Is the well-trodden path always the correct path? New insights into the role of footprint hydrocarbons during ant foraging.

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Abstract

Positive feedback and negative feedback are crucial mechanisms in the regulation of biological systems and whilst the role of positive feedback has been well studied in social insect networks, the role of negative feedback has not. This thesis presents evidence of a novel negative feedback mechanism at work during foraging by the ant *Lasius niger*. In a foraging context, cuticular hydrocarbons, passively deposited on substrate by ants as they walk, can act as a repellent cue. This effect can be measured through branch selection by foraging workers at a trail bifurcation. Evidence from both a highly controlled experimental set up and a more natural foraging context are provided to support this claim. The possible role of this signal in ant foraging networks is considered and comparisons with other known negative feedback mechanisms in ant foraging networks are drawn. Experiments with the ant *Monomorium pharaonis* were unable to show negative feedback signals being used to optimise food search tactics and this finding is evaluated in relation to the ecology of *M. pharaonis*. Circumstantial evidence is also presented in support of the hypothesis that *L. niger* workers can use alarm pheromones to recruit nest mates to unexplored territory. The need for further work testing this hypothesis is highlighted. The communication role of ant interactions during foraging is discussed and, based on new observations with *L. niger* workers, the position of the signaller relative to the recipient during the interaction is proposed as a possible component of the signalling mechanism. Further possible experiments based on both empirical observations and computational models are proposed to help augment the findings presented in this study.

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Declaration

Unless otherwise acknowledged in the text, all the material presented herein is the sole work of the author. None of the material has been presented for examination elsewhere.

Chapter 1 - Introduction

1.1 Negative and Positive Feedback in Biological Systems

Self regulating systems are found at all levels of biological organisation and the two main mechanisms controlling them are: positive feedback, where the result of a process is self amplifying; and negative feedback, where the result of a process is self inhibiting (Cinquin and Demongeot 2002a). These processes play a vital role in both intracellular (Kholodenko 2000) and intercellular (Freeman 2000), regulation and can function right up to the level of the ecosystem (Jouquet *et al.* 2006). In most biological systems negative feedback processes are far better understood than positive feedback (Cinquin and Demongeot 2002b) but unusually, the roles of negative feedback processes in the regulation of social insect societies are poorly understood whereas positive feedback processes have received much attention (Gadau and Fewell 2009). Social insects provide a very good model for studying self regulatory processes due to the logistical ease with which experiments can be carried out, but also because eusociality itself and the success of organisms that have adopted this strategy are inherently fascinating. Foraging is a particularly good context for such experiments because of the accessibility of the various components of the system (i.e. the workers have left the nest) but also because it has such a direct impact on colony fitness (Taylor 1978) and therefore success. Whilst the role of positive feedback in social insect foraging networks is well studied and understood, the role of negative feedback, particularly in relation to ant foraging networks is not. As our understanding of the role that positive feedback mechanisms play in ant foraging networks is unusually advanced, it would seem sensible to improve our understanding of the role negative feedback mechanisms. Developing a model where the roles of *both* negative and positive feedback are well understood could have wider implication in systems biology.

1.2 The Use of Positive Feedback Mechanisms During Foraging by Social Insects

1.2.1 Recruitment to Food by Social Insects (Other than Ants)

Recruitment, defined as "communication that brings nestmates to some point in space where work is required" (Hölldobler and Wilson 1990), relies on self propagating signals (i.e. positive feedback), and is key to the success of social insects. Perhaps the best known example of recruitment in insect societies is the "waggle dance" used by bees to recruit workers to a food source (Von Frisch 1967). At a basic level, waggle dancing workers recruit nest mates to a food source. These recruited workers can then return to the nest and amplify the signal. However, the waggle dance is a multi-component signal and can: indicate the presence of food; indicate the location of the food; indicate the type of food (via olfactory cues); and can stimulate foraging behaviour (using social or private information about food source). For a review see Grüter &

Farina (2009). Positive feedback mechanisms facilitating the recruitment of nestmates to food sources have also been studied in social wasps (Overmyer and Jeanne 1998), stingless bees (Nieh et al. 2003; Nieh 1998), bumblebees (Dornhaus and Chittka 1999), and termites (Traniello 1982).

1.2.2 Mass Recruitment to Food by Ants

Most ant species are central place foragers, collecting food from their home range and returning with it to a nest at a fixed location (Hölldobler and Wilson 1990). Frequently, workers will encounter food items that are too large or food sources that are too productive to be efficiently exploited by a single individual. When this occurs, ants can recruit nest mates to the new resource to help utilise or defend it. Mass chemical recruitment is one mechanism used by ant colonies to organise and regulate the efficient exploitation of spatially and temporally heterogeneous food resources that are too large for a single individual to harvest (Lach *et al.* 2010). On paths leading to food, mass-recruiting worker ants deposit attractive pheromones that recruit more workers to that path. These workers in turn lay trail pheromone, recruiting even more workers. Thus the input to the system (an ant depositing a pheromone) gives an output (ants being attracted) that results in further input (Szlep and Jacobi 1967). This leads to the signal being self-propagating and the formation of a positive feedback loop. The system does not rely on workers having experience of the food source as the pheromone trail can itself stimulate workers to leave the nest, guide them to the food source and also regulate the intensity of recruitment (Cassill 2003).

Complex colony level foraging processes can arise where there are competing trails and when individual workers exhibit a behavioural response that is a function of the amount of pheromone on the competing trails (Goss *et al.* 1989). Mass recruitment can lead to foraging effort being optimised in response to a heterogeneous environment. For example, a colony using mass recruitment to exploit two food sources of equal quantity and quality but different distances from the nest can “select” the nearer food source through mass recruitment without individuals having experience of both sources (Taylor 1978). At first workers will distribute more or less evenly between the paths leading to the two food sources but the round trip time for the nearer food source will be lower leading to a more rapid build up of trail pheromone on that path. As a consequence, a greater proportion of workers will take that path leading to the closer resource, further increasing the speed with which the trail pheromone builds up on that path, and diverting an even greater proportion of the foraging population to that resource. In a similar manner, a colony given a choice between two food patches equal distance from the nest but of uneven quantity or quality can “select” the most productive resource (Mailleux *et al.* 2003). If more workers are able to feed at once, or collect their required food load more quickly at one food source, the amount of pheromone deposited on that trail will build up more quickly leading to a proportionately greater number of individuals selecting that branch.

The optimisation of foraging through mass recruitment has been studied under the influence of a range of environmental, colony-level and individual-level influences. Environmental factors include: food quality (Taylor 1978; Beckers et al. 1990), food quantity (Mailleux *et al.* 2000, 2003), food type (Portha *et al.* 2004), food distance (Taylor 1978; Devigne & Detrain 2006) and trail geometry (Gerbier *et al.* 2008). Colony-level factors include: starvation (Traniello 1977, Mailleux *et al.* 2010), traffic congestion within the foraging network (Dussutour *et al.* 2004), colony size (Beekman *et al.* 2001) and short term fluctuations in colony foraging success (Gordon *et al.* 2007). Individual-level factors include: the role of U-turns on a trail (Beckers *et al.* 1992a), the role of bidirectional trail laying (Goss *et al.* 1989), worker physiology (Robinson et al. 2008), changes in the amount of pheromone deposited (Beckers *et al.* 1993) and the integrated use of visual landmarks (Evison *et al.* 2008). Examples of multiple attractive trail pheromones being used to organise mass recruitment have also been reported (Jackson *et al.* 2006, Dussutour and Nicolis 2009).

Mass recruitment is the foraging strategy employed by most ant species with large colony sizes. This trend has been linked to both foraging ecology (Beckers *et al.* 1989) and the limits imposed by colony size on the functional viability of a given foraging strategy (Planqué *et al.* 2010). The historical understanding of the mechanism was that it relied entirely upon the deposition and decay of ephemeral, attractive pheromones (Wilson 1962) but recently the importance of additional factors such as worker experience (Robinson et al. 2008; Provecho & Josens 2009; Grüter et al. 2010; Czaczkes et al. 2011) has been recognised.

1.3 The Use of Negative Feedback Mechanisms During Foraging by Insects

1.3.1 Ants

A system relying solely on positive feedback can get stuck in suboptimal states. An “overshoot” of workers arriving at a food source after the food is depleted has been reported in the ant *Solenopsis invicta* (Wilson 1962) and in the ant *Linepithema humile* workers are unable to switch to a shorter path to food once foraging on a longer route has become established (Goss *et al.* 1989). In extreme examples, “run away” positive feedback can result in ants becoming trapped on circular trails that they themselves are reinforcing each time they pass (Fourcassié & Deneubourg 1992).

Mathematical models have predicted that the introduction of a negative feedback mechanism into an ant foraging network that relies solely on positive feedback results in an increase in efficiency (Britton *et al.* 1998) but so far only three examples of negative feedback in ant foraging networks have been reported. A volatile chemical repellent has been found in the ant *Monomorium pharaonis* (Robinson et al. 2005) which when deposited on an unrewarding trail branch immediately after the bifurcation and has an effect on subsequent foraging decisions for 33 minutes (Robinson *et al*., 2008). An agent based model used to investigate the effect of this repellent on colony level foraging behaviour found that the foraging efficiency increased through ants having a improved ability to switch their foraging efforts to a new food source (Robinson et al. 2008). Negative feedback in relation to feeder crowding has been reported in the ant *Lasius niger* (Grüter *et al.* 2012). Here worker persistence resulted in the searching for and discovery of alternative food patches the exploitation of which could begin before the pheromone trail to the old patch had decayed. Negative feedback during foraging has also been reported in the ant *Lasius pallitarsis* in response to danger but no mechanism was proposed for how it was achieved (Nonacs, 1990).

Over twenty different exocrine glands have been found in the legs of ants (Billen 2009) many of which could be candidate sources for chemicals with a negative feedback function in a foraging trail context. Secretions from some of the exocrine glands in the legs of ants such as the Tibial Tendon Gland (Leuthold 1968), the Bitarsal Gland (Hölldobler and Palmer 1989) and the Pretarsal Footprint Gland (Billen *et al.* 2005) have already been associated with a trail communication function in ants.

1.3.2 Other Social Insects

Bees have a well documented negative feedback mechanism the “stop signal” (Nieh 1993, Pastor and Seeley 2005) which is targeted at individuals performing the waggle dance and is triggered by a range of factors including: poisoned food sources; overcrowding at feeders; and long waiting times for a successful forager to unload to a storer bee [as reviewed in Kirchner (1993)]. An elegant study by Nieh (2010) examined the stop signal use in response to danger (both alarm pheromone and mechanical disturbance) and found its use to be a modulatory process with the magnitude of signalling representing the magnitude of the danger.

A chemical negative feedback mechanism to avoid re-searching for food where other individuals have already searched is found in honeybees (Giurfa and Núñez 1992), and bumblebees (Schmitt and Bertsch 1990). When an individual lands on a flower, a volatile repellent cue is left, which subsequent individuals can then use to avoid wasting time and energy by landing on and probing flowers that have already been depleted of nectar. Wilms and Eltz (2008) provide strong evidence that the presence of “footprint hydrocarbons” and not a specific pheromone is the signal bees use. A chemical negative feedback agent has been found in termites where it inhibits gnawing (Reinhard and Kaib 1995).

1.3.3 Non-social insects

The negative feedback regulated mechanisms that induce the avoidance of recently searched or previously unrewarding areas can lead to an increase in foraging efficiency. This has been demonstrated in Carabid beetles where the avoidance of conspecific odour trails is an efficient search strategy (Guy *et al.* 2008). A chemical negative feedback mechanism in foraging has also been demonstrated in the larvae of the ladybird *Coccinella septempunctata* (Marks 1977) which leave repellent scent marks on the branches of host plants upon which previous searches were unrewarding.

1.4 The Remit of this study

1.4.1 Research Question

This study will aim to determine whether ants can use chemically mediated negative feedback mechanisms to increase the efficiency with which they either discover or exploit food sources. Specifically, it will focus on two predictions:

1. Ants detect chemical cues resulting from the passage of nestmates within a foraging arena. This information is used to improve their search strategy by avoiding areas where other ants are already searching for food [as seen in other insects (Marks 1977, Guy *et al.* 2008)].

2. Given a choice between competing recruitment trails, ants integrate information about background traffic levels into their decision making process. It has been suggested that trails with high levels of background marking may indicate that a food source has already been exploited and should therefore be avoided (Czaczkes et al. 2012).

1.4.2 Study Species

The study will focus on two ecologically dissimilar species, the Black Garden Ant (*Lasius niger*) and the Pharaoh's Ant (*Monomorium pharaonis*). Whilst the use of two species will facilitate only the qualitative comparison of results, such an approach will increase the probability of discovering novel negative feedback mechanisms in the limited time available. Studies drawing qualitative comparisons between two species are not unusual (Reznikova 1982; Taylor 1978; Beckers et al. 1990; Regnier & Wilson 1969; Jackson & Châline 2007).

M. pharaonis is an invasive, polydomous, polygynous species that does not discriminate between nest mates and non-nestmates (Hölldobler and Wilson 1990). The species is an opportunistic scavenger. At least three pheromones are known to be used during foraging, one attractive and long lasting, one attractive and short lasting and one repellent (Robinson et al. 2008). *M. pharaonis* are bidirectional trail layers and both fed and unfed workers lay trail pheromone by dragging their sting on the substrate. Marks are either discontinuous spots or continuous lines with the latter being displayed more often by fed ants (Jackson and Châline 2007). Workers are approximately 2mm in length so are too small for the trail laying behaviour to be observed with the naked eye. Seven different organic molecules have been shown to elicit trail following behaviour, see Parry & Morgan (1979), Morgan (2009) and Jackson & Châline (2007).

Lasius niger is a common monodomous and monogynous Palaearctic species, that farms aphids (Mailleux *et al.* 2000) and feeds opportunistically on insects (Portha *et al.* 2004). *L. niger* exhibits bidirectional trail laying but workers will only lay trails after having visited a food source and fed (Beckers *et al.* 1992b).

Trail pheromone marks are discontinuous spots on average 20 - 40mm apart (depending on food quality) (Beckers *et al.* 1993), and are deposited when the tip of the gaster is brought into contact with the substrate (Beckers *et al.* 1992b). This marking behaviour is easy to observe with the naked eye but exhibited only by about one third of foragers that have been in contact with food (Beckers *et al.* 1992b). The trail pheromone originates from the hindgut and has been identified (see Figure 1) as 3,4- dihydro-8-hydroxy-3,5,7-trimethylisocoumarin (Attygalle *et al.* 1987). The response of workers to different concentrations of trail pheromone (measured by the number of trail laying workers to have previously taken each of two competing branches) has been quantified (Beckers *et al.* 1993). The *L. niger* alarm pheromone has been identified as undecane (see Figure 1) and originates from the Dufour's gland (Maschwitz 1964).

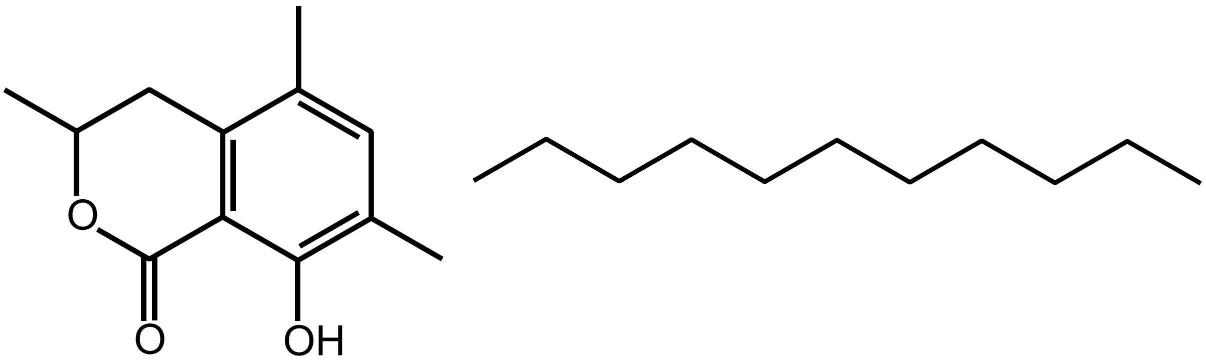


Figure 1 - The trail pheromone (3,4- dihydro-8-hydroxy-3,5,7-trimethylisocoumarin) (L) and alarm pheromone (undecane) (R) of *Lasius niger*.

Chapter 2 – General Materials and Methods

2.1 Ant Husbandry

2.1.1 Pharaoh's Ant

Three colonies of the Pharaoh's ant *Monomorium pharaonis* were available for experiments, each containing approximately 300 workers, multiple queens and brood. These colonies came from a population maintained under laboratory condition for eight years. Each colony was housed in a 22.5cm x 37cm plastic box with the vertical sides coated in Fluon®. A nest was provided constructed from two glass slides separated by four match sticks. The upper slide was coated in a sheet of translucent red acetate (transmission < 20% for λ < 600nm) and the nest cavity had a height of approximately 2mm (see Figure 2). The colonies were maintained at a temperature of 25°C (+/- 1°C) under a 12hour: 12hour light: dark cycle. Water was available to the colonies *ad libitum*.

The *Monomorium* colonies were fed on a two week cycle. In the first week they received 1M sucrose solution twice (Monday and Friday) and two chopped up meal worms (*Tenebrio molitor*) once (Wednesday). In the second week they received 1M sucrose solution once (Wednesday) and two chopped up meal worms twice (Monday and Friday). Colonies were starved prior to use in experiments, as detailed in the relevant methods section. These husbandry practices are equivalent to those already reported in the scientific literature (see Jackson et al. 2006; Robinson et al. 2005).

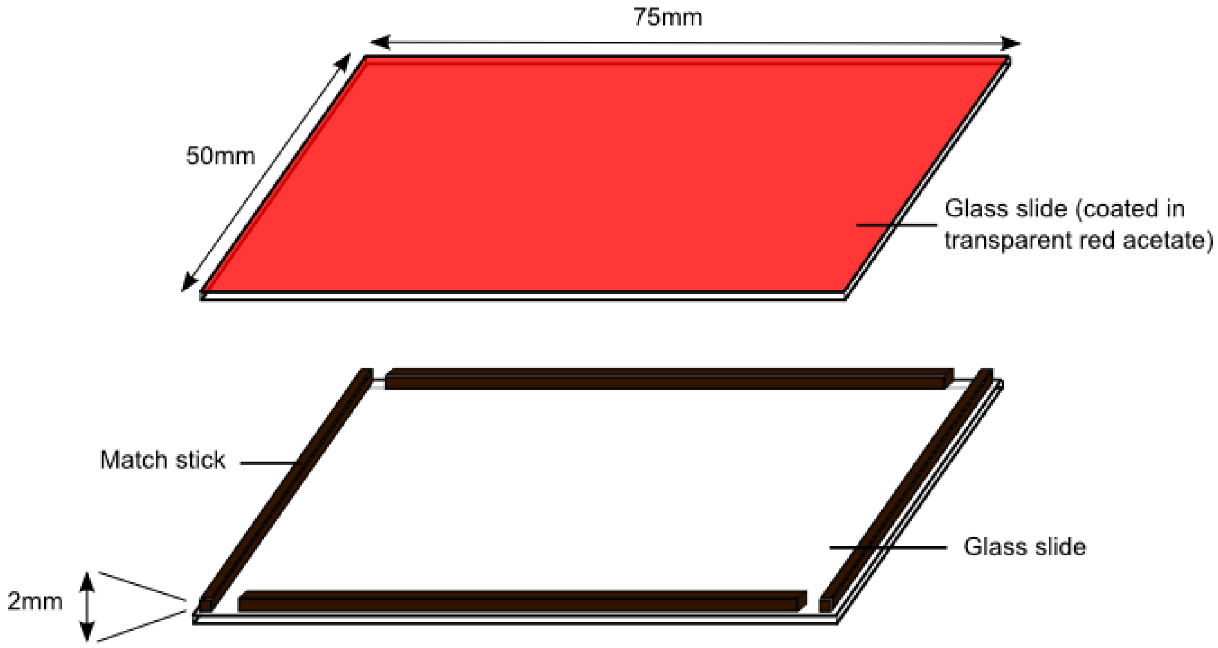


Figure 2 – Nest design for *Monomorium pharaonis*

2.1.2 Black Garden Ant

Seven colonies of the Black Garden Ant *Lasius niger* were available for experiments. Three of the colonies were established from queens collected during their mating flights in Cambridgeshire, 2011. Four colonies were queenless, two collected from North Yorkshire and two collected from West Yorkshire, all in May 2012. Colonies were required to have brood and a minimum of 150 workers for use in experiments. Each colony was housed in a 31cm x 47cm plastic box with the vertical sides coated in Fluon. A nest was provided constructed from a 9cm diameter Petri-dish with a 5mm deep layer of Gypsum plaster in its base. The sides and lid of the Petri-dish were coated in translucent red acetate (transmission < 20% for λ < 600nm) and access was via a 45mm long, clear plastic tube (see Figure 3). The colonies were maintained at a temperature of 18°C (+/- 2°C) and under a 12hour: 12hour light: dark cycle. Water was available to the colonies *ad libitum*.

The *Lasius* colonies were fed honey water (50% honey, 50% water) twice per week. Shredded dried chicken was provided once per week and a chopped up meal worm was provided once per week. Shredded, dried liver was provided to each colony once per month. Colonies were starved prior to use in experiments, as detailed in the relevant methods section. These husbandry practices are equivalent to those already reported in the scientific literature (see Beckers et al. 1992a; Devigne & Detrain 2002; Czaczkes et al. 2013).

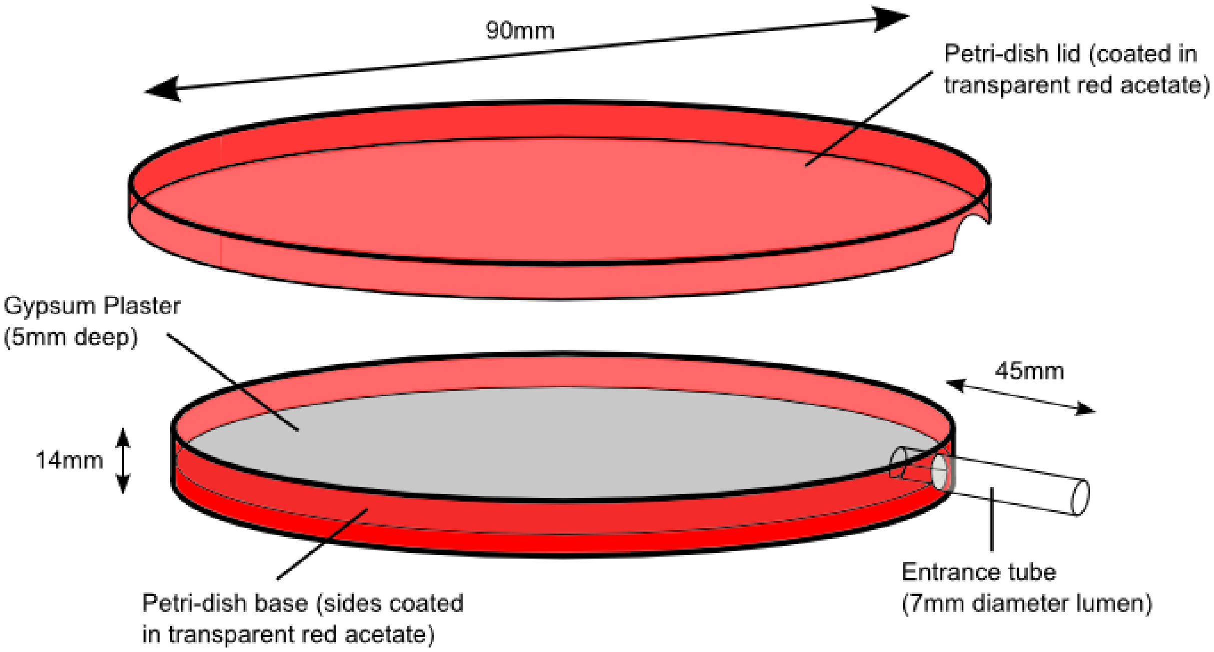


Figure 3 – Nest design for *Lasius niger*

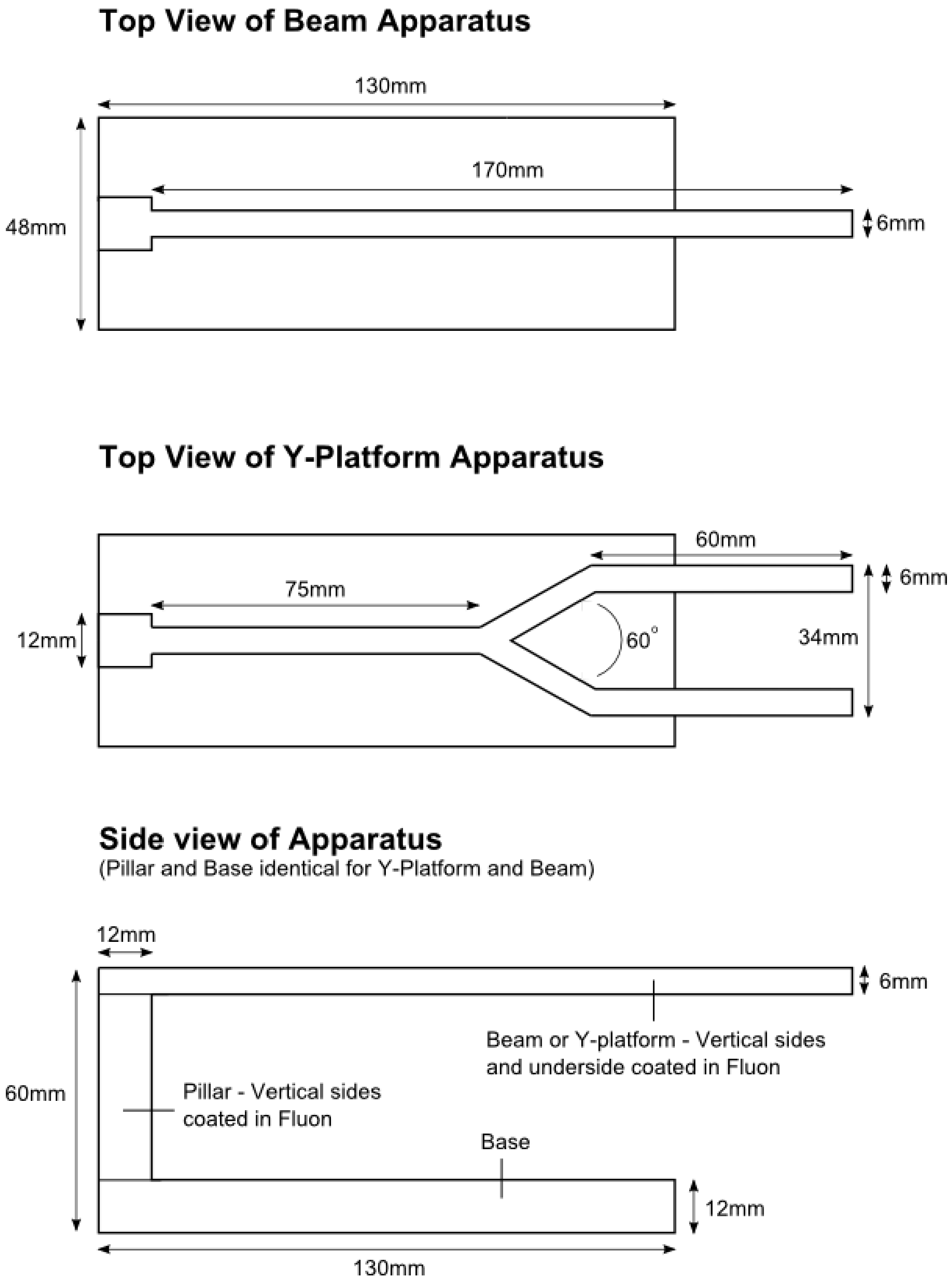
2.2 Apparatus

Two types of aerial platform manufactured from Perspex were used during experiments (see Figure 4 for details of their design). The Beam platform was 6mm wide and 170mm long. The vertical sides and underside were coated with Fluon® to restrict the ants to the upper surface. One end of the Beam was attached to a pillar and base which held the platform off the ground. The vertical sides of the pillar were also coated in Fluon®. How ants accessed the platform varied (see the relevant method section for details).

The second type of aerial platform was a Y-shaped platform. Each branch was 6mm wide and the bifurcation was symmetrical with an angle of 60°. The vertical sides and underside were coated with Fluon® to restrict the ants to the upper surface. The unbranched end of the Y-Platform was attached to a pillar and base which held the platform off the ground. The vertical sides of the pillar were also coated in Fluon®. How ants accessed the platform varied (see the relevant method section for details).

A branch width of 6mm was chosen to concentrate chemical deposits, and to increase the probability that an ant arriving at the bifurcation would be aware that the path split and also be more likely to detect any variation in chemical conditions on those two branches. Traffic congestion has been shown to play an important role in ant foraging decisions (Dussutour *et al.* 2004) but as traffic is only one directional in most experiments (or congestion is controlled where it is not), using narrow branches is not seen as problematic. After the bifurcation, both branches must deviate equally from the main axis of travel so as to not introduce any orientation bias (Gerbier *et al.* 2008). An angle of 60° degrees was chosen (30° deviation per branch) as it reflects the angles naturally occurring in the foraging trails of the Pharaoh's ant (Jackson *et al.* 2004, 2010), and is frequently used in other studies of ant foraging behaviour (Goss *et al.* 1989, Beckers *et al.* 1992a, Dussutour and Nicolis 2009) facilitating comparisons with this work.

Wherever paper was used to capture the chemical signature of the passage of ants clean, Eucalyptus Chlorine Free (ECF) Paper was used. This was to prevent chemicals such chlorine potentially affecting the captured chemicals or any behavioural response to them. The use of other types of paper has been criticised (Jackson *et al.* 2006).



Top View of Beam Apparatus

Top View of Y-Platform Apparatus

Side view of Apparatus

(Pillar and Base identical for Y-Platform and Beam)

Figure 4 – Design of aerial platforms used in experiments

2.3 Statistical methods

Analyses were carried out using R (R Core Team 2013). Details of the tests are given in the relevant chapters and their corresponding appendices.

For each *Monomorium* experiment in Chapter 3 and each *Lasius* experiment in Chapter 4 a generalized linear model (GLM) using a binomial error structure and a logistic link function was fitted and simplified. Where a negative binomial error structure was required for a GLM (see Chapter 5) the package “MASS” (Venables and Ripley 2002) was used to fit the model. These were simplified using the “lrtest()” function from the R package “lmtest” (Zeileis and Hothorn 2002). Analysis of Variance was used to compare population means for some of the results in Chapter 5.

For the *Lasius* experiment detailed in Chapter 6, generalized linear models using a binomial error structure and a logistic link function were fitted and simplified. Non nested models were compared using the Vuong’s Non nested Hypothesis Test (Vuong 1989). This test allows two models to be compared under the null hypothesis that both models are equally good at explaining the observed data. It uses a likelihood ratio based approach based on the Kullback-Leibler information criterion. The greater the magnitude of the test statistic, the better the fit of one model relative to the other. The test requires that the values of the response variable are the same for both models but otherwise works for nested and non nested models. In R, the Vuong’s Non nested Hypothesis Test is found in the package “pscl” (Jackman 2012). Some results in Chapter 6 required the comparison of between-group variances using the “leveneTest” function from the R package “car” (Weisberg and Fox 2010). Population means were then compared using Wilcoxon rank sum test.

Graphs which include histograms and the curves from logistic regression (Chapters 3 and 6) were produced using the R package “popbio” (Stubben and Milligan 2007).

Minimum adequate models are presented in the relevant results sections and details of simplification of the maximal model are given in the associated appendix.

Chapter 3 – When searching for food do workers of the Pharaoh's ant leave a repellent signal to help nestmates avoid researching unrewarding areas?

3.1 Ecological context of experiments.

Pharaoh's ants are opportunistic scavengers relying on the discovery and rapid exploitation of transient food sources (Sudd 1960). Foraging by this species has been shown to use multiple attractive pheromones (Jackson *et al.* 2006) and more recently it has also been shown that a repellent pheromone is used to mark unrewarding trail branches (Robinson et al. 2005) leading to a further increase in the efficiency of a colony's foraging network (Robinson et al. 2008) .

Whilst mechanisms leading to the efficient exploitation of a food source are well documented, mechanisms leading to the efficient discovery of food sources are poorly studied. This chapter presents an experiment to determine whether workers leave a chemical deposit whilst exploring (moving over unmarked substrate) that reduces the likelihood of subsequent nestmates re-exploring the same area. It also presents an experiment to determine whether a similar tactic is used by workers searching for food (moving over home range marked substrate in the absence of trail pheromone) to reduce the likelihood of nestmates re-searching unrewarding areas.

3.2 Set up for experiments

During experiments, the nest box of the Pharaoh's ant colony in use was set up next to a second box with Fluon® coated sides. Access to the second box was via a tube leading from the nest box to the apparatus in the test box. Preliminary analysis showed that the behaviour of Pharaoh's ants was affected by close proximity to nest mates. Left/Right choices at a bifurcation were not random if a naive ant arrived within 0.7 seconds of another ant having made a choice. The second ant was more likely to choose the branch previously taken by the first ant than expected (Pearson's Chi-Squared test with Yates' continuity correction: $n = 24$, $X^2 = 4.039$, d.f. = 1, $p = 0.045$). In order to eliminate this effect in the experimental system, the tube linking the nest and test boxes had the diameter of its lumen restricted to 2mm at the test box end thus reducing the rate at which ants arrived in the test box to less than one per second. In the test box, the entrance tube ended at the start (where the platform connects to the pillar) of the Beam apparatus during the set up phase or the Y- shaped Platform during the test phase. Ants were starved for a minimum of two days before being used to collect data.

3.3 Experiment M1- Does the passage of workers over unmarked substrate result in a chemical deposit that subsequent workers avoid?

Hypothesis: The passage of workers over unmarked substrate results in a chemical deposit that subsequent workers avoid.

Outline: The passage of workers was captured on ECF paper and presented to naive individuals as a choice test on a trail bifurcation. The behavioural response of the naive individuals was recorded.

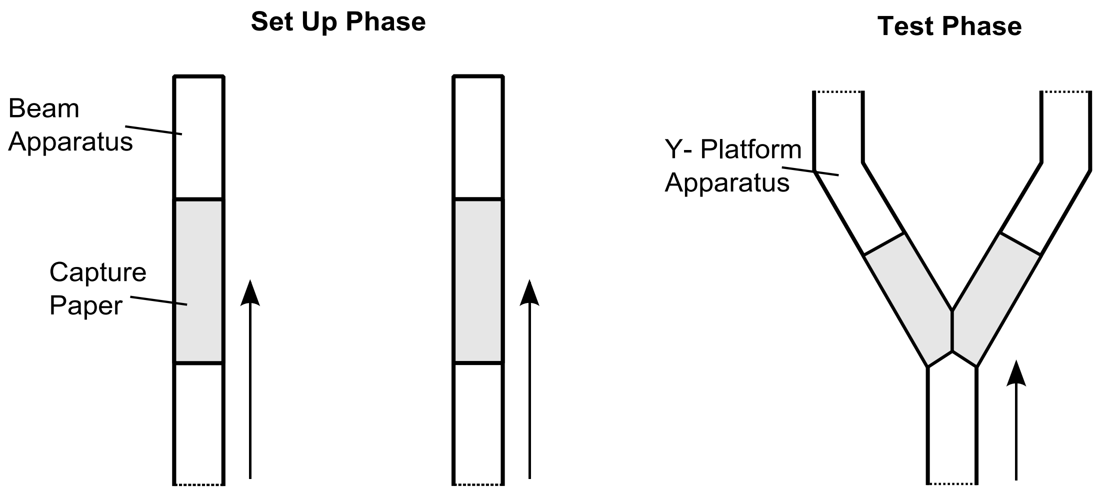
3.3.1 Methods: Set up Phase

The aim of the set up phase was to capture the chemical signature of the passage of ants on 6x20mm pieces of clean, ECF Paper (referred to hereafter as "capture paper"). This was achieved by placing a piece of capture paper on the Beam apparatus and allowing the ants to access it. Previous work has already shown that ants returning along an unrewarding trail can lay a repellent signal (Robinson et al. 2005) so in order to eliminate this effect, ants were only allowed to pass over the paper when travelling away from the nest, and were then removed from the apparatus using an aspirator (see Figure 5). The capture papers were only ever handled with gloves or forceps to prevent human contamination.

Each treatment required a different pair of capture papers to be set up and the number of ants to have crossed each piece depended upon the treatment (see Table 1). There is no *a priori* way to determine how the absolute difference (i.e. number of ants) and the relative difference (i.e. proportion of ants) between two trail branches affect the decision of an ant at the junction. In order to control for this, sixty ants were divided between sides A and B for each treatment, resulting in the absolute and relative difference between sides A and B for all treatments being directly proportional.

Table 1 – Treatments for *Monomorium* Experiments

Treatment	Number of ants to Side A	Number of ants to Side B	Absolute difference between sides	Relative difference (Absolute difference divided by total number of ants)
T1	0	60	60	1
T2	6	54	48	0.8
T3	12	48	36	0.6
T4	18	42	24	0.4
T5	24	36	12	0.2
T6	30	30	0	0



Capture paper Side A and B set up as per Table 1. Ants removed with an aspirator after crossing the paper.

Capture Papers A and B cut to shape and presented and one naive ant allowed to choose a branch.

Figure 5 – Experiment protocol for *Monomorium* experiments

3.3.2 Methods: Test Phase

During the test phase, the two appropriate capture papers from the set up phase were cut to shape and placed on the branches of the Y-maze at the bifurcation (see Figure 5). One naive ant was permitted to proceed to the bifurcation and choose a branch. The ant was deemed to have chosen a branch once all six tarsi were on one piece of paper. The ant was then isolated from its nest until all replicates of that treatment were completed for that colony. Capture papers were discarded after having been tested with one ant. Each replicate was completed in a maximum of twenty minutes (from the first set up ant arriving on the capture paper in the set up phase to the decision ant choosing a branch in the test phase).

Three colonies were used and four replicates of each treatment were performed with each colony (twelve replicates per treatment). Of these four replicates, in two the apparatus was oriented away from the observer, and in two the apparatus was oriented towards the observer. For each orientation Side A was left once and right once. Thus the experimental design was able to control for any bias arising for orientation cues such as light sources in the room or the location of the observer. For each replicate, new pieces of capture paper were set up. The apparatus was cleaned with 80% ethanol between replicates.

3.3.3 Methods: Analysis

For each replicate in the experiment, the total number of ants that had crossed the piece of capture paper presented on the right hand branch during the test phase was divided by sixty (i.e. divided by the sum that crossed the capture papers presented on the left and right hand branches). This gave a value equivalent to the proportion of ants that had crossed the right hand capture paper during the set up phase. For example, if six ants had crossed the piece of paper presented on the right hand branch (and fifty four the paper presented on the left hand branch), the proportion of ants to have previously crossed the right hand branch would be 0.1. “The proportion of ants to have previously crossed the right hand branch” was then used as an explanatory variable in a GLM with “branch choice by the naive test ant” as the response variable. Thus it was possible to analyse how the proportion of ants having previously taken the right had branch affected the probability of a naive ant also taking the right hand branch.

3.4 Experiment M2 - Does the passage of workers over home range marked substrate result in a chemical deposit that subsequent workers avoid?

Hypothesis: The passage of workers over home range marked substrate results in a chemical deposit that subsequent workers avoid.

Outline: The passage of workers was captured on home range marked ECF paper and presented to naive individuals as a choice test on a trail bifurcation. The behavioural response of the naive individuals was recorded.

3.4.1 Protocol

The protocol for the second experiment with the Pharaoh’s ant was identical to the protocol for the first in every respect apart from the use of *clean* ECF paper. In the second experiment, the capture paper had been left (away from food sources) in the nest box of the relevant colony for 24 hours prior to use in the experiment. This was to ensure that the capture paper would be explored and coated with home range markings (Robinson et al. 2005).

3.5 Results from Experiment M1 - Does the passage of workers over unmarked substrate result in a chemical deposit that subsequent workers avoid?

As the proportion of ants that had walked on the right hand piece of capture paper during the set up phase increased, the probability of a test ant taking the right hand branch in the test phase also increased (GLM: X^2= 18.613, d.f. = 1, p < 0.001) (see Figure 6).

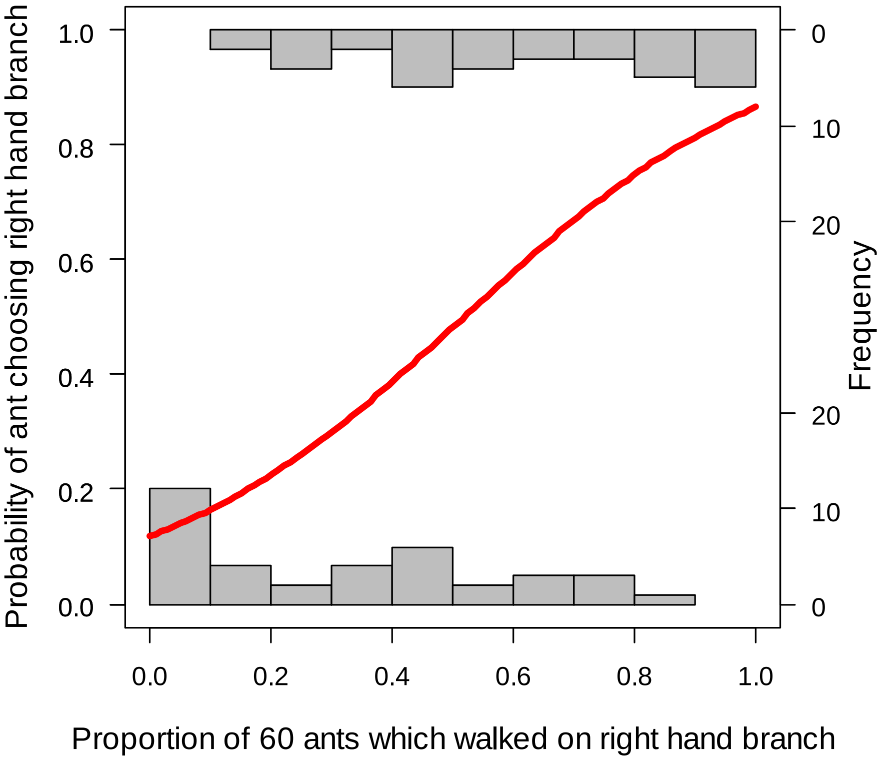


Figure 6 – The effect that the proportion of ants having previously taken the right hand branch has on the probability of a naive forager also taking the right hand branch. (GLM: X^2= 18.613, d.f. = 1, p < 0.001). The histogram represents the number of ants choosing the left hand branch (lower bins) or the right hand branch (upper bins) as the proportion of ant passages over the substrate on the right hand branch (relative to the total number of ant passages on both branches during the set up phase) increases. The line is the fit of the regression model.

3.6 Results from Experiment M2 - Does the passage of workers over home range marked substrate result in a chemical deposit that subsequent workers avoid?

Branch choice was explained by the proportion of ants that had walked over the substrate on the right hand branch during the set up phase; colony; and an interaction between these two terms (see Table 2).

Table 2 – Results of GLM from Experiment M2

Term	**Degrees of Freedom**	**X^2**	**Pr(>Chi)**
Colony	1	0.334	0.846
Proportion of ants to RHS	1	1.991	0.158
Colony : Proportion of ants to RHS	2	6.898	0.032

As can be seen from Table 2, only the interaction term made a significant contribution to model's fit. With two of the colonies, as the proportion of ants that had walked on the right hand piece of capture paper during the set up phase increased, the probability of an ant taking the right hand branch in the test phase also increased. However, with one colony, as the proportion of ants that had walked on the right hand piece of capture paper during the set up phase increased, the probability of an ant taking the right hand branch in the test phase decreased (see Figure 7).

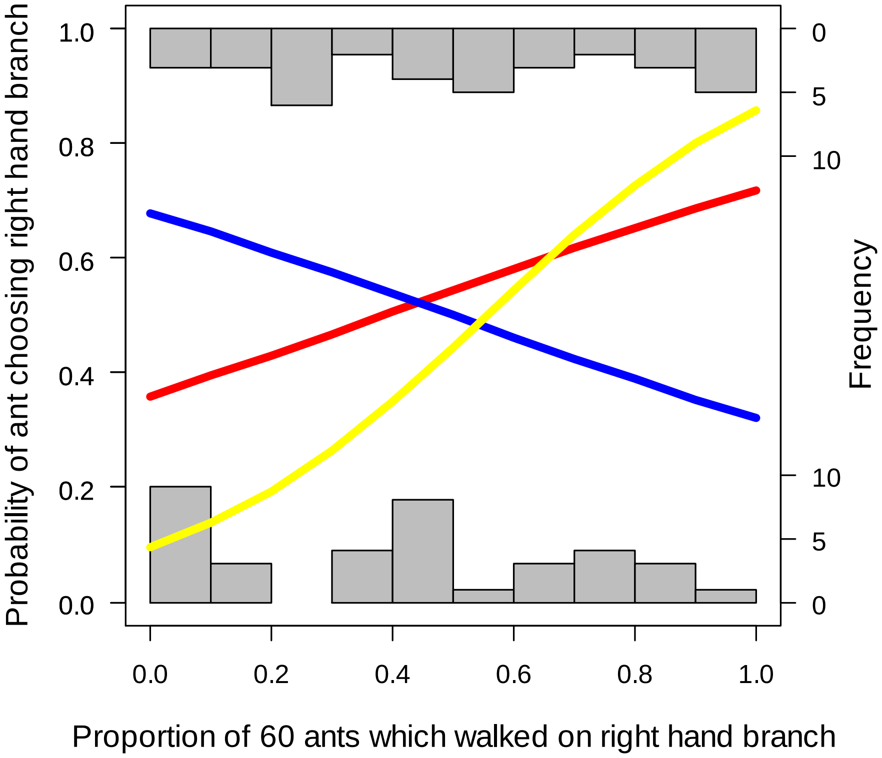


Figure 7 – The combined effect of "colony" and "the proportion of ants to have marked the substrate on the right hand branch" on the probability of a naive forager choosing the right hand branch. The histogram represents the number of ants choosing the left hand branch (lower bins) or the right hand branch (upper bins) as the proportion of ant passages over the substrate on the right hand branch (relative to the total number of ant passages on both branches during the set up phase) increases. The line is the fit of the regression model. The lines represent the fit for each colony in the regression model (see Table 2 for model fit).

3.7 - Discussion of *Monomorium* Results

Experiment M1 shows that as the proportion of ants to have previously crossed the substrate on the right hand branch increases, the probability of a naive ant that arrives at the bifurcation taking the right hand branch increases. This is strong evidence that the outwards passage of Pharaoh's ants on unmarked substrate leaves an attractive signal that affects the navigation of subsequent ants, contradicting the initial hypothesis.

Experiment M2 found a large amount of between colony variation in the effect of the passage of workers over home range marked substrate on the orientation of subsequent workers. It is not possible to conclude based on the available evidence whether the outwards passage of Pharaoh’s ants on home range marked substrate leaves a signal that can affect the navigation of subsequent ants, because there is so much between-colony variation. There is insufficient evidence to support the initial hypothesis.

The results from these experiments show that workers of the Pharaoh’s ant are recruited to unexplored substrate and cannot eliminate the possibility that the passage of workers over home range marked substrate also results in the presence of a chemical attractant. There is no evidence that *M. pharaonis* workers improve their search strategy by avoiding previously searched substrate. Instead it appears that the strategy employed is the rapid exploitation of newly discovered resources as previously suggested (Fourcassié & Deneubourg 1992; Fourcassié & Deneubourg 1994). By recruiting nest mates to unexplored areas, workers ensure that if a food source is discovered, exploitation of it can begin immediately. In order to investigate this process in more detail a smoked glass substrate (Jackson and Châline 2007) or video recording (Fourcassié & Deneubourg 1994) would be required.

Chapter 4 – Does the passage of *Lasius niger* over unmarked substrate in either the presence or absence of trail pheromone result in a chemical deposit that subsequent workers avoid, and if so, can the likely identity of this chemical be determined?

4.1 Ecological context of experiments

Home range marking has been shown to occur in *Lasius niger* passively through cuticular hydrocarbons rubbing off onto the substrate from tarsi (Lenoir *et al.* 2009). As *L. niger* are central place foragers, a greater density of workers is found closer to the nest and this leads to a home range marking gradient with areas closer to the nest having a greater concentration of "footprint hydrocarbons" (Devigne & Detrain 2006). An ant encountering unmarked substrate can be seen as "exploring" and there is evidence that *L. niger* can recruit nest mates to aid in the exploration of large patches of novel territory (although no trail laying behaviour was observed) (Devigne & Detrain 2002). This chapter presents experiments to determine whether workers leave a chemical deposit whilst exploring (moving over unmarked substrate) that reduces the likelihood of subsequent nestmates re-exploring the same area.

This chapter also presents experiments to examine whether whilst following a pheromone trail workers leave a chemical deposit (other than trail pheromone) that can affect the subsequent foraging decisions of nestmates. During all the experiments in this chapter, unless workers are being deliberately used to set up pheromone trails, they have been starved so that they will not exhibit pheromone trail laying behaviour (Beckers *et al.* 1992b).

4.2.1 General techniques for use in *Lasius* experiments.

All the experiments presented here had a set up phase and a test phase. The aim of the set up phase was to capture the chemical signature of the passage of ants travelling away from the nest on 6x20mm pieces of clean, ECF paper. "Capture" papers crossed by known numbers of ants could then be arranged at a Y-junction as a choice test for naive ants. For all experiments, capture papers and approach papers were only handled with forceps or gloves (to prevent contamination) and all apparatus was wiped with 80% ethanol between replicates. Colonies were starved for a minimum of three days before use.

4.2.1.1 General techniques (set up phase)

Each set up phase required the use of the Beam Apparatus which was placed in the nest box of the colony in use. Ants were able to access the beam via a 6mm wide strip of ECF "approach" paper. The approach paper was folded twice so that it rested against: the horizontal surface of the beam; the vertical surface at the back of the pillar; and the floor of the nest box (see Figure 8).

Approach paper was either unmarked, or marked with trail pheromone (depending upon the requirements of the experiment).

Capture papers were placed on the Beam so that they overlapped (by 10mm) with the approach paper. This ensured substrate continuity as the ant walked along the beam. A 20mm uncovered section of beam was left after the capture paper so ants could be brushed off the apparatus without disturbing the placement of either the capture paper or the approach paper (see Figure 8).

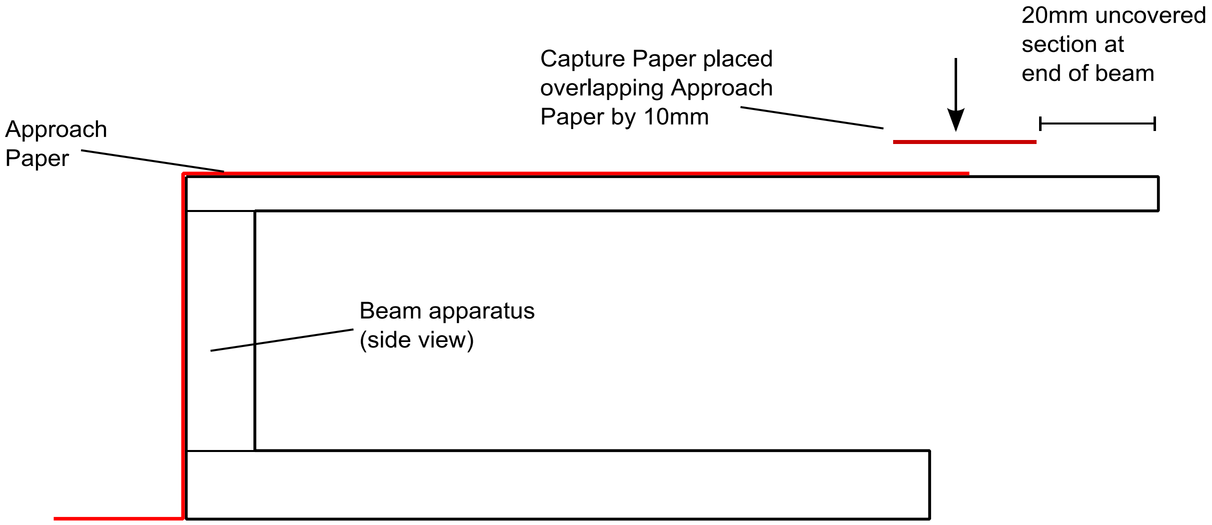


Figure 8 – Arrangement of approach paper and capture paper during the set up phase(s) of *Lasius* Experiments 1 – 3

If a pheromone trail was required on the approach paper an additional step was needed before capture paper was placed on the Beam Apparatus. The Beam Apparatus and a strip of approach paper were arranged in a Fluon® coated box. A 1M sucrose feeder was placed on the exposed end of the beam and four ants were transferred to the feeder from their nest box using a cocktail stick. The ants were allowed to feed and depart along the beam laying a pheromone trail. (These ants were kept isolated from their colony until the experiment was completed to prevent contamination of capture paper with trail pheromone). The sucrose feeder was then removed from the Beam Apparatus and a piece of capture paper placed on it as per Figure 8.

An inequality in the amount of trail pheromone on the two branches after the bifurcation would be likely to introduce a significant source of error to the experiments. To be certain that the two branches had the same amount, a fixed volume of known concentration would have to be applied to each. Unfortunately, however, the complex nature of the *L. niger* trail pheromone (see Figure 1) means that synthesizing it is beyond the scope of this project and unfortunately the chemical is not commercially available. As a consequence, the experiment must rely on a pheromone trail leading up to the bifurcation but not proceeding beyond it. Whilst this approach is a compromise, it is deemed suitable due to the use of discrete marks rather than a continuous pheromone trail by *L. niger*. Workers passing beyond the last pheromone mark on the approach paper (and onto the capture paper) are likely to behave as if they were still following a trail (and not as if they had passed beyond the end of one). This is because the 20mm length of the capture papers is well within the inter-mark distance of low density pheromone trails (Beckers *et al.* 1993).

The ants in the set up phases of experiment L1, L2, L3, L4, and L5 were only ever allowed to pass over the capture paper in one direction as a repellent pheromone has been shown to be used in some species to mark unrewarding trail branches (Robinson *et al.* 2005). If the *L. niger* workers had been allowed to return along the beam, they potentially could have behaved in a similar manner and contaminated the experiment.

4.2.1.2 General Techniques (test phase).

Each test phase required the use of the Y-Platform Apparatus which was placed in the nest box of the colony in use. Access was via a strip of approach paper folded as per Figure 8. Before a pair of capture papers could be placed on the Y-Platform, each had a corner removed (see Figure 9). The pair of cut capture papers was then arranged at the bifurcation overlapping the approach paper to ensure continuity of substrate (see Figure 9).

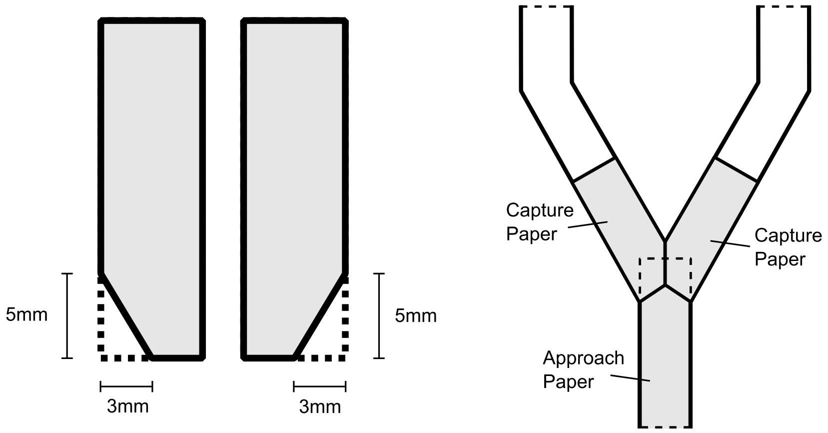


Figure 9 – Directions for cutting capture paper ready for presentation at the bifurcation (L). The arrangement of capture papers on top of approach paper (R).

Once the capture papers were arranged, one naive ant was permitted to proceed to the bifurcation and choose a branch. The ant was deemed to have chosen a branch once all six tarsi were on one piece of paper. The ant was then marked with a paint spot or isolated from its nest until all replicates of the treatment had been completed for that colony.

4.2.1.3 Replicates

For each replicate, new pieces of capture paper were set up. This was because through choosing a branch in the test phase, the naive ant was potentially changing the chemical conditions on that branch. Each replicate was completed in a maximum of twenty minutes (from the arrival of the first ant in the set up phase to the decision in the test phase) in order to reduce the likelihood that chemical evaporation would affect the results (Beckers *et al.* 1992a).

Four *L. niger* colonies were used and twenty four replicates of each experiment were performed with each colony (ninety six replicates per experiment in total). Of these twenty four replicates, during twelve, the main light source was on the left of the apparatus and in twelve it was on the right. Of these sets of twelve, during six the apparatus was oriented facing the observer and in six it was oriented away from the observer. Thus the experimental design was able to control for any bias arising for orientation cues such as light sources in the room or the location of the observer.

4.2.2 Experiment L1 - Do exploring workers prefer unmarked substrate, or substrate previously crossed by 20 workers?

Hypothesis: Whilst exploring, workers mark substrate as “explored”. Therefore, given a choice, naive workers would be more likely to choose the branch with unmarked substrate.

Outline: Naive ants approach a bifurcation on unmarked substrate and then choose between a branch that has substrate previously walked on by twenty (non trail laying) ants and a branch with substrate that has not been walked on by ants.

Unmarked approach paper and capture paper were arranged on the beam apparatus (see 4.2.1.1) and twenty ants were allowed to walk to the end of the beam before being brushed off (see Figure 10). The capture paper crossed by twenty ants, an unmarked piece of capture paper, and an unmarked piece of approach paper were presented on the Y- platform (see 4.2.2.2). One naive ant was permitted to proceed to the bifurcation along unmarked approach paper and choose a branch (see Figure 10). The time elapsed since the first ant arrived at the capture paper during the set up phase was also recorded. See Table 3 for controls.

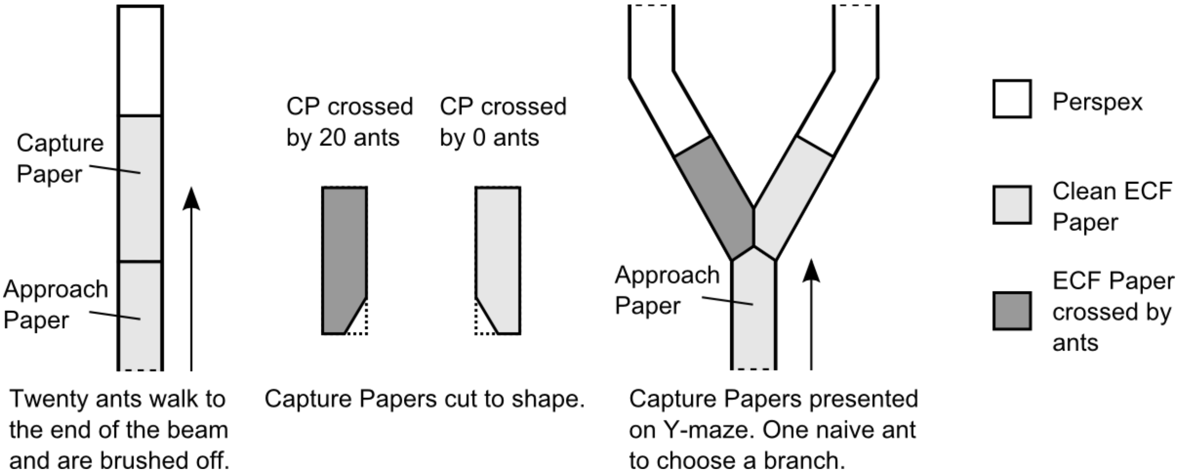


Figure 10 – The protocol for experiment L1

Table 3 - Controls to reduce the effect of environmental variables on the experiments L1-L6

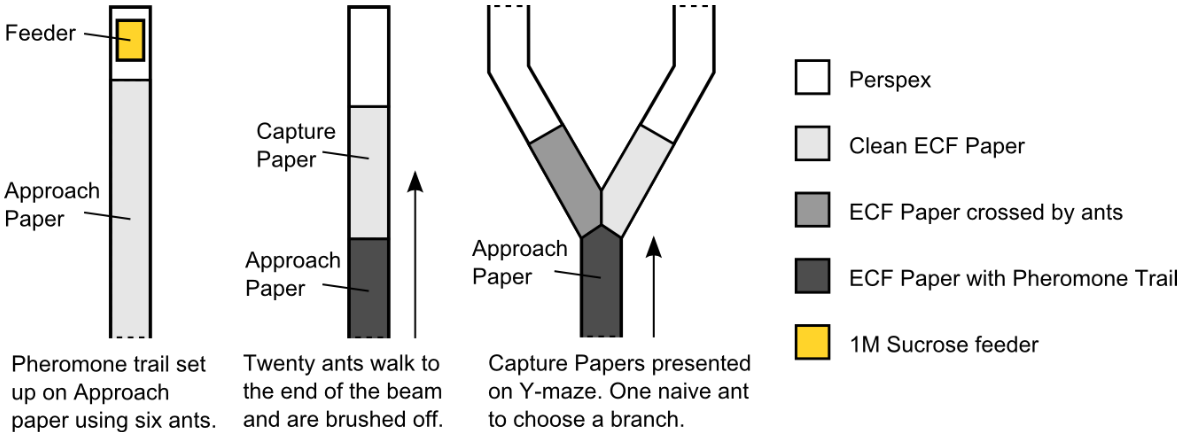
Side with paper crossed by: 20 ants (Exp L1, L2). Side with paper crossed by: 15 ants (Exp L4). Side set up first (Exp L3). Side with high home range marking concentration capture paper (Exp L5, L6).	Direction of Light Source	Travel relative to observer	Number of replicates
Left	Left	Towards	3
Left	Left	Away	3
Left	Right	Towards	3
Left	Right	Away	3
Right	Left	Towards	3
Right	Left	Away	3
Right	Right	Towards	3
Right	Right	Away	3

4.2.3 Experiment L2 - Do naive workers following a pheromone trail prefer a branch previously walked over by twenty unfed ants or a branch previously walked over by no ants?

Hypothesis: The passage of unfed (and hence not trail laying) ants along a pheromone trail results in a chemical deposit that subsequent ants can detect and avoid. Therefore, given a choice, naive workers would be more likely to choose the branch with unmarked substrate.

Outline: This experiment is identical to experiment L1 with the following exceptions. During the set up phase ants arrived at the capture paper by following pheromone trail marked approach paper (not unmarked paper). During the test phase the naive worker arrived at the bifurcation by following trail pheromone marked approach paper (not unmarked paper).

Capture paper and trail pheromone marked approach paper were arranged on the beam apparatus (see 4.2.1.1) and twenty ants were allowed to walk to the end of the beam before being brushed off (see Figure 11). The capture paper crossed by twenty ants, an unmarked piece of capture paper, and a trail pheromone marked piece of approach paper were presented on the Y- platform (see 4.2.1.2). One naive ant was permitted to proceed to the bifurcation along unmarked approach paper and choose a branch (see Figure 11). The time elapsed since the first ant arrived at the capture paper during the set up phase was also recorded. See Table 3 for controls.



Pheromone trail set up on Approach paper using six ants.

Twenty ants walk to the end of the beam and are brushed off.

Capture Papers presented on Y-maze. One naive ant to choose a branch.

Figure 11 – The protocol for experiment L2

4.2.4 Experiment L3 - Can a decay of the effect of the signal that was observed in experiment L2 be detected?

Hypothesis: The effect of the signal observed in experiment L2 decays quickly so naive ants given the choice between two branches sequential set up and each crossed by ten ants will be more likely to choose the branch set up first (i.e. the branch where the signal has been decaying the longest).

Outline: Naive ants approach a bifurcation by following a trail pheromone. They choose between two branches, both without trail pheromone but both having substrate that has previously been walked over by ten unfed ants.

Capture paper and trail pheromone marked approach paper were arranged on the beam apparatus (see 4.2.1.1) and ten ants were allowed to walk to the end of the beam before being brushed off (see Figure 12). This piece of capture paper was set aside and the process repeated. The two pieces of capture paper crossed by ten ants and a trail pheromone marked piece of approach paper were presented on the Y- platform (see 4.2.1.2). One naive ant was permitted to proceed to the bifurcation along unmarked approach paper and choose a branch (see Figure 12). See Table 3 for controls.

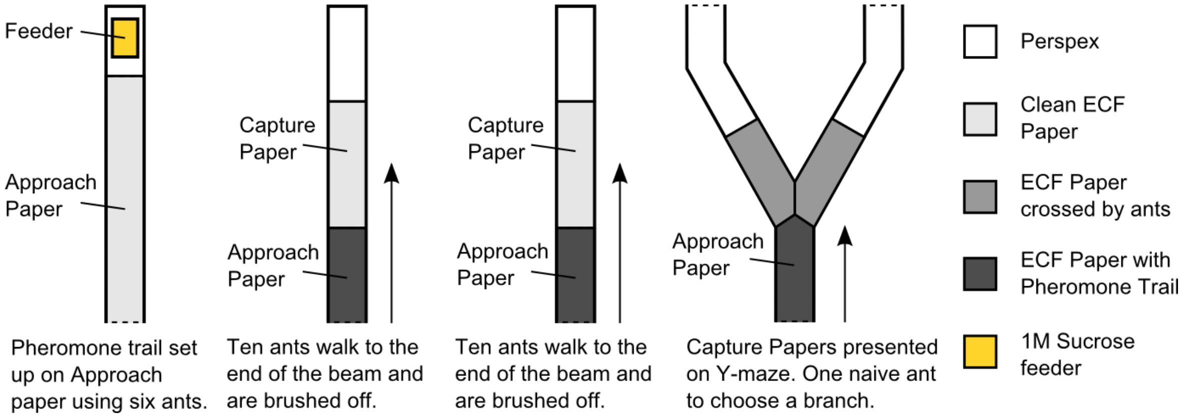


Figure 12 – The protocol for experiment L3

4.2.5 Experiment L4 - Is the effect observed in experiment L2 a result of paper crossed by unfed ants being repellent, or a result of unmarked paper being attractive?

Hypothesis: The effect of the signal observed in experiment L2 is a response to a repellent deposit left by the passage of ants over substrate, not unmarked substrate being attractive. Given a choice between paper crossed by fifteen ants and paper crossed by five ants, a naive ant will choose the paper crossed by five ants (i.e. the least repellent).

Outline: Naive ants approach a bifurcation by following a trail pheromone. They choose between two branches, both without trail pheromone but one having previously been walked over by fifteen unfed ants and the other with substrate that has previously been walked over by five unfed ants.

Capture paper and trail pheromone marked approach paper were arranged on the beam apparatus (see 4.2.1.1) and fifteen ants were allowed to walk to the end of the beam before being brushed off (see Figure 13). This piece of capture paper was set aside and a new piece of capture paper was placed on the beam. Five ants were allowed to walk to the end of the beam before being brushed off. The piece of capture paper crossed by fifteen ants, the piece of capture paper crossed by five ants and a trail pheromone marked piece of approach paper were presented on the Y- platform (see 4.2.1.2). One naive ant was permitted to proceed to the bifurcation along unmarked approach paper and choose a branch (see Figure 13). See Table 3 for controls.

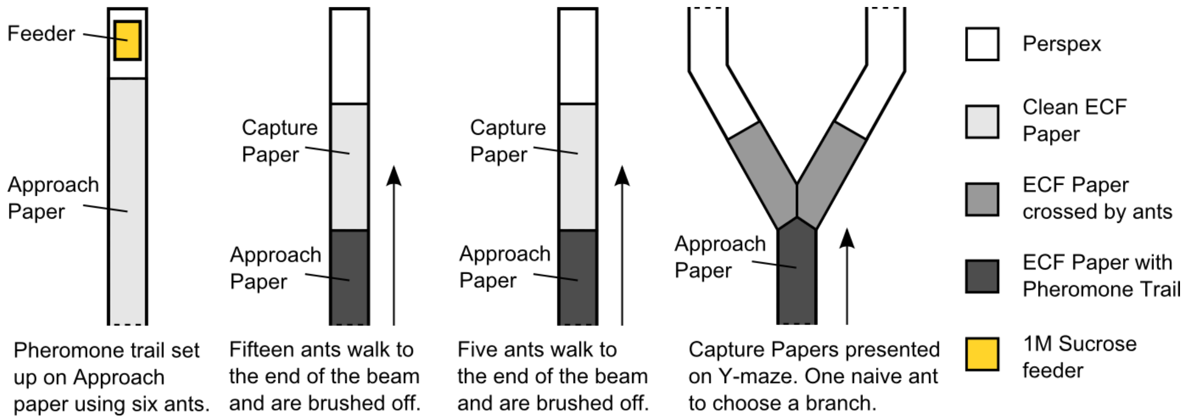


Figure 13 – The protocol for experiment L4

4.2.6 Experiment L5 - Can the effect observed in experiment L1 be explained by the presence of footprint hydrocarbons deposited by the twenty ants during the set up phase?

Hypothesis: Given a choice between a branch with a high footprint hydrocarbon concentration and a low footprint hydrocarbon concentration, naive workers will choose the branch with a high footprint hydrocarbon concentration. (Hydrocarbons attract exploring workers).

Outline: Capture paper was set up with a high home range marking concentration or a low home range marking concentration. These were presented on competing branches after a bifurcation and a naive ant allowed approach them on unmarked substrate and choose a branch.

Home range marking was collected on capture papers by leaving them in the nest box for twenty hours prior to use in the experiment. Papers were marked with either a "high" concentration (by being left 25mm from the nest entrance) or a "low" concentration (by being left 200mm from the nest entrance) (see Figure 14). A piece of high home range marking concentration capture paper and a piece of low home range marking concentration capture paper were presented on the Y-platform (see 4.2.1.2). One naive ant was permitted to proceed to the bifurcation along unmarked approach paper and choose a branch (see Figure 14). See Table 3 for controls.

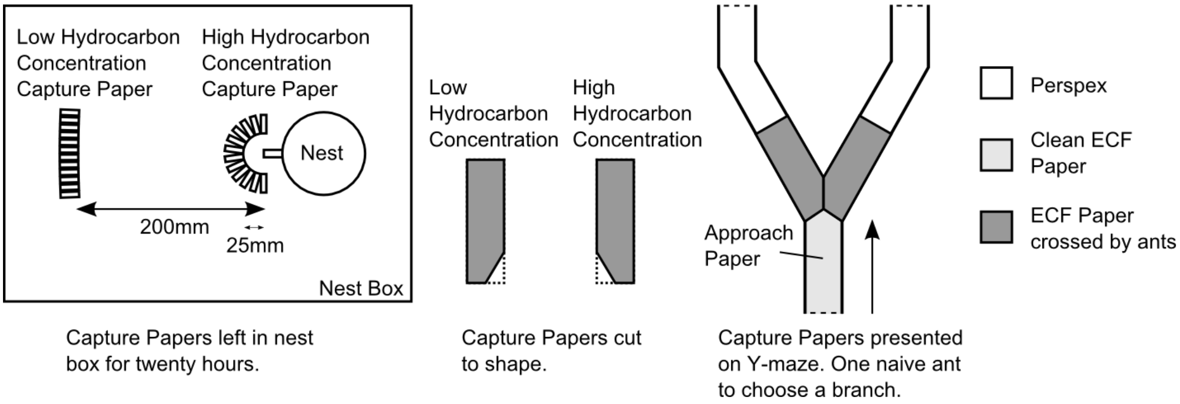


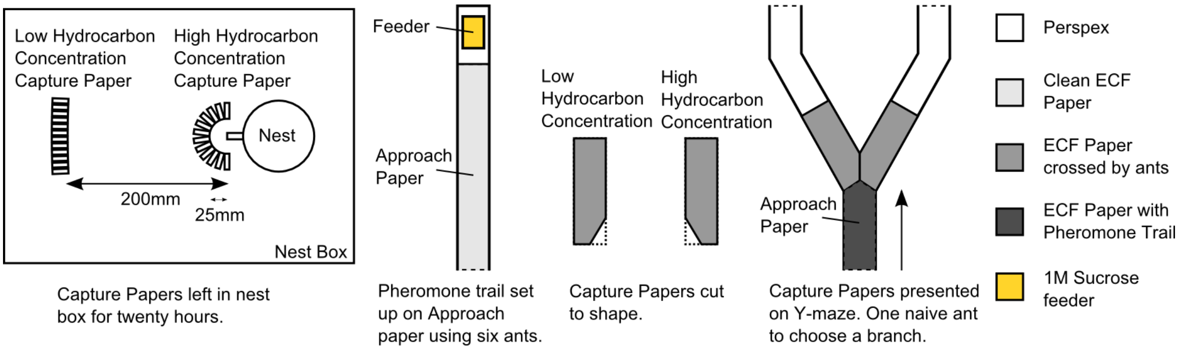
Figure 14 – The protocol for experiment L5

4.2.7 Experiment L6 - Can the effect observed in experiment L2 be explained by the presence of footprint hydrocarbons deposited by the twenty ants during the set up phase?

Hypothesis: Given a choice between a branch with a high footprint hydrocarbon concentration and a low footprint hydrocarbon concentration, naive workers following a pheromone trail will choose the branch with a high footprint hydrocarbon concentration. (Foragers, on their way to food, are repelled by footprint hydrocarbons).

Outline: Capture paper was set up with a high home range marking concentration or a low home range marking concentration. These were presented on competing branches after a bifurcation and one naive ant was allowed to approach them along trail pheromone marked substrate and choose a branch.

High home range marking concentration capture papers and low home range marking concentration papers were set up as detailed in 4.2.6. A pheromone trail was also set up on an approach paper. (See Figure 15). A piece of high home range marking concentration capture paper and a piece of low home range marking concentration capture paper were presented on the Y-platform (see 4.2.1.2). One naive ant was permitted to proceed to the bifurcation along trail pheromone marked approach paper and choose a branch (see Figure 15). See Table 3 for controls.



Capture Papers left in nest box for twenty hours.

Pheromone trail set up on Approach paper using six ants.

Capture Papers cut to shape.

Capture Papers presented on Y-maze. One naive ant to choose a branch.

Figure 15 – The protocol for experiment L6

4.2.8 Summary of the main differences between experiments L1-L6

Table 4 - Summary of the main differences between experiments L1-L6

Experiment	No. ants to side 1 (or home range marking concentration)	No. ants to side 2 (or home range marking concentration)	Side set up first	Pheromone present on approach paper?
L1	20	0	1	No
L2	20	0	1	Yes
L3	10	10	1	Yes
L4	5	15	2	Yes
L5	High	Low	N/A	No
L6	High	Low	N/A	Yes

4.3 Results

Details of the simplification of the statistical models used are given in Appendix 2.

4.3.1 Experiment L1 - Do exploring workers prefer unmarked substrate, or substrate previously crossed by twenty workers?

Naive ants were more likely to choose the branch with substrate previously walked on by twenty ants than the branch with unmarked substrate (GLM: X^2= 5.145, d.f. = 1, p = 0.023). Figure 16 (first column) shows the proportion of ants that chose either substrate type.

4.3.2 Experiment L2 - Do naive workers following a pheromone trail prefer a branch previously walked over by twenty unfed ants or a branch previously walked over by no ants?

Naive ants were more likely to choose the branch with unmarked substrate than the branch with substrate previously walked on by twenty ants (GLM: X^2= 4.205, d.f. = 1, p = 0.040). Figure 16 (third column) shows the proportion of ants that chose either substrate type.

4.3.3 Experiment L3 - Can a decay of the effect of the signal that was observed in experiment L2 be detected?

Branch choice was not affected by which branch had the substrate most recently walked on by ten workers (GLM: X^2= 1.110, d.f. = 1, p = 0.292).

4.3.4 Experiment L4 - Is the effect observed in experiment L2 a result of paper crossed by unfed ants being repellent, or a result of unmarked paper being attractive?

Naive ants were more likely to choose the branch with substrate previously walked on by five ants than the branch with substrate previously walked on by fifteen ants (GLM: X^2= 8.350, d.f. = 1, p = 0.004). Figure 16 (fourth column) shows the proportion of ants that chose either substrate type.

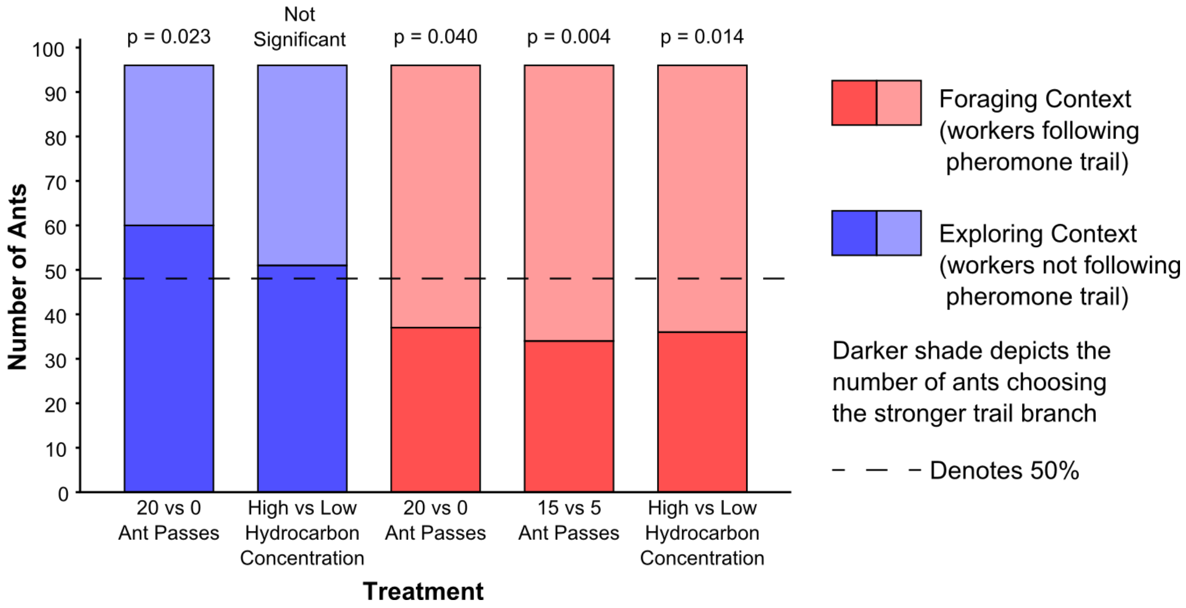


Figure 16 – The effect of substrate type on the bifurcation branch choices of naive workers of the ant *Lasius niger*. Each treatment presented a choice between: a branch with paper coated in a high footprint hydrocarbon concentration and a branch with paper coated in a low footprint hydrocarbon concentration; or a choice between two branches, each with substrate previously walked across by fixed number of nestmates (differing between the two branches). In three of the treatments (shown in red) a pheromone trail led up to the bifurcation whereas in the other two (shown in blue) unmarked substrate led up to the bifurcation.

4.3.5 Experiment L5 - Can the effect observed in experiment L1 be explained by the presence of footprint hydrocarbons deposited by the twenty ants during the set up phase?

Branch choice was not affected by which branch had the substrate with a high hydrocarbon concentration (GLM: X^2= 0.379, d.f. = 1, p = 0.538). Figure 16 (second column) shows the proportion of ants that chose either substrate type.

4.3.6 Experiment L6 - Can the effect observed in experiment L2 be explained by the presence of footprint hydrocarbons deposited by the twenty ants during the set up phase?

Naive ants were more likely to choose the branch that had substrate with a low hydrocarbon concentration (GLM: X^2= 6.075, d.f. = 1, p = 0.014). Figure 16 (fifth column) shows the proportion of ants that chose either substrate type.

4.4 Discussion

4.4.1 Evidence for negative chemotaxis as a response to footprint hydrocarbons by pheromone trail following workers.

Experiment L2 demonstrated that when workers of the ant *Lasius niger* are following a pheromone trail and reach a trail bifurcation, they are more likely to take a branch previously walked along by no ants than by twenty ants. The ants making the bifurcation decisions had not previously been given access to the Y-shaped platform so their choices could not have been influenced by experience (either positive or negative). The experiment was carefully designed to ensure that any possible orientation cue that could have introduced bias to the results was controlled (see Table 3). Additionally, each test ant was only used once. Together these measures ensured that no internal or external factor other than the capture papers on the two branches should have influenced the results. The result from experiment L2 therefore seems to indicate that there is something about the passage of ants along a pheromone trail that repels subsequent foragers. However, the result could also interpreted as indicating that unmarked substrate is attractive rather than the branch previously walked upon by ants is repellent. Experiment L4 demonstrated that an unmarked branch is not required to observe an uneven distribution of test ants at the bifurcation. Where naive ants were presented with a choice between a branch previously walked along by fifteen ants and another previously walked along by five ants, the naive ants were more likely to choose the branch with five ants. This supports the hypothesis that the passage of ants along a foraging trail leaves a weak repellent cue. During the set up phase of experiments L2 and L4 the ants were brushed off the apparatus after crossing the capture paper. This eliminates the possibility that the workers were returning and marking the capture paper as part of an unrewarding trail branch (Robinson *et al.* 2005). The repellent cue had to come from the outwards passage of ants and as olfaction is the primary sense of ants (Gronenberg and Hölldobler 1999), it is highly probable that a chemical cue is in use.

Experiment L3 demonstrated that where ten ants have previously walked along each branch, naive ants showed no preference for either. The removal of the term “side set up first” and interaction term between “decision time” and “side set up first” during model simplification indicates that the experimental design was not sensitive to decay of the effect of the repellent cue. If it had been, one would have expected that the later the decision time, the higher the probability that an ant would choose the side set up first.

Experiment L6 was carried out to determine whether “footprint hydrocarbons” can act as a repellent cue (and explain the effect observed in experiments L2 and L4). Although the result is only qualitative (comparing a high and a low concentration) it supports the hypothesis that in the presence of trail pheromone footprint hydrocarbons have a repellent effect on foraging ants.

Cuticular hydrocarbons have a low volatility (Lenoir *et al.* 2009, Czaczkes *et al.* 2012) so the result from experiment L3 would also support the hydrocarbon hypothesis.

Lasius niger has already been shown to use the background home range marking gradient to orient towards the nest when returning with food (Devigne & Detrain 2006). Perhaps the novel observation that hydrocarbons appear to repel trail following workers is merely an extension of the orientation behaviour. If the background home range marking gradient is being used to polarise pheromone trails, then travelling down the gradient would be the usual behaviour of an ant travelling from the nest to food. These results must be treated with some caution, however, because the pheromone trail did not continue after the bifurcation. Could it be instead that the results are explained by the ants behaving as if they have lost the pheromone trail? This question cannot be answered from the available data but it would seem unlikely that an ant having lost the trail it is following would orient away from areas where a greater number of nest mates had been. As mentioned in the introduction, the experiments were designed so that the capture papers were shorter than the inter mark spacing on a low density pheromone trail (Beckers *et al.* 1992b). Therefore an ant following a pheromone trail along the approach paper would not necessarily "expect" to encounter a pheromone mark on the capture paper.

4.4.2 Evidence for positive chemotaxis towards substrate recently explored by nestmates.

Experiment L1 demonstrated that when workers of the ant *Lasius niger* arrive at a trail bifurcation on unmarked substrate, they are more likely to take a branch previously walked along by twenty ants than by no ants. This refutes the hypothesis that exploring workers mark the substrate with repellent chemicals to avoid re-exploration. Instead, the result from experiment L1 supports other studies which have shown *Lasius niger* can perform mass exploration of novel territory without the use of trail pheromone (Devigne & Detrain 2002). The result from experiment L5 is surprising however, because no evidence was found to support the hypothesis that footprint hydrocarbons attract exploring workers. This contradicts the mechanism proposed by Devigne and Detrain (2002) to explain the regulation of mass exploration. The paper suggested that footprint hydrocarbons are used to recruit nestmates to unmarked areas and another study (Devigne & Biseau 2012) has shown that in a choice test *L. niger* workers preferentially choose home range marked substrate over unmarked substrate.

At first I considered the possibility that as my results are qualitative (comparing unknown "high" and "low" hydrocarbons concentrations) the difference between the two branches was insufficient for the ants to detect and choose between. However, an identical protocol (except for the use of trail pheromone) was used in experiment L6 and ants did choose between the two branches so this seems unlikely.

Closer examination of the results in Devigne and Detrain (2002) make a hydrocarbon hypothesis seem unlikely. The results show that when a new area becomes available, the maximum recruitment to it occurs very rapidly (in the first five minutes) and then gradually reduces over the next fifty minutes. As cuticular hydrocarbons have a low volatility and are deposited passively, it would seem more plausible for the recruitment to the novel area to build up slowly. Moreover, if hydrocarbons marked substrate is attractive, why would workers leave it to explore unmarked territory in the first place?

The difference between the hydrocarbon response presented here (experiment L5) and the Devigne and Biseau study (2012) can perhaps be explained by the context in which workers arrived at the choice test. In experiment L5, workers arrived at the bifurcation of their own volition (the Y-platform was in the nest box) whereas in the Devigne and Biseau study, ants were removed from their nest using forceps (Devigne, *pers. comm*.) and placed in an unmarked habituation arena before proceeding to the choice test. Being picked up and then placed (albeit carefully) in an unfamiliar location is no doubt stressful so it is perhaps unsurprising that workers chose to return to marked substrate.

During the set up phase for experiment L1 some ants were observed behaving erratically. An erratic ant moved more quickly than nest mates and also appeared to vibrate its gaster. Increased running speed has been reported as a behavioural response to alarm pheromone in *Lasius niger* (Bergström and Löfqvist 1970) and as the alarm pheromone (undecane) is secreted from the Dufour gland (Bergström and Löfqvist 1970, Attygalle *et al.* 1987), hypothesising that the erratically behaving ants were “alarmed” does not seem unreasonable. The experiments examining behaviour of ants on unmarked and home range marked paper in the presence and absence of trail pheromone are presented in Chapter 5.

Chapter 5 – the behaviour of ants on unmarked and home range marked substrate in the presence or absence of trail pheromone.

5.1 General Introduction

This chapter presents experiments designed to test whether *Lasius niger* workers behave in an alarmed fashion in response to unmarked substrate. It is worth noting however that the term “alarm behaviour” when applied to ants is used quite loosely in the published literature and can cover a wide variety of behavioural responses. For reviews of the behavioural responses, see Meer & Alonso (1998) and Parry & Morgan (1979).

Positive chemotaxis by *Lasius niger* workers has been demonstrated in response to “Giftsekret” (poison secretions) (Maschwitz 1964). The same paper also describes an alarm response behaviour in *Lasius niger* during which the alarmed ant extrudes a drop of moisture containing both alarm and toxic substances from the tip of its abdomen. However, I have only ever observed this behaviour in ants being held with forceps. Beyond positive chemotaxis, and increased running speed (Bergström and Löfqvist 1970), the behavioural response to alarm pheromone is poorly studied in *Lasius niger* but we can deduce some of the likely responses based on experiments with sister species. *Lasius claviger* individuals tended to move rapidly and “deliberately” towards the source of the alarm pheromone whereas the movement of *Lasius alienus* was more “erratic” (Regnier and Wilson 1969). These two strategies have been labelled as “panic alarm” (*Lasius alienus*) and “aggressive alarm” (*Laius claviger*). Aggressive alarm is associated with species that have high density of individuals within a nests leading to a “stand and fight” tactic whereas panic alarm is associated with species that have a low density of individuals within the nest leading to an “escape” tactic (Wilson & Regnier 1971). *Lasius niger* is an aggressive ant with very concentrated brood areas within the nest (Brian *et al.* 1965). We would therefore predict the alarm behaviour of this species to be increased speed, reduced path sinuosity and fewer stops.

5.2 Gaster Vibrations

Initial video analysis of the erratic behaviour showed that an erratically behaving ant was indeed raising and dropping its gaster (see Figure 17). The recording equipment was limited to twenty five frames per second so determining the exact frequency of the vibrations was not possible but a complete cycle (from the normal position, to raised, then dropped and back to normal) took no more than five frames (0.2 seconds). The video quality was also too low to determine the position of the legs or antennae during the cycle hence they have been omitted from the diagram (see Figure 17). The important feature of the behaviour was change in position of the gaster relative to the rest of the body. (When an ant is travelling quickly it sometimes appears that the gaster is moving up and down but it stays at a fixed angle relative to the body). It was not possible to determine from the videos, whether when dropped, the gaster touched the substrate upon which the ant was walking. If it did touch the substrate, the vibrations could have an additional recruitment function as seen in some other species of ant (Baroni-Urbani *et al.* 1988). Such vibrations are exhibited by workers of the ant *Camponotus rufifmur* in response to alarm and can attract nest mates (*personal observation*). The term “stridulation” is deliberately not used here as no sound recordings were made.

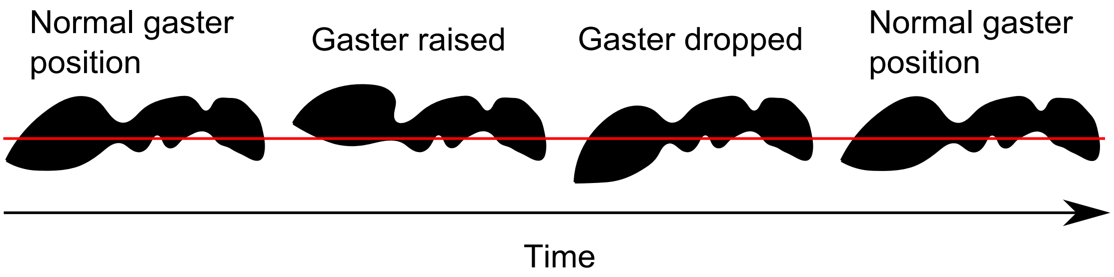


Figure 17 – Abdomen vibrations by workers.

5.3 Methods

Ants were videoed walking along a 100mm section of the beam apparatus. The equipment was set up so that the camera was parallel to the ground and perpendicular to the Perspex beam. A mirror was held pronated 45° from vertical behind the beam allowing the ant to be videoed from the side and from above simultaneously (see Figure 18).

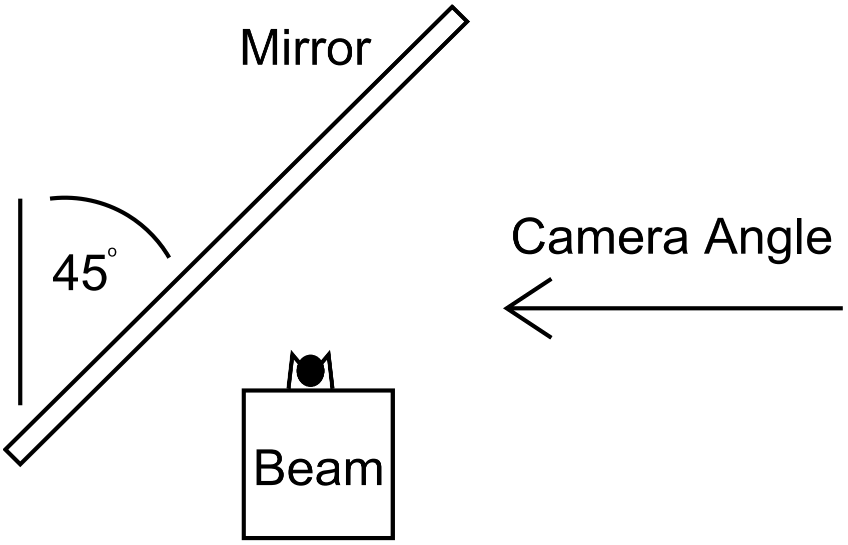


Figure 18 – Equipment set up allowing the ants’ behaviour to be recorded from above and below.

Fifty ants were videoed (ten ants from each of five colonies) and each ant was videoed under eight different conditions as the journey direction (away from or towards the nest), the presence or absence of footprint hydrocarbons and the presence or absence of trail pheromone varied (see Table 5).

Table 5 – Treatments for analysis of alarm response

Treatment	**Context – Is Trail pheromone present?**	**Substrate – Is Home range marking present?**	**Journey**
1	Yes	Yes	Away from nest
2	Yes	Yes	Towards nest
3	Yes	No	Away from nest
4	Yes	No	Towards nest
5	No	Yes	Away from nest
6	No	Yes	Towards nest
7	No	No	Away from nest
8	No	No	Towards nest

The test conditions were set up on strips of 6mm wide ECF paper. Home range marking was generated by leaving the strips of ECF paper in the relevant colony's nest box for twenty hours (Czaczkes et al. 2011) prior to use. Pheromone trails were set up using the methodology detailed in 4.2.1.1. Once set up, the strips of paper were placed on the beam apparatus (see Figure 19). The required worker was transferred to the end of the beam above the pillar using a cocktail stick.

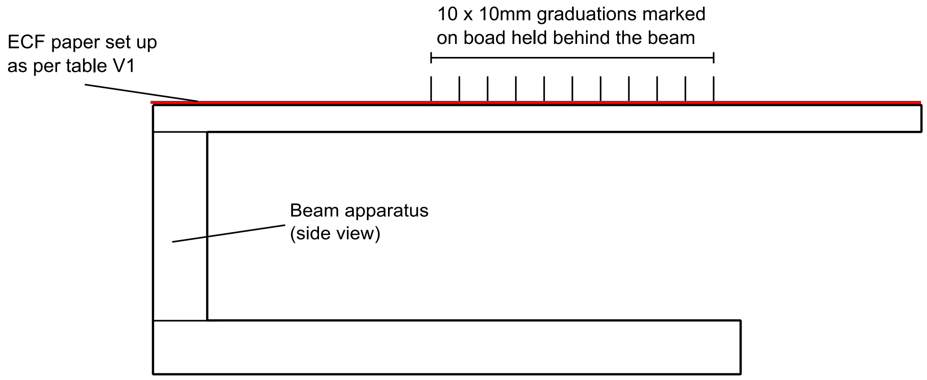


Figure 19 – Equipment set up for analysis of alarm behaviour

5.3.1 Video Analysis

All 200 video clips (eight per ant) were analysed blind to ensure no human bias was introduced into the procedure. Four aspects of the ants' behaviour on the beam that could indicate alarm were considered:

Speed – Speed was measured by recording the time it took for the ant to travel 100mm minus any time it spent stationary.

Abdomen Vibrations – Whether or not the abdomen vibrating behaviour (see Figure 17) occurred at any point in the 100mm was recorded.

Zigzags – The number of times during the 100mm the ant moved from one edge of the beam to the other.

Number of Stops – The number of times the ant stopped during the 100mm was recorded.

5.3.2 Statistical analysis

The “Number of Stops” and “Number of Zigzags” data sets were each analysed by fitting a Generalized Linear Model with a Negative Binomial error structure and log link function. (The large number of 0 values in all data sets lead to Poisson Regression model being overdispersed and therefore unsuitable). For the speed data, group means were compared using analysis of variance. For each model, journey (direction away from or towards the nest), substrate (the presence of absence of home range marking) and context (the presence or absence of trail pheromone) were considered as explanatory variables. All second and third order interactions between these terms were considered.

During simplification of the “Speed”, “Number of Zigzags” and “Number of Stops” models, the term “Context” was removed (see Results Appendix 3). The models assumed that each ant responds to the presence of absence of particular stimulus by amplifying or modulating a behaviour of interest. There is likely to be a range of possible worker responses influenced by factors such as age and nutritional levels. Therefore, when it transpires that one stimulus does not actually affect the behaviour, each ant is effectively being counted twice for the remaining stimuli (i.e. treatments 1=5, 2=6, 3=7, 4=8 in Table 5). This inflates the degrees of freedom in the models and introduces pseudoreplication. To avoid this, the data sets were halved and only the “pheromone absent” halves were considered.

The speed data set was transformed for normality and re-expressed as the square root of the original values.

5.4 Results

The Abdomen vibrating behaviour was only observed in fifty of the four hundred video recordings so occurred at too low a density for formal analysis using a statistical model. However, it is worth noting that seventeen of the observations were on substrate without home range marking or trail pheromone.

The presence of trail pheromone had no significant effect on an ant’s speed, the number of zigzags performed or the number of times it stopped.

Substrate and journey direction were the only terms to have a significant effect on ant speed. Ants moved more quickly on unmarked substrate than home range marked substrate (ANOVA

$F_{1,197} = 37.79$, $p < 0.001$) and independently, they also moved more quickly on the outwards than the return journey (ANOVA $F_{1,197} = 23.76$, $p < 0.001$) (see Figure 20).

Substrate and journey were the only terms to have a significant effect on the number of zigzags performed. Ants zigzagged more on home range marked substrate than unmarked substrate (NB: $\Theta = 4.98$, $X^2 = 8.389$, $p = 0.004$) and independently, they also zigzagged more on the outwards journey than the return journey (NB: $\Theta = 4.98$, $X^2 = 6.602$, $p = 0.010$) (see Figure 20).

Substrate and journey were the only terms to have a significant effect on the number of times an ant stopped. Ants stopped more on home range marked substrate than unmarked substrate (NB: $\Theta = 3.65$, $X^2 = 33.978$, $p < 0.001$) and independently, they also stopped more often on the return journey than the outwards journey (NB: $\Theta = 3.65$, $X^2 = 20.535$, $p < 0.001$) (see Figure 20).

Increased speed, fewer zigzags, and fewer stops all support the hypothesis that *L. niger* workers are alarmed by unmarked substrate.

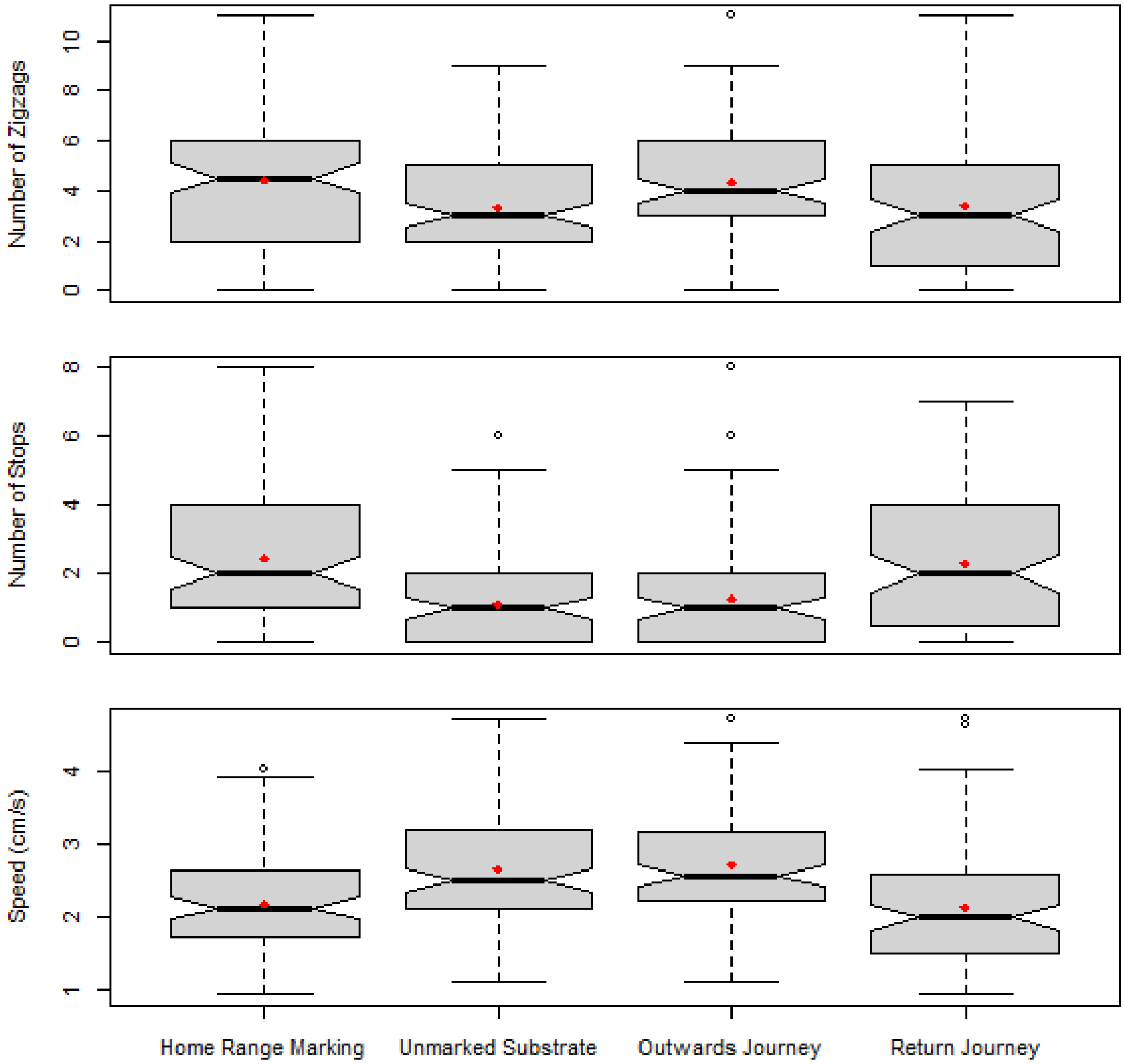


Figure 20 - The effect of the presence or absence of home range marking and the effect of travel direction on: the number of zigzags performed (row 1); the number of times an ant stopped (row 2); ant speed (row 3). Whiskers extend by 1.5 x IQR beyond 1st and 3rd quartile. Red dots represent the population means. Notches extend from the median to +/-1.58 IQR/sqrt(n).

5.5 Discussion

It could be argued that the variation in speed between home range marked and unmarked substrate observed here is merely a result of the corresponding variation in the number of stops and the number of zigzags. An ant making more zigzags is taking a longer path and therefore would be expected to take longer to walk the 100mm. The journey data is unusual however, because speed is highest on the outwards path but so too is the number of Zigzags. This is contrasted by the stopping data which correlates with speed (i.e. fewer stops and higher speed, more stops and lower speed) in response to both substrate marking and journey. Whilst the amount of time each ant was stopped was not included in the speed calculation, time when the ant was accelerating and decelerating to/from stopped was included. It was surprising that trail pheromone did not affect these behaviours.

It is of concern that previous work (Devigne & De Biseau 2012; Devigne & Detrain 2002) has not found a significant difference between worker speeds on home range marked and unmarked substrate but it is worth noting that there are large differences in reported speeds between studies (see Table 6). This study is the only one to record the speed of each ant under both conditions.

Table 6 – Comparison of published ant speed data

Measurement	**Devinge & De Bisseau 2012**	**Devigne & Detrain 2002**	**This study**
Speed on Home range Marked Substrate	0.68 cm/s n = 62	2.99 cm/s ± SE = 0.22 n = 77	2.18 cm/s ± SE = 0.07 n = 50
Speed on Unmarked Substrate	0.77 cm/s n = 46	2.4 cm/s ± SE = 0.16 n = 73	2.66 cm/s ± SE = 0.08 n =50

5.5.1 Was alarm pheromone a sensible hypothesis?

A rough calculation can be used to determine whether the use of alarm pheromone to recruit nest mates to unexplored territory is feasible. We can estimate the lifetime of a *Lasius niger* undecane deposit based on the data available for *Lasius claviger* (Regnier and Wilson 1968). It is important to emphasise at this stage that whilst closely related *Lasius* species often have very similar chemistry (Regnier and Wilson 1969, Bergström and Löfqvist 1970, Attygalle *et al.* 1987), only weak inference can be drawn when data collected from one species is used to examine hypotheses in another. The Dufour's gland undecane content in *Laisus claviger* is in the region of

2.48×10^{-6} g and an alarmed ant releases about 50% of this content (1.24×10^{-6} g). Relative molecular mass of undecane is 90 so this can be used to calculate the moles of undecane released (see Equation 1)

$$\frac{1.24 \times 10^{-6}}{90} = 1.38 \times 10^{-8} \qquad \text{(Equation 1)}$$

The number of moles released can then be multiplied by Avagadro's number to determine the number of molecules released (see Equation 2)

$$1.38 \times 10^{-8} \times 6.02 \times 10^{23} = 8.3 \times 10^{15} \qquad \text{(Equation 2)}$$

The evaporation rate of undecane from a capillary tube has been reported as 5.02×10^{12} molecules per second so this can be used to calculate how long 50% of the gaster contents will take to evaporate (see Equation 3)

$$\frac{8.3 \times 10^{15}}{5.02 \times 10^{12}} = 1652 \qquad \text{(Equation 3)}$$

This is equivalent to a lifetime of 27.5 minutes (7.5 minutes greater than the maximum time allowed for each replicate in Experiment L1)

Whilst the data presented in this chapter does support the initial hypotheses about the behaviour of *Lasius niger* when alarmed, the results are by no means conclusive. The possible use of alarm pheromone during exploration by *L. niger* warrants further investigation but as there are large numbers of exocrine glands in the legs of ants (Billen 2009), it is equally possible that the recruitment chemical comes from another source. Even if an "alarm" response is used to recruit nestmates to unmarked territory, it may not be underpinned by undecane. (I focussed on undecane because it is the major component of the Dufour's gland (Attygalle *et al.* 1987)). Experiments have shown that *Camponotus balzani* and *Camponotus sericeiventris* use 3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin (also the *Lasius niger* trail pheromone) as a long lasting trail orientation signal and use formic acid to stimulate recruitment (Kohl *et al.* 2003). The use of formic acid as a short term recruitment signal is worth considering.

Chapter 6 – The combined role of trail pheromone and footprint hydrocarbons in a real foraging context

6.1 Introduction

This chapter presents an experiment designed to determine whether ants integrate footprint hydrocarbon concentrations into their foraging decision if trail pheromone is also present after the bifurcation. Chapter 4 reveals strong evidence that *Lasius niger* workers integrate footprint hydrocarbons into the bifurcation decisions where no trail pheromone is present after the bifurcation, but the work required to examine any synergistic effect of trail pheromone and footprint hydrocarbons under strictly controlled conditions (i.e. through the use of synthesised trail pheromone) was unfortunately beyond the scope of this project (see 4.2.1.1). Instead, inspiration was taken from the seminal work of Deneubourg et al. (1990) and Beckers et al. (1993) and an alternative approach was adopted. These papers present a simple mathematical model that can be experimentally fitted allowing the bifurcation choice of a naive worker to be accurately predicted based upon the amount of pheromone on each of the competing branches.

This chapter presents an extension of the *Lasius niger* pheromone-only model of Beckers et al. (1993). The new model uses both the amount of trail pheromone and the amount of repellent footprint hydrocarbons on competing trail branches to predict the branch choice of a naive ant. The fit of the pheromone only and the combined models to experimental data is then compared under the null hypothesis that if footprint hydrocarbons are a cue used by foraging workers, the combined model will have a better fit than the pheromone-only model.

6.2 Method

A colony was given access to the Y-Platform apparatus with a 1M sucrose feeder on the left hand branch. Ants were allowed to explore the apparatus and feed. Approximately 300 seconds (mean = 305 seconds, standard deviation = 99 seconds) after the first trail laying forager had returned to the nest, the feeder was switched to the right hand trail branch. This was used to generate “competing” trail branches where one branch had false information about the presence of food. Ants were permitted to forage for a further thirty minutes. During this process the ants were filmed from the side. Switching the feeder branch was also important to try and reduce the probability of ants learning which branch had the feeder (Czaczkes et al. 2011; Czaczkes et al. 2013).

The video was then analysed and the branch choices of ants arriving at the bifurcation were recorded. A subject ant was deemed to have chosen a branch upon crossing one of the choice lines as detailed in Figure 21. The time at which the subject ant reached the bifurcation was recorded, as was an estimate of the chemical conditions on the two branches. The pheromone concentration is assumed to be proportional to the number of trail laying ants that have taken that branch (Beckers *et al.* 1992b) and the footprint hydrocarbon concentration is assumed to be proportional to the number of ants that have taken a particular branch.

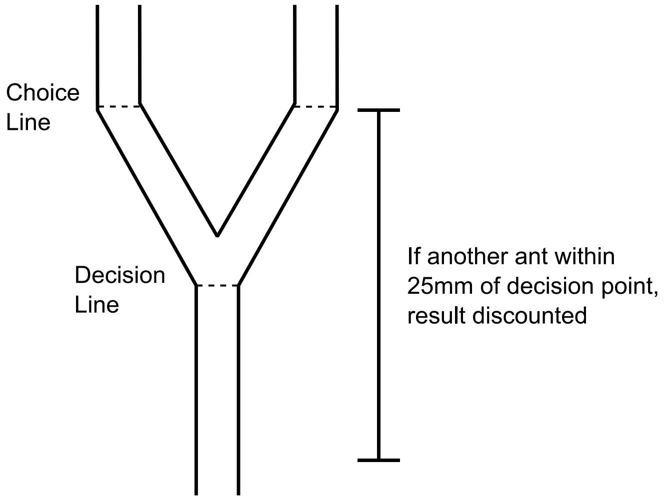


Figure 21 – Features of the Y-Platform used to analyse bifurcation choices.

Whether or not a subject ant was performing trail laying behaviour and whether the subject ant interacted with another ant was recorded. An interaction was deemed to have occurred when: an ant returning from the end of one trail branch met a subject ant travelling outwards from the nest head on, and both ants stopped for at least 0.28 seconds (7 frames of the video). There is some evidence to suggest that ants can communicate the location of food through tactile interactions (Reznikova & Ryabko 2001; Evison *personal communication*).

As it was the influence of the chemical conditions on competing trail branches that was of interest, decisions that were considered to have been biased by an external factor were not included. The bifurcation decision of a subject ant was excluded if:

- Before reaching the bifurcation, the ant had interacted with a returning ant. (These post-interaction bifurcation decisions are discussed in Chapter 7).
- Upon arrival at the decision line, another ant was within 25mm of the subject ant (see Figure 21).
- The subject ant did not cross the decision line travelling on the upper horizontal surface of the platform.
- Switching the feeder caused any obvious change in a subject ant's behaviour (e.g. it accelerated or started abdomen vibrations).

The protocol was repeated with six colonies giving a total of 142 decisions. For assessing the effect of chemical conditions on the bifurcation decisions, each decision was considered to be an independent data point. Even though during one replicate a subject ant could have made multiple trips to the feeder (and therefore more than one bifurcation decision), by choosing a branch the ant changed the chemical conditions on that branch (by depositing footprint hydrocarbons) and so could never have faced the same condition more than once. Each colony was only used once to ensure this.

6.3 Analysis

6.3.1 Analysis: The pheromone-only model

A mathematical model for describing the decision made by a worker at a bifurcation based on the amount of trail pheromone on the competing branches has already been published (Beckers *et al.* 1993). My aim was to observe ants foraging on a bifurcating trail and then compare their decisions to the existing pheromone only model and a new model which incorporates both trail pheromone and repellent hydrocarbons.

The pheromone only model is based upon earlier work by Deneubourg and colleagues (Deneubourg *et al.* 1990) and takes the form:

$$P_{right} = \frac{(k+R)^n}{(k+R)^n + (k+L)^n} \qquad \text{(Equation 4)}$$

Where:

P_{right} is the probability of an ant choosing the right hand branch after the bifurcation

R is the amount of pheromone on the right hand branch (measured by the number of trail laying ants to have taken that branch)

L is the amount of pheromone on the left hand branch (measured by the number of trail laying ants to have taken that branch)

k is a fitted parameter controlling the magnitude of the effect that a single unit of pheromone has on decisions

n is a fitted parameter controlling the linearity of the response

The equation is true where: $P_{right} = 1 - P_{left}$

Experimental fitting of Equation 4 with *Lasius niger* gave values of k=6 and n=2 (Beckers *et al.* 1993)

So for *Lasius niger*:

$$P_{\text{right}} = \frac{(6+R)^2}{(6+R)^2 + (6+L)^2} \qquad \text{(Equation 5)}$$

6.3.2 Analysis: The combined model

I would like to propose a new model which includes the original terms for the effect of pheromone, additional terms for the repellent effect of footprints and a term to convert pheromone and footprints into equivalent units.

$$P_{\text{right}} = \frac{(A*(6 + R))^2 + (B + G)^2}{(A*(6 + R))^2 + (A*(6 + L))^2 + (B + G)^2 + (B + D)^2} \qquad \text{(Equation 6)}$$

Where:

- G is the concentration of footprint hydrocarbons on the Left hand branch (measured by the number of ants to have walked along that branch)
- D is the concentration of footprint hydrocarbons on the Right hand branch (measured by the number of ants to have walked along that branch)
- B is a parameter controlling the magnitude of the effect that a single unit of footprints has on decisions (equivalent to the function of k in the pheromone model).
- A is a parameter which converts pheromone effect into the same units as hydrocarbon effect. (One unit of pheromone has a greater effect on ant decisions than one unit of hydrocarbons therefore we require pheromone to be expressed by a value larger than actual number of ants hence we multiply by A).
- $P_{\text{right}} = 1 – P_{\text{left}}$

6.3.2.1 Estimating B

The experiments from Chapter 4 demonstrated that where twenty ants have previously walked on the substrate from one branch and no ants have previously walked on the substrate from the other, naive ants have a 59/96 chance of taking the unmarked branch. We can use this to approximate the value that B (equivalent of R) should take in Equation 4 if fitted for the effect of footprints (assuming n=2).

Solving :

$$59/96 = \frac{(B+20)^2}{(B+20)^2 + (B+0)^2} \quad \text{gives a value of} \sim 33 \text{ for B} \qquad \text{(Equation 7)}$$

The experiments from Chapter 4 also demonstrated that where fifteen ants have previously walked on the substrate from one branch and five ants have previously walked on the substrate from the other, naive ants have a 62/96 chance of taking the branch with substrate walked on by five ants. We can use this to approximate the value that B (equivalent of R) should take in Equation 4 if fitted for the effect of footprints (assuming n=2).

And solving:

$$62/96 = \frac{(B+15)^2}{(B+15)^2 + (B+5)^2} \quad \text{give a value of} \sim 23 \text{ for B} \qquad \text{(Equation 8)}$$

Therefore considering $23 < B < 33$ would be sensible for the combined model (see Equation 6).

6.3.2.2 Estimating A

Trying to estimate A is more difficult. The experiments from Chapter 4 demonstrated that in order to achieve a 59/96 split of naive workers at the bifurcation one branch would have to have the chemical markings left behind by twenty ants whilst the other branch was unmarked. We can use Beckers' fitted model (Equation 5) to calculate (see Equation 9) how many trail laying ants would be required on one branch (given no ants on the other) in order to achieve a 59/96 split at the bifurcation.

$$59/96 = \frac{(6+R)^2}{(6+R)^2 + (6+0)^2} \qquad \text{(Equation 9)}$$

Solving for R give a value of 1.577

This means that in the hydrocarbon case 20 ants are required to achieve 59/96 whereas for trail laying ants, 1.58 ants are required to achieve 59/96 (see Equation 9). We might therefore expect the value of A to be in the region of:

$$\frac{20}{1.57} \approx 12.5 \qquad \text{(Equation 10)}$$

6.3.3 Comparing the pheromone only and combined models to experimental observations

If we calculate:

$$P_{right} = \frac{(12*(6+R))^2 + (28+G)^2}{(12*(6+R))^2 + (12*(6+L))^2 + (28+G)^2 + (28+D)^2} \qquad \text{(Equation 11)}$$

and fit it against ant decisions at the bifurcation, we find that there is a significantly higher probability that the observed data came from the combined model than the pheromone only model (Vuong Non-Nested Hypothesis Test-Statistic = 1.740, p = 0.041). See Table 7 and Figure 22 for details of the models' fits.

Table 7 – Comparison of pheromone-only and combined models

Model	**d.f.**	X^2	**P(>Chi)**
Pheromone	1	10.49	0.001
Combined	1	13.07	<0.001

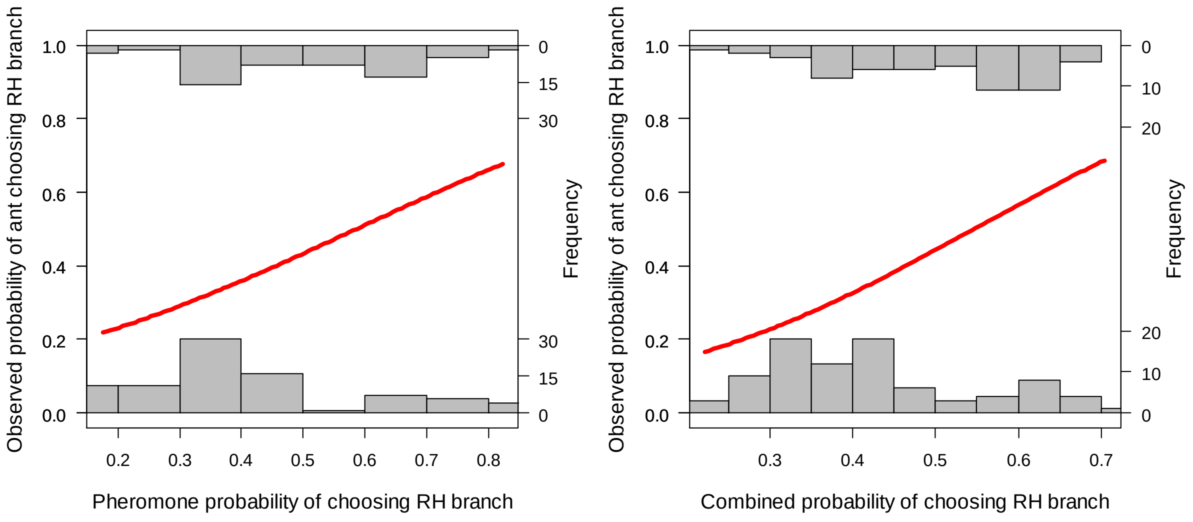


Figure 22 – The effect of pheromone probability (left) and combined probability (right) on the probability of an ant choosing the right hand branch. The fit of the regression lines is presented in Table 7.

This result supports the hypothesis that *Lasius niger* workers integrate information about both trail pheromone concentrations *and* home range marking into the foraging decisions. For sensitivity analysis of the combined model see Appendix 4. If the model fitting process is repeated using only the observations where there is trail pheromone on both branches (i.e. after the feeder switch from left to right), only the combined model still fits the data (X^2 = 5.575, d.f. = 1, p = 0.032).

6.4 Discussion

6.4.1 Pheromone Only Model vs Combined model.

The combined model was a better fit to the observed foraging decisions that the pheromone only model. This suggests that both trail pheromone and footprint hydrocarbon concentrations affect ant decisions at a trail bifurcation. The per ant effect of footprint hydrocarbons is much weaker than the per ant effect of trail pheromone and the global effect of footprint hydrocarbons is (approximately twelve times) weaker than the global effect of trail pheromone.

Other studies have reported that *Lasius niger* workers modulate their trail laying behaviour according to home range marking depositing more trail pheromone on areas with a high home range marking concentration than on areas with a low home range marking concentration (Devigne et al. 2004). This could be seen as evidence that the ants are compensating for the repellent nature of footprint hydrocarbons in a foraging context. Investigating further reveals that if the recruitment pheromone (but not the home range marking) is removed from a trail, experienced foragers deposit less trail pheromone on their outwards journey than their return journey. This difference is not observed in the absence of home range marking (Czaczkes et al. 2012). This result was interpreted as the high home range marking to pheromone concentration being symptomatic of a food source that had been heavily exploited. Workers were unwilling to lay a strong trail to a food source that might have expired. In contrast, having found that the food source was still viable, a strong pheromone trail was laid on the return to the nest.

The experiment presented in this chapter mimics a foraging context where a food source becomes depleted and a new one emerges. After the food has been switched from the left to the right hand branch, the left branch (with an established pheromone trail) is unrewarding whilst the right hand branch has a feeder. Under these conditions only the combined model fits the data suggesting that a gradual increase in the weak repellent effect of footprint hydrocarbons may have an important role in the abandoning of unrewarding trails. This observation cannot be explained by the decay of the trial pheromone on the first branch because all replicates were completed within the lifetime of the first trail pheromone mark (Beckers *et al.* 1993).

Chapter 7 – The communication role of interactions between workers during foraging

7.1 Introduction

Interactions between workers during foraging have been found to increase the probability that naive workers successfully locate a food source. In some species of wood ant, workers can accurately find food in complex mazes after interacting with an experienced ant even if all chemical cues are removed (Reznikova and Ryabko 2001). Experiments (also controlling chemical cues) have shown that *Lasius niger* worker are able to communicate the position of food in simple mazes but not complex ones (Evison *pers. comm.*). The communication mechanism is unknown. In the experiment presented in Chapter 6, workers were allowed to forage in a system where one source of social information about the location of food (trail pheromone and footprint hydrocarbons) was not necessarily accurate. This chapter compares the success of foragers that interacted with an experienced worker before making a bifurcation decision with the success of foragers that did not.

7.2 Method

See Chapter 6 for details of the protocol. An ant was deemed to have interacted if, before reaching the bifurcation, it met another ant returning from either trail branch and both ants stopped for at least 0.28 seconds (7 frames of the video). For ease of analysis, the decisions of subject ants that interacted with more than one experienced forager were discounted. The bifurcation decisions made by interacting ants also had to adhere to the independence rules listed in Chapter 6.

7.3 Results

One hundred and forty two bifurcation decisions where no interaction occurred were compared with forty four decisions where an interaction did occur. Ants that interacted with another ant before the bifurcation were more likely to choose the food branch than ants that did not interact before the bifurcation (X^2 = 7.242, d.f. = 1, p = 0.007) see Table 8.

Table 8 – Forager success and interactions

Interaction	Chose Food Branch	Chose Other Branch
Yes	32	12
No	71	72

Time since the start of the replicate is a potential measure of experience because there is a higher probability that subject ants arriving later in the replicate have previously visited the food. However, the average decision time between the interacting and non interacting groups was not significantly different (Wilcoxon rank sum test with continuity correction, W = 2914.5, p = 0.462).

Moreover, the average decision time between the food branch choosing and the non food branch choosing populations was not significantly different (Wilcoxon rank sum test with continuity correction, W = 4265, p = 0.870). This shows that experience (as measured by time) does not explain the increased probability of a subject ant choosing the food branch if it interacted with another ant before the bifurcation.

To ensure that the difference in probability of choosing the food branch between the interacting and non interacting groups was not simply due to a difference in the pheromone probability of choosing the food branch or the combined (pheromone and footprint) probability of choosing the food branch, the between group means of these two values were compared. The pheromone probability of choosing the food branch was not significantly different between the interacting and non interacting populations (Wilcoxon rank sum test with continuity correction, W = 2870.5, p = 0.381). The combined probability of choosing the food branch was also not significantly different between the interacting and non interacting populations (Wilcoxon rank sum test with continuity correction, W = 2815, p = 0. 293).

7.4 Interaction mechanism

It was observed that an ant returning from the food branch was more likely to pass the ant it interacted with on the opposite side (i.e. the side with the unrewarding branch) than expected (Exact Binomial Test: p = 0.014). Based on this observation I formed the hypothesis that a "push", similar to that observed in dense traffic regulating the flow of ants on narrow bridges (Dussutour *et al.* 2004) is an essential component of the message that experienced foragers give to inexperienced foragers about the reward status of a trail branch.

Hypothesis: On their way back to the nest, experienced foragers "push" nestmates they interact with towards the food branch (See Figure 23).

Result: There was no relationship between an ant being "pushed" and the likelihood of it choosing the food branch (X^2 with Yates' continuity correction = 0.068, d.f. = 1, p = 0.794).

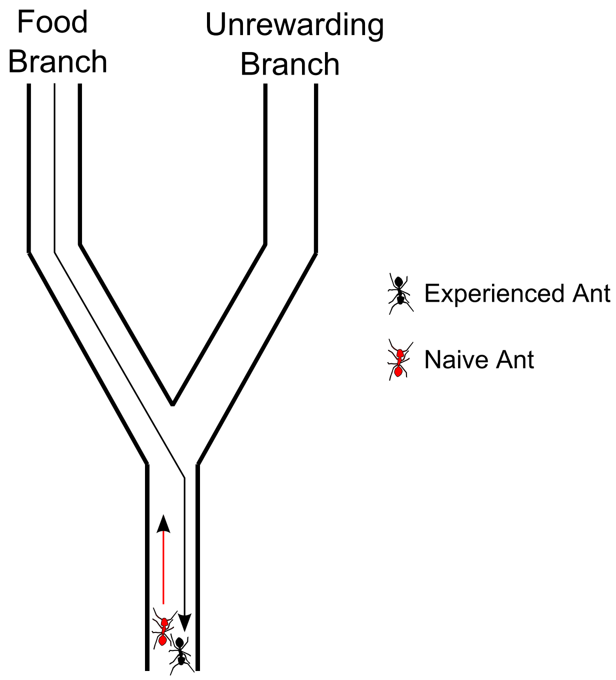


Figure 23 – Hypothesis: Experienced workers pass naive pass nestmantes on the unrewarding side and "push" them towards the food branch.

7.5 Discussion

Further data is presented here supporting claims that ants can communicate the location of food via tactile interactions (Reznikova & Ryabko 2001; Evison *pers. comm.*). The experiment presented here was not carried out under strictly controlled conditions (i.e. in the absence of trail pheromone) but variables, such as the amount of pheromone on competing branches cannot explain the higher probability of forager success if the individual interacted with an experienced forager before choosing a trail branch. The subject ants (those that made the bifurcation decision after the interaction) may have already visited a feeder during the experiment and so could use private information about the location of food to inform their foraging decisions (Grüter *et al.* 2010). However, because the feeder changed branch during the experiment, private information or social information in the form of trail pheromones did not necessarily represent the current location of food. In contrast, an ant returning from a trail branch would have current information about the reward status of that branch. Moreover, no significant difference in experience (as measured by trail laying behaviour or the amount of time elapsed since the start of the experiment) was found between the interacting and non interacting populations.

A possible mechanism for the information transfer during the interaction was also examined. The hypothesis that on their way back to the nest, experienced foragers “push” nestmates they interact with towards the food branch was tested. It was found that whilst an ant returning from the food branch was more likely to pass the “inexperienced” ant on the non-food side, “pushed” ants were not more likely to choose the food branch than ants that were not pushed. It worth emphasising again, that this experiment did not control the chemical conditions on the two branches. Such variation could potentially account for the discontinuity between being pushed and choosing the food branch. Other studies have shown that social insects preferentially use certain information types over others when making foraging decisions (Grüter *et al.* 2008, 2010). The relative importance ants assign to chemical social information and tactile social information is unknown. Further work is required to test the pushing hypothesis under more controlled conditions.

Chapter 8 – Discussion

8.1 Summary of Findings

This thesis has presented evidence that the passage of *Lasius niger* workers along a pheromone trail can affect the foraging decision of subsequent ants. Given a choice between two trail branches, naive workers following a pheromone trail up to the bifurcation will preferentially choose the branch with substrate walked on by fewer nestmates. Qualitative comparisons of foraging decisions where the bifurcation choice was instead between “high” and “low” home range marking hydrocarbon concentrations under the same procedure produced an equivalent effect with naive ants preferentially choosing the branch with substrate marked with a “low” home range marking concentration. This supports that hypothesis that footprint hydrocarbons act as a repellent cue during foraging (See Chapter 4).

A mathematical model was then devised to predict bifurcation choices based on both the repellent hydrocarbon and attractive pheromone concentrations on the two branches. This was compared with an existing model that predicts bifurcation choices based on trail pheromone alone. The combined model was more successful at predicting observed bifurcation choices than the pheromone only model (See Chapter 6).

Evidence was presented that the passage of workers over unmarked substrate (in the absence of trail pheromone) results in a cue that attracts subsequent workers. Qualitative comparisons giving naive workers a choice between “high” and “low” hydrocarbons concentrations failed to replicate this effect suggesting an alternative signal to footprint hydrocarbons (See Chapter 4).

Behavioural analysis was performed to test whether workers behaved in an alarmed manner when confronted with unmarked territory suggesting the possible use of alarm pheromone to recruit workers to unmarked territory. The results supported this theory but relied on assumed behaviours derived through comparison with sister species. Some of the results also conflicted with those presented in other studies so this finding should be treated with caution (See Chapter 5).

Observations were made that give further weight to the hypothesis that ants can communicate the position of a food source through tactile interactions. Ant position during interactions is tested as being a possible component of the signal (See Chapter 7).

Pharaoh’s ants were not found to use repellent cues to aid in the efficient exploration of novel territory or to use repellent cues to aid in the discovery of food sources within the existing territory (see Chapter 3).

8.2 Discussion of findings and further work

8.2.1 Discussion and Further Work: The repellent role of footprint hydrocarbons during foraging

Experiment L2 (Chapter 4) demonstrated that, upon arrival at a bifurcation, a naive ant that had been following a pheromone trail would preferentially choose substrate previously not walked on by ants to substrate walked across by twenty ants. This indicated that either unmarked substrate was attractive to ants or that there was something unattractive about substrate previously walked on by nest mates. Experiment L4 (Chapter 4) presented a bifurcation choice between: substrate previously walked across by five workers, or substrate previously walked across by fifteen workers. More naive ants than expected chose the branch previously walked across by five ants. This supports the hypothesis that there is a repellent cue left behind by the passage of ants and the effect observed in Experiment L2 was not an effect of blank paper. Experiment L6 (Chapter 4) presented a bifurcation choice between: substrate with a high home range marking (footprint hydrocarbon) concentration, and substrate with a low home range marking concentration. More naive ants than expected chose the branch with the low home range marking concentration. This result suggests that the home range marking chemicals (footprint hydrocarbons) are repellent to foraging ants and are the likely cause of the effects observed in experiments L2 and L4. The experiment described in Chapter 7 developed a mathematical model to predict the bifurcation choices of foraging ants based on both the amount of trail pheromone (measured by the number of trail laying ant passages) and the amount of footprint hydrocarbons (measured by the number of ant passages) on the competing branches. Empirical observations of foraging decisions at a bifurcation were made and the new model was compared to an existing pheromone only bifurcation choice model (Beckers *et al.* 1993). The combined model was significantly better at predicting the bifurcation choices than the pheromone only model, further demonstrating that *Lasius niger* workers integrate both information sources into their foraging decisions.

This is the first time that the repellent effect of footprint hydrocarbons during foraging has been reported in ants. A similar role of footprint hydrocarbons has been reported in bees (Wilms and Eltz 2008) and different roles of footprint hydrocarbons have been reported in ants. These roles include: a trail discrimination signal (Akino and Yamaoka 2005, Akino *et al.* 2005); nest marking (Lenoir *et al.* 2009); and a role in generating pheromone trail polarity (Devigne and Detrain 2006). Only three other negative feedback mechanisms involved in the regulations of ant foraging have been reported (Nonacs 1990; Robinson et al. 2005; Grüter et al. 2012). Some qualitative comparisons can be made between the chemical negative feedback mechanism reported by Robinson et al. and the mechanism reported here.

The repellent signal used by *M. pharaonis* is a pheromone (i.e. it is deposited to communicate a specific message to an intended recipient (Wyatt 2003)). In contrast, the *L. niger* repellent is merely a chemical cue passively deposited as the ant walks (Lenoir *et al.* 2009) without an intended message or recipient. In both cases the repellent signal lasts longer than the attractive pheromone used during the exploitation of a food source. In contrast, whilst the per unit response to the *M. pharaonis* repellent is stronger than the per unit response to the attractive pheromone, the per unit response to the *L. niger* repellent cue is much weaker than the per unit response to the corresponding trail pheromone [see Chapter 6 and (Robinson et al. (2008)]. This would seem to suggest that the two different repellent signals have two different roles within their associated foraging networks. The *M. pharaonis* repellent is used to mark unrewarding trail branches and it is possible that the *L. niger* repellent has a role in aiding the abandonment of crowded or recently depleted food sources (see Chapter 6, Giurfa & Núñez (1992); Lau & Nieh (2009), Czaczkes et al. (2012), Grüter et al. (2012), Townsend-Mehler & Dyer (2011).

Assessing the relative amount of trail pheromone and footprint hydrocarbons may also have a role as an optimal search strategy by naive foragers. By avoiding branches where the food quality (trail pheromone amount) to worker number (footprint hydrocarbons) ratio is low, ants could potentially increase the probability of choosing the path where a reward is more likely. Optimal search strategies involving the avoidance of conspecific odour trails has been demonstrated in Carabid beetles (Guy *et al.* 2008).

All the evidence that supports the footprint hydrocarbon hypothesis was gathered with experiments using a symmetrical bifurcation. This was done to remove any influence of trail geometry of foraging decisions (Jackson *et al.* 2004, Gerbier *et al.* 2008). It is possible that rather than being a ubiquitous repellent cue during foraging, variations in footprint hydrocarbon concentration are used by *Lasius niger* to give symmetrical trail junctions polarity (i.e. denoting which branch is most likely to take the forager away from the nest). At a trail junction, branches that lead away from the nest are likely to have a lower footprint hydrocarbon concentration than those that lead to the nest. This is because the same traffic volume will be shared between multiple paths each time the trail splits. A forager detecting a hydrocarbon concentration difference may therefore be using it merely to orient away from the nest and (because ants are central place foragers (Harkness and Maroudas 1985)) towards food. It has been established elsewhere (Devigne & Detrain 2006) that a footprint hydrocarbon gradient can give linear pheromone trails polarity and perhaps what is observed in this study is an extension of this process. Repeating the experiment using bifurcation with a range of asymmetries would help to address this question. It would also be interesting to examine whether a difference in footprint hydrocarbon concentration on two competing trail branches influences the bifurcation decisions of successful foragers returning from a food source to the nest (Evison *per. comm.*).

Many of the terms in the combined trail pheromone and footprint hydrocarbon bifurcation choice equation presented in Chapter 6 are crudely approximated, and sensitivity analysis (see Appendix 4) has shown that a range of values gives a better fitting model than the pheromone only model.

Artificially synthesising trail pheromone and using concentrations of known biological activity on competing trail branches alongside varying home range marking would facilitate a more formal fitting of the combined model. Once the underlying mechanism is formalised, questions relating to the role of the repellent within foraging networks can be addressed with more confidence. Specific questions such as the role in optimal path selection or abandonment of unrewarding food source can be addressed using Monte Carlo methods or agent based simulation (Beckers *et al.* 1992a, Grüter *et al.* 2012).

A very recent paper (Perna *et al.* 2012) has suggested that the non-linear type of models used by Goss et al. (1989), which were adapted in Chapter 6, do not reflect the ant response to trail pheromone as accurately as Webber's Law. Further work would also need to evaluate these competing hypotheses.

8.2.2 Discussion and Further Work: Recruitment of workers to unmarked territory

Measuring the behavioural response of *Lasius niger* workers to a synthetic source of the alarm pheromone undecane (Maschwitz 1964) would give an indication of the reliability of the results presented and conclusions drawn in Chapter 4. To confirm that undecane is released in response to unmarked territory, substrate should be collected and analysed using Mass Spectrometry or Gas Chromatography. Such approaches are frequently taken (Martin & Drijfhout 2009; Lenoir et al. 2009).

8.2.3 Discussion and Further Work: The communication role of trail interactions during foraging

Several studies have shown that ants can communicate the position of a food source through tactile interactions (Reznikova & Ryabko 2001, Evison *pers. comm.*) but in order for these to gain greater credibility the mechanism for how this is achieved needs to be determined. This study has shown that position of ants relative to each other may be a component of the signal and the pushing behaviour described in Dussutour *et al.* (2004) is proposed as a mechanism. Parallels can also be drawn with the honeybee waggle dance where the orientation of a dance relative to gravity contains information about the location of a food source (Von Frisch 1967). The position of audience bees relative to the dancer is also thought to be important (Rohrseitz and Tautz 1999). A communicative role of stridulations or antennation during an interaction is perhaps the most promising direction for further enquiry (Evison, *pers. comm.*) and high quality video recording would be required to facilitate this.

8.3 The link between ecology and foraging strategy

Recruitment to unmarked territory is also clearly a key part of the foraging strategy of *Monomorium pharaonis* [see also Sudd (1960), Fourcassié and Deneubourg (1992;1994)]. By recruiting nestmates to new areas, the exploitation of any food resources that are discovered can begin immediately. The use of a distributed central place foraging system (facilitated via polydomy) improves the likely success of such a “rush in and grab” tactic whilst also providing resilience if danger is encountered (Debout and Schatz 2007). Once a food source is secured, multiple pheromones can then be used to regulate its exploitation (Robinson, Green, *et al.* 2008, Robinson, Ratnieks, *et al.* 2008).

The ecology of *Lasius niger* is rather different as it is a species that farms aphids (Banks and Nixon 1958) and so relies more on the efficient exploitation of stable food resources than on opportunism. This is highlighted by the important role played by experience during foraging (Grüter *et al.* 2010, Czaczkes *et al.* 2011, 2013). This thesis presents evidence suggesting that a complementary repellent signal (footprint hydrocarbons) is working alongside trail pheromone and experience to regulate to foraging of this species.

8.4 Final note

This study has succeeded in its aim of contributing to our understanding of the role of negative feedback in ant foraging networks. It has raised as many (if not more) questions than it has answered, emphasising just how valuable an approach it has turned out to be. As our understanding of the synergistic role of positive and negative feedback in ant foraging networks increases, the power of the ant foraging network model as a general tool for Systems Biology will also increase. Moreover, the early work by Goss et al. (1989) gave rise to the field of Ant Colony Optimisation (ACO) algorithms (Dorigo *et al.* 2006) and it remains to be see what practical applications could result from extensions to their work such as this.

Appendix 1 – further detail relating to the results presented in Chapter 3

1.1 Monomorium Experiment 1

The maximal model was:

Choice ~ (Colony + Time + Proportion of ants to RHS + Side set up first) ^ 2

During model simplification, terms were removed in the following order:

Table 9 – Experiment M1 model simplification

Term	d.f.	X^2	Pr(>Chi)
Colony : Time	-2	-3.6700	0.1596
Proportion of ants to RHS : Time	-2	-0.3314	0.5648
Time : Side set up first	-1	-1.1761	0.2781
Proportion of ants to RHS : Side set up first	-1	-2.3531	0.1250
Time	-1	-1.6486	0.1991
Colony : Side set up first	-2	-3.8076	0.1490
Side set up first	-1	-1.0129	0.3142

1.2 *Monomorium* Experiment 2

The maximal model was:

Choice ~ (Colony + Time + Proportion of ants to RHS + Side set up first) ^ 2

During model simplification, terms were removed in the following order:

Table 10 – Experiment M2 model simplification

Term	d.f.	X^2	Pr(>Chi)
Colony : Side set up first	-2	-0.06061	0.9701
Proportion of ants to RHS : Colony	-2	-0.32339	0.8507
Time : Side set up first	-1	-0.39070	0.5319
Proportion of ants to RHS : Side set up first	-1	-0.60990	0.4348
Time : Colony	-2	-1.20502	0.5474
Colony	-2	-0.80189	0.6697
Side set up first	-1	-0.93365	0.3339
Time: Proportion of ants to RHS	-1	-3.08544	0.0790
Time	-1	-0.01530	0.9015

Appendix 2 - further detail relating to the results presented in Chapter 4

2.1 Experiment L1 - Do exploring workers prefer unmarked substrate, or substrate previously crossed by 20 workers?

The maximal model was:

Choice ~ (Colony + Time + Date + Apparatus Orientation + Side with paper crossed by 20 ants + Decision time) ^ 2

During model simplification, terms were removed in the following order:

Table 11 – Experiment L1 model simplification

Variable	**d.f.**	**X^2**	**Pr(>Chi)**
Colony : Date	-3	-1.1894	0.75554
Time : Apparatus Orientation	-1	-0.0379	0.84562
Decision time : Date	-1	-0.0485	0.82572
Decision time : Side with paper crossed by 20 ants	-1	-0.1577	0.69129
Decision time : Time	-1	-0.4298	0.51208
Colony : Side with paper crossed by 20 ants	-3	-1.5880	0.66211
Time : Side with paper crossed by 20 ants	-1	-1.5066	0.21966
Colony : Apparatus Orientation	-3	-3.6866	0.29735
Decision time : Apparatus Orientation	-1	-0.4776	0.48953
Time : Date	-1	-0.8367	0.36035
Colony : Time	-3	-7.2361	0.06474
Colony : Decision Time	-3	-1.5921	0.66119
Apparatus Orientation: Side with paper crossed by 20 ants	-1	-2.1399	0.14351
Apparatus Orientation: Date	-1	-2.6924	0.10083
Date : Side with paper crossed by 20 ants	-1	-2.5182	0.11254
Apparatus Orientation	-1	-0.0645	0.79954
Colony	-3	-2.8803	0.41046
Time	-1	-0.0409	0.83977
Date	-1	-0.1282	0.72030
Decision Time	-1	-1.6255	0.20232

2.2 Experiment L2 - Do naive workers following a pheromone trail prefer a branch previously walked over by twenty unfed ants or a branch previously walked over by no ants?

The maximal model was:

Choice ~ (Colony + Time + Date + Apparatus Orientation + Side with paper crossed by 20 ants + Decision time) ^ 2

During model simplification, terms were removed in the following order:

Table 12 – Experiment L2 model simplification

Variable	d.f.	X^2	Pr(>Chi)
Apparatus Orientation: Side with paper crossed by 20 ants	-1	-0.0015	0.96891
Time: Decision time	-1	-0.0108	0.91722
Colony: Date	-3	-4.0866	0.25226
Time: Side with paper crossed by 20 ants	-1	-0.2150	0.64290
Date: Decision time	-1	-0.5739	0.44873
Time: Apparatus Orientation	-1	-0.6550	0.41832
Colony: Side with paper crossed by 20 ants	-3	-3.8966	0.27285
Date: Side with paper crossed by 20 ants	-1	-2.7589	0.09671
Decision time: Side with paper crossed by 20 ants	-1	-2.3910	0.12203
Apparatus Orientation: Date	-1	-3.7959	0.05138
Colony: Apparatus Orientation	-3	-4.9762	0.17355
Colony: Time	-3	-6.9687	0.07290
Date: Time	-1	-0.1315	0.71691
Apparatus Orientation: Decision Time	-1	-3.3635	0.06666
Colony: Decision Time	-1	-5.6420	0.13039
Date	-1	-0.2601	0.61003
Decision Time	-1	-0.6962	0.40407
Colony	-3	-2.1377	0.54431
Orientation	-1	-1.2661	0.26050
Time	-1	-2.3700	0.12368

2.3 Experiment L3 - Can a decay of the effect of the signal that was observed in experiment L2 be detected?

The maximal model was:

Choice ~ (Colony + Time + Date + Apparatus Orientation + Decision time + Side set up first) ^ 2

During model simplification, terms were removed in the following order:

Table 13 – Experiment L3 model simplification

Variable	**d.f.**	**X^2**	**Pr(>Chi)**
Date: Decision Time	-1	-0.0089	0.92489
Colony: Apparatus Orientation	-3	-4.1352	0.24724
Time: Apparatus Orientation	-1	-0.0416	0.83838
Date: Time	-1	-0.0460	0.83010
Decision Time: Side Set Up First	-1	-0.0989	0.75315
Apparatus Orientation: Decision Time	-1	-0.2942	0.58754
Time: Side Set Up First	-1	-0.3995	0.52734
Colony: Time	-3	-5.5645	0.13483
Date: Orientation	-1	-0.0018	0.96581
Colony: Side Set Up First	-3	-1.7239	0.63163
Date: Side Set Up First	-1	-0.8022	0.37043
Colony: Decision Time	-3	-3.7542	0.28926
Apparatus Orientation: Side Set Up First	-1	-1.6830	0.19453
Colony: Date	-3	-7.3089	0.06268
Time: Decision Time	-1	-3.4274	0.06412
Apparatus Orientation	-1	-0.0678	0.79459
Colony	-3	-2.8373	0.41739
Date	-1	-0.8538	0.35547
Decision Time	-1	-1.1559	0.28231
Side Set Up First	-1	-1.1097	0.29215
Time	-1	-3.7846	0.05173

2.4 Experiment L4 - Is the effect observe in experiment L2 a result of paper crossed by unfed ants being repellent, or a result of unmarked paper being attractive?

The maximal model was:

Choice ~ (Colony + Time + Date + Apparatus Orientation + Side with paper crossed by 15 ants + Decision time) ^ 2

During model simplification, terms were removed in the following order:

Table 14 – Experiment L4 model simplification

Variable	d.f.	X^2	Pr(>Chi)
Date: Apparatus Orientation	-1	-0.0196	0.88855
Date: Decision Time	-1	-0.0558	0.81318
Apparatus Orientation: Decision time	-1	-0.0869	0.76815
Time: Side with paper crossed by 15 ants	-1	-0.3888	0.53294
Apparatus Orientation: Side with paper crossed by 15 ants	-1	-0.3012	0.58313
Decision time: Side with paper crossed by 15 ants	-1	-0.5093	0.47543
Colony: Side with paper crossed by 15 ants	-3	-7.4076	0.05998
Decision time: Time	-1	-0.4516	0.50158
Colony: Date	-3	-3.1512	0.36889
Decision time: Colony	-3	-6.8148	0.07804
Date: Side with paper crossed by 15 ants	-1	-1.0541	0.30457
Apparatus Orientation: Colony	-3	-3.7409	0.29083
Time: Colony	-3	-7.7481	0.05152
Date: Time	-1	-0.0612	0.80456
Apparatus Orientation: Time	-1	-0.8124	0.36740
Decision Time	-1	-0.0007	0.97923
Apparatus Orientation	-1	-0.0405	0.84041
Colony	-3	-0.4777	0.92377
Date	-1	-0.0786	0.77922
Time	-1	-0.2632	0.60794

2.5 Experiment L5 - Can the effect observed in experiment L1 be explained by the presence of footprint hydrocarbons deposited by the 20 ants during the set up phase?

The maximal model was:

Choice ~ (Colony + Apparatus Orientation + Side with “Far” type paper) ^ 3

During model simplification, terms were removed in the following order:

Table 15 - Experiment L5 model simplification

Variable	d.f.	X^2	Pr(>Chi)
Colony : Apparatus Orientation: Side with “Far” type paper	-3	-1.26297	0.7379
Colony : Side with “Far” type paper	-3	-0.44136	0.9316
Apparatus Orientation : Colony	-3	-1.58125	0.6636
Apparatus Orientation : Side with “Far” type paper	-1	-0.39468	0.5298
Apparatus Orientation	-1	-0.04342	0.8349
Colony	-3	-2.52159	0.4714
Side with “Far” Type paper	-1	-0.37937	0.5379

2.6 Experiment L6 - Can the effect observed in experiment L2 be explained by the presence of footprint hydrocarbons deposited by the 20 ants during the set up phase?

The maximal model was:

Choice ~ (Colony + Apparatus Orientation + Side with “Far” type paper) ^ 3

During model simplification, terms were removed in the following order:

Table 16 - Experiment L6 model simplification

Variable	d.f.	X^2	Pr(>Chi)
Colony: Apparatus Orientation: Side with “Far” type paper	-3	-0.93825	0.8162
Colony: Side with “Far” type paper	-3	-1.27862	0.7342
Apparatus Orientation: Side with “Far” type paper	-1	0.00000	1.0000
Apparatus Orientation: Colony	-3	-3.08626	0.3785
Apparatus Orientation	-1	-0.17985	0.6715
Colony	-3	-0.89308	0.8271

Appendix - 3 further detail relating to the results presented in Chapter 5

3.1 Speed

The speed data was expected to be normally distributed but was found to have slight positive skew. As it is the effect of different treatments on ant speed, not the speed value itself that is of interest, the data were transformed by taking the square root of each value. The transformed data were normally distributed (Shapiro-Wilk Normality Test, W = 0.996, p-value = 0.311).

The maximal model was:

Speed ~ (Context + Substrate + Journey) ^ 3

During model simplification, terms were removed in the following order:

Table 17 – Speed Model Simplification1

Term	d.f.	Sum of Squares	F	Pr(>F)
Context : Substrate : Journey	-1	-0.000472	0.0085	0.92643
Context : Substrate	-1	-0.000142	0.0026	0.95958
Context : Journey	-1	-0.086451	1.5640	0.21183
Journey : Substrate	-1	-0.199325	3.6059	0.05831
Context	-1	-0.057609	1.0422	0.30795

After all results in the “Foraging” Context had been removed the maximal model was:

Speed ~ (Substrate + Journey) ^ 2

During model simplification, terms were removed in the following order:

Table 18 – Speed Model Simplification 2

Term	d.f.	Sum of Squares	F	Pr(>F)
Substrate : Journey	-1	-0.1096	2.1629	0.143

3.2 Zigzags

The maximal model was:

Number of Zigzags ~ (Context + Substrate + Journey) ^ 3

During model simplification, terms were removed in the following order:

Table 19 – Zigzag Model Simplification 1

Term	**d.f.**	**X^2**	**Pr<(Chi)**
context: journey: Substrate	-1	0.6725	0.4122
journey: Substrate	-1	0.4302	0.5119
context: Substrate	-1	0.6266	0.4286
context: journey	-1	0.6397	0.4238
context	-1	0.0000	0.9960

After all results in the "Foraging" Context had been removed the maximal model was:

Number of Zigzags ~ (Substrate + Journey) ^ 2 Theta: 4.98 (Std. Err.: 1.37)

During model simplification, terms were removed in the following order:

Table 20 – Zigzag Model Simplification 2

Term	**d.f.**	**X^2**	**Pr<(Chi)**
journey: Substrate	-1	0.0132	0.9086

3.3 Number of Stops

The maximal model was:

Number of Stops ~ (Context + Substrate + Journey) ^ 3

During model simplification, terms were removed in the following order:

Table 21 – Stops Model Simplification 1

Term	**d.f.**	**X^2**	**Pr<(Chi)**
context: journey: Substrate	-1	0.0338	0.8542
journey: context	-1	0.0261	0.8717
context: Substrate	-1	0.1489	0.6996
Substrate: journey	-1	2.6745	0.1020
context	-1	0.3015	0.5830

After all results in the "Foraging" Context had been removed the maximal model was:

Number of Stops ~ (Substrate + Journey) ^ 2 Theta: 3.72 (Std. Err.: 1.35)

During model simplification, terms were removed in the following order:

Table 22 – Stops Model Simplification 2

Term	**d.f.**	**X^2**	**Pr<(Chi)**
journey: Substrate	-1	0.9456	0.3308

Appendix 4 - further detail relating to the results presented in Chapter 6

4.1 Sensitivity testing for combined model

The effect of changing A and B on the fit of the combined data [values given are p (>Chi)].

Two expected values of B were considered (“23” from Chapter 6 Equation 8 and “33” from chapter 6 Equation 7). “28” was considered because it was half way between the expected values and “15” and “45” were chosen to give an idea of how the combined model behaved outside of the expected range. Chapter 4 has given an indication of the effect each unit of footprint hydrocarbons has on decisions at the bifurcation so testing outside of the range “15-45” cannot be justified.

Where “na” shown where the combined model was not a better fit to the data than the pheromone only model.

Table 23 – Combined model Sensitivity analysis Pr<(Chi)

		Value of A				
		8	**10**	**12**	**14**	**16**
Value of B	**15**	0.0001	0.0001	0.0007	0.0004	0.0005
	23	na	0.0002	0.0007	0.0004	0.0005
	28	na	na	0.0003	0.0004	0.0005
	33	na	na	0.0007	0.0004	0.0005
	45	na	na	na	na	na

The corresponding p values from a Vuong Non-Nested Hypothesis Test between combined model and pheromone model.

Table 24 – Combined model Sensitivity analysis Vuong Non-Nested Hypothesis Test

		Value of A				
		8	**10**	**12**	**14**	**16**
Value of B	**15**	0.0427	0.0277	0.0191	0.0205	0.0194
	23	na	0.0412	0.0255	0.028	0.0258
	28	na	na	0.0410	0.0352	0.0321
	33	na	na	0.0409	0.0454	0.0410
	45	na	na	na	na	na

4.2 Interactions

Variance in decision times between interacting and not interacting groups was not significantly difference (Brown-Forsythe test, $_{F1,185}$ = 3.809, p = 0.053).

Variance in decision times between interacting and not interacting groups was not significantly different (Brown-Forsythe test, $_{F1,185}$ = 3.320, p = 0.070).

Variance in pheromone probability of taking the food branch between interacting and not interacting groups was not significantly different (Brown-Forsythe test, $_{F1,185}$ = 0.032, p = 0.858).

Variance in the combined probability of taking the food branch between interacting and not interacting groups was not significantly different (Brown-Forsythe test, $_{F1,185}$ = 0.013, p = 0.910).

References

Akino, T., Morimoto, M., and Yamaoka, R., 2005. The chemical basis for trail recognition in *Lasius nipponensis* (Hymenoptera: Formicidae). *Chemoecology*, 15 (1), 13–20.

Akino, T. and Yamaoka, R., 2005. Trail discrimination signal of *Lasius japonicus* (Hymenoptera: Formicidae). *Chemoecology*, 15 (1), 21–30.

Attygalle, A.B., Vostrowsky, O., Bestmann, H.J., and Morgan, E.D., 1987. New chemicals from the dufour gland of the formicine ant *Lasius niger* (Hymenoptera:Formicidae). *Insect Biochemistry*, 17 (1), 219–225.

Banks, C.J. and Nixon, H.L., 1958. Effects of the ant, *Lasius niger* L., on the feeding and excretion of the bean aphid, *Aphis fabae* Scop. *Journal of Experimental Biology*, 35, 703–711.

Baroni-Urbani, C., Buser, M.W., and Schilliger, E., 1988. Substrate vibration during recruitment in ant social organization. *Insectes Sociaux*, 35 (3), 241–250.

Beckers, R., Deneubourg, J.L., and Goss, S., 1992a. Trails and U-turns in the selection of a path by the ant *Lasius niger*. *Journal of Theoretical Biology*, 397–415.

Beckers, R., Deneubourg, J.L., and Goss, S., 1992b. Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). *Insectes Sociaux*, 72, 59–72.

Beckers, R., Deneubourg, J.L., and Goss, S., 1993. Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *Journal of Insect Behavior*, 6 (6), 751–759.

Beckers, R., Deneubourg, J.L., Goss, S., and Pasteels, J.M., 1990. Collective decision making through food recruitment. *Insectes Sociaux*, 37 (3), 258–267.

Beckers, R., Goss, S., Deneubourg, J.L., and Pasteels, J.M., 1989. Colony size, communication, and ant foraging strategy. *Psyche*, 96, 239–256.

Beekman, M., Sumpter, D.J., and Ratnieks, F.L., 2001. Phase transition between disordered and ordered foraging in Pharaoh's ants. *PNAS*, 98 (17), 9703–6.

Bergström, G. and Löfqvist, J., 1970. Chemical basis for odour communication in four species of *Lasius* ants. *Journal of Insect Physiology*, 16, 2353–75.

Billen, J., 2009. Occurrence and structural organization of the exocrine glands in the legs of ants. *Arthropod Structure & Development*, 38 (1), 2–15.

Billen, J., Thijs, B., Ito, F., and Gobin, B., 2005. The pretarsal footprint gland of the ant *Amblyopone reclinata* (Hymenoptera, Formicidae) and its role in nestmate recruitment. *Arthropod Structure & Development*, 34 (2), 111–116.

Brian, M. V, Hibble, J., and Stradling, D.J., 1965. Ant pattern and density in a southern English heath. *The Journal of Animal Ecology*, 34 (3), 545–555.

Britton, N.F., Stickland, T.R., and Franks, N.R., 1998. Analysis of ant foraging algorithms. *Journal of Biological Systems*, 6 (4), 315–336.

Cassill, D., 2003. Rules of supply and demand regulate recruitment to food in an ant society. *Behavioral Ecology and Sociobiology*, 54, 441–450.

Cinquin, O. and Demongeot, J., 2002a. Roles of positive and negative feedback in biological systems. *Comptes Rendus Biologies*, 325 (11), 1085–95.

Cinquin, O. and Demongeot, J., 2002b. Positive and negative feedback: striking a balance between necessary antagonists. *Journal of Theoretical Biology*, 216 (2), 229–41.

Czaczkes, T.J., Grüter, C., Ellis, L., Wood, E., and Ratnieks, F.L.W., 2013. Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. *The Journal of Experimental Biology*, 216 (2), 188–97.

Czaczkes, T.J., Grüter, C., Jones, S.M., and Ratnieks, F.L.W., 2011. Synergy between social and private information increases foraging efficiency in ants. *Biology Letters*, 7 (4), 521–4.

Czaczkes, T.J., Grüter, C., Jones, S.M., and Ratnieks, F.L.W., 2012. Uncovering the complexity of ant foraging trails. *Communicative & Integrative Biology*, 5 (1), 78–80.

Debout, G. and Schatz, B., 2007. Polydomy in ants: what we know, what we think we know, and what remains to be done. *Biological Journal of the Linnean Society*, 90, 319–348.

Deneubourg, J.L., Aron, S., Goss, S., and Pasteels, J.M., 1990. The self-organizing exploratory pattern of the Argentine ant. *Journal of Insect Behavior*, 3 (2), 159–168.

Devigne, C. and De Biseau, J.C., 2012. The differential response of workers and queens of the ant *Lasius niger* to an environment marked by workers: ants dislike the unknown. *Behavioural Processes*, 91 (3), 275–81.

Devigne, C. and Detrain, C., 2002. Collective exploration and area marking in the ant *Lasius niger*. *Insectes Sociaux*, 49 (4), 357–362.

Devigne, C. and Detrain, C., 2006. How does food distance influence foraging in the ant *Lasius niger*: the importance of home-range marking. *Insectes Sociaux*, 53 (1), 46–55.

Devigne, C., Renon, A.J., and Detrain, C., 2004. Out of sight but not out of mind: modulation of recruitment according to home range marking in ants. *Animal Behaviour*, 67 (6), 1023–1029.

Dorigo, M., Birattari, M., and Stutzle, T., 2006. Ant colony optimization. *IEEE Computational Intelligence Magazine*, (November).

Dornhaus, A. and Chittka, L., 1999. Insect behaviour: Evolutionary origins of bee dances. *Nature*, 401 (2), 38.

Dussutour, A., Fourcassié, V., Helbing, D., and Deneubourg, J.L., 2004. Optimal traffic organization in ants under crowded conditions. *Nature*, 428 (4), 70–73.

Dussutour, A. and Nicolis, S.C., 2009. The role of multiple pheromones in food recruitment by ants. *The Journal of Experimental Biology*, 212 (15), 2337–48.

Evison, S.E.F., Petchey, O.L., Beckerman, A.P., and Ratnieks, F.L.W., 2008. Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. *Behavioral Ecology and Sociobiology*, 63 (2), 261–267.

Fourcassié, V. and Deneubourg, J.L., 1992. Collective exploration in the ant *Monomorium pharaonis* L. *In*: J. Billen, ed. *Biology and Evolution of Social Insects*. Leuven University Press, 369–373.

Fourcassié, V. and Deneubourg, J.L., 1994. The dynamics of collective exploration and trail formation in *Monomorium pharaonis*: experiments and model. *Physiological Entomology*, 19, 291–300.

Freeman, M., 2000. Feedback control of intercellular signalling in development. *Nature*, 408 (16), 313–319.

Von Frisch, K., 1967. *The dance language and orientation of bees.* Belknap Press, Cambridge.

Gadau, J. and Fewell, J., 2009. *Organization of insect societies: from genome to sociocomplexity*. Harvard University Press.

Gerbier, G., Garnier, S., Rieu, C., Theraulaz, G., and Fourcassié, V., 2008. Are ants sensitive to the geometry of tunnel bifurcation? *Animal Cognition*, 11 (4), 637–42.

Giurfa, M. and Núñez, J.A., 1992. Honeybees mark with scent and reject recently visited flowers. *Oecologia*, 89 (1), 113–117.

Gordon, D.M., Holmes, S., and Nacu, S., 2007. The short-term regulation of foraging in harvester ants. *Behavioral Ecology*, 19 (1), 217–222.

Goss, S., Aron, S., Deneubourg, J.L., and Pasteels, J.M., 1989. Self-organized shortcuts in the Argentine ant. *Naturwissenschaften*, 76, 579–581.

Gronenberg, W. and Hölldobler, B., 1999. Morphologic representation of visual and antennal information in the ant brain. *The Journal of Comparative Neurology*, 412 (2), 229–40.

Grüter, C., Balbuena, M.S., and Farina, W.M., 2008. Informational conflicts created by the waggle dance. *Proc. R. Soc. B.*, 275, 1321–7.

Grüter, C., Czaczkes, T.J., and Ratnieks, F.L.W., 2010. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. *Behavioral Ecology and Sociobiology*, 65 (2), 141–148.

Grüter, C. and Farina, W.M., 2009. The honeybee waggle dance: can we follow the steps? *Trends in Ecology & Evolution*, 24 (5), 242–7.

Grüter, C., Schürch, R., Czaczkes, T.J., Taylor, K., Durance, T., Jones, S.M., and Ratnieks, F.L.W., 2012. Negative feedback enables fast and flexible collective decision-making in ants. *PloS one*, 7 (9), e44501.

Guy, A.G., Bohan, D.A., Powers, S.J., and Reynolds, A.M., 2008. Avoidance of conspecific odour by carabid beetles: a mechanism for the emergence of scale-free searching patterns. *Animal Behaviour*, 76 (3), 585–591.

Harkness, R. and Maroudas, N., 1985. Central place foraging by an ant (*Cataglyphis bicolor* Fab.): a model of searching. *Animal Behaviour*, 916–928.

Hölldobler, B. and Palmer, J.M., 1989. A new tarsal gland in ants and the possible role in chemical communication. *Naturwissenschaften*, 76 (8), 385–386.

Hölldobler, B. and Wilson, E.O., 1990. *The Ants*. Cambridge, Massachusetts: Belknap Press.

Jackman, S., 2012. pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory.

Jackson, D.E., Bicak, M., and Holcombe, M., 2010. Decentralized communication, trail connectivity and emergent benefits of ant pheromone trail networks. *Memetic Computing*, 3 (1), 25–32.

Jackson, D.E. and Châline, N., 2007. Modulation of pheromone trail strength with food quality in Pharaoh's ant, *Monomorium pharaonis*. *Animal Behaviour*, 74 (3), 463–470.

Jackson, D.E., Holcombe, M., and Ratnieks, F.L.W., 2004. Trail geometry gives polarity to ant foraging networks. *Nature*, 432 (16), 907–9.

Jackson, D.E., Martin, S.J., Holcombe, M., and Ratnieks, F.L.W., 2006. Longevity and detection of persistent foraging trails in Pharaoh's ants, *Monomorium pharaonis* (L.). *Animal Behaviour*, 71 (2), 351–359.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., and Lepage, M., 2006. Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. *Applied Soil Ecology*, 32 (2), 153–164.

Kholodenko, B.N., 2000. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *European Journal of Biochemistry*, 267 (6), 1583–8.

Kirchner, W.H., 1993. Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. *Behavioral Ecology and Sociobiology*, 33 (3), 169–172.

Kohl, E., Hölldobler, B., and Bestmann, H.J., 2003. Trail pheromones and Dufour gland contents in three Camponotus species (*C. castaneus, C. balzani, C. sericeiventris*: Formicidae, Hymenoptera). *Chemoecology*, 13 (3), 113–122.

Lach, L., Parr, C., and Abbott, K.L., 2010. *Ant Ecology*. Oxford University Press.

Lau, C.W. and Nieh, J.C., 2009. Honey bee stop-signal production: temporal distribution and effect of feeder crowding. *Apidologie*, 41 (1), 87–95.

Lenoir, A., Depickère, S., Devers, S., Christidès, J.P., and Detrain, C., 2009. Hydrocarbons in the ant *Lasius niger*: from the cuticle to the nest and home range marking. *Journal of Chemical Ecology*, 35 (8), 913–21.

Leuthold, R.H., 1968. A tibial gland scent-trail and trail-laying behavior in the ant *Crematogaster ashmeadi* Mayr. *Psyche*, 75, 233–48.

Mailleux, A.C., Buffin, A., Detrain, C., and Deneubourg, J.L., 2010. Recruiter or recruit: who boosts the recruitment in starved nests in mass foraging ants? *Animal Behaviour*, 79 (1), 31–35.

Mailleux, A.C., Deneubourg, J.L., and Detrain, C., 2000. How do ants assess food volume? *Animal Behaviour*, 59 (5), 1061–1069.

Mailleux, A.C., Deneubourg, J.L., and Detrain, C., 2003. Regulation of ants' foraging to resource productivity. *Proc. R. Soc. B.*, 270, 1609–16.

Marks, R.J., 1977. Laboratory studies of plant searching behaviour by *Coccinella septempunctata* L. larvae. *Bulletin of Entomological Research*, 67 (2), 235–41.

Martin, S. and Drijfhout, F., 2009. A review of ant cuticular hydrocarbons. *Journal of Chemical Ecology*, 35 (10), 1151–61.

Maschwitz, U., 1964. Gefahrenalarmstoffe und Gefahrenalarmierung bei sozialen Hymenopteren. *Zeitschrift fur Vergleichende Physiologie*, 47, 596–655.

Vander Meer, R.K. and Alonso, L.E., 1998. Pheromone directed behavior in ants. *In*: R.K. Vander Meer, M. Breed, M. Winston, and K.E. Espelie, eds. *Pheromone Communication in Social Insects*. Westview Press, Boulder C.O., 159–192.

Morgan, D.E., 2009. Trail pheromones of ants. *Physiological Entomology*, 34 (1), 1–17.

Nieh, J.C., 1993. The stop signal of honey bees: reconsidering its message. *Behavioral Ecology and Sociobiology*, 33 (1), 51–56.

Nieh, J.C., 1998. The food recruitment dance of the stingless bee, *Melipona panamica*. *Behavioral Ecology and Sociobiology*, 43, 133–145.

Nieh, J.C., 2010. A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology*, 20 (4), 310–5.

Nieh, J.C., Contrera, F.A.L., and Nogueira-Neto, P., 2003. Pulsed mass recruitment by a stingless bee, *Trigona hyalinata*. *Proc. R. Soc. B.*, 270, 2191–6.

Nonacs, P., 1990. Death in the distance: mortality risk as information for foraging ants. *Behaviour*, 112 (1), 23–35.

Overmyer, S.L. and Jeanne, R.L., 1998. Recruitment to food by the German yellowjacket, *Vespula germanica*. *Behavioral Ecology and Sociobiology*, 42 (1), 17–21.

Parry, K. and Morgan, E.D., 1979. Pheromones of ants: a review. *Physiological Entomology*, 4 (2), 161–189.

Pastor, K.A. and Seeley, T.D., 2005. The Brief Piping Signal of the Honey Bee: Begging Call or Stop Signal? *Ethology*, 111 (8), 775–784.

Perna, A., Granovskiy, B., and Garnier, S., 2012. Individual rules for trail pattern formation in Argentine ants (*Linepithema humile*). *PLoS Computational Biology*, 8 (7), e1002592.

Planqué, R., Van den Berg, J.B., and Franks, N.R., 2010. Recruitment strategies and colony size in ants. *PloS one*, 5 (8), e11664.

Portha, S., Deneubourg, J.-L., and Detrain, C., 2004. How food type and brood influence foraging decisions of *Lasius niger* scouts. *Animal Behaviour*, 68 (1), 115–122.

Provecho, Y. and Josens, R., 2009. Olfactory memory established during trophallaxis affects food search behaviour in ants. *The Journal of Experimental Biology*, 212, 3221–7.

R Core Team, 2013. R: A language and environment for statistical computing. *R Foundation Statistical Computing*.

Regnier, F.E. and Wilson, E.O., 1968. The alarm-defence system of the ant *Acanthomyops claviger*. *Journal of Insect Physiology*, 14, 955 – 970.

Regnier, F.E. and Wilson, E.O., 1969. The alarm-defence system of the ant *Lasius alienus*. *Journal of Insect Physiology*, 15, 893–898.

Reinhard, J. and Kaib, M., 1995. Interaction of pheromones during food exploitation by the termite *Schedorhinotermes lamanianus*. *Physiological Entomology*, 20, 266–272.

Reznikova, J.I., 1982. Interspecific communication between ants. *Behaviour*, 80 (1), 84–95.

Reznikova, Z.I. and Ryabko, B., 2001. A study of ants' numerical competence. *Computer and Information Science*, 5, 111 – 126.

Robinson, E.J.H., Green, K.E., Jenner, E.A., Holcombe, M., and Ratnieks, F.L.W., 2008. Decay rates of attractive and repellent pheromones in an ant foraging trail network. *Insectes Sociaux*, 55 (3), 246–251.

Robinson, E.J.H., Jackson, D.E., Holcombe, M., and Ratnieks, F.L.W., 2005. Insect communication: "no entry" signal in ant foraging. *Nature*, 438, 442.

Robinson, E.J.H., Ratnieks, F.L.W., and Holcombe, M., 2008. An agent-based model to investigate the roles of attractive and repellent pheromones in ant decision making during foraging. *Journal of Theoretical Biology*, 255 (2), 250–8.

Robinson, E.J.H., Richardson, T.O., Sendova-Franks, A.B., Feinerman, O., and Franks, N.R., 2008. Radio tagging reveals the roles of corpulence, experience and social information in ant decision making. *Behavioral Ecology and Sociobiology*, 63 (5), 627–636.

Rohrseitz, K. and Tautz, J., 1999. Honey bee dance communication: waggle run direction coded in antennal contacts? *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, 184 (4), 463–470.

Schmitt, U. and Bertsch, A., 1990. Do foraging bumblebees scent-mark food sources and does it matter? *Oecologia*, 82 (1), 137–144.

Stubben, C.J. and Milligan, B.G., 2007. Estimating and analyzing demographic models using the popbio package in R. *Journal of Statistical Software*, 22 (11).

Sudd, J.H., 1960. The foraging method of Pharaoh's ant, *Monomorium pharaonis* (L.). *Animal Behaviour*, 8, 67–75.

Szlep, R. and Jacobi, T., 1967. The mechanism of recruitment to mass foraging in colonies of *Monomorium venustum* Smith, *M. subopacum* ssp. *Phœnicium* Em., *Tapinoma israelis* For. and T. *Simothi*. *Insectes Sociaux*, 14 (1), 25–40.

Taylor, F., 1978. Foraging behavior of ants: theoretical considerations. *Journal of Theoretical Biology*, 71, 541–565.

Townsend-Mehler, J.M. and Dyer, F.C., 2011. An integrated look at decision-making in bees as they abandon a depleted food source. *Behavioral Ecology and Sociobiology*, 66 (2), 275–286.

Traniello, J.F.A., 1977. Recruitment behavior, orientation, and the organization of foraging in the carpenter ant *Camponotus pennsylvanicus* DeGeer (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, 2 (1), 61–79.

Traniello, J.F.A., 1982. Recruitment and orientation components in a termite trail pheromone. *Naturwissenschaften*, 69, 343–345.

Venables, W.N. and Ripley, B.D., 2002. *Modern applied statistics with S*. Fourth Edi. Springer, New York.

Vuong, Q.H., 1989. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica: Journal of the Econometric Society*, 57 (2), 307–333.

Weisberg, S. and Fox, J., 2010. *An R companion to applied regression*.

Wilms, J. and Eltz, T., 2008. Foraging scent marks of bumblebees: footprint cues rather than pheromone signals. *Naturwissenschaften*, 95 (2), 149–53.

Wilson, E.O., 1962. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith) 1. The Organization of Mass-Foraging. *Animal Behaviour*.

Wilson, E.O. and Regnier Jr, F.E., 1971. The evolution of the alarm-defense system in the formicine ants. *American Naturalist*, 105 (943), 279–289.

Wyatt, T.D., 2003. *Pheromones and animal behaviour: communication by smell and taste*. Cambridge University Press.

Zeileis, A. and Hothorn, T., 2002. Diagnostic checking in regression relationships. *R news*.