations.

Syndromes of cooperative transport in ants

Cooperative transport, also referred to as group retrieval, group transport or cooperative carrying, is common but far from universal in ants. It is known in at least 40 genera in different subfamilies of the Fomicidae (Hölldobler & Wilson 1990; Moffett 1992, 2010). Although no formal comparative analysis has been carried out, this strongly suggests that cooperative transport has evolved multiple times in ants. There is also much variation in apparent sophistication and effectiveness. The cooperative transport abilities of particular ant species lie on a continuum from never occurring to highly specialised, efficient and rapid. For convenience, we categorize cooperative transport in ants into three general syndromes: uncoordinated transport, encircling coordinated transport, and forward-facing coordinated transport. For a summary, see table 15.1.

Example species	General description	Example image
Uncoordinated Cooperative Transport		
Myrmica rubra, Formica lugubri, F. rufa, Daceton armigerum, Ectatomma ruidum, Anoplolepis longipes. See (Chauvin 1950, Sudd 1965, Moffett 1992)	Slow transport with frequent, long lasting deadlocks. All ants attempt to drag the item towards the destination – ants at the back do not attempt to lift and walk forward.	 Uncoordinated transport in a *Formica* species. A deadlock has occurred during transport, with the ants on either side of the beetle pulling in opposite directions.
Encircling Coordinated Transport		
Pheidologeton diversus, *Carebara pygmaeus, Pheidole oxyops*, *P. pallidula, Aphaenogaster cockerelli,Paratrechina longicornis, Lasius neoniger* See (Moffett 1988, Robson & Traniello 1998, Czaczkes et al. 2010)	Rapid transport with deadlocks mostly absent. Ants at the leading edge drag, ants at the back lift, push or carry. Large items retrieved cooperatively often make up a sizeable proportion of total retrieved biomass. Recruitment for cooperative transport is rapid. The need for recruitment is assessed by tractive resistance of the item.	Top—*Pheidole oxyops* cooperatively transporting a stingless bee (*Melipona scutellaris)*. Bottom—The Longhorn Crazy Ant *Paratrechina longicornis* cooperatively transporting royal brood (right).  
Forward-Facing Coordinated Transport		
Eciton burchelli, E. hamatum, Dorylus wilverthi, Leptogyns borneensis See (Franks 1986, Franks, Sendova-Franks, & Anderson 2001)	Rapid transport with no deadlocks. All carrying ants face in the direction of carriage. A leading ant, usually large, straddles the item and lifts. Other ants, usually smaller, join behind the leading ant, also straddle the item, help lift and reduce rotation (see figure 15.1). Workers join until the item is moving at standard column walking speed.	 Eciton army ants cooperatively transporting a centipede segment. Note the larger submajor with very long legs at the front. *Image reproduced with permission of Ammonite (www.ammonite.co.uk)*

Table 15.1 - The three syndromes of cooperative transport

In uncoordinated transport, item movement is characterised by frequent deadlocks in which ants pull in opposite directions resulting in no forward motion (Sudd 1965; Moffett 1986, 1992; Pratt 1989). These deadlocks are resolved by random changes in the composition, orientation or behaviour of the group members, which indicates lack of coordination (Sudd 1965). Sudd (1965), in an extensive study of cooperative prey transport by *Myrmica rubra* and *Formica lugubris*, both of which perform uncoordinated transport, found three discrete stages to transport. Transport begins when the first ants find the food item (stage one), but then stops as more ants find the item and deadlock occurs (stage two). Deadlock can last up to ten minutes, until random changes cause the deadlock to end. The third stage is characterised by higher speed and path straightness than the first and second stages, implying better organisation of the carriers who have by chance brought themselves into an effective alignment. However, no evidence was found of specific cooperative behaviour: ants did not synchronise their pulling efforts and often pulled in opposite directions. A burst of motion occurred when ants by chance attempted to pull the item in the same direction. Whilst the ants "agreed" about the general direction the item is to be moved in, they "disagreed" on how to achieve this. Nonetheless, ants did not assemble randomly around the food item, but over time came to be more evenly spaced around the item. Eventually, the groups of ants could also exert larger forces than individual ants could alone. Thus, whilst uncoordinated, this syndrome of cooperative transport can be useful for dislodging snagged items or for slowly retrieving items too large for a single ant.

In the second syndrome, encircling coordinated transport, ants are recruited to a food item, encircle it, and quickly transport the item back to the nest once a sufficient number of ants have assembled to move the item (e.g *Pheidologeton diversus ,* M. Moffett, pers. com., *Leptogenys diminuta* (Maschwitz & Steghaus-Kovac 1991), *Pheidole oxyops* (Czaczkes et al. 2011b), *Pheidole pallidula* (Toffin 2003)*, Aphaenogaster cockerelli* (Hölldobler et al. 1978; Berman et al. 2011)*, Paratrechina longicornis* (T. Czaczkes, pers. obs.). Deadlocks are not a conspicuous feature, except briefly if the item becomes snagged along the route. During encircling cooperative transport, ants at the front of the item lift and pull or drag the item whilst walking backwards, ants at the back of the item lift the item and walk forwards, and ants at the sides lift or drag and walk sideways. These are distinct subtasks (*sensu* Anderson & Franks 2001) which must be carried out concurrently, and thus the ants engaged in encircling cooperative transport constitute a team (Anderson & Franks 2001). See table 15.2 for a further discussion of the various definitions of teams.

Individual scouts assess the need for cooperative transport by first trying to move an item, and if the item cannot be moved recruitment is initiated by the scout (Hölldobler et al. 1978; Traniello 1983; Detrain & Deneubourg 1997; Daly-Schweitzer et al. 2007). The number of ants transporting the item is often adjusted to the size of the item (Traniello 1983; Traniello & Beshers 1991; Robson & Traniello 1998) although this is not mediated by the discoverer's recruitment behaviour. Numbers of transporting ants can be reduced by ants leaving the item, and can be increased if the item is not being moved or is not moved rapidly as this results in transporting ants leaving the item and initiating further recruitment (Robson & Traniello 1998). The availability of space around the perimeter of the item also limits the number of transporting ants (Moffett 1988). Having more carriers around an item results in higher transport speeds, up to a point (Moffett 1988; Cerdá et al. 2009; Czaczkes et al. 2011b).

Food items are the primary targets of cooperative transport. In species which perform coordinated transport, large proportions of a colony's food by mass can be retrieved via cooperative transport (e.g 72% in *Aphaenogaster senilis* (Cerdá et al. 1998b), 85% in *Lasius neoniger* (Traniello 1983), 78% in *P. oxyops* (Czaczkes et al. 2011b), 88% in *Pa. longicornis* (chapter 14)). Other cooperatively transported items include stones removed during nest excavation and large waste items such as beetle carapaces (T. Czaczkes, pers. obs. in *P. oxyops*), large soil particles to be used in nest construction (Moffett 1987) and enslaved *Myrmecocystus* honey pot ant repletes (Hölldobler 1981b). Large brood items are also moved cooperatively, and Moffett (1992) suggests that this may be the original purpose for which cooperative transport evolved, given that even ant species that show no cooperative transport of food have brood items much larger than workers, such as the pupae of queen ants, that they need to be able to move rapidly during colony emergencies (see figure 15.1).

In the third syndrome, forward-facing cooperative transport, one ant lifts and carries the item from the front whilst facing forwards, and one or more other ants join along the item in a line also facing forward (Franks 1986; Franks et al. 2001)(see figure 15.2). Forward-facing cooperative transport has been described only in army ants, but in three genera on three continents: the Neotropical *Eciton* army ants (Franks 1986), the African driver ant species *Dorylus wilverthi* (Franks et al. 1999), and the Asian *Leptogenys borneensis* (C. von Beern, unpublished data, video evidence available on request). Except in *L. borneensis*, which is monomorphic, carrying groups are frequently composed of a larger ant straddling and lifting the item from the front and one or more smaller ants, which also straddle and lift the item, from the middle or rear. Transport begins with the single front carrier. Other ants then join the group, assisting by lifting and carrying from behind, which reduces rotational forces and drag

(Franks 1986). Additional ants may join the back of the item, increasing transport speed, until the transport speed approaches the normal marching speed of the column (Franks 1986) (see figure 15.2). Thus, matching of ant number to prey size also occurs in forward-facing coordinated transport (Franks 1986, Franks et al. 2001). In *Eciton* the front ants are often sub-majors, which have longer legs than medias but shorter mandibles than the majors, and are a specialised carrier or porter caste (Franks 1986). The groups of forward-facing carriers are also often described as a team (Franks 1986, Franks et al. 1999).

Name / proponent	Description	Teams require	Examples from sports
Different castes working concurrently (Hölldobler & Wilson 1990)	"teams … can be defined as members of different castes that come together for highly coordinated activity in the performance of a particular task"	• Multiple individuals working towards the same goal • concurrently performed subtasks • members in non-interchangeable roles	American Football, Rugby. Different non-interchangeable 'castes' (fast runners e.g wingers in rugby, large tacklers, e.g props in rugby) work concurrently, performing different subtasks (wingers receive passes and score tries, props tackle opposing teammates)
Different sub-tasks being performed concurrently (Anderson & Franks 1999)	"A team task requires different subtasks to be performed concurrently for successful completion." A team is a group of individuals performing a team task. Individuals not only have to work concurrently, they must also coordinate their different contributions.	• Multiple individuals working towards the same goal • concurrently performed subtasks	Basketball, polo Different subtasks (e.g.shoot guard or centre in Basketball) performed concurrently, but no extreme physical differentiation of players.
Daily parlance (Moffett 2010)	Any group of individuals that work towards a single goal. Synonymous with 'cooperating group'.	• Multiple individuals working towards the same goal	Tug-of-war, Bowling, relay running In a tug-of-war players perform identical tasks concurrently. In bowling and relay running players perform identical roles, and do so singly.

Table 15.2 – Definitions of a 'team' range from the highly restrictive necessity for different castes, to the highly inclusive definition used in daily parlance. The highly restrictive definition includes very few natural examples, limited only to new and old-world army ants. The highly inclusive definition encompasses all cooperating groups, and so is perhaps too uninformative for scientific discourse.

Figure 15.1 - *Carebara simalurensis* cooperatively transporting a large brood item. Notice how the ants lift the item using the underside of their heads and front legs. A similar behaviour is displayed by *P. diversus.* In contrast, ants carrying items individually grasp with their mandibles, as shown by the ant on the right which is transporting a small brood item. *Image copyright Mark W. Moffet / Minden Pictures.*

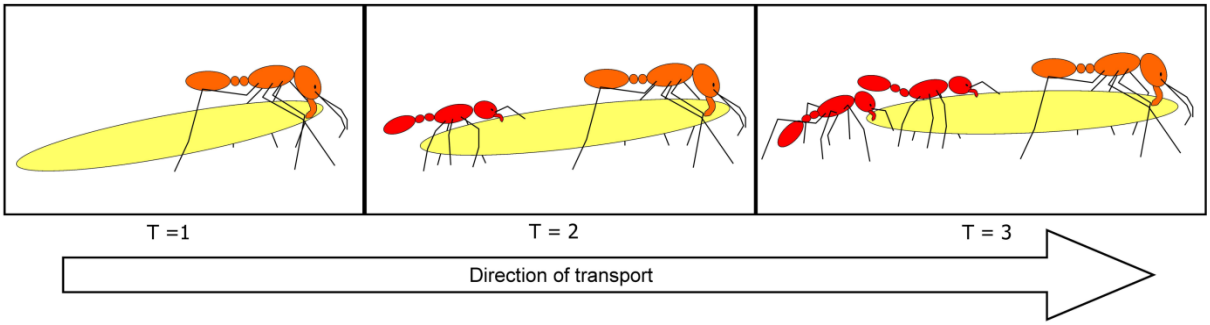

Figure 15.2 - Forward-facing cooperative transport. At T = 1 a larger worker begins to lift and drag the item forward, but due to the weight of the item, and drag and rotational forces, transport is slow. At T = 2 a smaller worker, sensing a slow-moving item, joins behind the larger worker and assists in lifting, thereby reducing rotational forces and drag, and allowing the item to move faster. If the item is still moving below a threshold transport speed another smaller worker might join in T = 3 and assist in transport.

Adaptations and preadaptations to cooperative transport

Adaptations for cooperative transport

Early writers (Grasse 1934; Rabaud 1937; Chauvin 1950) concluded that during cooperative transport the transporting ants behave identically to individual ants, taking no notice of the actions of the other ants. Indeed, Sudd (1960a) concluded after his study of cooperative transport in *Pheidole crassinoda* that "the behaviour of individuals in a transporting group appears to contain no element of behaviour that were not shown by single transporting ants" (Sudd 1965). However, more recent results demonstrate that this is not the case. In *Eciton burchelli* not only is there a specialist porter caste for carrying large loads (Powell & Franks 2005, 2006) but workers also possess behavioural rules that refine cooperative transport. For example, ants joining a team in which their strength is greater than that needed to move the item efficiently soon disengage from the item and leave the group (Franks et al. 2001). *Pheidologeton diversus* workers transport items individually by grasping with their mandibles, but during cooperative transport groups of ants lift the item by pushing against it with their front legs and head (Moffett 1988)(see figure 15.1). In *Formica schaufussi* the scout ant which discovers a large food item and recruits nestmates maintains the cohesion of the recruited ants – if the scout ant is removed whilst leading the recruits to the food item, the group disbands and foraging is abandoned (Robson & Traniello 2002).

Adaptations for cooperative transport outside carriage – the example of recruitment

Behaviours additional to the actual moving of the item may also be under selection as part of cooperative transport. One example of this is the recruitment of nestmates to an item. Recruitment specialised for cooperative transport is a good example of such an adaptation, and can be contrasted with well studied recruitment to aphid patches, and their laboratory equivalent: the sucrose syrup feeder. Many ants utilize semi-permanent replenishing food sources at specific locations, such as aphid patches. Naïve ants can be recruited to such food sources by pheromone trails, but as nestmate ants make repeated visits to the food source and the food source is long-lived, accurate trail pheromone following may not be essential. In addition, experienced ants can use route memories to relocate the feeding site (Harrison et al. 1989; Grüter et al. 2011). Thus, in *Lasius niger,* a species that recruits mostly to aphid patches and does not perform cooperative transport, pheromone trails last for up to 20 hours (Evison et al. 2008), but are followed with relatively low accuracy (62-70% accuracy at a T-bifurcation (Grüter et al. 2011). By contrast, in cooperative transport recruitment is to a single point,

which places a premium on accurate trail following. In *P. oxyops*, which relies heavily on cooperative transport, 85% of recruits chose the correct branch at T-bifurcation on a fresh trail (Czaczkes & Ratnieks 2012) laid by a single ant that discovered the food item, could not move it, and so laid a trail back to the nest. The need for accurate trail following, combined with the fact that a long-lived trail is not needed, has resulted in the convergent evolution of trail pheromones that evaporate rapidly, with complete decay of the item-discoverer's trail occurring in just 5-7 minutes (*Aphaenogaster albisetosus,* (Hölldobler et al. 1978) *, P. oxyops* (Czaczkes & Ratnieks 2012), *Pa. longicornis* (chaper 14)). In contrast, the trail of mass recruiting non-cooperatively transporting ants may last much longer: up to 24 hours in *L. niger* (Evison et al. 2008), up to 48 hours in *Monomorium pharaonis* (Jackson et al. 2006)). A short-lived trail pheromone may indeed be adaptive for cooperative transporters, as once items have been removed they do not replenish, and so continued recruitment to a location would serve no purpose and could even increase the exposure of workers to risks outside the nest. A short-lived trail and high accuracy may also be an adaptation to cooperative hunting of large mobile prey (Maschwitz & Steghaus-Kovac 1991; Witte et al. 2010).

Ants which rely on cooperative transport must recruit sufficient workers to move a food item before other colonies of their own or other species, or indeed non-ant competitors, find the item (see next section). Thus, some ant species that use cooperative transport can decrease the time needed to recruit a transport team using local recruitment, either by emitting an air-born attractant pheromone (Hölldobler et al. 1978; Traniello 1983, chapter 14) or by workers intercepting a pheromone trail to the nest and following it towards the food item (Czaczkes & Ratnieks 2012).

Distribution of ants around a transported item

The distribution of ants around a transported item is also far from random. Some species tend to carry items by the corners, which increases speed of transport (Czaczkes et al. 2010), and by the front and back, avoiding the side (Sudd 1965, Czaczkes et al. 2010). These non-random arrangements are driven by ants preferentially leaving unappealing grasping points (in this case, side sections of an item), and preferentially joining onto more appealing grasping points (in this case, corner sections) (Czaczkes et al. 2010). More ant-power is usually deployed at the front, and less at the back, as demonstrated during team transport by army ants (Franks et al. 1999). Where large-bodied worker castes are not available, multiple monomorphic ants can arrange themselves to produce this pattern by having more carriers at

the front than the back (Czaczkes et al. 2010). The use of minors collectively as a 'plastic supercaste' (Franks 1986) allows greater flexibility when foraging on unpredictable food sources (Traniello 1989) given that worker demography cannot change rapidly according to short-term needs and that maintaining a standing supply of specialist castes is expensive (Oster & Wilson 1978; Bourke & Franks 1995).

Adaptive behaviours that are not adaptations

Behaviours that make cooperative transport more efficient need not necessarily be adaptations for cooperative transport. As mentioned above, during cooperative transport (except team transport and the derived transport of *Pheidologeton* and *Carebara*) ants at the front walk backwards dragging the item, while ants at the back of the item walk forwards whilst lifting and carrying (table 15.1) (Moffett 1992, Czaczkes et al. 2010). This might at first be considered an adaptation to cooperative transport, but may in fact simply be a behaviour carried over from individual transport. When ants transport an item individually they lift and carry light items facing forward, but drag heavy items whilst facing backwards (Sudd 1960). The same rules may be used during cooperative transport: if the item is not in motion, or moving slowly, the item is grasped and dragged. If the item is moving rapidly, and thus is "easy" for a joining ant to move, the item is grasped and lifted (figure 15.3). As ants first join the front of the item, and only then begin to join the back (Czaczkes et al. 2010), these rules would result in efficient cooperation without any new adaptations to the cooperative situation. Likewise, groups of ants are capable of rotating an item so that it assumes a low drag orientation (Czaczkes & Ratnieks 2011). This behaviour, whilst beneficial in that it reduces drag forces and so reduces energy expenditure, is probably not a specific adaptation to cooperative transport as it can arise from the same rules utilised by an individual forager (see figure 15.4): on encountering a large item, ants attempt to drag it to the nest. This will cause the item to pivot around the point of highest drag, resulting in a reorientation. Some behaviours used by individual foragers are also useful during cooperative transport, and could be considered preadaptations which, while facilitating the emergence of cooperative transport, were not specifically evolved in a cooperative transport context.

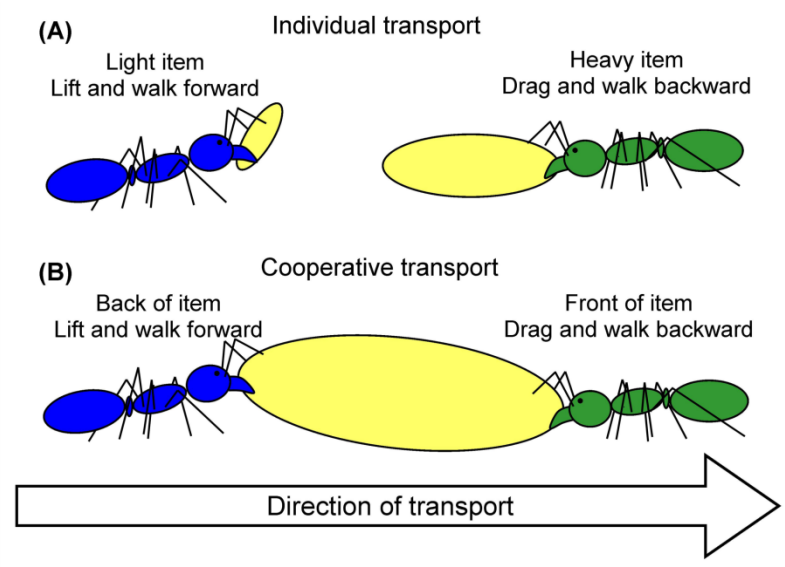

Figure 15.3 - One potential adaptation distinguishing coordinated from uncoordinated cooperative transport. During individual transport ants carrying light loads lift the item and walk forward. When transporting heavy loads individual ants walk backwards and drag. By relaxing the transition from lifting and walking forward to dragging backward when multiple ants are transporting an item, ants would be able to begin assisting in cooperative transport even when the item is being moved slowly enough to trigger a switch to walking backwards and dragging if the item was being individually transported.

What adaptations do coordinated transporters show during cooperative transport?

Perplexingly, distinguishing behavioural adaptations which allow coordinated cooperative transporters to be especially effective during transport has proven difficult. One possibility is that the willingness of ants to grasp items by the sides and walk sideways, a behaviour never observed for long during individual transport, is such an adaptation. Another possible adaptation is a relaxation of the switching between walking forward and lifting when carried items are light and dragging and walking backwards when items are heavy (figure 15.3). During coordinated cooperative transport, ants joining the back of an item assist by lifting and walking forward, even if the item is moving slower than their normal walking speed. During individual transport, when items are being moved too slowly, the ant switches from lifting and walking forward to dragging backwards (Sudd 1960a). How individuals sense that an item is being cooperatively transported, so that this switch should not be made, is unknown. Lastly, the specialised carrying posture used by *Pheidologeton* and *Carebara* during cooperative transport (Moffett 1988 – see figure 15.1) is a clear adaptation to cooperative transport.

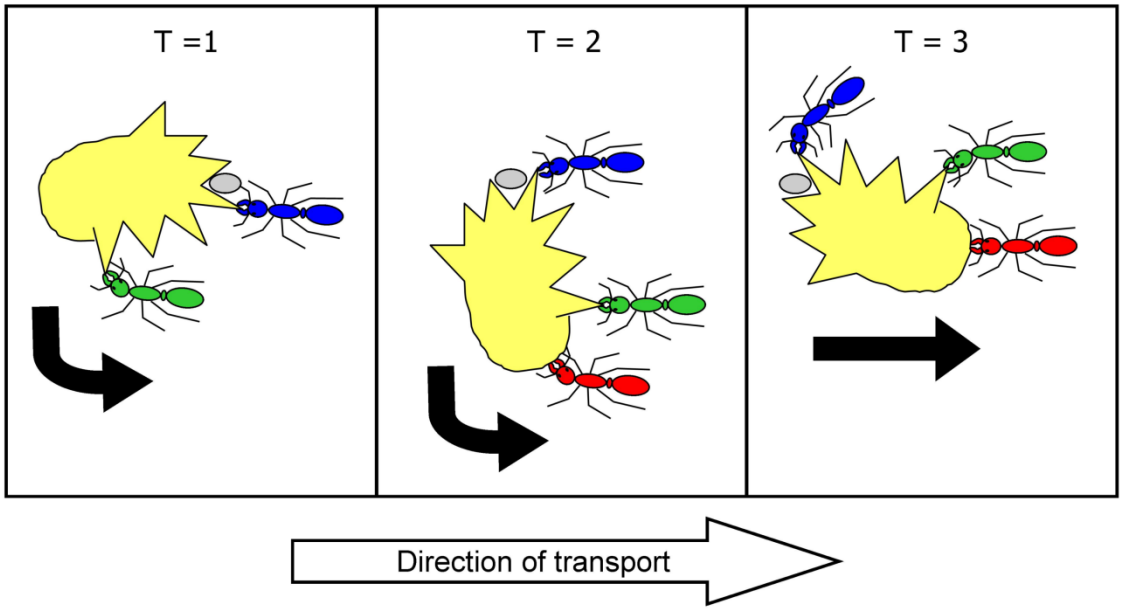

Figure 15.4 - The turning of a food item reduces drag and facilitates cooperative transport – an adaptive behaviour that is not an adaptation. Ants assemble around an item and attempt to move it in the direction of transport (T = 1). However, the item is snagged (small grey oval). The blue ant attempts to pull the item, to no avail. The green ant, by pulling the item, causes it to pivot around the point where it is caught. In T = 2 the item is still somewhat snagged, and the pulling of the blue and green ants cannot dislodge the item. However, the pulling of the red ant causes the item to pivot again. In T = 3 the item is in an orientation that reduces drag, and transport proceeds. Image based on Czaczkes & Ratnieks (2011).

The ecology of cooperative transport – why do it?

Perhaps the most obvious benefit of cooperative transport is to retrieve items larger than cannot be retrieved by an individual worker. By transporting food items cooperatively, ants become in effect a larger organism (Carroll & Janzen 1973; Cerdá et al. 1998b; Hölldobler & Wilson 2009). However, many ant species that do not perform cooperative transport also forage on large food items. Why then is cooperative transport needed, and why do only some ant species use cooperative transport?

Ants that forage on large food items without cooperative transport either recruit *en masse* to the item and feed *in situ*, even in rare cases bring brood to the item to feed (Masuko 1990), or dissect the item and carry parts back individually (Djiéto-Lordon et al. 2001; Richard et al. 2001; Yamamoto et al. 2009). Large food items will eventually have to be dissected in order to be consumed, and so we must ask why it is preferential to dissect items in the nest rather than *in situ*. Once sufficient individuals reach the prey item it is effectively dominated, and unavailable to competitors (Hölldobler et al. 1978; Adams & Traniello 1981; Traniello 1983). However, dissection can take several hours (Yamamoto et al. 2009), leading to the risk

that the item will be discovered and dominated by superior competitors. Cooperative transport is important for ants which cannot win direct competition, as it assists them in scramble competitions by allowing rapid retrieval of the item before larger ants can remove the food item, or ants with large colonies can recruit *en masse* to the item (Hölldobler et al. 1978, Traniello 1983, Traniello 1987, Traniello & Beshers 1991). Cooperative transporters mostly retrieve medium or large items, but very large items are not transported as these cannot be moved swiftly enough and so would often be lost to competitors (Cerdá et al. 1998). However, these arguments do not fit the army ant situation as well, given that army ants perform cooperative transport even though they face little competition and also dissect prey items *in situ*. We suggest that in army ants cooperative transport is not specifically for removing items more rapidly to avoid competition, but to allow carriers to keep up with the dense flow of traffic and so reduce the possibility of traffic jams. As army ants often raid over long distances with extended trails (Schneirla 1933), reducing time and energetic costs may be significant (see below). Whilst useful for avoiding competition and increasing retrieval speed in ground foraging species, cooperative transport is very rare in arboreal species. Cooperative transport on branches is risky, as cooperatively transporting groups are more likely to fall off branches than individuals (Yamamoto et al. 2009).

Cooperative transport may also provide energetic benefits. In some, but not all, cooperatively transporting species cooperative transport has been found to be super-efficient (Hölldobler et al. 1978, Franks 1986, Moffett 1988, Franks et al. 1999). That is, the loads transported by a group could not be transported as rapidly (or in some cases at all) by the same ants individually, no matter how the item was fragmented. For example, two cooperating workers might be able to carry three times the load weight that a single worker could. This increase in efficiency might result from a reduction in drag as the item is lifted, or from a reduction in rotational forces, or both. As a similar number of ants are required to dissect the item *in situ* or in the nest, transporting the item to the nest may result in less ants having to be recruited and having to travel to the item.

Transporting food items back to the nest may act as a form of task partitioning, making use of idle nest-based workers by allocating the task of dissection to nest workers whilst foragers can return to foraging or other tasks (Moffett 1987). Even more effective task partitioning resulting from retrieval of large food items is the ‘dissection’ and consumption of food items by brood, which cannot do any other task. Similarly, the time-consuming task of dissection can be delayed by bringing food to the safety of the nest, were dissection can take place once foraging is over.

Lastly, cooperative transport may be used when fragmentation is not an option. Ants may use cooperative transport to remove tough waste material, such as stones or beetle carapaces, from the nest (T. Czaczkes pers. obs.). Moffett (1992) has also suggested that cooperative transport may be widespread when transporting royal brood, which can be large and is obviously non-divisible. He gives the example of *Carebara simalurensis* (figure 15.2), which transports brood using a highly derived coordinated transport (i.e. using the specialised behaviour of carrying by lifting with the head and forelegs), but does not cooperatively transport food items (Moffett 1992).

Cooperative transport elsewhere in nature

Cooperative transport is frequent in both ants and humans. Although it is not their exclusive domain, there are very few reports or anecdotal accounts of cooperative transport in other animals. Social spiders (*Anelosimus eximius*) have been reported to cooperatively move prey items from the outskirts of their communal web towards the centre (Vakanas & Krafft 2004). The spiders weave and then tension a strand of "traction silk" between the web and the prey. The prey is then cut free of the web, causing it to move in the direction of the traction silk. On one occasion, one spider cut the web, one pulled the prey, a third pushed, and a fourth lifted the prey item to prevent it getting stuck on the web. This seemingly advanced team transport is, however, extremely slow (about 1cm per minute) and occurs over very short distances (about 10cm in total) within what is effectively the nest. There seem to be no specific recruitment behaviour signals. Spiders are attracted via vibrations in the web caused by the prey and perhaps by other spiders.

In some dung beetles, such as *Canthon cyanellus* , males and females cooperate in rolling a dung ball (Fabre 1911; Halffter 1997). Females may be attracted by a solitary male rolling a dung-ball, although males will also recruit females using long-range pheromone signals even if rolling has already been completed before a female is attracted. The organisation of the pair is non-random, with the male occupying the energetically more demanding pushing role on 85% of occasions (Favila 1988). However, it is important to note that the male can roll the ball on his own. Whilst having the female may save the male some energy, cooperative transport has probably arisen more as an adjunct to pair formation than for ergonomic benefits and is not a necessity. Similarly, burying beetles (*Necrophorus spp.*) transport carcasses from patches of hard ground to patches of soft ground for burying. This can be performed individually, with the beetle crawling underneath the carcass and, whilst

lying with its back to the ground, levering the carcass forward with its legs. If a mate arrives during transport the pair can cooperate in the transport of the carcass, but again cooperative transport has probably arisen as an adjunct to pair formation than due to the necessity for increase ergonomic benefit (Milne & Milne 1976).

Moffett (1987, 2010) provides second-hand reports of rodent litter mates cooperatively conveying food and of various canid and felid species jointly moving food to shady spots. However, none of these behaviours seem to be common, transport is reported as inefficient and uncoordinated, and we have found no reports published in peer-reviewed literature.

Whilst cooperative transport by individual animals is rare in nature, cooperative transport is in fact extremely common in eukaryotes, but on a microscopic scale. Intra-cellular transport of vesicles is often performed by multiple molecular motors pulling a single vesicle along microtubules (Gross et al. 2002). Much as in ants and humans, multiple motors can achieve greater power than individuals, allowing the transport of heavier loads and more rapid load transport (Klumpp & Lipowsky 2005; Lipowsky et al. 2010). Cooperative transport by multiple molecular motors also allows longer range transport: molecular motors will unbind from microtubules sporadically due to thermal noise, so larger groups of transporting motors greatly reduce the probability that all motors become disengaged at once (i.e., cause increased reliability), causing transport to stop (Lipowsky et al. 2010).

Why is cooperative transport so rare outside ants and humans? Clearly, many animals are excluded from this behaviour as they are not social. Even amongst cooperating groups there is often much conflict amongst group members (Smith & Szathmáry 1995), arising from a conflict between maximising individual fitness and collective benefits. In eusocial insects, whilst there may be conflict over reproduction (Visscher 1996; Ratnieks et al. 2006; van Zweden et al. 2007), there is seldom conflict over where resources should be brought to, as all eusocial insects are central place foragers and almost all food items must be brought back to the nest. Selection for cooperation has led to many social insects developing complex and sophisticated communication mechanisms in order to increase colony foraging efficiency (von Frisch 1967; Wilson 1972; Seeley 1995). Why then do we not see cooperative transport in the other eusocial insects? Bees collect mainly liquids and powders (nectar and pollen) for which there is no need for cooperative transport. Likewise termites either live inside their food source, or cut organic matter into pieces of suitable size for individual transport. Wasps do forage on individual prey items, for which competition may be high. However wasps fly, and the coordination of cooperative transport by two flying carriers might be particularly difficult, especially as an error could result in the item being dropped during flight and likely lost.

Indeed, whilst humans use multiple boats or engines to transport items over land or water, the first successful trial of flying cooperative transport was only achieved very recently (Mellinger et al. 2010), and we are not aware of any large-scale or commercial applications. The constraint against cooperative transport in flight also seems to apply to us. Ants appear to be predominant in the animals in their use of cooperative transport as they have a suite of attributes which make cooperative transport both possible and useful, namely central place foraging among cooperating individuals from the same nest, foraging on foot, and utilizing large food items in habitats with competitors including other ants. The species which do evolve coordinated cooperative transport are those that need to secure items before more dominant species find the item, or species, usually army ants, which benefit from greater transport efficiency when moving items along long trails.

Cooperative transport in robots and simulations

Roboticists have been attempting to achieve cooperative transport by robots for over 20 years (Eustace et al. 1993; Bay 1995). Cooperative transport in ants is attractive to roboticits not only because they are the only non-human that effectively transport large loads, but also due to the nature of social insect organisation. The rules used by individual workers can be simple, and so robots based on ants need not be over-complicated. Ants also work in flexible groups, and group performance is generally robust and not greatly affected by changes in the number of individuals or whether all are functioning. In addition, and of great importance, ant groups are self organized and do not require remote control or overseeing (Kube & Bonabeau 2000, Berman et al. 2011). Cooperative transport in ants is also scalable in the number of transporters, is effective at transporting a large range of items, and does not require previous knowledge about the payload to be transported. However, apart from these very general properties, implementations of ant-inspired designs do not generally take inspiration from the specific behaviours of ants (Ratnieks 2008). In an exceptional case, Berman et al. (2011) studied the behaviour of *A. cockerelli* in the lab, and modelled transport in a simulation using qualitative data from their biological studies. They observed, as in previous studies on ants (Sudd 1960a, 1965), that ants respond to difficulties during carriage by changing their orientation or grasping location. By implementing such behaviour in simulated robots they found that carriage speed increased over time, much as in ants, as individual carriers align themselves in better configurations. However, inspiration is often a two way process, and engineers working on the problem of cooperative transport can provide inspiration for

biologists. By formalising the task of collective box-pushing by multiple robots, Kube and Bonabeau (2000) pose useful questions about cooperative transport. Some of the answers to their questions are known, but others merit future study. Among the questions they raise are: Is worker behaviour in group transport different than in solitary transport? How do several ants cooperate and coordinate their actions to actually transport the item? How does a group of transporting ants handle deadlocks, caused either by the environment or by agonistic behaviours of other transporters? Although partial answers to some of these questions are addressed above, all would benefit from further, formalised study.

Studies of simulated robots tasked with transporting large items can also inform biologists on the evolution of cooperative transport. Groβ & Dorigo (2008) created simulated robots that can move and grasp, but cannot communicate with, or even sense, other simulated robots. By using evolutionary algorithms that select for increased distance that an item is moved, the behaviour of robots was allowed to evolve over multiple rounds of selection. Groβ and Dorigo investigated whether individuals engaged in cooperative transport can benefit from behaving differently from those engaged in solitary transport. Robot behaviour evolved both in the situation where they had to individually move an object as far as possible, with the object being light enough for one robot to move, and in the situation where the box was too heavy for an individual robot to move, so that multiple robots were needed. They found that robots evolved for cooperative transport did indeed perform better than those which were evolved for individual transport. However, robots evolved for individual transport could nonetheless perform cooperative transport, demonstrating that simple rules designed for individual retrieval can result in cooperative transport, as has been suggested in ants (Chauvin 1950; Sudd 1960a; Czaczkes & Ratnieks 2011)(see above). Groß & Dorigo (2008) also demonstrated that communication amongst individuals during cooperative transport need not be direct, but can arise via individuals changing the state of the environment other individuals interact with, a process known as stigmergy (Grasse 1934). Such evolutionary experiments demonstrate clearly that whilst cooperative transport can arise from behaviours selected for by individual transport, selection specifically for cooperative transport abilities can result in more effective cooperative transport. This echoes the case in real ants, were uncoordinated transport can arise from multiple individuals acting as if they were performing individual transport, but coordinated transport, with its associated adaptations, is more effective.

Directions for future study

Cooperative transport is, in our opinion, an understudied topic worthy of further attention. Not only does it provide inspiration for engineers and roboticists, but it also provides an easily manipulated platform for studying the self-organisation of groups. Many questions about cooperative transport remain unanswered, and puzzling facts remain unexplained. Some closely related species demonstrate very different cooperative transport abilities: *Lasius niger*, for example, does not seem to perform cooperative transport, whilst *L. neoniger* performs efficient cooperative transport of large loads, and indeed collects 85% of its food this way (Traniello 1983). What behavioural traits, adaptations or features are needed to allow cooperative transport to occur? Under which circumstances does coordinated cooperative transport evolve? How do ants sense when they should attempt to assist in cooperative transport or attempt to retrieve an item as an individual? *Pheidologeton diversus* may prove to be an ideal study organism to answer this question, as workers switch between dragging or lifting items using their jaws during individual retrieval to lifting with their head and forelegs during cooperative transport (see figure 15.1). Their body posture effectively signals the state they perceive they are in.

It may also be that the recruitment system of an ant species allows or precludes cooperative transport: lack of recruitment will prevent ants from achieving cooperative transport, but mass recruitment (with long lasting pheromones), could result in maladaptive recruitment to items long gone. This could perhaps be offset by the use of a ‘stop’ or ‘no entry’ signal (Robinson et al. 2005).

Whether or not group members communicate during cooperative transport is still an open question. Stigmergy is likely to play a large role in organisation, as are rules regarding avoiding crowding by both fellow carriers and parts of the item being carried (Czaczkes et al. 2011b). However, it is possible that some method of quorum-sensing (Pratt et al. 2002) is employed, either to regulate the number of carriers around an item and prevent further recruitment (Hölldobler et al. 1978), or to signal a change from individual to cooperative transport.

Cooperative transport is a behaviour which is very amenable to study: it is conspicuous, easily manipulated and provides a system in which cooperation and organisation of groups of two to over a hundred can be studied. As cooperative transport does not require direct communication between group members, it is a useful tool for researchers interested in decentralised systems, providing emergent properties which arise within minutes and are

performed reliably. Roboticists are beginning to take more direct inspiration from the cooperative transport behaviours of ants, and biologists can in turn take inspiration from work of roboticists on this topic. We hope that this review will provide a useful introduction for others. We look forward to new studies from both biological and engineering perspectives, and studies that combine the two.

Acknowledgements

We thank Mark Moffett, Katja Rex, the Ludwig Maxillian University of Munich Behavioural Ecology group and two anonymous reviewers for comments on previous versions of the manuscript. TC was funded by a BBSRC studentship.

Part 3 - Final discussion, references and appendices

Chapter 16: Final discussion

The organisation of foraging in social insects is a large and highly complex topic. In this thesis I have examined several aspects of foraging organisation: the deposition and use of trail pheromones, the role of non-pheromone signals and cues, and the role of route memory in foraging. Naturally, these topics lead to wider questions, for example how route memories are formed, or how ants navigate around their environment, which would exceed the scope of this these, but see Collett & Collect (2002) or Collett *et al.* (2003) for reviews. Thus, this discussion will attempt to focus on what this thesis adds to the field, and what questions remain to be answered.

The deposition and use of trail pheromones

A large part of the thesis deals with the deposition and use of trail pheromones by ants. The result in chapters 4, 5, 6, 7, 8 and 13 present, at their most basic level, a series of newly described behavioural rules regarding the use of trail pheromones. These new findings are significant additions to our understanding of foraging organisation in ants. Moreover, as results began to accumulate it became apparent that the convenient story of ant organisation as "simple units following simple rules resulting in emergent complexity" is something of a myth. As discussed in chapter 10, the results of my own research and that of many others in the field have continually demonstrated both the complexity of the individual units – the ants – and the complexity of the rules they follow.

Route memory in foraging organisation

Whilst the role of memory was discounted by early authors, and insect societies were even described as Markovian, or memory-free, superorganisms (Lumsden 1982), this thesis presents individual memory as a highly important factor in foraging organisation. Such memories can be very long lasting: in *Formica rufa,* after overwintering, ants return to foraging sites visited the previous autumn after a dormancy of four to six months (Rosengren & Fortelius 1986). Data presented here (chapter 4) and elsewhere (Lubbock 1884; Harrison et al. 1989; Aron et al. 1993; Salo & Rosengren 2001; Grüter et al. 2008) demonstrate that individual memory is often followed in preference to social information, emphasising the importance of memory. In addition, I demonstrate in this thesis that route memories interact with trail

pheromones (chapters 3, 5 and 7) and home range markings (chapters 5 and 6), and that important collective foraging behaviours, such as switching from a less productive to a more productive food source, is not nececarily mediated by trail pheromones, and thus in these cases is presumably based on route memory (chapter 9).

The results presented in this thesis indicate that the role of memory in foraging organisation of ants has been underestimated. Classic models of ant foraging and colony decision making completely ignore memory, and model only trail pheromone following (Deneubourg et al. 1983; Beckers et al. 1990; Sumpter & Beekman 2003; Detrain & Deneubourg 2008). Many of these models are based on ants such as *Lasius niger*, where route memories are known to take precedence over trail pheromones, and interact in a complementary manner with trail pheromones. Thus, patterns which are classically explained by trail pheromone following may also be explained by evoking route memories. For example, the finding that ant colonies can become so fixated upon a food source that they cannot to switch to newly discovered food sources, could be explained by the majority of ants having a well developed route memory to the initial food source. The selection of less crowded paths could also be stabilised by route memory, with ants remembering only successful path choices, and not those resulting in a U-turn. A similar mechanism, combined with a rule causing ants to perform more U-turns the more oblique their angle to their goal, could also result in the straightest possible route to and from a food source. These possibilities are all highly amenable to experimentation.

Cooperative transport

When I began to write up the first cooperative transport manuscript, I was surprised to find such a charismatic topic in academic disarray. Bits and pieces of research had been performed since the 1960s, with a periodicity of about 10 years, but this sub-field of myrmecology seemed surprisingly neglected. The research conducted in chapters 11, 12, 13 and 14 provides new insights into the organisation of cooperative transport, and how specialisation on this behaviour selects for a specialised recruitment process. Yet more important for the field is the review of cooperative transport (chapter 15) which brings some unity and order to this disparate topic. I hope that the publication of this review, along with the other papers, provides a basis from which to work, and so encourages further work on this rewarding and neglected topic.

Future directions

The organisation of foraging in ants is far from being fully understood. Many questions arose during the course of these studies, and only few of them could be answered experimentally over this period. I hope to tackle some of these in my future career.

Dr Grüter and I noticed soon after gathering the data for chapter 4 that a large gap exists in the foraging organisation literature: that ants do indeed choose the stronger pheromone trail at a bifurcation in a manner proportional to the relative strengths of the two branches has yet to be shown experimentally, with naturally laid trails. The classic paper by Hangartner (1969b), which all other papers refer to, used gland dissections, at concentrations which are likely to be much higher than those on natural trails. I have discussed this problem with many colleagues, and am pleased to say that a PhD student at the Ludwig-Maximilian University in Munich has taken up the challenge. Whilst still working with gland extracts, W. von Thienen, working with the Argentine ant *Linepithema humile* and the fungus-eating ant *Euprenolepis procera*, has shown that ants do indeed choose the stronger path in a proportional manner under a range of biologically meaningful pheromone concentrations. Ideally such experiments would be conducted with naturally deposited pheromones, as dissected glands vary considerably in their size and strength (S. Jones, pers. comm) but these preliminary results suggest that our assumptions about trail following still apply.

In light of this, we might expect ants to be using a "copy when uncertain" strategy (Laland 2004). We demonstrate in chapter 7 that uncertainty increases when ants are faced with multiple, alternating bifurcations – a situation commonly faced by ants foraging in vegetation. Using such a path allows us to test whether ants rely more heavily on trail pheromones when their uncertainty increases, and so follow trail pheromones over route memory. The results from chapter 7, showing increased accuracy in the presence of trail pheromones, suggest this would be the case, but in this experiment the information sources did not conflict. The results from chapter 7 also led to new specific hypotheses, for example that pheromone following will be stronger on the first T-bifurcation (where more errors occurred) than the second.

As route memory seems to play a much more important role in foraging organisation than it is currently attributed, one key piece of data which affects this organisation is missing: It is as yet unknown how rapidly ants can switch, or rewrite, their route memories. For example, if an ant visits a feeder on one branch of a bifurcation twice successfully, but then on subsequent visits food is found only on the other branch of the maze, how many visits are

required for the ant to begin searching in the new feeder location? The level of individual route memory flexibility will dramatically affect how the colony allocates its work force between multiple limited rate feeders. Indeed, the results from chapter 9 demonstrate that trail pheromones do not explain the re-allocation of ants to the more productive feeder. Whilst it is possible, as the agent based simulations show, that this result appears purely due to disappointment, followed by a random walk, I predict that efficiency would be significantly increased if ants remembered successful foraging trips, and on their next visit headed directly in that direction. Of course, in nature the situation may be more dynamic, and food sources may vary over time. By integrating success or failure over multiple visits, ants could choose the most rewarding feeder. I have collected preliminary data on memory switching both at the University of Sussex and at the LMU in Munich (see appendix A). The results suggest that as ants make repeatedly successful visits to a feeder they require more disappointments to switch to a new feeder. This, in turn, suggests that the results of multiple visits are integrated. More data are required to be able to make confident statements, along with related data, such as whether this pattern attenuates at higher visit numbers, what role trail pheromones may play in switching, and how food location switching affects pheromone deposition.

Likewise, the role of the two negative feedback components – a reduction in pheromone deposition under crowded conditions (chapter 8) and a reduction in pheromone deposition in the presence of high pheromone levels (chapter 7) – must be explored. I predict that these components will help ant colonies maintain foraging flexibility, and that the first will allow colonies to choose the least limited path to a feeder even if the bottleneck is far from the choice point. The possibility that trail pheromones promote route learning by path confinement and by acting as a learning signal are also worthy of examination.

Final remarks

Perhaps unsurprisingly, I am ending my doctoral studies with more questions than I began with. I have enjoyed studying the behaviour of ants immensely, and cannot imagine a better group of study organisms. Frankly, I have been spoilt by myrmecology, and studying other organisms will forever seem a slow, drab affair by comparison. However, I will try to keep an open mind, and am sure that to get a true understanding of social insect organisation working with bees at least, if not wasps or termites, is a valuable addition. Nonetheless I fear I shall always remain a myrmecologist at heart. I am currently waiting to hear back from several grant proposals, and hope that the world will let me continue working on this fascinating and rewarding topic.

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Appendix A: Pilot studies into the memory plasticity of *Lasius niger*

Introduction and aims

Whilst many ants famously rely on trail pheromones to guide naïve nestmates to a from food sources (Hölldobler & Wilson 1990), once an ant has visited a food source several times, it is likely to rely primarily on route memory for navigation (Beugnon & Fourcassie 1988; Aron et al. 1989; Harrison et al. 1989; Grüter et al. 2011). A reliance on route memory can potentially exlain patterns attributed to organisation via pheromone trails, such as colonies being unable to switch food sources mid-exploitation (Beckers et al. 1990). A reliance on route memory could also maintain and enhance the formation of patterns put in place by other mechanisms, such as the selection of the shortest possible route to a feeder (Beckers et al. 1992b) or the selection of the least crowded route (Dussutour et al. 2006). However, most models of ant behaviour do not take route memory into account (Deneubourg et al. 1983; Beckers et al. 1990; Sumpter & Beekman 2003; Detrain & Deneubourg 2008). Whilst it is known that ants can form route memories very rapidly (Grüter et al. 2011), and that the speed of learning depends on the path complexity (Czaczkes et al. 2012a), many parameters required for a realistic model of ant foraging are missing. One such key parameter is how rapidly ants can reform memories in light of a changing environment. The primary aim of these pilot studies was to discover how rapidly an ant could learn to stop searching for a feeder at a depleted location, and instead switch its path to the location of a newly discovered feeder. The response of ants to a switch in feeder location in terms of pheromone deposition was also explored.

Methods

Two pilot studies were performed. The first was performed by T. Czaczkes in the Laboratory of Apiculture and Social Insects at the University of Sussex in 2010. 6 *Lasius niger* colonies were tested – for care instructions see chapter 2. Starved colonies were connected via a drawbridge to a T maze of dimensions identical to that of the maze used in chapters 4, 13 and 14. The T maze was overlaid with fresh strips of white printer paper. A 1 mol sucrose feeder was placed at one end of the maze, and a group of 5-10 ants were allowed onto the apparatus and

individually marked with paint dots on the abdomen whilst drinking at the sucrose feeder. Ants were allowed to make a either 1, 3 or 5 visits to the feeder, after which the feeder was repositioned on the other arm of the T maze. Whenever an ant deposited pheromone on the maze the paper overlay was removed and replaced with a fresh overlay, thus forcing ants to rely entirely on their own route memory. Ants crossing one of the decision lines 3cm from the centre of the bifurcation were considered to have made a decision to go left or right. The decisions made by ants both before the feeder switch, and for up to 10 visits after the switch, were recorded. All ants used in an experiment were excluded from future experiments.

The second experiment was performed by H. Windley and S. Ocasio Ortiz at the Ludwig Maximilian University in Munich in 2011. *L. niger* colonies were housed in variable sized plastic containers along with the nest-soil with which they were excavated, and fed three times per week on honey and crickets. Colonies were starved for 4-5 days prior to each experiment. The apparatus was identical to that used in the previous pilot study. Data on path choice was taken identically to the previous pilot study. The number of pheromone depositions on the 3cm section of the T maze before the bifurcation (stem) and in a 3cm section of the T maze after the bifurcation (head) were counted on both outward and return journeys. Ants were allowed to make 1, 3, 5 or 10 visits to the feeder before the feeder location was switched.

Datasets from the two pilot studies were analysed separately. For the method of statistical analysis, see chapter 2. All results reported are post Benjimini-Hochberg corrections.

Results

Ants learn, showing a greater proportion of correct decisions in later visits (GLMM, $Z = 10.05$, $P < 0.0001$)(see figure A1, and table A1 for sample sizes). I found a significant effect of treatment: ants with only one previous visit to the feeder made fewer errors than ants which had made 3 (GLMM, $Z = -0.5869$, $P < 0.0001$) or 5 (GLMM, $Z = -6.875$, $P < 0.0001$) previous visits. There was, however, no difference in the amount of errors made by ants with 3 or 5 previous visits to the feeder (GLMM, $Z = -1.164$, $P = 0.245$), although I did find a significant interaction between treatment and visit between treatments 3 and 5, showing that ants on treatment 5 switch searching locations later than those in treatment 3 (GLMM, $Z = 2.588$, $P = 0.01159$).

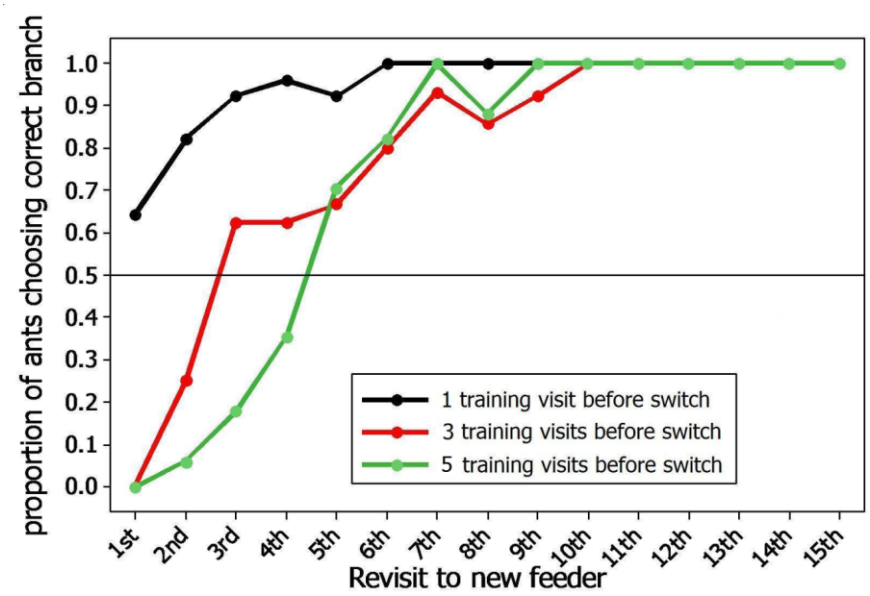


Figure A1 – proportion of ants in the first pilot study choosing the branch where they had previously found a sucrose feeder.

visit after switch	1 training visit	3 training visits	5 training visits
1	15	15	15
2	15	15	2
3	14	15	15
4	14	15	15
5	14	14	15
6	13	14	15
7	6	14	15
8	2	13	15
9	1	12	13
10	0	8	8
11	0	6	4
12	0	2	3
13	0	0	2
14	0	0	2
15	0	0	2

Table A1 - Number of measurements in the first pilot study for each visit after the location of the feeder was changed, for each treatment

Second pilot study

The small sample size of treatment 10, coupled with the high variability in the data, make statistical analysis of this dataset problematic.

Decision making

Once again, ants learn over time (visit is a significant factor, GLMM, Z = 7.016, P < 0.0001)(see figure A2 and table A2 for sample sizes). There was no significant interaction between visit and treatment (Z < 1.97, P > 0.12). Some differences were found between the treatments: ants in treatment one made fewer errors than those in treatment five (Z = -2.703, P = 0.0172). There was a trend in this direction comparing treatment one and three (Z = -1.979, P = 0.0797). There was no difference between treatment one and ten (Z = -0.318, P = 0.7503). Treatment 3 was not different to treatment 5 (Z = -1.103m P = 0.3005) or treatment ten (Z = 1.103m P = 0.3005). There was a trend towards ants in treatment five making more errors than in treatment 10 (Z = 1.825, P = 0.085).

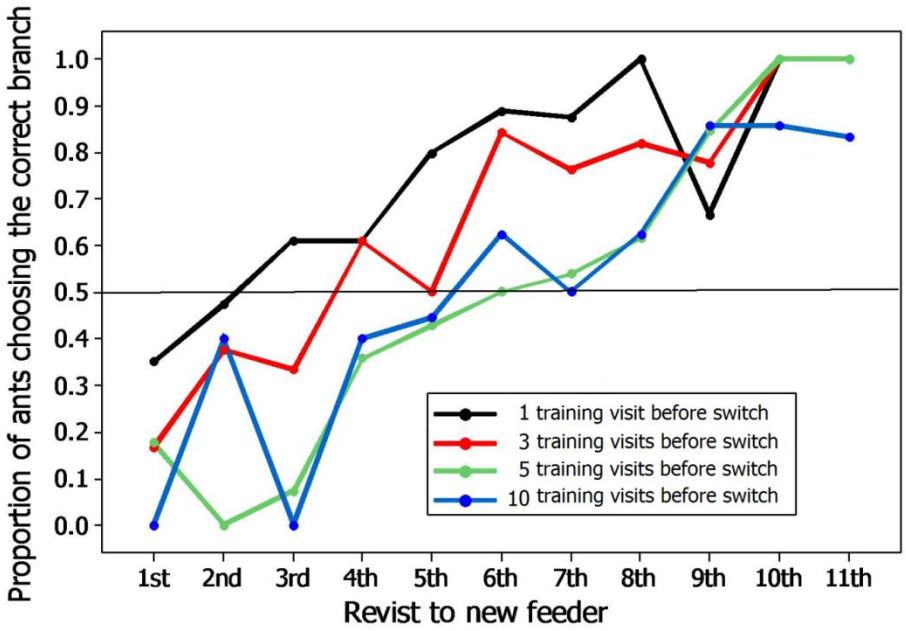


Figure A2– proportion of ants in the second pilot study choosing the branch where they had previously found a sucrose feeder

visit after switch	1 training visit	3 training visits	5 training visits	10 training visits
1	20	24	17	10
2	19	24	14	8
3	18	24	14	8
4	18	23	14	8
5	15	20	14	8
6	9	19	14	8
7	8	17	13	8
8	7	11	13	8
9	6	9	13	7
10	2	1	2	6
11	0	0	1	6

Table A2 – Number of measurements in the second pilot study for each visit after the location of the feeder was changed, for each treatment

Pheromone depositions

The low sample sizes preclude a sensible statistical analysis of the data. However, I present graphs of the collected data in figure A3.

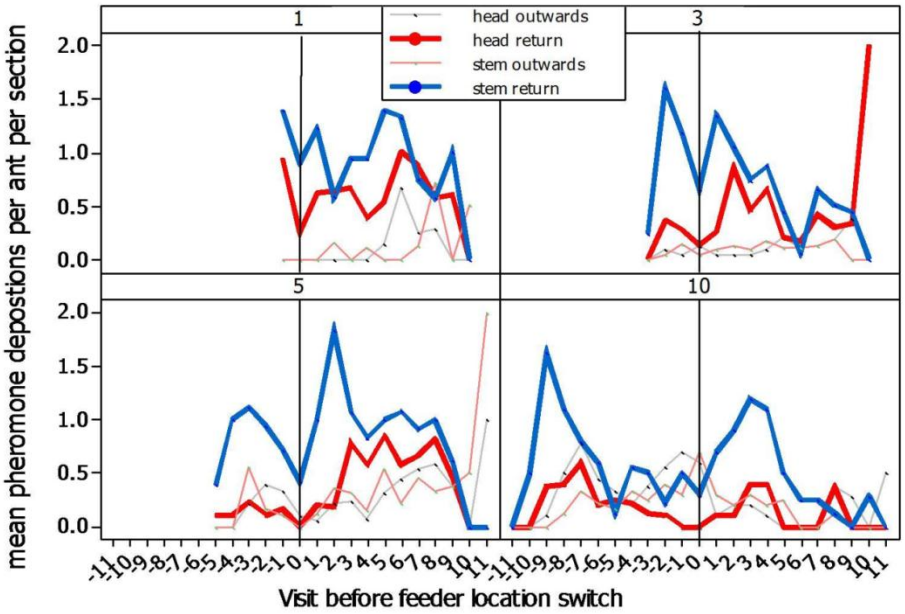


Figure A3 – pheromone depositions by number of training visits before feeder location switch. Connect lines for returning ants, which deposited most pheromone, are bolder. Depositions may be on a section of the stem or a section of one of the branches (the head) of the T maze. Ns can be very low, especially in the higher visit numbers.

Preliminary conclusions and discussion

The results from both pilot studies demonstrate that the speed at which ants switch their searching location depends on how many successful visits were previously made to the original feeder location. Ants with few previous visits to a feeder require X + 1 visits on average to switch their searching to a new feeder location from an old location that was visited X many times. These results have implications for how route memories will affect colony level foraging. The ants integrate the experience of past visits so as to decide where to search for food. Thus, ants could in principal learn to search for food first at feeders that are more reliably productive. The effective productivity of a feeder for an ant depends on two factors: the actual productivity of the feeder, and how often the feeder is exploited. By integrating over multiple visits, individual ants will eventually settle on the feeder that provides it with the highest reliability. At a colony level, this should result in different ants 'specialising' at different feeders, with the number of ants 'specialising' on each feeder depending on the actual productivity of the feeder.

Future directions

Data on memory flexibility after many training visits is critical. It is possible, and indeed seems likely, that after a certain number of visits ants will stop becoming more heavily 'fixated' on a feeder, as otherwise ants which visit a feeder for many weeks would require an equal number of weeks to stop searching that location – a clearly maladaptive strategy.

It is also as yet unclear what the role of trail pheromones is in the formation and plasticity of route memories. I have show in chapter 5 that trail pheromone can act as a reassurance to route memories, and I chapter 7 that trail pheromones affect route learning. It could be that once a pheromone trail has developed to location, this pheromone trail would disrupt learning to another location, as ants which stray off the strongly marked trail would lose the "reassurance" they are on the right path (see chapter 5). Thus, they may be slow to begin searching at a new feeding location. In this experiment we always removed trail pheromones from the apparatus. If this hypothesis is true, we would expect ants to require more visits to a new feeding location before their searching location switches if trail pheromones were allowed to remain on the maze. On the other hand, it has been suggested (Collett & Collett 2002) that trail pheromones might act as a training signal, enhancing the

speed at which routes are memorised. I provide some supporting evidence for this in chapter 7. It may thus be that if trail pheromones were present on the maze that ants will begin searching at the new feeder location sooner. As the “reassurance” effect of trail pheromones seems to be an all-or-nothing effect (chapter 5), I am inclined to predict that indeed switching would occur sooner, or at least not later, if trail pheromones were not removed from the apparatus. The answer to this question can easily be discovered experimentally.

Appendix B: pairwise comparisons for experiment 3, chapter 7

In this experiment trail pheromone was allowed to accumulate on the trail, and removed either i) after each visit, ii) only on the ants' final visit, or iii) never, in order to ascertain whether the presence of pheromone assists in route learning. The model included the terms 'bifurcation' and 'treatment' as explanatory variables. Treatment has five levels: experience + pheromone (trip 6), experience, no pheromone (trip 7), only pheromone (naïve), pheromone always removed, and experience + pheromone (trip 7). We found significant interactions between some of the treatment comparisons and bifurcation, and in other treatment comparisons we found no effect of bifurcation. We summarise the significant findings below.

- Ants which never had access to pheromone are less accurate than ants that always had access to pheromone, on both bifurcations (Z= -3.030 P = 0.00815).
- On their last visit, ants which never had access to pheromone are less accurate than ants that had access to pheromone in all but the last visit (Z = -2.922 P = 0.0326)
- Naïve ants with trail pheromone are more accurate than ants that had access to pheromone in all but the last visit (Z = -2.480 P = 0.02196).
- Ants that had access to pheromone in all but the last visit are less accurate on their last visit than on their penultimate visit (Z = -4.269 P = 0.000196)
- Significant interaction between bifurcation and experienced ants with no pheromone on only the 7th visit Vs ants with pheromone on all visits (Z = -2.584 P = 0.01956)
  -No difference on firsT-bifurcation (see table 1 below)
  -On second bifurcation ants with experience but no pheromone on the 7th visit are less accurate than ants that always had access to trail pheromones (see table 2 below)
- Significant interaction between bifurcation and naïve ants with pheromone Vs ants that always had access to trail pheromones (Z = -3.170 P = 0.00762):
  -On firsT-bifurcation naïve + phero more accurate (see table 1 below),
  -on second bifurcation naïve + phero less accurate (see table 2 below)
- Interaction between bifurcation and experienced ants with no pheromone on only the 7th visit Vs ants that never had access to trail pheromones (Z= -2.584 P= 0.01956):
  -on firsT-bifurcation ants which never had access to trail pheromones are less accurate than experienced ants with no pheromone on only the 7th visit (see table 1),
  -no difference in accuracy on the second bifurcation (see table 2)
- Interaction between bifurcation and ants which never had access to trail pheromone Vs naïve ants with pheromone (Z = -3.170 P= 0.00762)
  -on firsT-bifurcation ants which never had access to trail pheromone are less accurate than naïve and with pheromone (see table 1)
  -no difference on second bifurcation (see table 2)

Table key:

- FullPhero trip 7 = pheromone never removed from path
- MemTest trip 7 = pheromone removed from path only on the 7th visit
- MemTest trip 6 = the same ants as MemTest trip 7, but on the visit before, where pheromone remains on the path
- NoPhero trip 7 = pheromone always removed from path
- Naïve + phero = ants that have never visited the food source before, on a path with trail pheromone

Table 1 - Bifurcation 1

Compared to X, Y is…	FullPhero trip 7	MemTest trip 7	MemTest trip 6	NoPhero trip 7	Naïve + phero
FullPhero trip 7	N/A	Not different Z = 0.052 P = 0.9586	Not different Z = -1.536 P = 0.156	More accurate Z= 2.972 P = 0.00493	Less accurate Z= -2.411 P =0.0199
MemTest trip 7	Not different Z = -0.052 P = 0.9586	N/A	Not different Z = -1.563 P = 0.1475	More accurate Z = 2.862 P = 0.00526	Less accurate Z = -2.415 P = 0.0199
MemTest trip 6	Not different Z = 1.536 O = 0.1556	Not different Z = 1.563 P = 0.1475	N/A	More accurate Z= 4.199 P < 0.00001	Not different Z= -0.493 P = 0.6220
NoPhero trip 7	Less accurate Z = -2.972 P = 0.0105	Less accurate Z = -2.862 P = 0.0158	Less accurate Z|= -4.199 P< 0.0001	N/A	Less accurate Z= -5.737 P < 0.00001
Naïve + phero	More accurate Z = 2.411 P = 0.0265	More accurate Z= 2.414 P =0.0263	Not different Z = 0.493 P = 0.622	More accurate Z = 5.737 P < 0.00001	N/A

Table 2 - Bifurcation 2

Compared to X, Y is…	FullPhero trip 7	MemTest trip 7	MemTest trip 6	NoPhero trip 7	Naïve + phero
FullPhero trip 7	N/A	More accurate Z= 2.937 P = 0.00828	Not different Z= 0.906 P= 0.3650	More accurate Z = 2.875 P= 0.01010	Borderline More accurate Z = 2.292 P = 0.0548
MemTest trip 7	Less accurate Z = -2.937 P = 0.00674	N/A	Less accurate Z= -2.476 P = 0.0275	Not different Z= -0.146 P = 0.88404	Not different Z = -1.443 P = 0.1861
MemTest trip 6	Not different Z= -0.906 P = 0.36500	More accurate z = 2.476 P = 0.02215	N/A	More accurate Z = 2.397 P= 0.02754	More accurate Z= 1.629 P = 0.1723
NoPhero trip 7	Less accurate Z= -2.875 P = 0.00674	Not different Z = 0.146 P = 0.88407	Less accurate Z = -2.397 P = 0.0275	N/A	Not different Z= -1.319 P = 0.1872
Naïve + phero	Less accurate Z = -2.292 P = 0.02737	Not different Z= 1.443 P = 0.18626	Not different Z = -1.629 P = 0.1292	Not different Z = 1.319 P = 0.23411	N/A

Appendix C: Supplementary information for Chapter 8: Negative feedback in ants: crowding results in less trail pheromone deposition

Appendix C part 1 – analysing the effect of trail crowding in terms of proportion of individual ants depositing trail pheromone, and trail pheromone depositions per depositing ants, for individually followed focal ants

This analysis was performed to ascertain the relative importance of ants reducing the number of pheromone depositions they perform, and the proportion of ants that decide to stop depositing pheromone at all.

Proportion of ants depositing trail pheromone at least once

We found a significant interaction between both ant treatment and visit number, and path width and visit number, on the probability of ants depositing trail pheromone ($P < 0.0001$, $Z = -7.003$ and $P = 0.00031$ and $Z = -3.779$ respectively, see figure A below). The probability of individual ants depositing pheromone decreases with visit number on both narrow and wide trails, but this reduction in deposition probability occurs faster on narrow trails. Likewise, when few ants are on the trail the probability of pheromone deposition decreases with visit number on narrow trails ($P < 0.0001$, $Z = -6.722$), but not on wide trails ($P = 0.455$, $Z = -0.747$).

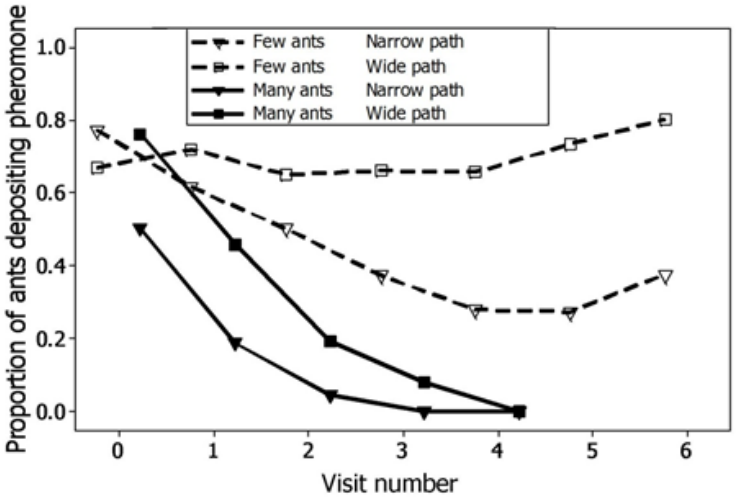


Figure A - the proportion of ants performing at least one trail pheromone deposition as a function of path width, number of ants on the trail, and visit number.

Number of pheromone depositions per journey by ants depositing pheromone at least once

Conversely to the above, when we consider depositions per depositing ant, we find no effect of path width (P = 0.56, Z = -0.754) and a smaller effect of ant number: Deposition rates are reduced on later visits (P < 0.0001, Z = -9.541), and this effect is stronger when many ants are present (P < 0.0001, Z = -4.534, see figure B below).

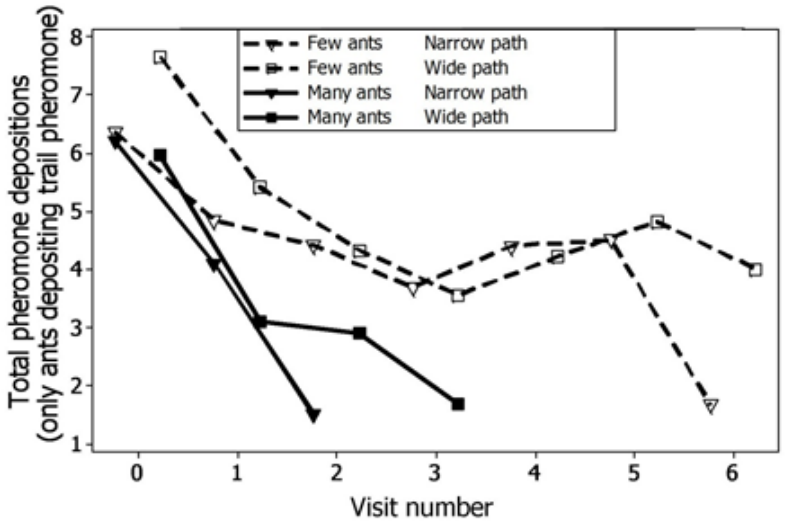


Figure B - Average depositions per journey of ants which deposit pheromone at least once, as a function of path width, number of ants allowed onto the path, and visit number.

Appendix C part 2 - analysing the effect of trail crowding in terms of proportion of individual pheromone depositions per and pheromone depositions per depositing ant, for all ants.

In the main text we present data showing that when many ants are allowed onto a path there are more total depositions on wide paths than narrow paths. Conversely, when few ants are allowed onto the trail path width has no effect on total pheromone depositions (see figure A below).

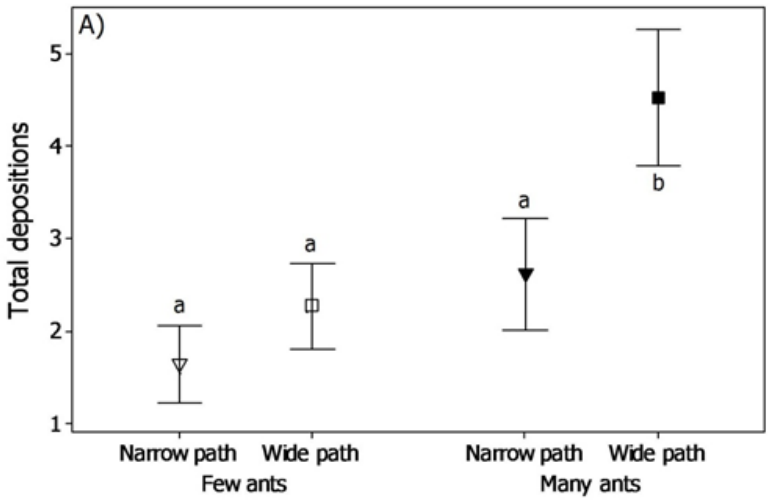


Figure A - The effect of path width and number of ants on a path on total pheromone depositions on the first 4cm of the path.

Here, we analyse that data in terms of depositions per ant, proportion of ants depositing pheromone, and depositions per depositing ant.

We find more depositions per ant when the number of ants on the trail is limited ($Z = -3.189$, $P = 0.00191$) and on wide as compared to narrow paths ($Z = -2.269$, $P = 0.0233$)(see figure B). More head-on collisions between ants result in less depositions per ant ($Z = -3.386$, $P = 0.00142$). This pattern could either be due to fewer ants depositing pheromone at all, or depositing ants depositing less pheromone, or both. This is explored below.

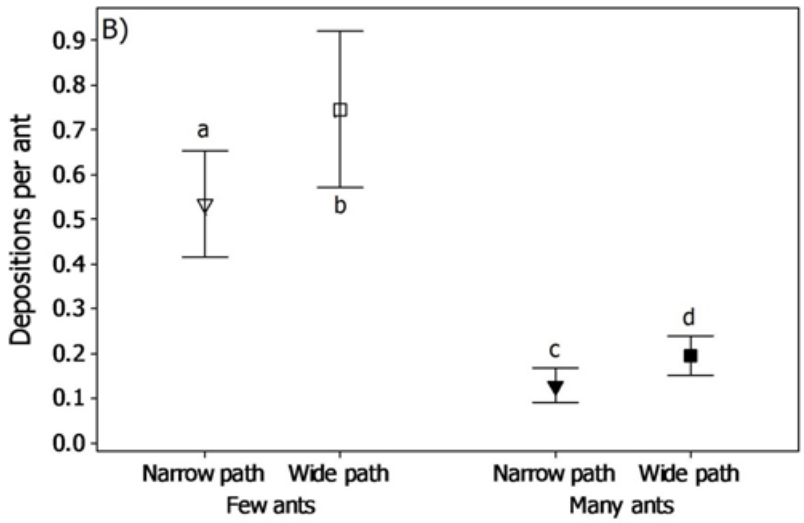


Figure B - The effect of path width and number of ants on a path on number of depositions per ant on the first 4cm of the path.

A higher proportion of ants deposit pheromone when few ants are allowed onto the path (P = 0.00771, Z = -2.89), although we find no effect of path width or number of collision on the proportion of ants depositing pheromone (P = 0.593, Z = -0.534 and P = 0.182, Z = -1.488 respectively) (see figure C below).

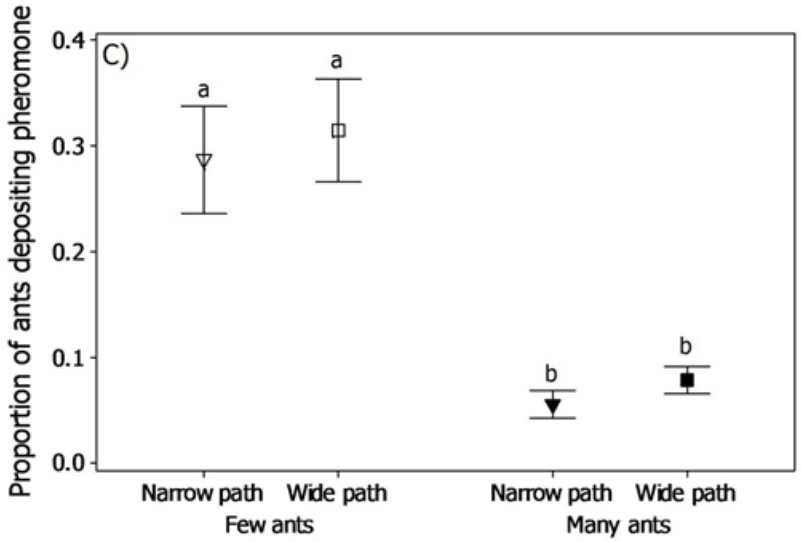


Figure C - The effect of path width and number of ants on a path on proportion of ants depositing pheromone, on the first 4cm of the path.

Conversely, when considering pheromone depositions per depositing ant path width had no effect (Z = -0.956, P = 0.339), although surprisingly depositing ants deposited slightly more

pheromone on when many ants were allowed onto the path (Z = 2.98, P = 0.0039) (see figure D below). This result is puzzling and not in line with our other findings. It may perhaps be explained by noting that not all ants deposit equal amounts of pheromone. Indeed, Beckers et al. (1992a) found that the two ants which laid most pheromone accounted for 80-90% of all pheromone deposited. Our data do not show such extreme heterogeneity. However, if such 'champion' trail laying ants are also less likely to stop depositing pheromone in response to high encounter rates the observed pattern would emerge, as we have also shown that ants modulate the number of pheromone depositions deposited only weakly, and mostly modulate total pheromone depositions on a trail by deciding whether to deposit pheromone at all.

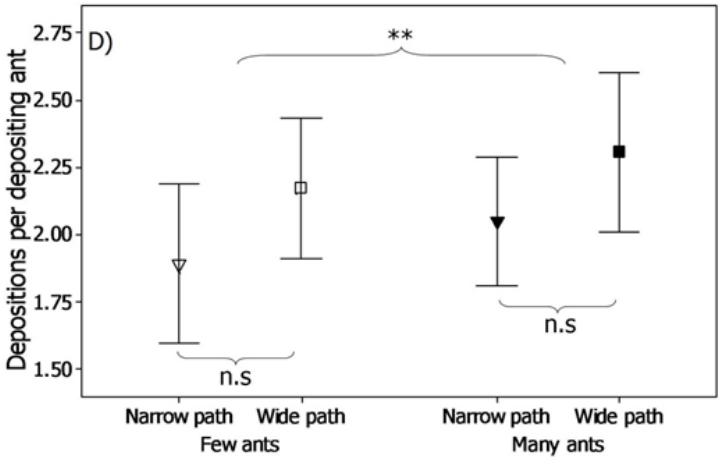


Figure D - The effect of path width and number of ants on a path on number of pheromone depositions performed by ants that performed at least once deposition, on the first 4cm of the path.

Appendix C part 3 – simulating crowding using 'artificial ants'

In the main text we describe how the presence of 'artificial ants' in the form of glass beads can affect pheromone deposition behaviour in *Lasius niger*. We report that both the colour of the beads (black or clear) and whether the beads were coated by nestmate cuticular hydrocarbons (CHCs) affect the total pheromone depositions per journey of the ants. The results of a pairwise comparison of the treatments is presented in table A below .

So as to explore the relative contribution of ants varying the number of pheromone depositions they perform, or choosing not to deposit pheromone at all, we also analysed the effect of the treatments in those terms. Pairwise comparisons of treatments for the proportion of ants depositing pheromone, and the number of depositions per journey for ant which deposited pheromone at least once, are presented in tables B and C respectively, and the results displayed graphically in figures A and B below.

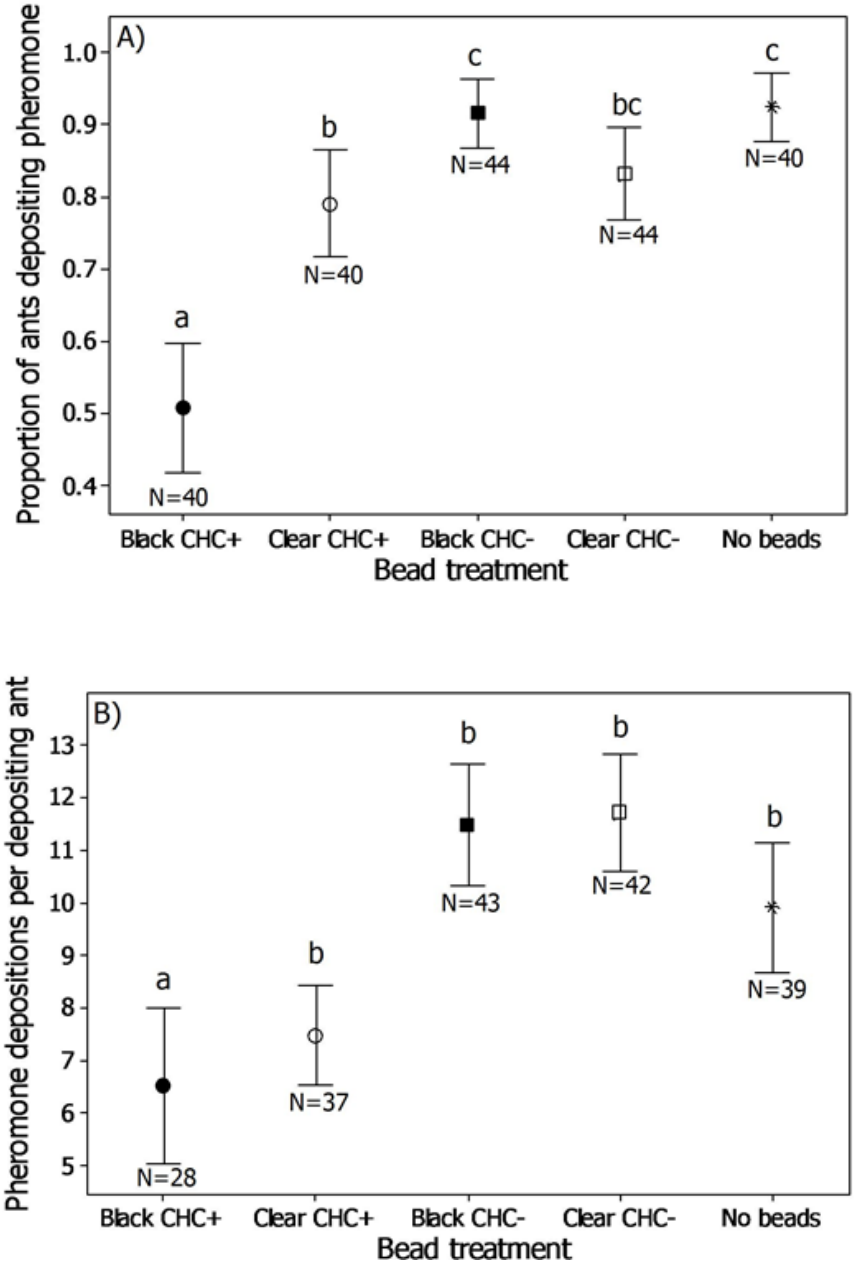
Overall, there were clear differences between the treatments. The presence of black CHC+ beads reduced the average pheromone depositions of ants, the proportion of foragers that deposited trail pheromone and the number of pheromone depositions per ant which deposited pheromone compared to all other treatments (figure 8.3 in main text, figures A & B here, and tables A-C below). The presence of clear CHC+ beads causes a reduction in the average pheromone depositions of ants and the proportion of ants depositing pheromone compared to all treatments except black CHC+ beads (figure 8.3 in main text, figure B and table B below). The amount of pheromone deposited by ants that did deposit at least once was lower when black CHC+ beads were present compared to all other treatments (Figure B and Table C below). However, the non-significance of the difference between black CHC+ and clear CHC+ is driven by three of the ten colonies, which show no difference. If these colonies are excluded from the analysis, we find no difference between black CHC and clear CHC ($P = 0.6102$, $Z = 0.132$), and significant differences between clear CHC and the other three treatments (vs. Black CHC- $P < 0.0001$, $Z = 5.976$, vs. Clear CHC- $P < 0.0001$, $Z = 4.959$, vs No Beads $P = 0.0004$, $Z = 3.607$). Thus it is uncertain whether colour plays a role in ascertaining whether a contacted item is a fellow forager.

Table A	Effect	Z value	P value
Black CHC vs Clear CHC	Less pheromone depositions	-5.4	< 0.0001
Black CHC vs Black Blank	Less pheromone depositions	-10.7	< 0.0001
Black CHC vs Clear Blank	Less pheromone depositions	-8.84	< 0.0001
Black CHC vs No Beads	Less pheromone depositions	-10.8	< 0.0001
Clear CHC vs Black Blank	Less pheromone depositions	-4.7	< 0.0001
Clear CHC vs Clear Blank	Less pheromone depositions	-2.87	= 0.005
Clear CHC vs No Beads	Less pheromone depositions	-4.15	< 0.0001
Black Blank vs Clear Blank	No difference	1.42	= 0.153
Black Blank vs No Beads	No difference	-0.286	0.775
Clear Blank vs No Beads	No difference	-1.635	0.128

Table B	Effect	Z value	P value
Black CHC vs Clear CHC	Less ants deposited pheromone	-3.836	< 0.00032
Black CHC vs Black Blank	Less ants deposited pheromone	-5.575	< 0.0001
Black CHC vs Clear Blank	Less ants deposited pheromone	-4.45	< 0.0001
Black CHC vs No Beads	Less ants deposited pheromone	-5.91	< 0.0001
Clear CHC vs Black Blank	More ants deposited pheromone	-2.226	0.00434
Clear CHC vs Clear Blank	No difference	-0.579	0.5625
Clear CHC vs No Beads	Less ants deposited pheromone	-2.045	0.234
Black Blank vs Clear Blank	No difference	1.762	0.0975
Black Blank vs No Beads	No difference	-0.266	0.7903
Clear Blank vs No Beads	No difference	-1.972	0.0607

Table C	Effect	Z value	P value
Black CHC vs Clear CHC*	Less depositions per ant (No difference)	-2.70 (0.132)	0.0347 (0.610)
Black CHC vs Black Blank	Less depositions per ant	-3.755	0.00087
Black CHC vs Clear Blank	Less depositions per ant	-3.096	0.0098
Black CHC vs No Beads	Less depositions per ant	-3.756	0.000863
Clear CHC vs Black Blank*	No difference	-1.07	0.564
Clear CHC vs Clear Blank*	No difference	-0.366	0.714
Clear CHC vs No Beads*	No difference	-1.114	0.547
Black Blank vs Clear Blank	No difference	0.723	0.587
Black Blank vs No Beads	No difference	-0.069	0.945
Clear Blank vs No Beads	No difference	-0.776	0.547

Table 1 - A) Did the pheromone depositions of ants (including journeys in which ants did not deposit pheromone) differ between treatments? B) Did the proportion of ants depositing pheromone differ between treatments? C) Did the number of pheromone depositions by ants that deposited pheromone differ between treatments? All tests are Generalised Linear Mixed effect Models (GLMMs). Beads placed on the trail were either black or clear, and either blank or coated in nestmate cuticular hydrocarbon (CHC). Results displayed graphically in figure 3 in the main text, and figures A and B below. *If three colonies in which the Clear CHC treatment had no effect are removed and the data reanalysed, the difference between Black CHC vs Clear CHC becomes non-significant, and the differences between Clear CHC and the other three treatments become significant, with less deposition per ant in the Clear CHC treatment.



Figures A and B) The effect of bead treatment on A) the proportion of ants depositing trail pheromone and B) pheromone depositions per depositing ant. Beads could either be black or clear, and either covered by CHCs (CHC+) or not (CHC-), or could be absent altogether, resulting in five separate treatments: black CHC+, black CHC-, Clear CHC+, clear CHC-, and control (no beads). Dots represent means, whiskers 95% C.I. Treatments headed by the same letter are not significantly different. N = number of ants contributing to this group. Note the big change in the results of Clear CHC+.

Appendix D: Supplementary information for chapter 9: Negative feedback enables fast and flexible collective decision-making in ants

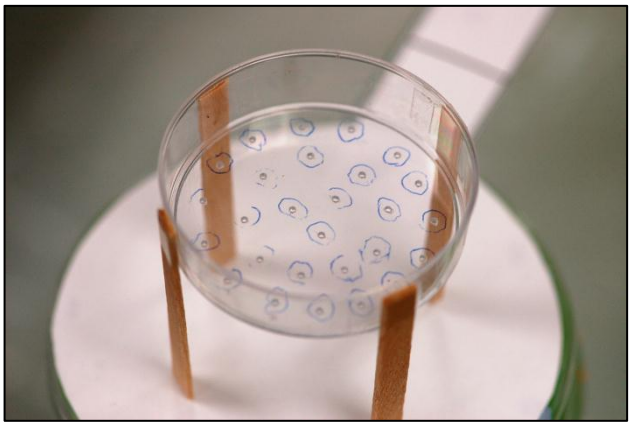


Figure S1. Photo showing the feeder (petri-dish, 5cm diameter) standing on 2cm wooden legs. The feeder contained 1M sucrose solution. Ants could gain access to the solution via 1mm feeding holes (27 in this situation).

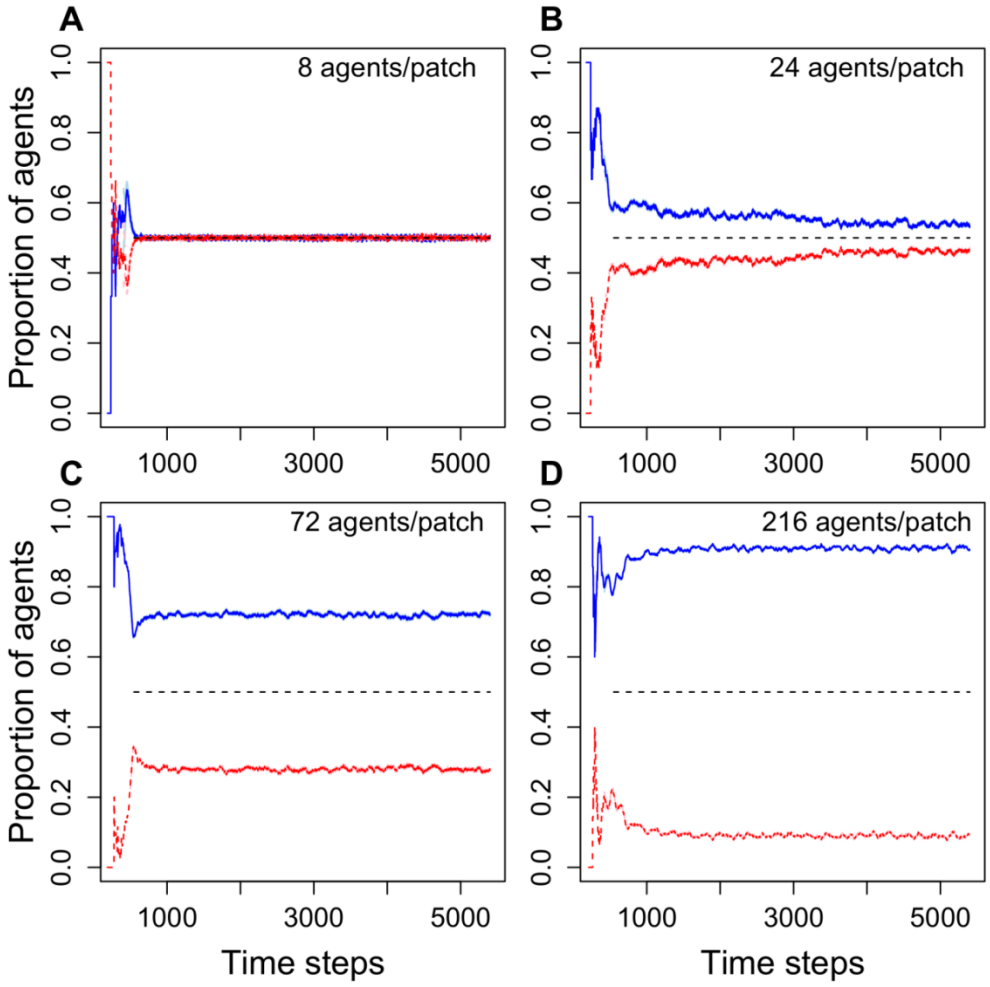


Figure S2. Model 1 with the different behavioural states being updated in reversed sequence (unloading agents -> recruiting agents -> dissatisfied agents -> feeding agents -> foraging agents -> idle agents). Proportions of agents visiting two identical food patches each with space for 8, 24, 72 or 216 foraging agents. The blue line represents the patch that had more agents after 600 time steps, the red line the other. The dashed black line indicates an equal distribution of agents at both feeders. Data averaged from 30 simulations in each situation. The standard deviation (StDev) is shown in light blue and pink. However, since the StDev is very small it is difficult to see by eye.

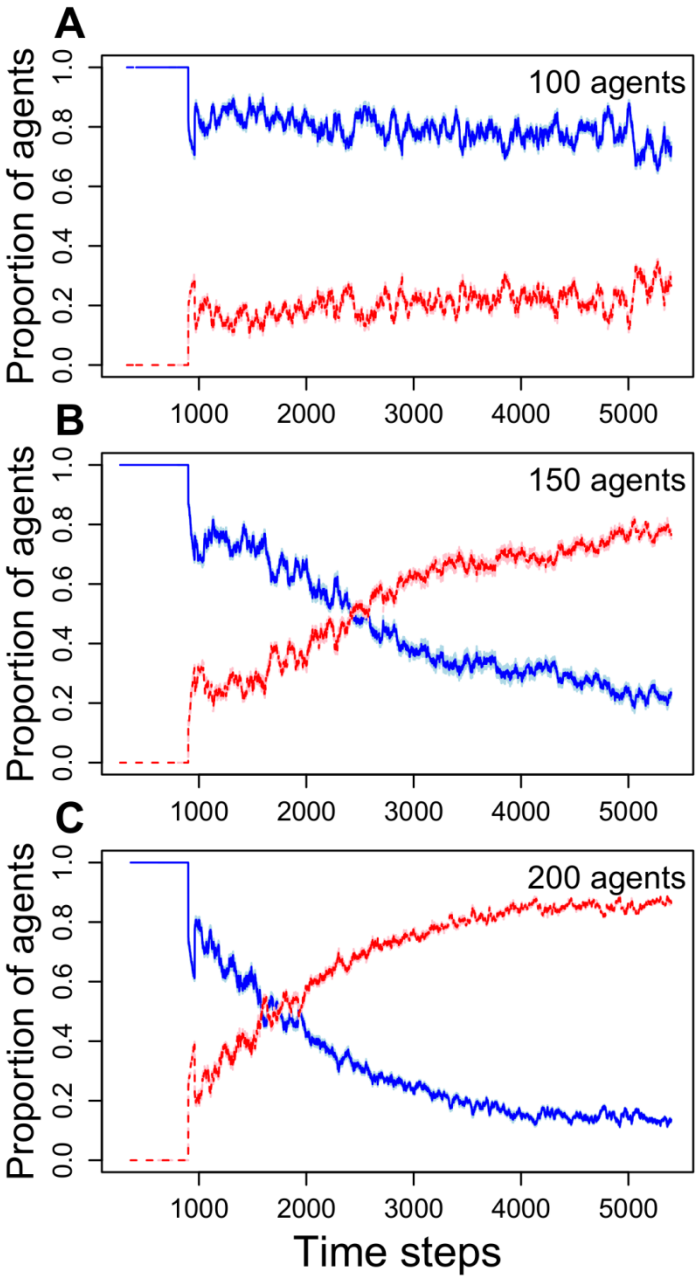


Figure S3. Smallest colony size still showing flexibility under high crowding conditions (8 vs. 24 agents). Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 900 times steps after agents started foraging at the first food patch (blue line). Data averaged from 10 simulations in each situation. The StDev is shown in light blue and pink. However, since the StDev is very small it is difficult to see by eye.

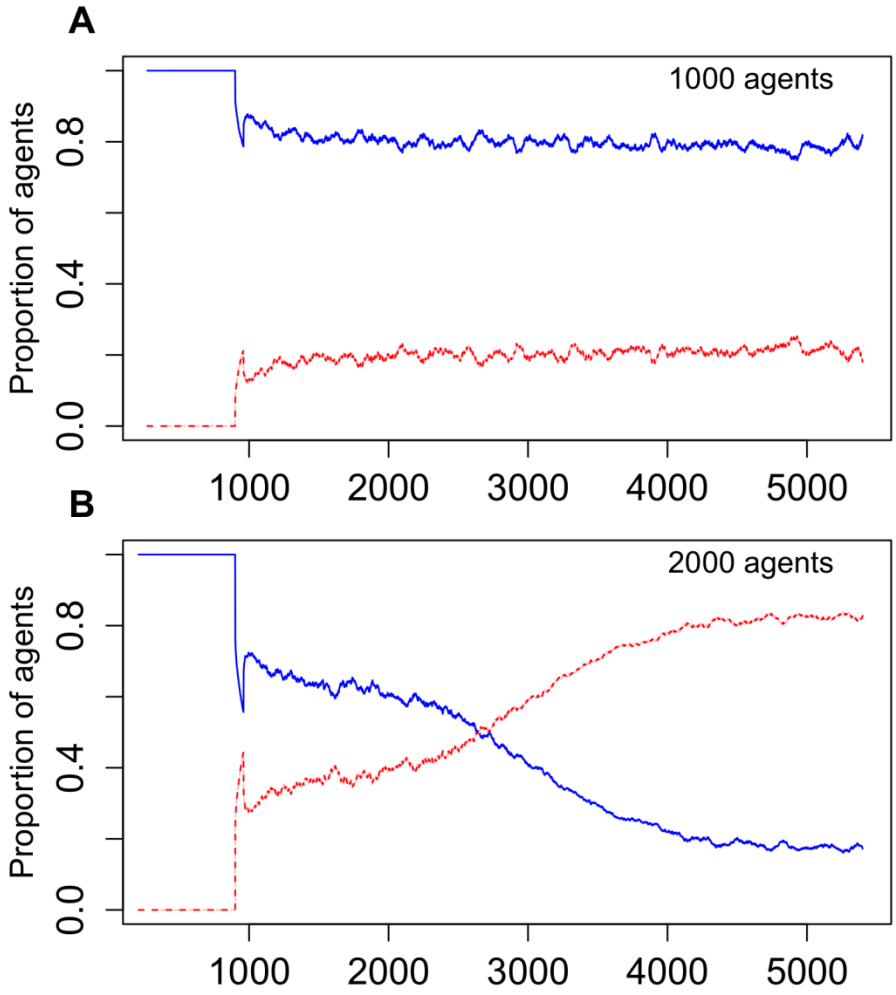


Figure S4. Colony size needed for flexibility under low crowding conditions (72 vs. 216 agents). Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 900 times steps after agents started foraging at the first food patch (blue line). Data averaged from 10 simulations in each situation. The StDev is shown in light blue and pink. However, since the StDev is very small it is difficult to see by eye.

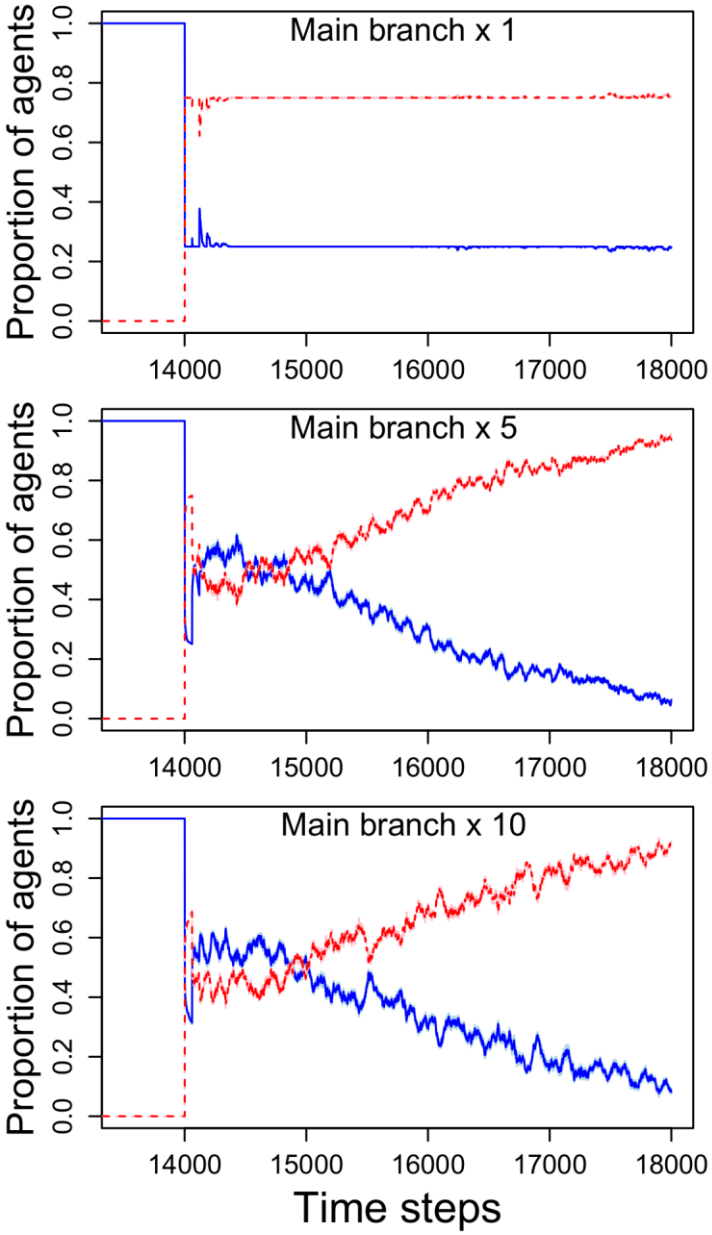


Figure S5. The effect of the main branch length on flexibility under high crowding conditions (8 vs. 24 agents). Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 14000 time steps after agents started foraging at the first food patch (blue line). The delay of 14000 time steps was chosen because it guaranteed that agents discovered the first food source by random walks even if the main branch was 10 times longer than by default. A main branch length x 10 corresponds to approximately 2 m. The instantaneous switch shown in (A) is caused by a large number of dissatisfied agents occupying the second food patch after 14000 time steps. However, with a longer main branch the dissatisfied agents are distributed over a larger area. Data averaged from 10 simulations in each situation. The StDev is shown in light blue and pink.

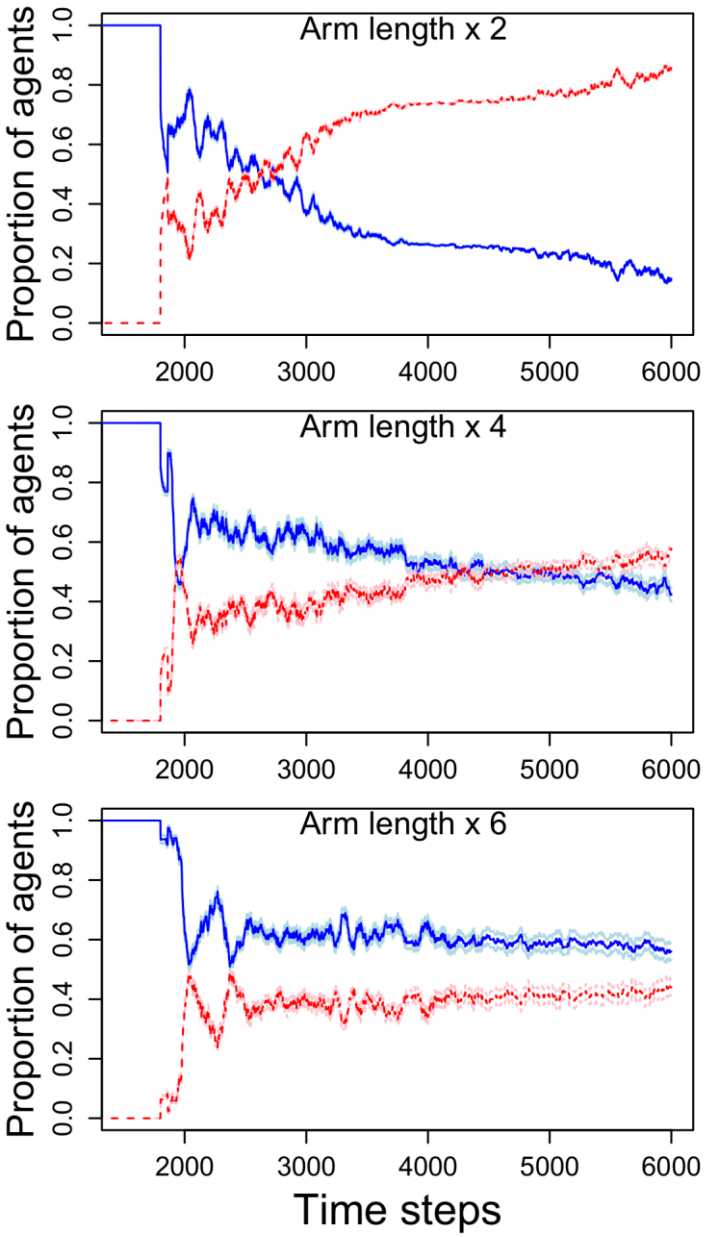


Figure S6. The effect of the arm length on flexibility under high crowding conditions (8 vs. 24 agents). Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 1800 time steps after agents started foraging at the first food patch (blue line). The delay of 1800 time steps was chosen because it guaranteed that agents discovered the first food source by random walks even if the arm length was 6 times longer than by default. An arm length x 6 corresponds to approximately 2 m. Data averaged from 10 simulations in each situation. The StDev is shown in light blue and pink.

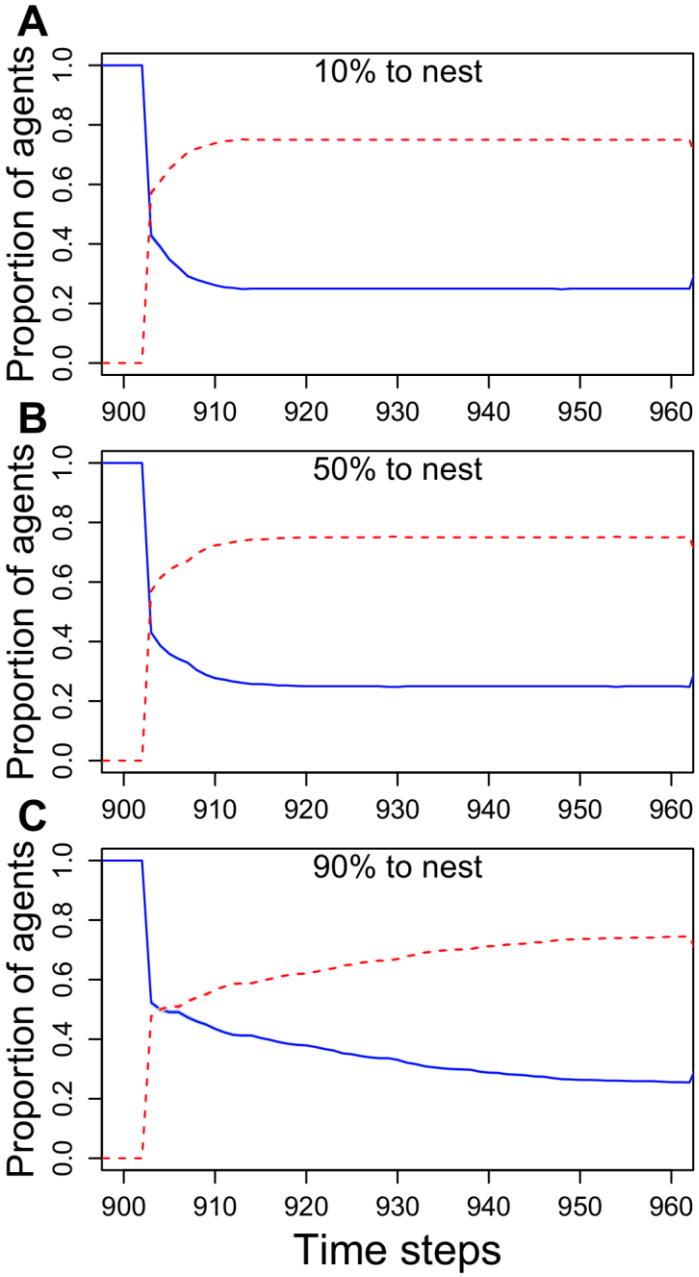


Figure S7. The effect of the probability of dissatisfied agents to walk to the nest versus to the second feeder on collective flexibility under high crowding conditions (8 vs. 24 agents). Proportions of agents foraging at the two food patches, in which the second patch (red line) allowed three times as many agents to feed simultaneously but was made available 900 time steps after agents started foraging at the first food patch (blue line). This model slightly differed from the main model in that dissatisfied ants did not perform a random walk but had a certain probability to either walk on a direct path to the nest or to the second food source (probabilities were 10% vs. 90%, 50% vs. 50, 90% vs. 10%). If both feeders were crowded, dissatisfied agents walked back to the nest and then became "foraging agents" again. Data averaged from 10 simulations in each situation. The StDev is shown in light blue and pink.

Appendix E: Comparing actual distributions of ants at the corners of experimental food items to expected distributions

The aim here is to create a series of models to describe how many distinct corners are occupied when 4 ants carry a square food item when they have no preference for occupying the corners. We then compare the results of these models with the results of experiments using real ants and determine whether they are significantly different, and thus whether real ants are more or less likely to occupy a corner than would be expected if they had no preference for occupying corners.

Description of the problem

The sides of the square food item were divided in 5 sections of equal length (figure A1). During an experiment, one can record whether an ant chooses a sector belonging to a corner (sectors A, B, C and D) or not (sectors O).

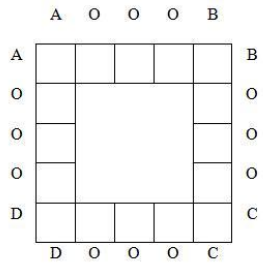


Figure A1

We define *k* as number of corners occupied by at least one ant. In terms of probability, our problem is similar to describing the probability of picking '*k*' distinct letters (excluding O) in the following set: {A,A,B,B,C,C,D,D,O,O,O,O,O,O,O,O,O,O,O,O}.

We can define three situations:

- Letter picked are immediately replaced (e.g. sectors can be occupied by more than one ant).
- Letter picked are removed (e.g. sectors can be occupied by only one ant).
- Letter picked are removed and if a corner letter is picked (A, B, C or D) the other corner letter is removed (e.g. sector and corner can be occupied by only one ant).

Situation with replacement

This situation is equivalent to having *n=4* independent trials with each trial resulting in one of 5 possible outcomes (picking either A, B, C, D or O). This is equivalent to describing a multinomial distribution. For notation, event 'A' (picking the square at one of edge A) occur 'a' times (and respectively for other letter)

Knowing:

- Event A, B, C and D occur with the same probability p_c*=0.1* (p_c : probability of joining one of the corners).
- Event O occurs with probability q_c*=1-4*p_c*=0.6* (q_c : probability of joining any non-corner sector).

We have:

$$P_1(A = a, B = b, C = c, D = d, O = o) = \frac{n!}{a!\, b!\, c!\, d!\, o!} {p_c}^{a+b+c+d} q^o$$

The equation can be interpreted as the number of permutations where the same event happens (in this situation order is not important) times the probability of such an event happening.

The goal is to determine the probability density function of observing *k* distinct corners sectors occupied. As an example for 1 corner occupied we are looking for: $P(A \neq 0, B = 0, C = 0, D = 0)$ which can be viewed as a sum of multinomial with $= [1\ to\ 4]$. If we define $\phi_1(k)$ as the probability of observing *k* corner occupied, we derived $\phi_1(k)$ for each values of *k*:

$$\phi_1(0) = {q_c}^4$$
$$\phi_1(1) = 4\,[(p_c + q_c)^4 - {q_c}^4]$$
$$\phi_1(2) = 6\,[(2p_c + q_c)^4 - 2(p_c + q_c)^4 + {q_c}^4]$$
$$\phi_1(3) = 4\,[(3p_c + q_c)^4 - 3(2p_c + q_c)^4 + 3(p_c + q_c)^4 - {q_c}^4]$$
$$\phi_1(4) = (4p_c + q_c)^4 - 4(3p_c + q_c)^4 + 6(2p_c + q_c)^4 - 4(p_c + q_c)^4 + {q_c}^4$$

If ants do display a preference we expect the value of p_c to differ from 0.1 .

Situation without replacement and no preferences for corner sector

In this situation when one sector is occupied it will not be available for subsequent individuals. Because there is no replacement, the probability of one happening will not follow an exactly multinomial distribution.

If we define:

- K: number of distinct corner occupied
- C: total number of sectors occupied at a corner

We have:

$P(K=k, C=c) = \frac{4!}{(4-c)!2^{ck}} 2^k \frac{12!}{(12+c-4)!} \frac{1}{T}$

with $T = 20 * 19 * 18 * 17$

Because each corner is equivalent, this probability must be multiplied by the number of permutation resulting in the same outcome: $\frac{4!}{(4-k)!(c-k)!(2k-c)!}$.

Finally, the probability of having '*k*' distinct corners occupied is given by:

$$\varphi(k) = P(K=k) = \sum_{c=k}^{\min(2k,4)} \frac{4!}{(4-k)!\,(c-k)!\,(2k-c)!} \frac{4!}{(4-c)!\,2^{c-k}} 2^k \frac{12!}{(12+c-4)!} \frac{1}{T}$$

The 'simplicity' of this formulation is allowed by the restriction that each corner can be carried by at most 2 individuals.

Situation without replacement and a preference for some sectors

We consider the same situation as above but with a preference for either corner sectors or non-corner sectors such that $p_c \neq .1$.

Given the complexity of finding a general analytical formulation, but the limited number of possible outcomes (K ranging between 0 and 4), we simply derived all the probabilities, depending on *p*, of each event. To illustrate the logic behind this, we present an example:

We define:

p_c: probability of carrying one of the two sectors of a corner (if there is no preference p_c=0.1)

x=20p_c and y=20-4x

We then derive the probability of 2 corners being occupied:

First let consider the probability of the distribution: $P(A=1, B=1, C=0, D=0)$

- One possibility of obtaining such a result is by drawing {*a,b,o,o*} in this order:

$$P(\{a,b,o,o\}) = \frac{x^2 y \left(y - \frac{y}{12}\right)}{20 \times \left(20 - \frac{x}{2}\right) \times \left(20 - \frac{2x}{2}\right) \times \left(20 - \frac{2x}{2} - \frac{y}{12}\right)}$$

- Alternatively the same outcome with different order {*a,o,b,o*}

$$P(\{a,o,b,o\}) = \frac{x^2 y \left(y - \frac{y}{12}\right)}{20 \times \left(20 - \frac{x}{2}\right) \times \left(20 - \frac{x}{2} - \frac{y}{12}\right) \times \left(20 - \frac{2x}{2} - \frac{y}{12}\right)}$$

And so forth for each order.

Then we consider: $P(A=2, B=1, C=0, D=0)$ using the same logic. And finally we count all the possibilities of having the same outcome but with different corners occupied.

The process is tedious but gives us the possibility of drawing an analytical expected distribution ϕ_2 for each value of p_c ranging between *0 and 0.25* (total avoidance of corner, up to total avoidance of non-corner). We also note that φ is a special case of ϕ_2 for $p_c = 0.1$.

Situation without replacement, a preference for some sectors, and the two sectors of each corner can only be occupied by one ant

This situation is similar to the previous one, but corners cannot be co-occupied. Using the same example:

First let us consider the probability: $P(A = 1, B = 1, C = 0, D = 0)$

- One possibility of obtaining such a result is by drawing {*a,b,o,o*} in this order:

$$P(\{a, b, o, o\}) = \frac{x^2 y\left(y - \frac{y}{12}\right)}{20 \times (20 - x) \times (20 - 2x) \times \left(20 - 2x - \frac{y}{12}\right)}$$

- Alternatively the same outcome with different order {*a,o,b,o*}

$$P(\{a, o, b, o\}) = \frac{x^2 y\left(y - \frac{y}{12}\right)}{20 \times (20 - x) \times \left(20 - x - \frac{y}{12}\right) \times \left(20 - 2x - \frac{y}{12}\right)}$$

And so forth for each order.

In this situation, $P(A = 2, B = 1, C = 0, D = 0)$ cannot happen (same corner occupied twice). Finally we count all the possibilities of having the same outcome but with different corners occupied.

Again the process is tedious but gives us the possibility of drawing an analytical expected distribution φ_3 for each value of p_c ranging between *0 and 0.25* (total avoidance of corner, up to total avoidance of non-corner). Having an analytical expectation for the distribution of occupied corners, we can perform a maximum likelihood estimation of the parameter p_c. Finally, having estimated p_c , given that there are 2 corner sectors per corner and there are 12 non-corner sectors and 8 corner sectors, we can show that the preference ratio for corner sectors over side sectors equal: $r = 12\left({}^{p_c}/_{2}\right)/\left(1 - 8\left({}^{p_c}/_{2}\right)\right)$. Thus r = 5.4.

Appendix F: Supplementary information for chapter 13: Pheromone trails in the Brazilian ant *Pheidole oxyops*: extreme properties and dual recruitment action

Part 1 (Experiment 2): Effect of distance to nest entrance on discovery of food items – frequency of food items in each trial

The data presented in figure 13.3 are pooled from three separate trials in which experimental food items (individually numbered 5x5mm squares of cheese) were placed at regular intervals in circles at varying distances from a relatively isolated *Pheidole oxyops* colony. The frequency of food items varied between trials and distances, as detailed in the table below. Distances of 2, 4 and 6 meters were tested twice. The replicated distances, all from trial 3, are presented in the last two columns.

Distance from nest	Circumference	Trial	Number of food items	Food items per meter	Number of food items (replicate)	Food items per meter (replicate)
0.5	3.13	1	4	1.27		
1	6.28	1	8	1.27		
2	12.57	1,3	16	1.27	6	0.48
3	18.85	1	24	1.27		
4	25.13	1,3	32	1.27	12	0.48
5	31.42	2	20	0.64		
6	67.70	2,3	40	1.1	18	0.48
8	50.27	3			26	0.52
10	62.83	3			30	0.48

part 2 (Experiment 4): Effect of recruitment and the pheromone trail on the movement of food items: modelling the random build-up of ants at a food source

This part of the experiment aims to estimate the time required for a certain number of ants to find a food item by chance, without any form of recruitment. We placed a 15X15mm food item 2 meters from a *Pheidole oxyops* nest and waited until an ant found the item. Every ant that found the item was immediately removed, and the time at which the ant had found the item, measured from the moment the item was presented, and the order of the ant (whether it was the first ant to discover the food, the second ant, and so on) was noted. This continued until 10 ants were captured or 25 minutes had elapsed from the beginning of the

trial. If 25 minutes elapsed without 10 ants being captured, the remaining positions were given a value of 25 minutes. This will have the effect of underestimating the time required for 10 ants to reach the food item. Thus, the results represent a conservative estimate (erring on the side of more rapid discovery).

Using this data, we built a linear mixed-effect model which attempts to predict the time an ant was captured at by the discovery order of the ant. This model had an intercept of 141.8 and a slope of 92.4 seconds per ant which discovers the item (see figure below). The average number of ants required to begin moving the item and to move it 5cm was 2.2 and 6.6 ants, respectively. Using the model of random ant build-up, we can interpolate how many seconds would be required for those two events to happen without any recruitment. This interpolation provides the figures 345.2 and 747.3 seconds for first movement of the item and movement of the item 5cm, respectivly (see figure below).