The organisation of polydomous nesting in wood ant colonies: behaviour, networks, foraging and resource redistribution.

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PhD

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April 2015

Abstract

Social behaviours are an important component of evolutionary success. This is perhaps most evident in the societies of social insects: the interactions between individuals underlie the organisation of their highly complex, and highly successful, societies. An important socially organised determinant of ecological success for social insects is nesting strategy. Many ant species can have a polydomous nesting strategy: a polydomous colony inhabits several spatially separated, but socially connected, nests. How this complex nesting strategy is organised is largely unknown. I undertook a series of studies to investigate how polydomous colonies of the ecologically important red wood ant *Formica lugubris* are organised at the individual, nest and colony levels. I found that resources are redistributed locally, between nests, within polydomous wood ant colonies. Further investigation showed that this local resource redistribution is mediated by individual workers treating other nests of the colony as food sources. I also investigated the role that nests which do not appear to be foraging are playing in polydomous wood ant colonies. I found that these non-foraging nests, rather than having a specialised role, are part of the colony expansion process. I explored the importance of resource acquisition to individual nests by investigating the effect that position in the colony nest-network has on the survival, reproduction and growth of nests within a polydomous colony. I found that position within a dynamic nest-network was an important determinant of life-history success for individual nests in wood ant colonies. My results suggest that little behavioural innovation is needed for a colony to become polydomous, and indicate benefits a polydomous nesting strategy may provide a colony. These results highlight how understanding the proximate mechanism and development of a social behaviour, can give important insights into the ultimate function and evolution of a life-history strategy.

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Acknowledgements

I owe a large number of people a debt of gratitude for lending me their time, resources and brain-power during the course of my PhD. First thanks must go to my supervisor, Elva Robinson. Elva has been consistently generous with her time and support in all matters. I have learnt a lot during my PhD and this in a large part to Elva's guidance and insight. I am particularly grateful for Elva's forbearance: while, I learnt, the appropriate use of; commas, and other grammatical-motifs.

I have also been supported academically by Training Advisory Panel: Dan Franks, Chris Thomas and Jon Pitchford. Throughout my PhD they have given useful advice and guidance and offered a valuable fresh perspective on my work. Some of the work in this thesis was conducted in collaboration with Dan Franks. Dan has consistently shown an interest, and been generous with his time, supporting the work in this project, for which I am very grateful.

I also had the pleasure of being involved in the Theoretical Ecology, Ecology and Evolution groups during my PhD. I am thankful for useful comments I have received when presenting at these meetings, and the interesting things I learnt while other presented. I would also like to express my gratitude for the many instances of kind support from staff in both YCCSA and the department of Biology.

I would like to gratefully acknowledge the financial support I have received from the National Environmental Research Council, who funded this work.

The National Trust were a CASE partner on this project, and I am grateful for their support. I would like to specifically thank Carl Hawke for his interest, advice, and support during this project. I would also like to thank Chris Milner and the staff and volunteers at the Longshaw Estate. Their support and interest were instrumental in making my fieldwork both productive and enjoyable. I would also like to thank Jonathan Pattrick and Darren Chin who assisted me in the field for chapters 3 and 5 respectively.

I have been lucky enough to be part of an active and supportive research group. Past and present members, namely: Duncan Procter, Zoe Cook, Yi-Huei Chen and Philip Buckham-Bonnett, have provided valuable insights to my work, and they have also been an important part of making my office an enjoyable place to work. I must also thank others I have shared an office with over the years- especially: Sarah Collings, Celina Wong and Ben Russell- for interesting discussions, and putting up with me for so long.

Finally, I am grateful for the support of my parents and family. Their consistent interest and support have been invaluable, in the truest sense of the word, throughout my academic life.

Author Declaration

I hereby declare that this submission is entirely my own work, except where due acknowledgement is given. This work has not been presented for an award at this, or any other, university.

Chapter 2 has been published in *Insectes Sociaux* (Ellis and Robinson 2014) and is presented as published. References in the text to ‘unpublished work’ have been replaced with reference to the appropriate chapter in this thesis.

Chapter 3 has been published in *Behavioral Ecology* (Ellis et al 2014) and is presented as published. References in the text to ‘unpublished work’ have been replaced with reference to the appropriate chapter in this thesis. Additionally, spelling and grammar has been converted from American English to British English.

Chapter 4 is in preparation for submission to *Behavioural Ecology and Sociobiology*.

Chapter 5 is in preparation for submission to *PLOS ONE*.

Chapter 6 is in preparation for submission to *Proceedings of the Royal Society B***.**

Additional supervision for the work presented in chapters 3 and 6 was provided by Daniel W. Franks. D Franks is acknowledged as an author on these publications.

Chapter 1: Introduction

1.1 General Introduction

Behaviour evolves and functions in the social, as well as the ecological, environment. Perhaps the most extreme examples of the organisation and evolution of behaviours in the social environment are found in the complex eusocial societies of social insects. The simple, self-organised behaviours, which govern many aspects of collective behaviour in hymenopteran colonies, are intriguing examples, not only of how evolution can act within social systems, but also how a complex system can be organised.

In recent decades the idea of an ant colony as a collection of closely related sisters living in a single nest with a single queen has been found to be insufficient in almost all ant species (Heinze 2008). An important example of how this idea has been challenged has been the revelation that in a large number of ant species a single colony inhabits several spatially separated, but socially connected, nests (Debout et al. 2007; Robinson 2014). This dispersed nesting system, called polydomy, has evolved many times independently within the ants, but its proximate organisation and ultimate function remain largely unknown. In this thesis I present a series of studies investigating the organisation of the polydomous nesting strategy in the red wood ant *Formica lugubris*.

1.2 Polydomy

Polydomy is important because it has the potential to profoundly influence both the social system of a colony, and the way that colony interacts with the rest of ecosystem. A colony spatially separated between different nests faces a variety of challenges not experienced by colonies inhabiting a single nest (monodomous colonies). For example, whereas the individuals in a monodomous colony have at least the potential to interact, in a spatially separated colony subsections of the colony will not come into contact. An important consequence of this is that ants in different nests are likely to have different access to resources, as food is rarely spread evenly through the environment. Redistributing resources between spatially dispersed parts of the colony is a problem not encountered in monodomous colonies. A polydomous colony will, therefore, require behavioural mechanisms not needed by colonies inhabiting a single nest, to redistribute resources between nests.

The challenge of organising a dispersed nesting colony has had to be overcome several times in ants. Polydomy has evolved several times independently in ants; the nesting strategy has been reported in over 150 ant species from all the major ant subfamilies (figure 1.1; Debout et al. 2007). Polydomy does not fit a particular ecological or phylogenetic niche (figure 1.1) and the benefits the strategy provides a colony remain unclear (Debout et al. 2007; Robinson 2014). The ecological benefits a polydomous nesting strategy may provide an ant colony are discussed in more detail in chapter 2.

Regardless of the benefits provided by dispersed nesting, an important challenge that a polydomous colony needs to overcome is how to redistribute resources between the nests of the colony. Many behaviours in social insect colonies are direct towards resource redistribution; foraging behaviours, for example, transfer resources from the environment into the nest, and brood care behaviours transfer resources from the workers to the brood. In a polydomous colony an important part of this resource redistribution system is the transfer of resources between nests. I define a polydomous colony on the basis of resource sharing between nests (discussed in more detail below); understanding the organisation of this resource redistribution is therefore vital to properly understanding this widespread and important nesting strategy.

Defining colony boundaries

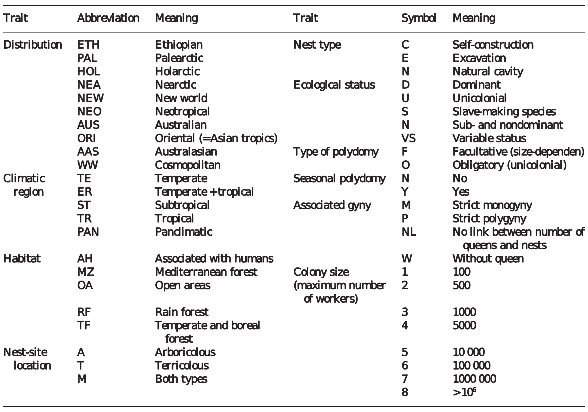
In this thesis I define a polydomous colony as two or more spatially separated, but socially connected, nests (Debout et al. 2007). A nest in the context of a polydomous colony is defined as any structure that houses workers and brood (Debout et al. 2007), any other structure is considered an outstation (Anderson & Mcshea 2001). The spatial separation between the nests within a polydomous colony must be larger than the distance between chambers within a nest to be considered as separate nests (Debout et al. 2007).

The social connection between nests within a polydomous colony can be defined in several ways. Some studies define a polydomous colony on the basis of resource redistribution between nests (e.g. McIver 1991; Pfeiffer & Linsenmair 1998; Holway & Case 2000; Van Wilgenburg & Elgar 2007). Defining a colony on the basis of resource exchange has the advantage of representing a direct, and ecologically relevant, connection between the nests involved. Sharing resources means that nests can be considered as in some sense a single unit, at least in ecological terms. For the studies presented in this thesis I use this ecological, functional, definition of polydomy.

Rather than directly measuring resource exchange between nests, many studies use aggression and spatial-clustering to infer polydomy (e.g. Cerdá et al. 2002; Santini et al. 2011; Moffett 2012). Using aggression and clustering implies a definition of polydomy

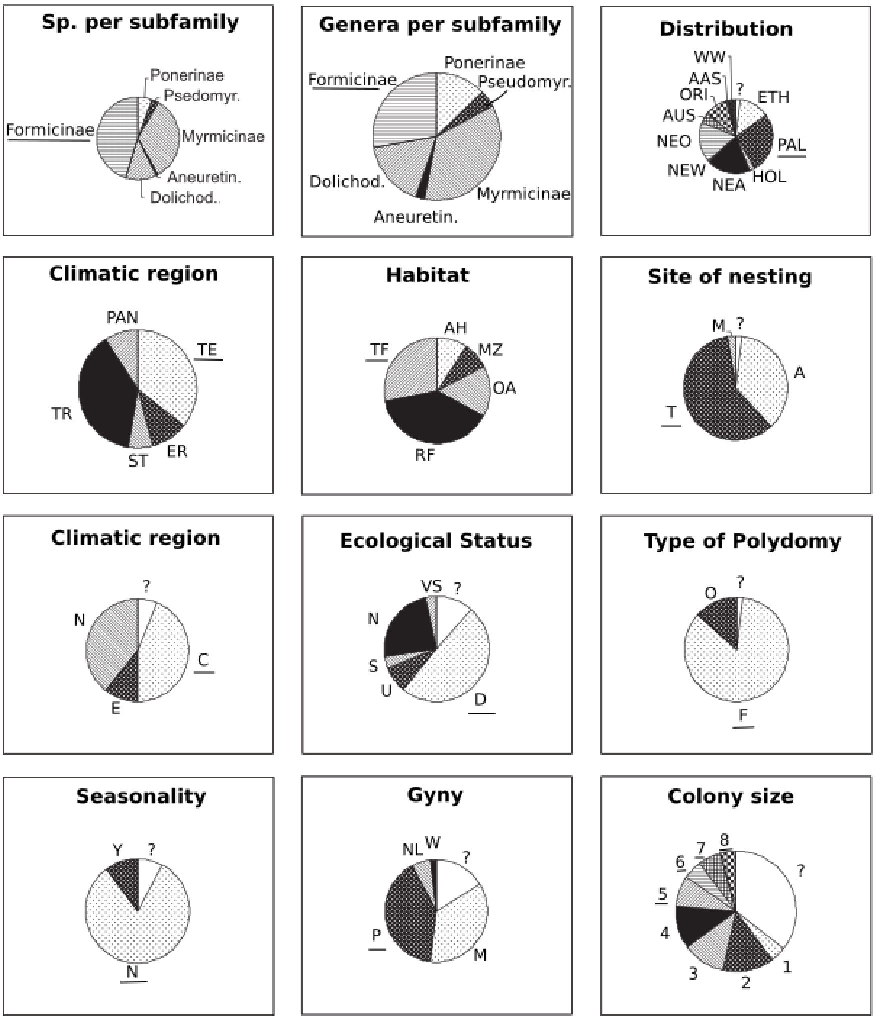
Figure 1.1 Traits associated with polydomous nesting in ants. Adapted from (Debout et al. 2007).

a) Key. ‘Type of Polydomy’ defines if a species is always reported as polydomous (obligatory) or has been reported as both polydomous and monodomous (facultative). Gyny refers to the number of queens in a nest: monogynous colonies have only one queen, polygynous colonies have more than one queen.



Trait	Abbreviation	Meaning	Trait	Symbol	Meaning
Distribution	ETH	Ethiopian	Nest type	C	Self-construction
	PAL	Palearctic		E	Excavation
	HOL	Holarctic		N	Natural cavity
	NEA	Nearctic	Ecological status	D	Dominant
	NEW	New world		U	Unicolonial
	NEO	Neotropical		S	Slave-making species
	AUS	Australian		N	Sub- and nondominant
	ORI	Oriental (=Asian tropics)		VS	Variable status
	AAS	Australasian	Type of polydomy	F	Facultative (size-dependen)
	WW	Cosmopolitan		O	Obligatory (unicolonial)
Climatic region	TE	Temperate	Seasonal polydomy	N	No
	ER	Temperate +tropical		Y	Yes
	ST	Subtropical	Associated gyny	M	Strict monogyny
	TR	Tropical		P	Strict polygyny
	PAN	Pandimatic		NL	No link between number of queens and nests
Habitat	AH	Associated with humans		W	Without queen
	MZ	Mediterranean forest	Colony size (maximum number of workers)	1	100
	OA	Open areas		2	500
	RF	Rain forest		3	1000
	TF	Temperate and boreal forest		4	5000
Nest-site location	A	Arboricolous		5	10 000
	T	Terricolous		6	100 000
	M	Both types		7	1000 000
				8	$>10^6$

b) Distribution of traits in polydomous ants. In each chart, the underlined trait is that category (or categories) that contains *Formica lugubris*.



based upon a shared space. Lack of aggression, and close proximity, between nests suggests a level of mutual tolerance and indirect resource sharing, perhaps implying a shared descent, and membership of the same colony. Given in-depth species-specific information, aggression can be a useful way to assess how colony boundaries change on a broad, landscape, scale. However, in the studies presented here, I am interested in the organisation of polydomy at the scale of interactions between individual nests, for this I need a definition of colony boundaries which is robust at this fine scale: resource exchange between nests is likely to be a more accurate way to assess this than a shared space.

A final commonly used way to assess colony boundaries is based on genetic information, and the relatedness between individuals in nests. The advantage of basing colony boundaries on relatedness is that it gives a direct insight into the way that selection is acting between colonies. However, a disadvantage of basing colony boundaries on relatedness is that historical population changes may obscure ecologically important effects. Polydomous colonies of many species tend to reproduce by budding, which produces patches of nests with a shared descent (e.g. Sundström et al. 2005). However, at the local-scale competition will be between groups of nests cooperating by sharing resources, rather than being based on competition between groups of nests with different shared descents. As I am interested in the organisation of polydomy at the local-scale between individual nests resource redistribution may be a more useful way to represent the level at which selection acts on this trait.

In some cases colony boundaries (defined by either: resource exchange, aggression or relatedness) become so broad that the colony is considered to be a supercolony. Helanterä et al. (2009) define a supercolony as ‘an extreme form of polydomy, where the colony is so large that direct interactions between workers from separated nests become impossible’. A unicolonial population is, in turn, usually defined as a whole population consisting of a single supercolony (Helanterä et al. 2009). Supercolonies are an interesting concept and may have important evolutionary implications (e.g. Kennedy et al. 2014). Supercolonies are particularly common in invasive species outside of their native range (e.g. Vogel et al. 2010; Hoffmann 2014). In this thesis I concentrate on the organisation of the dispersed nesting strategy in smaller polydomous colonies. However, as supercolonies are simply ‘an extreme form of polydomy’ (Helanterä et al. 2009), in some cases the factors influencing the organisation of polydomy on a small scale may scale up to the broader, supercolonial, scale.

1.3 The *Formica rufa* group

The members of the *Formica rufa* group (red wood ants) are interesting species in which to study polydomy because they show both intraspecific and interspecific flexibility in nesting strategy. The nesting strategies of the red wood ant group are discussed in detail in chapter 2. An advantage of studying polydomy within the red wood ant group is that many aspects of their biology have been well-studied. Using a well-studied species as a model is advantageous because many important aspects of the natural history of the group are well understood: this acts as a useful basis for studying a complex strategy such as polydomy.

The red wood ant group is generally considered to consist of 6 species: *Formica rufa* (Linnaeus, 1761), *Formica aquilonia* (Yarrow, 1955), *Formica lugubris* (Zetterstedt, 1838), *Formica paralugubris* (Seifert, 1996), *Formica polyctena* (Foerster, 1850) and *Formica pratensis* (Retzius, 1783). Recent genetic analysis has suggested that a cryptic species, previously identified as *F. lugubris*, may be present in the Eastern Swiss Alps (Bernasconi et al. 2011), however this has not been confirmed. There also appears to be hybridisation between species in the group (e.g. Seifert et al. 2010; Kulmuni & Pamilo 2014), however, to our knowledge, the *F. lugubris* studied in this thesis are not a hybrid population. All members of the group are morphologically and behaviourally similar. The group is usually considered to be monophyletic, with *F. truncorum/F. frontalis* as a sister clade (Bernasconi et al. 2011). Some recent phylogenies have suggested that *F. truncorum*/*F. frontalis* are part of the *F. rufa* clade, but the resolution of these two species in the tree was low perhaps biasing the results (Goropashnaya et al. 2012). In the studies presented in this thesis we assume that the *F. rufa* group are a monophyletic clade of 6 species (not including *F. truncorum* and *F. frontalis*). We use the terms *F. rufa* group, red wood ants and wood ants interchangeably.

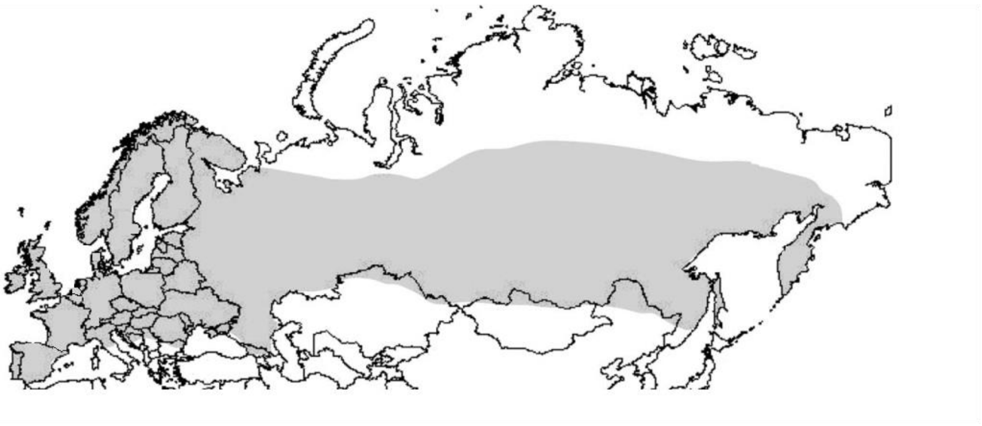


Figure 1.2 The distribution of the *F. rufa* group in Eurasia. Adapted from Goropashnaya et al. (2004).

The red wood ants are found in woodland and forest across much on Northern Eurasia (figure 1.2). The main food source for the red wood ants is honeydew collected by farming homopteran colonies in the canopy of trees (Rosengren & Sundström 1991), this reliance is likely to be an important factor limiting the wood ants to forest habitats. The wood ants are the dominant invertebrate predator in Eurasian woodlands and probably represent the final ant successional stage in mature forests (Punttila 1996; Punttila & Haila 1996; Vepsäläinen et al. 2000). The group are characterised by their large nest-mounds constructed of pine needles and other leaf-litter built above subterranean chambers (figure 1.3). These mounds are likely to be an important component of the group's success, as they provide a well-defended, dry habitat with a relatively stable temperature in which to raise brood.

Figure 1.3 Polydomous *Formica lugubris* colony.

Wood ants are a keystone species in woodland across much of Northern Eurasia. As dominant predators the presence of wood ants can profoundly affect the communities of ants (Mabelis 1984; Savolainen & Vepsäläinen 1989) and other invertebrates in the areas around their nests (Laakso & Setälä 2000; Punttila et al. 2004; Wardle et al. 2011). The nests themselves are constructed from leaf-litter and other detritus which can affect the nutritional and physical properties of the soil in a variety of ways (e.g. Kristiansen & Amelung 2001; Lenoir et al. 2001; Domisch et al. 2007). The nests the also act has hosts to a variety of myrmecophiles (Robinson & Robinson 2013; Harkonen & Sorvari 2014) including the UK biodiversity priority species *Formicoxenus nitidulus* (JNCC 2010; Parmentier et al. 2014). Understanding the nesting habits of this important keystone species is important to preserve natural woodland ecosystems (Mabelis 2007).

All studies presented in this thesis are conducted on a population of *Formica lugubris* in central England. In Britain there are currently three species of red wood ant: *F. rufa* concentrated in the south of England and Wales, *F. lugubris* mainly found in central and northern England and Scotland and *F. aquilonia* in the north of Scotland (BWARS 2015). The range of *F. lugubris* does overlap with both other red wood ant species. The historical range boundaries of the species are difficult to assess as nests were often moved to provide food for pheasants on shooting estates (Yarrow 1955). *Formica lugubris* are particularly variable in their nesting strategy; with polydomous populations found in England, parts of Finland and parts of Switzerland and monodomous populations reported in Ireland, parts of Finland and parts Switzerland (chapter 2). This variability in nesting strategy makes them an interesting species in which to examine the organisation of polydomous colonies.

1.4 The Longshaw Estate

All studies presented in this thesis were conducted at the Longshaw Estate in central England (N53° 18’ 33’’, E-1° 36’ 96’’). The estate was chosen for use in this study for several reasons. Firstly, the estate has a large and healthy *Formica lugubris* population; a survey in winter 2011/2012 found over 900 nests over only part of the site (figure 1.4). Secondly, the estate has a mixture of habitats within a relatively small area (discussed in more detail below). Thirdly, most of the site is grazed by sheep and some cattle during the spring and summer; this means that there is very little undergrowth which facilitates locating wood ant nests and observational studies. Finally, the site mangers (the National Trust) are committed to conserving the natural woodland ecosystem, and have a particular interest in the wood ants. For example, on the basis of my survey of the area plans for forestry work at the site were amended to preserve areas with particularly high concentrations of wood ants.

The whole estate is approximately 650 ha^{-1}, but all studies were conducted in a smaller 95 ha^{-1} area within the site. This smaller area was selected because it had a large number of *F. lugubris* nests, in a range of habitats. Other areas of the site were less amenable to study consisting of a mixture of moorland, steep sided valleys and monoculture silver birch (*Betula* spp.) woodland. The silver birch forest may represent an early successional stage as it is on area which was historically the site of quarrying activity. The silver birch woodland contains few *Formica lugubris* nests and those which are present tend to be smaller the nests in other areas of the site (*personal observation*).

The study area is on a west facing slope between 260m and 350m above sea level. The area contains a variety of habitats. There are several patches of historic forestry plantation, which were originally established at the site in the 1920s. These plantation areas consist of dense woodland, the majority of the trees are Scots pine (*Pinus sylvestris*), with some larch (*Larix* spp.) also present. Between these plantations are areas of open woodland pasture, with some large trees scattered in open areas of grazed pasture. At the Longshaw estate the scattered trees are a mixture of oak (*Quercus* spp.) and sycamore (*Acer pseudoplatanus*). There are also some small areas of deciduous woodland with a mixture of tree species. *Formica lugubris* are found over all of this area but tend to be concentrated around the edge of the forested areas (figure 1.4). I observed ants foraging to oak, sycamore, larch, scots pine and silver birch trees, in approximately the proportion which they are found at the site (figure 1.5).

Figure 1.4 Map of the study area within the Longshaw estate with the locations of *Formica lugubris* nests marked. Nests are marked with a circle. The nest locations are based on a survey conducted over the winter 2011/2012. The are 908 nests present within this 9.5 ha^{-1} area. Average nest size was 64 cm x 58 cm x 21 cm (diameter x diameter x height).

Wood ants have no predators that regularly lead to the abandoning of a nest in England. At the Longshaw estate there is some predation of ants by the green woodpecker (*Picus viridis*) but their feeding does not lead to the destruction of the nest. I occasionally encountered nests which had been partially excavated, probably by badgers (*Meles meles),* this did not always lead to the nest being abandoned and the damage was often rapidly repaired. The only other substantial threat to the ant nests was from human activity. Ants near to paths were often disturbed by passers-by; however no colonies used in this study were adjacent to busy pathways.

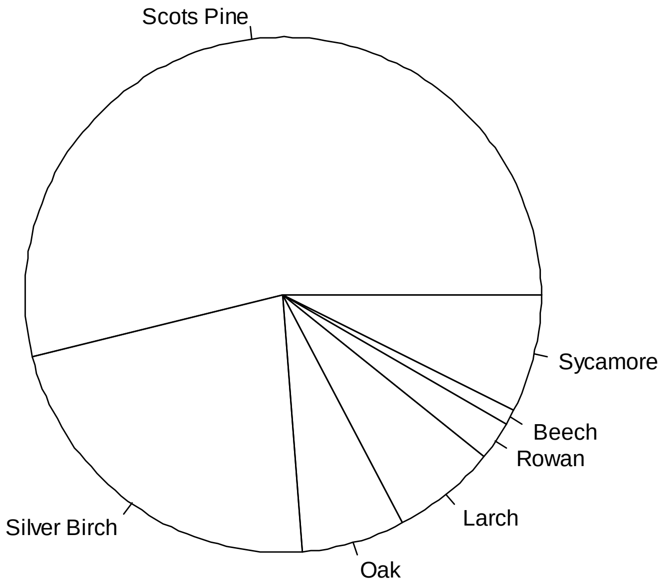


Figure 1.5 Species of trees foraged to by *Formica lugubris* at the Longshaw estate. Figure is based on the species of trees foraged to by the ten colonies used for network analysis in chapter 3.

1.5 Thesis outline

In this thesis I present a series of studies investigating the relationships between nests in polydomous *Formica lugubris* colonies. Chapter 2 reviews what is known about polydomy in the red wood ant group. The review highlights the relationship between polydomy and polygyny in the group; discusses the possible ecological advantages polydomy may provide the group; and discusses the potential consequences a dispersed nesting strategy may have for the wood ant social structures. A key question arising from this review was the extent to which a polydomous wood ant colony is centrally organised at the colony-level. In chapter 3 we use network analytic techniques to investigate if resource redistribution in polydomous colonies is organised at the colony-level, or the local, internest, level. Based on the results in chapter 3 we hypothesised how the behaviours of individual ants could lead to resource redistribution through the colony. In chapter 4 we tested this hypothesis, investigating how the behaviour of individual ants relates to the observed pattern of resource redistribution through the colony. Examination of polydomous colonies in chapter 3 and 4 had shown that some nests within the colonies do not appear to be foraging. In chapter 5 we investigated the possible roles of these non-foraging nests, and suggest a mechanism by which polydomous colonies may expand. Chapters 3, 4 and 5 suggest a mechanism by which resources are redistributed between nests within polydomous colonies, and a mechanism by which polydomous colonies may expand through the environment. In chapter 6 we use these results to inform an investigation of how the polydomous nest-networks change over time, and how this affects the life-history of nests within the polydomous colonies.

Chapter 2: Polydomy in Red Wood Ants

Abstract

Polydomy, a single colony spread between multiple nests, is a widespread life-history strategy in ants. The mechanisms by which a polydomous colony functions, and the fitness benefits this nesting strategy provides, remain poorly understood. Here we review what is known about polydomy in the well-studied and ecologically important *Formica rufa* group. We focus particularly on the ecological fitness benefits polydomy may provide to members of the *F. rufa* group. We discuss the well-documented association in this group between polygyny (multiple queens in a colony) and polydomy, and how this relationship may favour colony reproduction by budding. We argue that although polygyny and reproduction by budding may drive a colony to spread between multiple nests, the maintenance of prolonged communication between these nests needs further explanation in terms of fitness benefits. The potential benefits of polydomy in the *F. rufa* group are discussed, specifically how polydomy may help a colony: exploit resources, dominate spaces, or lower the cost of stochastic nest destruction. The potential consequences of polydomy for the social organisation of a colony are explored. We also highlight gaps in current knowledge, and suggest future research directions.

2.1 Introduction

The Formicidae are remarkable for the variation they show in many aspects of their life history. This variation is particularly pronounced in their nesting habits, from the huge underground complexes of leafcutter ants, to the small improvised nests of Argentine ants and the temporary bivouacs of army ants. In all cases the nesting strategy has a profound effect on the way the species interact with their environment (Rockwood 1976; Holway & Case 2000; Franks & Fletcher 1983).

A common, but largely understudied, nesting strategy is polydomy. A colony is polydomous if it is spread between two or more spatially separated, but socially connected, nests (Debout et al. 2007; figure 2.1). Following Debout et al. (2007), a nest is defined as any structure containing both workers and brood. Polydomy appears to have evolved multiple times in ants, and is found in all the major subfamilies across all continents (Debout et al. 2007). However the benefit that this nesting strategy brings remains largely obscure as it occurrence is not limited to any particular biome, habitat or dominance level (Debout et al. 2007). Polydomous nesting, like other nesting strategies, is likely to have a fundamental effect on how a species interacts with the environment.

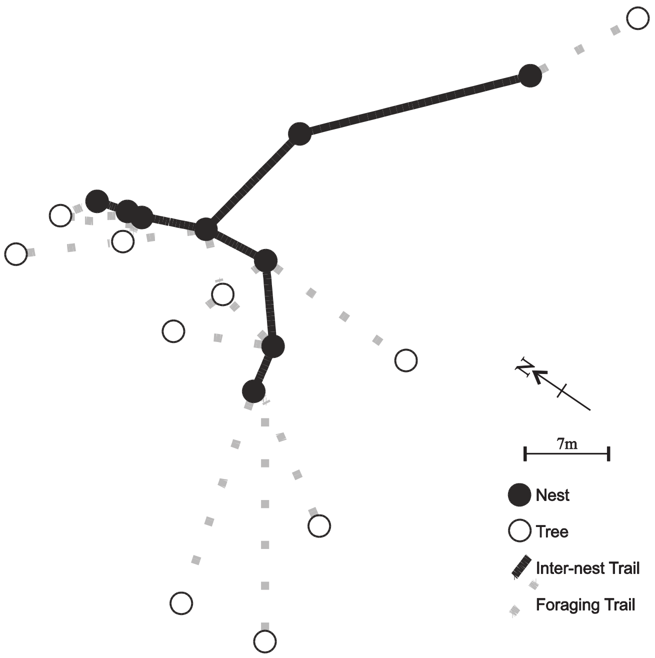


Figure 2.1 An example of the layout of a polydomous *Formica lugubris* colony. The map was constructed by measurement of the trails (defined as a distinct path with more than 10 workers in 40cm) of ants travelling between nests, and between nests and trees. (chapter 3) This colony was mapped in July 2012 at the Longshaw estate, Peak District, UK (*SE pers. obs.*).

Ants of the *Formica rufa* group are particularly interesting with regards to nesting strategy as this strategy is flexible within species and variable between species. The *F. rufa* group (hereafter red wood ants) consists of six morphologically and behaviourally very similar species (Bernasconi et al. 2011; listed in table 2.1). Species of this group are characterised by their nesting habits: they build large nests composed of subterranean chambers and

	Monodomous	Polydomous /Unicolonial	Monogynous/ Weakly Polygynous	Polygynous
Formica rufa	England [1,2,3] Finland[4, 5, 6]		Finland[7, 8,9, 10, 11] Germany[12] Sweden[13] Switzerland[14]	England[13,15]
Formica aquilonia	Sweden[16]	Finland[4,5,17,18,19,20,21,22,23] Switzerland [24]		Finland[4,8,9,10,11,18,20,21,22, 23, 25] Northern Ireland[25] Russia[26] Scotland[25, $] Switzerland [24, 25]
Formica lugubris	Finland[4] Ireland[27] Switzerland[24]	Canada[28#] England[29,30, $] Finland[6] Switzerland[24]	Finland[8,9, 11, 25] Ireland[25] Switzerland[24]	Switzerland[24, 25] England[29] Russia[26]
Formica paralugubris		Italy[31] Switzerland[24,32*,33*, 34]		Italy[31] Switzerland [24,32*,33*, 34]
Formica polyctena	Finland[35] Netherlands[36]	Finland[4,5,23] Germany[12] Netherlands[36,37] Poland[38]		Finland[4,7,6,8,9,10,11, 23] Germany[39] Netherlands[36] Switzerland[14]
Formica pratensis	Finland[4]		Finland [4,9,10,11] Germany[12]	Netherlands[40] Switzerland[14]

Table 2.1 Summary of geographical spread of polydomy and polygyny, separated by the artificial boundary of national borders. '#' indicate an artificial introduction, '*' indicate instances when studied as *F. lugubris* but now shown to have been *F. paralugubris* and '$' is personal observation by the authors. References: 1-(Camlitepe & Stradling 1998), 2-(Skinner 1980a), 3- (Elton 1932), 4-(Rosengren & Pamilo 1983), 5-(Punttila 1996), 6-(Rosengren 1971), 7-(Sundström et al. 2005), 8-(Kilpeläinen et al. 2005), 9-(Punttila & Kilpeläinen 2009), 10- (Helanterä & Sundström 2007), 11-(Pamilo 1990), 12-(Wellenstein 1973), 13-(Collingwood 1979), 14-(Keller 1991), 15-(Skinner 1980b), 16-(Buhl et al. 2009), 17-(Rosengren et al. 1987), 18-(Sorvari et al. 2008), 19-(Sorvari & Hakkarainen 2005) 20-(Sorvari & Hakkarainen 2004), 21-(Pamilo et al. 2005), 22- (Sorvari & Hakkarainen 2007), 23-(Rosengren 1985), 24-(Bernasconi et al. 2005), 25-(Pamilo et al. 1992), 26-(Mäki-Petäys et al. 2005), 27-(Breen 1979), 28-(Storer et al. 2008), 29-(Gyllenstrand & Seppä 2003), 30-(Sudd et al. 1977), 31-(Fortelius et al. 1993), 32-(Cherix 1980), 33-(Chapuisat & Keller 1999), 34-(Bernasconi et al. 2006), 35-(Rosengren & Sundström 1987), 36-(Mabelis 1979b), 37- (Mabelis 1984), 38-(Szczuka & Godzińska 1997). 39-(Horstmann 1982), 40-(Brian 1983)

and above-ground domes of pine needles and other leaf litter (Punttila & Kilpeläinen 2009; figure 2.2). The size and complexity of these nests means they are likely to be costly to build (and therefore costly to abandon) which may be an important limitation on the life history of these species. The number of these nests a colony has varies considerably both within and between species. For example, while *F. rufa* and *F. pratensis* have only been observed as monodomous (the whole colony in one nest), others such as, *F. aquilonia, F. lugubris* and *F. polyctena* have been observed to be polydomous in some areas but monodomous in others (table 2.1).

Figure 2.2 A polydomous *Formica lugubris* colony. Peak District, UK. (S. Ellis)

An extended form of polydomy, called unicoloniality, in which there is no intraspecific aggression within entire populations has been found in one *F. rufa* group species: *F. paralugubris* (Chapuisat & Keller 1999; Helanterä et al. 2009). Unicoloniality can result in very large colonies of hundreds of nests (Cherix 1980). This review focuses on the smaller, more common, multicolonial, polydomous populations.

Red wood ants are the dominant invertebrate predator in woodland across much of northern Eurasia (Savolainen & Vepsäläinen 1988). As such they have a significant impact at multiple ecosystem levels affecting ant and other arthropod community structures (Mabelis 1984; Punttila et al. 1994; Sudd & Lodhi 1981; Punttila et al. 2004; Kilpeläinen et al. 2005), and acting as a food source, and competitor, to vertebrates (Haemig 1992; Rolstad et al. 2000; Otto 2005). The nests themselves act as a habitat for a variety of myrmecophiles and nest associates (Laakso & Setälä 1998; Otto 2005; Mabelis 2007; Robinson & Robinson 2013); and the chemical structure of the soil within, and around, the nests is changed by the collection of large amounts of leaf-litter into a warm dry environment (Lenoir et al. 2001; Wardle et al. 2011). Additionally, red wood ants can impact the growth of trees through their main food source of aphid herding (Rosengren & Sundström 1991). These interactions are mediated by the position of the nests within the habitat (Sudd & Lodhi 1981; Sudd 1983). Spreading the colony through multiple nests will change the arrangement of the nests in the environment, and therefore affect the way a colony interacts with its surroundings. Understanding the causes and consequences of polydomy is therefore important to understanding how these keystone species interact with their environment.

This review focuses primarily on the relationship between polydomous nesting strategy and the ecology of the *F. rufa* group, specifically how the particular ecology of the *F. rufa* group may encourage polydomy and what ecological fitness benefits polydomy may provide the members of the group.

2.2 The relationship between polydomy and polygyny

It has been suggested that polydomy is simply an ‘epiphenomenon’ of having multiple reproducing queens in the same nest (polygyny) in the red wood ant group (Rosengren & Pamilo 1983). Analysis of nesting habits within the red wood ant group reveals there is a significant association between polygyny and polydomy (GLMM, $t=48.5$, $df=2$, $p<0.01$; table 2.2). However this correlation does not necessarily imply causation; it could be that both are being selected for by similar ecological conditions (Debout et al. 2007).

Polygyny can affect the way new colonies are founded. Red wood ants use two main methods of dispersal: temporary social parasitism and reproduction by budding (e.g. Rosengren et al. 1993). Red wood ants do not perform truly independent dispersal and are always reliant on an existing workforce to raise the first batch of workers. This suggests that establishing a nest is difficult (as it requires help from a hijacked workforce). Temporary social parasitism involves a young queen entering the nest of another species, usually members of the *F. fusca* group, usurping the queens position and hijacking the workforce (Bourke & Franks 1995). This method of colony foundation does not require a nest to have more than one mated queens within a nest at the same time, so it is possible for both polygynous and monogynous red wood ant colonies. Reproduction by budding is a very common dispersal method in red wood ants. When budding, a fragment of the colonies workers and queens leave the nest on foot and found a new colony, which is clearly only possible if the colony is polygynous, at least temporarily (Bourke & Franks 1995).

The chief advantage of reproduction by budding is that it is probably a much safer method of colony foundation than temporary social parasitism (e.g. Rosengren & Pamilo 1983). Temporary social parasitism presents risks, not least of which will be finding a suitable host colony to invade. As wood ants dominate their habitat they outcompete other ant species, including the species they parasitise (Mabelis 1984), making potential nest sites even more limited (Rosengren et al. 1993). Even once the host has been located the process of infiltrating the colony, and eliminating the existing queen, is likely to be very risky, and has been observed to frequently result in the death of the invading queen (Hölldobler & Wilson 1990).

	Monogynous		Polygynous	
Monodomous	5	*F. rufa* (1) *F. lugubris* (3) *F. pratensis* (1)	1	*F. rufa* (1)
Polydomous	0		6	*F. aquilonia* (2) *F. lugubris* (2) *F. polyctena* (2)

Table 2.2 The relationship between domy and gyny in populations of the red wood ant group. For analysis, a wood ant population was defined as the occurrence of a given species in one of five broad geographical regions; Fennoscandia (Sweden and Finland), Britain (England, Wales and Scotland), Ireland, North-Western Europe (Germany and the Netherlands) and the Alps & Jura (Switzerland and Italy). Populations were included only if information on both gyny and domy was available (n=12). Table 2.2 shows the number of regions observed exhibiting the properties of a particular category; the species included are also shown with the number of regions in which they show this strategy in brackets. Unicolonial populations were excluded. We investigated the relationship between gyny and domy using a generalised linear mixed model with a binomial error structure. Domy is used as the dependent variable, gyny and species as fixed effects; species region was included as a random effect to allow for the same species appearing in multiple geographic regions. Statistics were performed in R 2.14.0 using the MASS package (R Development Core Team 2011).There is a significant association between gyny and domy in the red wood ant group (GLMM, t=181.1. df=2, p<0.01).

In contrast, budding allows queens to travel with a protective retinue of workers to a prebuilt nest (Keller 1995). Additionally, budding will allow the new nest to immediately outcompete independent foundresses in the same area (Keller 1995). Indeed habitat domination is sometimes argued as the ultimate reason for red wood ants to be polygynous (Rosengren & Pamilo 1983). These benefits are likely to be particularly important in a nest-site-limited environment because there will be much higher competition for the few available nest sites.

In addition to affecting the way a colony disperses, polygyny also has complex effects on relationships within the colony. In simplistic terms, as the number of reproducing queens increases, the intracolony relatedness will approach zero, which will decrease the inclusive fitness returns to workers. Increased competition for resources and worker attention in a polygynous colony is also likely to impose a cost on reproductive queens. However, despite these costs, it is becoming increasingly clear that polygyny is the predominant social system in ants, which suggests that it does bring a fitness benefit (Keller 1995).

An ecological pressure which may favour the evolution and maintenance of polygyny is nest-site limitation (Debout et al. 2007). For wood ants an important nest-site limiting factor is likely to be temperature (discussed below). If the availability of nest sites is limited in the environment, the success of dispersing queens founding a new colony will be low (Rosengren & Pamilo 1983; Rosengren et al. 1993). This may favour queens who are philopatric and return to the natal nest after mating because, even though their lifetime reproductive success is lowered compared to an independently founding queen, the chance of successful independent foundation is so low that it becomes beneficial to settle in the natal nest (Rosengren & Pamilo 1983; Rosengren et al. 1993). Analysis of within-nest genetic relatedness indicates that this regularly occurs in red wood ant colonies (e.g. Chapuisat et al. 1997). As the number of queens in a nest rises, the success of each queen will decrease until it becomes more beneficial to disperse even with a very low probability of success, so a frequency- and density-dependent equilibrium is established between proportions of dispersing and philopatric queens (Rosengren & Pamilo 1983; Pamilo 1990). It should be noted, however, that even philopatry does not ensure reproductive success: it was found that in a highly polygynous unicolonial *F. paralugubris* population in Switzerland only 42% of inseminated queens survive in a nest after the mating flight (Fortelius et al. 1993).

Queen philopatry could also benefit the resident workers and established queens of the receiving colony in a weakly polygynous system. They may benefit by inclusive fitness as the returning queens are likely to be related, offsetting the costs of the potential loss of some individual reproductive success (Pamilo 1990). Additionally if there is reproductive skew among queens (observed in *F. aquilonia*: Pamilo et al. 2005, well studied in closely related non red wood ant *F. fusca* Bargum & Sundström 2007; Bargum et al. 2007) it suggests some queens dominate reproduction. If this is the case the costs of taking on new queens may not result in any substantial loss of the reproductive success of resident, and presumably dominant, queens (Keller 1995). Reproductive skew could also benefit workers by increasing their relatedness to the reproductive queens active at a particular time (Bargum & Sundström 2007). Workers may benefit by increased longevity of the colony, allowing the colony to survive beyond the lifespan of individual queens, which is especially important if nests sites are limited (Keller 1995).

Nest-site limitation may therefore be an important selective force for both polygyny and reproduction by budding (Rosengren & Pamilo 1983). This is supported by a well-documented association between polygyny and budding in ants (Keller 1991; Sundström et al. 2005). In red wood ants this trend is particularly pronounced, with many studies on a variety of species showing polygyny accompanied by high spatial genetic viscosity suggestive of reproduction by budding (e.g. *F. paralugubris* Chapuisat et al. 1997; *F. aquilonia* Pamilo et al. 2005; *F. lugubris* Bernasconi et al. 2005).

Reproduction by budding provides the link between polygyny and polydomy. If, once the new bud nest has formed, it maintains contact with the mother nest, the colony has become polydomous (Pedersen & Boomsma 1999). In some instances polydomy has been considered purely as an intermediate stage between budding and true separation (Pedersen & Boomsma 1999). However the stability of red wood ant polydomous networks (Rosengren 1985), despite the ecological costs likely to be associated with polydomy (such as spread of disease and parasites through the network or intracolonial internest competition), suggests that there is an ecological benefit of polydomy in this group. The exact nature, and extent, of these costs has not been studied, and is an important area of future research. Budding may be the proximate reason for a colony spreading between multiple nests, but does not provide an ultimate explanation for its maintenance. This is supported by the fact that outside the red wood ant group there are many examples of polydomy in monogynous species, suggesting that there must be an ecological benefit to the maintenance of polydomy, even in the absence of polygyny (Bourke & Franks 1995; Debout et al. 2007).

2.3 Ecological advantages of polydomy

The nesting strategy of a species is expected to be adapted to the habitat in which it lives. There have been many proposed explanations for how polydomous nesting may help a species to take advantage of its environment, they can be broadly categorised as those that emphasise the importance of; exploiting a resource, exploiting a space and spreading risks. These explanations, and the way they may apply to red wood ants, are discussed below.

I. Exploiting a resource

Polydomy may benefit a colony by increasing its ability to exploit a resource. A resource is defined as ‘anything provided by the environment to satisfy the requirements of a living organism’ (Lawrence 2005). There are two resources that are particularly important to the life-history of red wood ants: food and nest sites.

a) Efficient foraging

By becoming polydomous, a colony may be able to efficiently exploit, or defend, spatially separated resources. In contrast a monodomous colony could only be expected to efficiently exploit a limited number of resources before costly travel times, make the exploitation economically unrewarding. For it to be advantageous for a species to become polydomous to exploit a food source, that food source must be fixed spatially and relatively stable temporally. The advantage of building an additional nest nearer to a food resource is that travel times can be reduced, therefore increasing colony foraging efficiency and reducing exposure to predators (Debout et al. 2007). Additionally, having a nest near a food source may also be the most efficient way to defend a stable, but patchily distributed, food source from competitors (Hölldobler & Lumsden 1980).

Red wood ants are very reliant on stable food sources (Rosengren & Sundström 1991). Along with many other species of ant, red wood ants have a mutualistic relationship with aphids, maintaining aphid herds as a source of carbohydrate-rich honeydew (Rosengren & Sundström 1991). The aphid herds are both spatially fixed (they will tend to stay in the same tree) and temporally stable (the herds will persist, at least during the course of a season). It may therefore be worthwhile for the colony to build an additional nest near this food resource to more efficiently exploit and protect it. The importance of stable food sources to red wood ants is demonstrated by their very high route fidelity and site allegiance. Many studies have found that individual ants show very high loyalty to a particular trail and nest; this fidelity is maintained even over winter hibernation (Rosengren 1971; Rosengren & Fortelius 1986; Rosengren & Fortelius 1987; Rosengren & Sundström 1987; Gordon et al. 1992). Site fidelity is only beneficial if the food source can be expected to persist. Polydomy as a means of resource exploitation at least has the potential to act within this system.

Some observations support the idea of red wood ants using polydomy to exploit resources. For example it has been found in both England and Finland that ant activity is highest on trees nearest the nest (Sudd 1983; Rosengren & Sundström 1991). This is not necessarily evidence of polydomy for resource exploitation, as it could be that ants feed on the trees because they are nearest, rather than because they have chosen to site their nests near good foraging trees. It does, however, suggest a link between nest location and foraging activities.

The link between foraging to stable food sources and nesting in red wood ants has been further examined by investigating the foundation of new nests. It was found that after destruction of a nest, *F. aquilonia* formed polydomous nests on the foraging trails of the destroyed nest (Rosengren 1971). Even without nest destruction, 64% of new nests in a *F. polyctena* colony over a five year period were formed on previously existing foraging trails

(Mabelis 1979b). Both of these results could indicate that new nests are preferentially formed closer to food sources.

Similarly, links between the direction in which new nests bud, and the location of stable food sources has been suggested. If sugar is sprinkled in the territory of *F. polyctena*, the colony will form outstations (nest-like structures but without brood: Anderson & Mcshea 2001) and in a few cases true nests (Mabelis 1979b). In the same way after forest clear-cuts *F. aquilonia* colonies bud into many small nests, with a skew towards the edge of the uncut forest, suggesting they may be budding towards the food sources in the woodlands (Sorvari & Hakkarainen 2005). Budding towards food has also been observed during experimental manipulation in other ant species (Holway & Case 2000; Lanan et al. 2011).

Polydomy does seem to be related to resource exploitation in red wood ants, however further investigation is necessary to ascertain if it is the ultimate reason for polydomy, or simply a by-product of an organisation which was actually formed for another purpose. A better understanding of the costs associated with long foraging trails, the relationship between the layout of the food sources and the nest arrangement, and an understanding of how foraged food is shared through the colony would give a clearer picture of the relationship between stable food sources and polydomy. It should be noted that there do not appear to be consistent differences between the habitats of monodomous and polydomous red wood ant populations. Further investigation is necessary to see if there is a reason that polydomy might be advantageous for foraging in some areas but not others.

b) Monopolising nest sites

The distribution of an ant population within a space will be limited by the number of available nest sites. If nest-site availability is the limiting factor in the environment then it may be advantageous for a colony to become polydomous to take advantage of available nest sites in the vicinity, as an investment in future colony growth. The value of nest sites to wood ants is likely to be high because, as discussed above, in a nest-site–limited environment independent colony foundation is likely to have a low success rate. It may therefore be beneficial to reproduce by colony budding; polydomy can facilitate this by allowing a colony to occupy locally available nest sites (Banschbach & Herbers 1996a).

An important factor which may limit wood ant nest sites in an environment is local temperature. Temperature is important because it affects brood development speed and can therefore have a direct impact on fitness (Huey & Berrigan 2001). Wood ants may be expected to face a trade-off between temperature and foraging. To forage efficiently the nests need to be in woodland, or at least near trees (which will shade the nest), whereas to have the best temperature for brood development they need high insolation. Indeed shade tolerance is often considered a hallmark of different species in an otherwise very behaviourally and ecologically similar group. For example, studies in Finland have repeatedly found that polygynous, polydomous *F. aquilonia* is usually found in shady forest interiors, while mongynous monodomous (in Finland) *F. lugubris* is usually found around forest edges and in younger forest stands (Punttila 1996; Punttila & Kilpeläinen 2009). This could, however, be due to other differences in the ecology of these species rather than a direct consequence of shade tolerance. Differences in shade preference in different species suggest that there is a link between temperature and wood ant life history, even if it is not a direct result of shade tolerance.

Wood ants have been demonstrated to control the temperature of their nests, cooling in summer and warming in the winter (Rosengren et al. 1987). Being able to control temperature in this way may suggest wood ant nest sites are less reliant on the level of insolation. However metabolic heat production will only work efficiently once the nest has reached a certain size. It may therefore be important to found new nests in sunny areas, which may be limited (Rosengren et al. 1987). The availability of suitably insolated sites near trees may therefore be an important nest-site limiting factor in the environment for wood ants.

If temperature, or another environmental influence, means nest-sites are limited in the environment for wood ants it may bring fitness benefit to polydomy. In a nest-site–limited environment polydomy is a way to monopolise a rare resource. To fully understand how scarce nest-sites are more information is needed on the environmental variables that combine to create a good nest-site for wood ants, and importantly the trade-off between foraging and insolation. Once the variables limiting wood ant nest sites in the environment are understood, then the relationship between nest-site limitation and polydomy can be investigated.

II. Exploiting a space

Instead of polydomy being used to directly exploit a resource, it could be used to occupy a space in which resources may occur in the future. Exploitation of space could take two main forms; dispersed central place foraging and territorial defence.

a) Dispersed Central Place Foraging

Social insects are often considered to be central place foragers, they collect food from the environment and return it to a central place: the nest (Ydenberg 2007). Central place theory predicts how far and often an animal should forage from a 'central place', given the trade-off between searching for food and transporting food, in order to maximise the rate of energy gain (Ydenberg 2007). However, polydomous colonies can escape this limitation by spreading their nests over a larger area, in a strategy known as dispersed central place foraging (Schmolke 2009; Cook et al. 2013).

Rather than focusing on exploitation of the resource itself (above), the strategy of dispersed-central place foraging allows a colony to exploit a space over which the resource may occur. This is particularly relevant to species that have widely dispersed and unpredictable food sources, for example species that prey on other arthropods. The advantages of polydomy in these situations have been demonstrated theoretically: polydomous colonies have significantly higher foraging success if food is scattered, but if food is clumped they have equal success to monodomous colonies (Schmolke 2009; Cook et al. 2013).

Red wood ants, however, show limited reliance on arthropod prey and scavenged food due to their much more important relationship with aphids. Indeed not only is up to 94% of colony foraging directed towards honeydew (Rosengren & Sundström 1991), but of the remaining 5% of foraged material, the majority comes from the aphid herds (Cherix 1980; Cherix 1987; Robinson et al. 2008). The reliance of red wood ant colonies on dispersed prey may actually be low. The benefits of dispersed central place foraging derive from increased ability to collect spatially and temporally unpredictable food sources (Cook et al. 2013), as red wood ants do not meet this assumption it suggests that the benefits of dispersed central place foraging may not apply to red wood ants.

b) Territorial defence

Polydomy may also allow more efficient exploitation of space in other ways. Having a network of connected nests dispersed throughout a colonies territory may allow more efficient defence, by decentralising the workers and spreading them more evenly through the territory (Hölldobler & Lumsden 1980).

Red wood ants are usually thought of as ecologically dominant and territorial (Savolainen & Vepsäläinen 1988), which would mean they could benefit from dispersed defence. However some studies suggest otherwise: models have shown that for species with patchily distributed, stable food sources, such as red wood ants, it may actually be more beneficial to defend only the resources rather than the territory itself (Hölldobler & Lumsden 1980). Resource defence, as opposed to territorial defence, has been observed in red wood ants. If two colonies of *F. polyctena* are lured to an area with food baits they will fight, but when the food is removed the fighting will rapidly abate (Mabelis 1984) suggesting that it is the resource rather than the territory that is being defended. Interestingly in spring, before the aphid herds are established, territorial conflict is much more widespread (Mabelis 1984), perhaps reflecting their greater reliance on scavenging and hunting at this time of year.

Some observations do support the use of polydomy for territory defence in red wood ants. For example, *F. polyctena* colonies in the Netherlands have been observed founding new nests at the same time as neighbouring colonies are expanding their territory (Mabelis 1979b). This may suggest that the colony is consolidating its territory by increasing the number of polydomous nests (Mabelis 1979b). However, this observation could also be ants moving to defend a resource rather than the space itself.

In conclusion, it is uncertain if red wood ants are truly territorial, and if they are, whether the benefit gained by dispersed defence outweighs the high costs of nest foundation and maintenance. This could be investigated by manipulating nest densities to create areas of high nest density (high competition environment) and areas of low nest density (low competition environment). If polydomy is a means of territorial defence, a higher degree of polydomy would be expected in areas of high competition compared to areas of low competition.

III. Spreading Risks

Polydomy could also provide benefits beyond improving exploitation of the environment (be that resources or space): it could also improve colony survival by spreading risks associated with the habitat through multiple spatially separated nests. For example, observation of a *F. polyctena* colonies in the Netherlands suggests that when attacked by conspecifics they may abandon the nests, retreating to a different nest in the same colony (Mabelis 1979c), a behaviour which has been observed in other polydomous ant species (Dahbi et al. 2008).

Red wood ants, however, do not fit the trend which might be expected for species with a high risk of nest destruction. It might be expected that monogynous populations would be more polydomous than polygynous populations because the potential costs of stochastic nest destruction are expected to be higher in monogynous than polygynous species. This is the opposite of what is actually found (table 2.2) suggesting that risk spreading may not be a major ecological benefit of polydomy in this group.

The only significant biological threats which may result in destruction of red wood ant nests come from predation by vertebrates (Otto 2005), but little investigation has been made of the extent of predation and its impact on the ants. Overall, the risks in the environment for an ecologically dominant species with stable well-constructed nests and very high worker numbers may not be high enough to make avoiding destruction a significant benefit of polydomy in the *F. rufa* group. The relationship between risk and polydomy could be investigated more completely by manipulating the destruction of nests in an area and observing the affect this has on the nesting strategy of the colonies. This manipulation could involve, for example, selectively damaging nests in an experimental plot. This treatment could be used on species with flexible nesting habits or to compare the response of species with different nesting strategies.

2.4 Consequences of polydomy for red wood ant colonies

As well as the adaptive benefits that polydomy brings to red wood ant colonies it also has the potential to affect them profoundly in other ways. Ant societies show many complex behavioural, social and kinship interactions and by spatially separating sections of the society into different nests these interactions could be changed, both within and between nests of the colony.

Consequences for conflicts within the colony

An important and well-studied interaction in social hymenopteran societies is the queen-worker-conflict over sex ratio of offspring (Trivers & Hare 1976). In a polygynous colony, sex ratio is affected by a wide variety of factors, such as queen or worker control of sex-ratio, number of queens and interqueen relatedness (Pamilo 1990; Bourke & Franks 1995). The complexity of relationships between these factors is highlighted by the wide range of sex-ratios found even within a single species of red wood ant. For example, polygynous polydomous populations of *F. polyctena* and *F. aquilonia* in Finland have been found to have even sex ratios at some locations and female-biased ratios at others (Pamilo & Rosengren 1983). The physical separation between nests in a polydomous colony can result in these factors, such as number of queens and size of worker population, differing between nests. This adds an extra level of complexity to the conflict.

An effect of polydomy on this conflict is manifested in the case of split sex ratios. In a population of a polygynous species there are likely to be differing numbers of queens within different colonies and therefore differing optimal sex ratios. Different colonies in the population will compete to manipulate the population sex ratio to their preferred optima, until eventually colonies will be producing single-sex brood in an attempt to produce their population-level optima, leading to different colonies producing different sexes of offspring (Bourke & Franks 1995). In a polygynous, polydomous colony such as those of red wood ants, this split sex ratio could occur between nests within the same colony. This has been observed between nests in polydomous *F. polyctena* colonies, though interestingly not between nests in polydomous *F. aquilonia* colonies (Pamilo & Rosengren 1983). The reason for the difference in behaviour of these two species is unknown. The prevalence of intracolony split sex ratios in other red wood ant species has not been investigated, and an interesting approach might be a comparison of sex ratio strategies between colonies in monodomous populations and between nests within polydomous colonies.

A second conflict within insect societies is between investing resources in worker offspring or sexual offspring (called the resource allocation conflict). Generally, in a polygynous colony, workers will favour queen replacement before queens (Debout et al. 2007). This is because, due to the asymmetric relatedness within the colony, workers are likely to be more closely related to the new queens (who are the offspring of a full-sister, life-for-life relatedness= 0.375) than the existing queens are to the new queens (who are their offsprings, offspring life-for-life relatedness= 0.25) (Bourke & Chan 1999). Polydomy may impact on this conflict by resulting in differences in resource availability at different nests of the colony (Debout et al. 2007). There are few models of the resource allocation conflict so inferring the effect of polydomy is difficult. In red wood ants it has been found that proportion of nests producing sexuals decreases with increasing degree of polydomy (Pamilo & Rosengren 1983); this could be a consequence of resource allocation conflict, but it has not been tested. To understand this problem fully the relationship between the number and sex of sexual offspring produced by different nests within a colony, and the nest properties (such as size and amount of foraging) needs to be investigated.

Consequences for colony communication

An important consequence of having a highly polygynous colony is that both intranest and internest relatedness will decrease over multiple generations and tend towards 0. For example, polygynous *F. aquilonia* colonies in Finland were found to have intranest relatedness between 0.01 and 0.09, which is very low compared to the 0.75 expected of full sisters in hymenoptera (Pamilo 1982). Low intranest relatedness may result in difficulty differentiating colony members from alien conspecifics. Colony-mate–recognition is important for maintenance of territory and defence of food sources (Hölldobler & Wilson 1990). Ants recognise colony mates using colony-specific cuticular hydrocarbons profiles, which are derived from a combination of genetic and environmental cues (Hölldobler & Wilson 1990). In polygynous *Formica fusca* (which is closely related to the red wood ants) increasing intracolony genetic diversity has been shown not to translate to increased cuticular hydrocarbon diversity (Helanterä et al. 2011). The colony odour is therefore likely to be strongly influenced either by environmental cues, or maintained by mixing of individual odours to create a colony ‘Gestalt’ odour (Helanterä et al. 2011). The close phylogenetic relationship, and shared life-history features, of *F. fusca* with the red wood ants suggests that a similar mechanism is likely to be present in the group. Environmental cues have been found to be important for colony-mate recognition in the *F. rufa* group: experimentally divided *F. aquilonia* colonies will rapidly develop mutual aggression, and this discrimination is faster and results in higher aggression if the colonies are fed different foods (Sorvari et al. 2008). This may represent a problem for polydomous colonies, because different nests may have different environmental conditions which could lead to problems with colony-mate recognition.

An important way that colony odour could be maintained in polydomous species is through internest movement (Dahbi et al. 1997). By workers moving, and carrying other workers and brood, between nests colony odour can be constantly updated and maintained (Dahbi et al. 1997). In polydomous *Cataglyphis iberica* colonies, low levels of cuticular hydrocarbons have even been suggested as the cue to transport a worker between nests (Dahbi et al. 1997). This mechanism is at least possible in polydomous red wood ants: transport of workers and brood between nests has regularly been observed and the existence of a specialised transporter class has been suggested in *F. polyctena* (Rosengren 1971). However, the nature and function of this transporter class has not been fully investigated.

Internest movement is also likely to be important for redistribution of resources and workers through the colony (McIver 1991; Cook et al. 2013). Studies of monodomous red wood ant colonies show that while foraging, workers show very strong site allegiance and route fidelity (Lamb & Ollason 1994; Gordon et al. 1992). A similar mechanism, based on site and route fidelity, is likely to be involved in internest communication although it remains untested. This theory could be tested by direct, and thorough, examination of the movement of individual workers along internest trails.

2.5 Future Directions

There remain many gaps in our understanding of polydomy, a fundamental life history trait in the *F. rufa* group. An important area of investigation is to uncover the true ecological benefits and costs that polydomy brings to these species. An understanding of the mechanisms by which polydomy is created and maintained is a useful first stage in this process.

Another interesting area of inquiry is the relationship between polydomy and unicolonality. It has been proposed that unicolonial populations emerge when the evolutionary pressures selecting for polygyny and polydomy combine in the right situation (Helanterä et al. 2009). By this model unicolonial populations are polydomous populations which have not reached either natural or competitive boundaries (Helanterä et al. 2009). However the selection pressures that favour a unicolonial population rather than a multicolonial polydomous population are unclear. Red wood ants are a good study system for addressing this question due to their gradient from monodomy to unicoloniality within a small phylogenetic and ecological range.

Polydomy also raises questions about the unit of selection in ant colonies. There is a long history of interest into the appropriate level at which to investigate selection in ants; whether it is the gene, the individual, or the colony (Bourke & Franks 1995). Polydomy adds another, intermediate, unit of selection: the nest (Debout et al. 2007). How this fits into the larger picture of selective forces is an interesting area of investigation. This is particularly interesting in red wood ants because of the flexibility in nest number, which may result in different selective forces acting at different colony locations.

The effect of polydomy on the way colonies interacts with their environment also has very important consequences. Red wood ants are keystone species in woodlands across northern Eurasia (Mabelis 2007). Understanding the relationship between the nests of a colony, and how this influences their relationship to the woodland ecosystem, will allow better decisions on forestry and habitat management to help protect and preserve these species and their forest ecosystem.

2.6 Conclusions

Polydomy is a fundamental life history trait in the red wood ants, and as such is a valid area of study in its own right. In the red wood ants it appears that polygyny, via reproduction by budding, has acted as the starting point for the development of polydomy. However, the maintenance of polydomy as a life history trait in the face of costs associated with internest movement, suggests that it gives colonies a fitness advantage. The nature of this advantage is likely to be associated with their foraging habits. For species which, like wood ants, forage on spatially and temporally stable food sources the ecological advantages of polydomy are likely to be related to the exploitation and monopolisation of resources over a larger area, maximising foraging efficiency and colony nutrient intake. As wood ants rely so heavily on spatially and temporally stable food sources the possible benefits polydomy may provide for exploiting a space (as opposed to specific resources in that space) are likely to be limited. Similarly, the environmental risks to an ecologically dominant species in large well-constructed nests is likely to mean that risk spreading may not be a strong pressure promoting polydomy in these species. Different ecological, and especially competitive, conditions at different locations may explain why red wood ants show such flexibility in nesting strategy, but this remains untested.

Polydomy is widespread in ants, but is not associated with any one particular ecological niche (Debout et al. 2007). It may be that ants in general have the potential for polydomy, but the advantages and disadvantages this nesting system provides may rely heavily on the ecological circumstances of the species or species group. For example, risk spreading may not be an important pressure on red wood ants but it is likely to be a very strong pressure on a tramp species such as *Linepithema humile*. Similarly, the effects of polydomy on the foraging behaviour of ecologically dominant honeydew farming species such red wood ants are likely to be very different from the effects on the foraging of a subdominant scavenger such as *Cataglyphis iberica*. In all cases, as this example of red wood ants illustrates, polydomy has the potential to profoundly affect how the colony functions and interacts with its environment.

Chapter 3: Resource redistribution in polydomous ant nest-networks: local or global?

Abstract

An important problem facing organisms in a heterogeneous environment is how to redistribute resources to where they are required. This is particularly complex in social insect societies as resources have to be moved both from the environment into the nest and between individuals within the nest. Polydomous ant colonies are split between multiple spatially separated, but socially connected, nests. Whether, and how, resources are redistributed between nests in polydomous colonies is unknown. We analysed the nest-networks of the facultatively polydomous wood ant *Formica lugubris*. Our results indicate that resource redistribution in polydomous *F. lugubris* colonies is organised at the local level between neighbouring nests, and not at the colony-level. We found that internest trails connecting nests that differed more in their amount of foraging were stronger than trails between nests with more equal foraging activity. This indicates that resources are being exchanged directly from nests with a foraging excess to nests which require resources. In contrast, we found no significant relationships between nest properties, such as size and amount of foraging, and network measures such as centrality and connectedness. This indicates an absence of a colony-level resource exchange. This is a clear example of a complex behaviour emerging as a result of local interactions between parts of a system.

3.1 Introduction

Resources are usually spread unevenly through the environment and an important task for many animal species is to redistribute these resources in response to local need. For example, the mammalian body uses the circulatory system to redistribute oxygen through the body, birds may bring food from the environment back to their nest (Krebs et al. 1977) and humans build complex transport networks to move goods to where they are needed (Guimerà et al. 2005). The mechanisms by which these systems function, and how they are organised, profoundly affects their efficiency and robustness to change.

Redistribution of information and resources is particularly challenging for social insects because of the multiple-stages through which resources have to be transferred. Food, for example, is often transported by foraging workers from the environment back to the nest, then passed from foraging workers to nest workers, and from these workers to the queens and brood. An additional level of complexity is present if a colony is polydomous. Polydomous colonies are spread between multiple spatially separated nests, socially connected by trails of ants travelling between them (Debout et al. 2007). In a polydomous colony, resources may need to be redistributed between the different nests of the colony, as well as through all the other stages common to social insect colonies.

Polydomy is a widespread life-history strategy in ants and is thought to have convergently evolved multiple times in a wide variety of ant genera (Debout et al. 2007). However the mechanism by which the polydomous system functions, and the benefits it provides the colony, remain poorly understood (Debout et al. 2007). Polydomy has the potential to have a profound effect on how a colony relates to the environment (Van Wilgenburg & Elgar 2007; Debout et al. 2007; Cook et al. 2013; Ellis & Robinson 2014). Being distributed through the environment allows a colony to exploit resources, such as food and sunlight, over a larger area. Over a larger area environmental heterogeneity is likely to mean that, at least at temporarily, some nests will have more of a particular resource than others. Whether resource redistribution occurs, and the mechanism by which it works, is important to understanding how the colony functions.

The redistribution of resources at the global, colony, level has to be mediated by the local interactions between individual nests. The relationship between global and local effects can be investigated using network analysis. Polydomous ant colonies are analogous to networks, with nests as nodes, and the trails between nests as connections (Cook et al. 2014). Many tools have been developed to study networks (Newman 2003a; Croft et al. 2008). These tools allow investigation of how local interactions relate to a broader global pattern; in this case how communicating trails between nests relate to the organisation of the polydomous colony.

We used network analysis to investigate how resources are redistributed through polydomous *Formica lugubris* colonies. *Formica lugubris* is a member of the *F. rufa* species group (sometimes known as the red wood ants); and are the dominant invertebrate predators in woodland across much on Northern Eurasia. They are particularly useful for investigating polydomous nesting because polydomy is flexible both within species and between species (Ellis & Robinson 2014). For example, *F. lugubris* has been reported as monodomous at locations in Finland (Rosengren & Pamilo 1983), Switzerland (Bernasconi et al. 2005) and Ireland (Breen 1979), but polydomous in England (Sudd et al. 1977; Gyllenstrand & Seppä 2003), and at other locations in Switzerland (Bernasconi et al. 2005) and Finland (Rosengren 1977).

Polydomous wood ant colonies form distinct trails of ants travelling between these nests; workers carry food, nesting material, brood and queens along these trails in both directions (Rosengren & Pamilo 1983). It is unknown how polydomous red wood ants organise the redistribution of resources through the colony. Understanding how these resources are being redistributed through the colony is an important part of understanding the adaptive advantage polydomy may bring the colony. The primary means of resource redistribution through a polydomous colony is likely to be along the trails between the nests. These connections are therefore the key to understanding how resources are redistributed between nests. The patterns of connections between nests, and how this pattern relates to the properties of the nests themselves will reflect how resources are redistributed through the colony. In this study we investigate these internest connections. Specifically we consider two interlinked questions: 1) how is resource redistribution mediated at the local-level between nests? and 2) how do the local interactions relate to the colony-level redistribution of resources?

3.2 Methods

Study species and field site

The study was conducted on a large *Formica lugubris* population in the Longshaw Estate, Peak District, England (53º 18.55 N, -1º 36.16 W) in July and August 2012. There are no other members of the *F. rufa* group at the site. The 0.95 ha^{-1} site contains a mix of open sparsely planted grassland, deciduous woodland, mixed woodland and the remains of historic scots pine plantations. A survey over winter and spring 2011/12 found a total of 921 *F. lugubris* nests on the site (fig 1.4).

Ants of the *F. rufa* group build distinctive above-ground mounds of pine needles and other leaf litter, over extensive subterranean chambers. These nests can be large, up to a meter in height, and can contain from hundreds to millions of workers (SE *pers. obs.*). If polydomous, a colony will form distinct trails of ants travelling between these nests. Distinctive nests and clear trails are an advantage of using *F. lugubris* as it means that the networks can be readily and reliably mapped.

The location of wood ant nests is likely to be particularly influenced by two environmental factors, the location of food in the environment, and the temperature of the nest-site. A distinctive feature of red wood ant foraging is their reliance on spatially and temporally stable food sources. Red wood ants, along with many other ant species, farm homopterans for honeydew (Hölldobler & Wilson 1990); this actually provides the majority (up to 94%) of the colonies' nutrient intake (Rosengren & Sundström 1991). For wood ants this farming is usually of aphid herds in trees (Rosengren & Sundström 1991). In addition to foraging for honeydew in trees, wood ant colonies also hunt and scavenge for arthropods in the canopy, including a large proportion of their protein intake from feeding on the aphids themselves (Cherix 1987; Robinson et al. 2008). The positions of trees in the landscape may influence nest layout not only through affecting the foraging structure but also by shading the nests. Insolation is an important environmental variable for red wood ants (Rosengren et al. 1987; Punttila 1996; Punttila & Kilpeläinen 2009; Sorvari & Hakkarainen 2009). The relationship between insolation and the internal temperature of ant nests is complex, as higher insolation is likely to mean higher temperatures, but also higher variation in temperature (Sorvari & Hakkarainen 2009). Additionally, large wood ant nests can control their internal nest temperature through metabolic heat production, but smaller nests cannot (Rosengren et al. 1987). In general, more insolated, and therefore warmer, nests are likely to have a higher brood development rate (at least in smaller and newly-founded nests), but they will be further from trees, which may lower their foraging efficiency.

Network Mapping

We constructed maps of the trail system between and around nests. We are interested in the function of this internest communication. Therefore, for the purpose of this study, a colony is defined by communication (i.e. regular exchange of workers, brood and other resources) between nests, rather than with reference to intercolony aggression which has been used in previous studies (Sorvari & Hakkarainen 2004). Ten polydomous networks were mapped over the site (table 3.1). Colonies were chosen for this analysis based on a preliminary colony survey conducted during the early summer. The largest ten networks from this survey were selected for analysis unless, in the period between the preliminary survey and mapping, they were obscured by the growth of understory vegetation or reduced by destruction of nests in the network, in which case the next largest unmapped colony was used.

Colony	Number of Nests	Number of Internest Trails	Foraged Trees	No. of non-foraging nests
1	22	22	38	10
2	10	10	4	6
3	21	30	18	10
4	14	17	4	10
5	14	15	9	6
6	7	6	6	1
7	10	10	14	3
8	9	8	10	1
9	13	13	8	8
10	20	26	7	10

Table 3.1 Details of the polydomous networks used in this study (maps; appendix A)

All mapping was performed during mid-late summer when colonies have reached their largest extent (Mabelis 1979a) and in warm, sunny conditions to minimize the effect of temperature and weather-based variation in trail activity (Rosengren 1977). The layout of nests, trees and trails was mapped from the compass bearing of the trails and length of trails measured using a trundle-wheel (example: figure 3.1, further examples in appendix A). In addition we recorded: internest trail activity, foraging trail activity, nest population and canopy cover over each nest. The activity on a trail was measured as distance along a central portion of the trail needed to find 10 ants (in the absence of confounding features such as groups of workers carrying prey). This measure has an advantage over rate-based measures because it is not affected by the speed at which the ants are moving, and can be readily converted to the useful measure of number of ants per meter of trail.

The strength of a trail is an important consideration for much of the analysis in the study. How much a trail is used gives an indication of how valuable it is to the nests involved, and an estimate of the amount of resource exchange occurring along the trail. Trail strength (S) is estimated as the total number of ants travelling along a trail between nests a and b taking into account the size of the nests at each end of the trail. Multiplying the number of ants per meter (w) by the length of the trail (l) gives an estimate of the amount of resource exchange

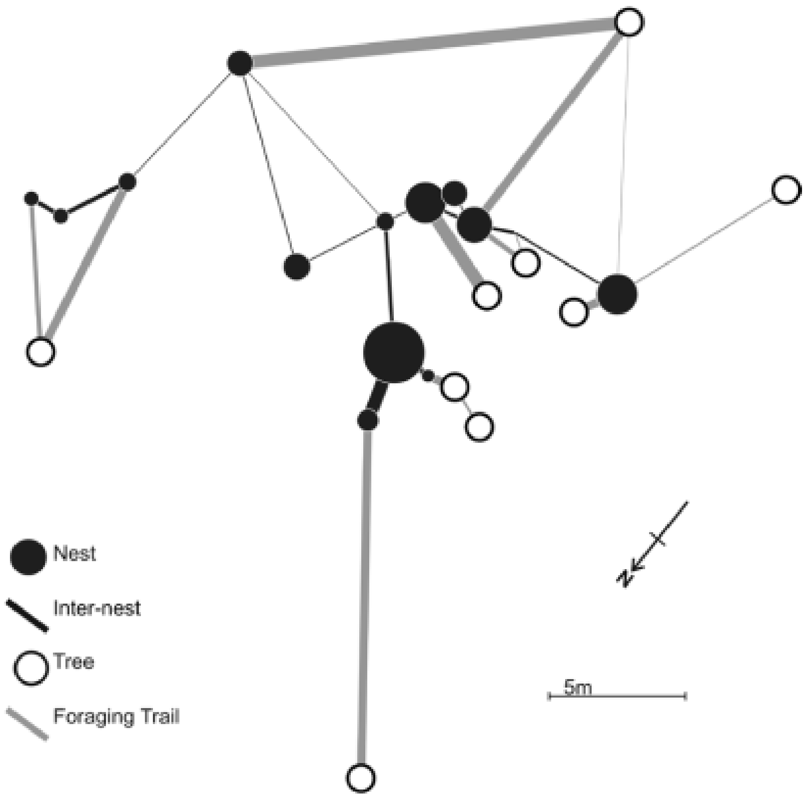


Figure 3.1 Example of a polydomous network (colony 5; see table 3.1) used in this study. Area of a black circle indicates the nest size and the width of trails indicates their strength. All trees and wood ant nests in the area represented are shown on the map.

(or at least the potential amount) occurring between nests, but does not give a good impression of the value of the trail to the nests because it does not take into account the sizes of the nests at each end of the trail. The sizes of the connected nests will strongly affect the number of workers available to travel along the trail, masking the relative value that trail to the nests as a channel for resource exchange. We account for this by dividing the total number of workers on the trail by the mean population of the nests (see below) the trail is connecting ($p_{a,b}$). The calculation of the strength of the trail between nests between nests a and b is shown in equation 1.

$$S_{a,b} = \frac{w.l}{p_{a,b}} \qquad [1]$$

The worker-population of wood ant nests can be accurately estimated using a mark-release-recapture method based on marking after surface disturbance (Chen & Robinson 2013), however it is time-consuming and disruptive to the nests. We used the mark-release-recapture method to calibrate a quicker, but less accurate, estimate of nest population calculated from nest-mound volume (Chen & Robinson 2013). We measured the volume of all the nests as half the volume of an ellipsoid based on measurement of two perpendicular diameters and nest height (Chen & Robinson 2013). One nest per colony was randomly chosen to calibrate volume measurement with a mark-release-recapture estimate of nest population. For greater reliability these calibration measurements were pooled with equivalent data from a separate study (SE *unpublished*) using smaller networks at the same site (n=15). We fitted a linear regression to give a site-specific relationship between nest volume and estimated nest-population (Linear Regression: $R^2=52.7$, df=1,24, $p<0.001$). We used the values of the regression to give estimates of the nest-population of each nest. To avoid ambiguity we will use nest size to refer to the population size of a nest, rather than its physical size.

Distinct trails of ants form between nests and trees with aphid herds. The majority of the ants in these trails are likely to be foragers, collecting honeydew from the aphids and then returning with it to the nests (Gordon et al. 1992). The number of ants from a nest visiting a foraging tree is therefore a measure of the amount of foraging (or potential amount of foraging) being performed by a nest. We define a foraging trail as a clear trail (more than 10 ants in 40 cm) from a nest to a tree. The number of ants on a foraging trail was measured in the same way as for the internest trails. Foraging trail strength was calculated as the number of ants on the trail divided by the population of the nest the trail originates from; this is to control for the internal demand of the foraging nest. The amount of foraging performed from a nest was calculated by summing the strengths of all of the foraging trails connected to a nest. This measure only uses the foraging trails to trees and does not take into account any foraging being performed elsewhere, for example in the leaf litter. However it is likely that the proportion of nutriment provided by the aphid herds is very high (up to 94% has been suggested: Rosengren & Sundström 1991) as they are a source of both honeydew and protein for the colonies (Cherix 1980; Cherix 1987; Robinson et al. 2008). Using the strength of the trails to trees as a measure of amount of foraging will take into account the majority of the food that a nest collects. Nests are considered ‘non-foraging’ if they do not form trails to any trees, this does not necessarily mean that the nests perform no foraging at all, simply that they do not form foraging trails directly to aphid bearing trees.

The amount of foraging performed by a nest can be used to calculate the foraging differential of an internest trail. The foraging differential is the difference in amount of foraging performed by the nests connected by a trail. In analysis of foraging differentials, trails between two non-foraging nests are excluded because the foraging differential is always 0 and is therefore unsuitable for analysis.

The amount of insolation received by a nest is largely determined by the canopy cover over the nests. Canopy cover over nests was estimated using digital photographs taken vertically 30cm above the highest point of the nest. ImageJ (Rasband 2012)was then used to count the number of dark pixels (black/white intensity threshold=255) in the 8-bit version of the image to give the percentage canopy cover (for a similar method see; Robinson et al. 2008).

Network Analysis

This study investigates the relationship between the nest and trail properties and network structural properties. All network analysis was performed in R (R Development Core Team 2011), using the igraph (Csardi & Nepusz 2006) and nortest packages (Gross & Ligges 2012). Three main nest-level network parameters were measured for the analysis: connectedness, centrality and assortativity. These measures allow us to ask biologically meaningful questions about the position of the nests in the network.

Connectedness is a measure of how much resource exchange a nest is facilitating. It can be measured simply as degree: the number of other nests connected to the nest. It can also be calculated as weighted degree which is the sum of the strength connections to the other nests (Croft et al. 2008). We use both measures.

Centrality is a measure of the extent to which a nest occupies an important position in the network (Newman 2003b). We use two network metrics, node-betweenness and closeness, to estimate the centrality of a nest to the network. Node-betweenness measures the amount of information flow though a node and is measured as the total number of shortest paths between all pairs of nests in the network that pass through the nest. If ants were travelling freely and optimally through the network, nests with the highest node-betweenness would be passed through most often. It was calculated both by considering all trails as equal strength (unweighted node-betweenness) and by taking into account the strengths of the trails when calculating the shortest path (weighted node-betweenness). Closeness is a measure of how many trails must be passed along from a nest to reach all other nests in the network. So ants starting from a nest with high closeness can reach all other nests in the network by travelling along fewest trails. This was calculated as both a simple count and weighted by trail strength.

Trail-betweenness is a measure of optimal flow through a particular trail in the network. As node-betweenness measures the number of shortest paths passing through a node, so trail-betweenness measures the number of shortest paths passing through a particular trail. We also calculated trail-betweenness and weighted trail-betweenness for the internest trails.

Assortativity measures the extent to which nests with a particular property are connected in the network (Newman 2003b). We calculated both unweighted and weighted (by trail strength) network association using Newman's assortativity coefficient *r* (Newman 2003a). We examined the assortativity of nest size, amount of foraging and weighted degree (called degree correlation) within the networks.

To account for autocorrelations we used a null model based on 1000 node-label permutations using the quadratic assignment procedure (QAP). This preserves the network structures while nest or trail properties are randomized (Croft et al. 2011). Where analysis is performed on pooled data from all the colonies, randomizations were constrained to within each colony. All significance values based on network measures were calculated using QAP. All analyses not based on QAP fit the assumptions of the statistical test used. All quoted values are mean±standard error.

3.3 Results

Local Structure

Our results clearly show that strength of an internest trail is related to the foraging properties of the nests that it connects rather than being related to any colony-level network properties. The strength of internest trails gives an indication of how resource exchange is facilitated at a local level, between individual nests. Trail strength is a measure of the investment a nest puts into the connection to another nest. Analysis of trail strengths is therefore representative of the value a nest places on a particular trail, which gives insights into how the trails are being used. By examination of the network maps, internest trails can be split into three categories: those between two foraging nests (F-F; 28% of trails), those between a non-foraging and a foraging nest (nF-F; 50% of trails) and those between two non-foraging nests (nF-nF; 22% of trails). There is no significant relationship between the type of trail and the strength of a trail (ANOVA, F=1.13, n=177, p=0.664). However, there is a significant positive correlation between the foraging differential (the difference in amount of foraging performed at nests at each end of the trail) and the strength of the trail on nF-F trails (Pearson: r=0.36, n=79, p=0.019; figure 3.2). There is no significant correlation between foraging differential and trail strength on F-F trails (Pearson: r =0.04, n=44, p=0.464). If the data from nF-F trails and F-F trails are combined there is no significant relationship between foraging differential and trail strength (Pearson: *r*=0.2, n=123, p=0.126).

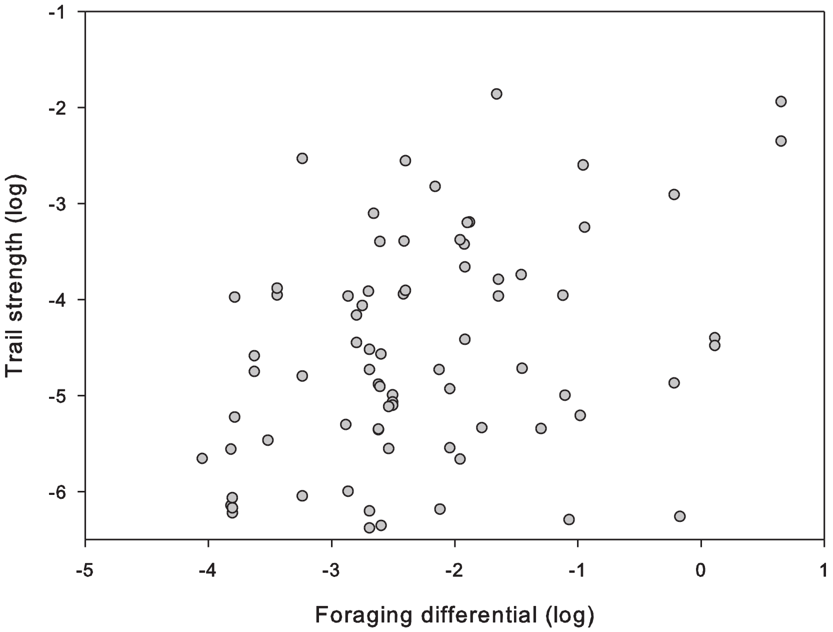


Figure 3.2 Relationship between foraging differential and trail strength. There is a weak but significant positive correlation between the variables (Pearson: ρ =0.36, n=79, p=0.015). Axes are logged for presentation due to the large range of values of both foraging differential and trail strength.

The length of a trail is likely to be important for ants travelling between nests. Overall, F-F trails are significantly longer than other types of trail (F-F: 6.72 ±1.33m, nF-F: 3.18 ±0.24m, nF-nF: 2.61 ±0.40m; ANOVA: F=7.80, n=177, p=0.001). For nF-F trails, longer trails are both significantly stronger (Pearson: r =0.30, n=77, p=0.013) and have a significantly higher foraging differential (Pearson: r =0.12, n=79, p=0.042) than shorter trails. However, F-F trails show no significant relationship between trail length and either trail strength (Pearson: r =0.32, n=44, p=0.26) or foraging differential (Pearson: r =0.04, n=44, p=0.75).

There is evidence of significant positive assortment by weighted degree at least within some networks (table 3.2). This is probably in part a consequence of the relationship between foraging differential and trail strengths. Assortment by weighted degree is indicative of clusters of high resource exchange within the network.

Colony-level structure

We investigated the relationship between the colony network structure and properties of the nests that make up the colony by examining correlations between network structure variables (connectedness and centrality) with nest properties (size, canopy cover and amount of foraging). We found no significant relationship between the network structure and any of

Colony	*r*	*p*
1	0.57	<0.001*
3	0.28	0.231
4	0.43	0.003*
5	0.20	0.282
6	0.53	0.024*
7	0.25	0.234
8	0.67	0.018*
9	0.62	0.063 •
10	0.13	0.627
11	0.46	0.021*

Table 3.2 Weighted degree correlation of the polydomous *F. lugubris* colonies, *r* is Newman's Assortativity coefficient; a positive value shows positive assortment. An asterisk indicates p<0.05 and a dot indicates p<0.1. All p-values have been adjusted with a Bonferroni correction to control for repeated assortativity tests on the same colony (appendix B).

the nest variables (appendix B). Similarly there is no significant association by either size or amount of foraging (appendix C).

The number of nests in a colony might be expected to be linked to environmental and internal colony variables. However there is no significant relationship between the number of nests, and the mean canopy cover over the nests of the colony (Spearman: ρ=122, n=10, p=0.48). Similarly there is no significant relationship between the number of nests in the colony and the size of the nests in the network (Pearson: *r*=0.31, df=8, p=0.76). It was not necessary to use quadratic assignment procedure for these nest number statistics as they are not network related.

The strength of a trail is a measure of actual flow of ants within the polydomous network; it might therefore be expected to relate to the trail-betweenness which is a measure of optimal flow through the network. However, there is no significant relationship between trail strength and trail-betweenness in any of the networks (appendix D). Similarly there is no significant relationship between the type of trail and either trail-betweenness or weighted trail-betweenness in any of the networks (appendix E).

More restricted flow of workers through the network could occur if workers from a particular nest use the foraging trails from neighbouring nests. In the case of nF-F trails, workers from the nF nest could use the foraging trails from the F nest; this would increase the amount of foraging the F nest is carrying out, relative to its size. The number of extra-foragers should scale with the size of the nF nest, resulting in a relationship between the size of the nF nest on the trail and the relative amount of foraging from the F nest. However, there is no significant relationship between the size of the nF nests and the relative amount of foraging occurring from the F nest on nF-F trails (Pearson: *r*=0.06, n=79, p=0.24). This suggests that moving from internest trails to foraging trails is unlikely to play a significant role in resource redistribution, at least on nF-F trails.

Relationship between nest variables

Nest size, canopy cover over the nest (as a proxy for insolation) and amount of foraging are ecologically important nest traits. The relationships between these variables were analysed within the context of the network. The results suggest that the most important variable is the difference between a nest foraging or not foraging. Foraging nests are larger (72,630 ±23,900 workers vs. 22,760 ±4,923 workers: F vs. nF), and in darker areas (30 ±2.6% canopy cover vs. 21 ±2.4% canopy cover: F vs. nF) whereas non-foraging nests are smaller and in lighter areas (foraging and nest size: ANOVA, F=7.09, n=139, p=0.001; foraging and canopy cover: ANOVA, F= -3.5, n=139, p=0.003; figure 3.3). There is no significant relationship between the canopy cover and size of a nest (Pearson: r=0.12, n=139, p=0.084). Larger foraging nests do not forage proportionally less than smaller foraging nests (Pearson: r=-0.08, n=76, p=0.233). Similarly, foraging nests show no significantly relationship between amount of foraging and canopy cover (Pearson: r = -0.01, n=76, p=0.613).

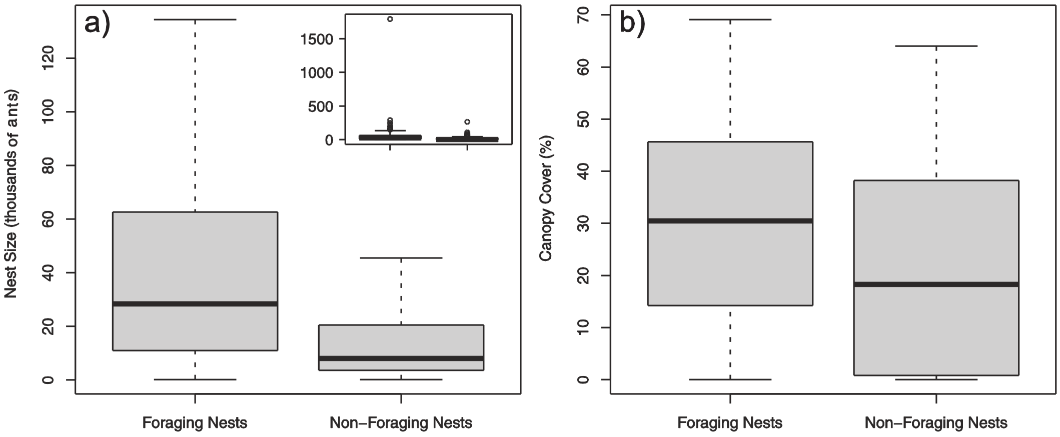


Figure 3.3 Summary of relationships between nest variables, in both figures n=139. a) Foraging and nest size, without outliers (inset with outliers). b) Foraging and canopy cover.

3.4 Discussion

Our study shows that *Formica lugubris* polydomous nest-networks are structured around exchange of foraged resources between pairs of nests, rather than at the level of the colony. This is evident in the positive relationship between internest trail strength and foraging differential and the absence of a relationship between trail-betweenness (a measure of optimal movement through a network) and trail strength. Both results suggest that individual ants are not moving through the whole network to redistribute resources but rather travelling only locally to nests to which they are directly connected. This is supported by the relationships between trail length and the other trail properties. In a colony based around local resource exchange it would be worthwhile to construct long trails between distant nests only if there is an important gain to be made from the connection. This is what we found in the *F. lugubris* networks. In this case, the gain is probably resource exchange, as this pattern is only observed in the nF-F trails. There is no evidence that workers from nF nests use the foraging trails of their neighbours, suggesting that resource exchange is occurring at the nest, rather than on the foraging trails from the nest. Further study is needed to establish the mechanism of this resource exchange, and how it relates to the movement of individual workers.

If the network is structured around colony-level resource exchange, a correlation would be expected between nest properties (such as size or amount of foraging) and network variables (such as centrality and connectedness). For example, in a colony optimized to redistribute foraged resources, foraging nests might be expected to be well-connected, because they are acting as a hub from which other nests collect resources. Or it might be expected that non-foraging nests show higher centrality, as they are acting as a link between separate foraging patches and maintaining colony cohesion. Our finding, that there is no relationship between any of these variables, suggests a lack of colony-level organisation of resource redistribution in polydomous *F. lugubris* colonies.

The lack of colony-level organisation is further highlighted by the lack of relationship between the number of nests in a colony and either canopy cover or sizes of nests in the network. It might be expected there is an optimum number of nests for a colony dependent on external (insolation) or internal (size) conditions. The absence of relationship between degree of polydomy and canopy cover is interesting as it is inconsistent with previous work on wood ants which has suggested a link between polydomy and insolation (Sudd et al. 1977; Sorvari & Hakkarainen 2005). Indeed it has been argued that a higher degree of polydomy is important to survival in deeper woodland (Punttila 1996). However the difference in findings between the studies may be caused by difference in the habitats. For example, in contrast to many previous studies (e.g. (Punttila 1996), this study was undertaken in the absence of any other members of the *F. rufa* group. Further investigation is needed to establish if this lack of relationship between canopy cover and degree of polydomy is just a local pattern or a more general feature of wood ant ecology.

The concept of a network built around local interactions shares features with other aspects of wood ant life history. Previous studies of monodomous colonies have found that foragers display a high degree of site allegiance and route fidelity (Rosengren 1977; Rosengren & Fortelius 1986; Gordon et al. 1992). Polydomy in *F. lugubris* could function by a similar mechanism based on workers showing loyalty to a particular nest and providing food for, or taking food from, neighbouring nests. This mechanism would result in the observed pattern of higher numbers of workers visiting (or visiting from) nests with a foraging excess. A particularly clear pattern would be expected between foraging and non-foraging nests, as the non-foraging nests have no other substantial source of food. This pattern is what we found in the *F. lugubris* polydomous networks.

Similar mechanisms to those implied by our results have been used in theoretical models of polydomy (Schmolke 2009; Cook et al. 2013). In these models workers are loyal to a particular nest and treat other nests of the colony as food sources. This mechanism would create a network based on the interactions between partly-autonomous nests rather than a colony-level organisation. A related mechanism has been observed in other ant species based on a transporter class specializing in carrying resources along internest trails (Dahbi et al. 1997; Pfeiffer & Linsenmair 1998), further investigation is necessary to distinguish between these mechanisms in *F. lugubris*.

Route fidelity is a feature of foraging in many ant species, particularly species which rely heavily on honeydew for nutrition (Rosengren 1977; Tilles & Wood 1986; McIver 1991; Quinet & Pasteels 1996; Gordon 2012). The wide phylogenetic distribution of this mechanism may suggest that trail fidelity is an efficient way to forage for spatially and temporally stable food sources. For nests in a polydomous colony, other nests in the network may act as spatially and temporally stable food sources, which would make it beneficial to ‘exploit’ them using a similar mechanism to stable food sources. Resource redistribution in polydomous ant colonies may, therefore, be an example of the adaption of existing behaviours to new tasks, in this case foraging behaviours to being used to facilitate resource exchange.

The lack of colony-level organisation suggests a certain level of autonomy for nests within the network. This nest autonomy also has the potential to facilitate division of labour between nests in the network. Similarly to within the colony itself, where workers often specialize at different tasks (e.g. foraging, brood care etc.) nests within a colony may specialize at, for example, foraging, or brood production. Division of labour may explain the presence of so many non-foraging nests in our *F. lugubris* polydomous networks. For example, the smaller and better insolated non-foraging nests are likely to have a different internal temperature to the larger, shaded, foraging nests, perhaps providing a better temperature for brood development. Similarly, non-foraging nests could be important for collection of other resources which the colony needs such as nesting material. It is also important to note that our definition of 'non-foraging' nest does not necessarily mean that a nest is not foraging at all, just that it is not forming foraging trails to trees. It may be that smaller 'non-foraging' nests are actually playing an important role as bases for scavenging and hunting arthropod prey. This contrasts with studies of polydomy in some other ant species which have been observed to build smaller nests, without brood, near to honeydew sources as temporary bases for foragers (McIver & Steen 1994; Lanan et al. 2011; Csata et al. 2012; Lanan & Bronstein 2013). In these colonies there is a clear division of labour between the 'foraging bases' and the permanent, brood-rearing, nests. Further investigation is needed to establish the extent and role of division of labour in polydomous colonies

The concept of 'nest traits' as opposed to 'colony traits' raises interesting questions about levels of selection in this species. The level at which selection acts is an important question in the study of evolution. In social insects the problem becomes even more complex by the addition of colony level selection, as well as selection on the individual, and ultimately the gene (Bourke & Franks 1995). Polydomous colonies have the potential for yet another level of selection: the nest (Banschbach & Herbers 1996b; Debout et al. 2007). In this system it certainly seems like there is the potential for nest level selection. Nests in the *F. lugubris* network seem to show a certain degree of autonomy: at least in terms of acquisition of resources, each nest appears to be acting either independently, or only with neighbours. This raises the intriguing possibility of nests which are 'better' at collecting resources than others. This might result in increased production of brood by some nests, which (depending on the levels of brood and queen exchange between nests) may result in a selective advantage to gathering resources at the expense of the rest of the colony. This may be manifested in the non-foraging nests found in the *F. lugubris* polydomous networks. Rather than providing an adaptive benefit to the colony, the non-foraging nests could be parasitic upon the effort of the foraging effort of the rest of the colony i.e. non-foraging nests are a cheating strategy in polydomous colonies. The non-foraging nests may be smaller simply because they are completely reliant on other nests for resources and perhaps this strategy may only be possible if the nest has a small population. However further study, especially of the level of brood and queen exchange between nests, is needed to establish if the conditions for nest-level selection are being met by polydomous colonies.

Some studies of polydomous networks have found evidence of efficient and robust nest-network organisation at the colony level (e.g. Aron et al. 1990; Latty et al. 2011). Analysis of the polydomous networks of a variety of ant species (including *F. lugubris*) has suggested that the networks are locally and globally efficient for resource transportation (Cook et al. 2014). One of the characteristics of locally and globally efficient networks is the pattern of many local connections with a few longer connections (Watts & Strogatz 1998). The longer trails may represent an adaptation to increase the robustness of the entire nest-network: this is indicative of a higher, colony-level organisation of polydomy (Cook et al. 2014). In the current study, there is no relationship between the strength and length of trails between pairs of foraging nests; these longer trails may be the trails playing an important role in maintaining colony cohesion and adding a measure of robustness to the networks. Longer connections which increase network efficiency and robustness have been found in other systems including termite nest galleries (Perna et al. 2008), bottlenose dolphin social networks (Lusseau 2003) and Trinidadian guppy social systems (Croft et al. 2004). In these examples, the relationship between local connections and the global organisation has significant implications for the structure of the communities. In wood ant polydomous networks the link between the local internest interactions and colony-level social organisation has comparably significant implications for how the colony functions, and how the colony reacts to changes in the environment, which makes it an important area for further investigation.

Local interactions which build up to more complex, colony-level, behaviours are a recurring theme in the study of social insects. The raiding patterns of army ants (Franks et al. 1991), house-hunting in *Temnothorax albipennis* (Robinson et al. 2011) and honey bee comb formation (Camazine 1991), to name only a few have all been shown to be driven by the interactions in behaviour of individuals, rather than by any central control or planning. This is not limited to social insects and has been found in many other biological systems (e.g. vertebrate movement: (Couzin & Krause 2003); human decision-making: (Krause et al. 2010); plant growth: (Leyser 2011). This self-organised pattern appears to reflected in the polydomous nesting strategy of *F. lugubris* as the behaviour seem to be mediated by the local interactions between individual nests, with no central organisation and limited colony-level structure.

Chapter 4: Inter-nest food sharing within wood ant colonies: a simple behavioural mechanism promotes nesting strategy plasticity

Abstract

Resource sharing is an important cooperative behaviour in many animals. Sharing resources is particularly important in social insect societies, as division of labour often results in most individuals including, importantly, the reproductives, relying on other members of the colony to provide resources. Sharing resources between individuals is therefore fundamental to the success of social insect colonies. Resource sharing is complicated if a colony inhabits several spatially separated nests, a nesting strategy common in many ant species (polydomy). Resources must be shared not only between individuals in a single nest, but also between nests. We investigated the behaviours facilitating resource redistribution between nests in a dispersed-nesting population of wood ant *Formica lugubris*. We marked ants, in the field, as they transported resources along the trails between nests of a colony, to investigate how the behaviour of individual workers relates to colony-level resource exchange. We found that workers from a particular nest 'forage' to other nests in the colony, treating them as food sources. Workers treating other nests as food sources means that simple, existing foraging behaviours are used to overcome the complex problem of resource redistribution in a distributed system. It may be that this simple behavioural mechanism facilitates the evolution of this complex life-history strategy.

4.1 Introduction

Resource sharing is a fundamental form of cooperative behaviour. The benefits of resource sharing can be direct, such as an increase in the growth or survival of offspring provisioned by a parent (Ydenberg 2007), or more indirect, such as increased access to resources provided by foraging in a group (Waite & Field 2007). In eusocial insect societies, resource sharing behaviours are vital to the survival and fitness of a colony. Only a small proportion of the individuals within a colony are usually involved in the collection of resources, so it is important for the colony to effectively redistribute these resources throughout the rest of the colony, especially to the brood and reproductive individuals. A variety of complex collective behaviours are involved in facilitating, and regulating, within-colony resource redistribution (e.g. Boi et al. 1999; Robinson et al. 2009; Sendova-Franks et al. 2010; Mersch et al. 2013)

For a social insect colony, resource redistribution becomes more complicated if a single colony inhabits several spatially separated nests. This distributed nesting strategy, called polydomy, is common in ants; it is found in at least 150 species representing all the major ant subfamilies (Debout et al. 2007). In a polydomous ant colony, not only do resources have to be redistributed within a single nest but also between nests (Robinson 2014). As with all collective patterns in social insects, this global resource redistribution must be achieved by the distributed behaviours of individual workers.

In polydomous colonies of the red wood ant *Formica lugubris,* resource redistribution between nests occurs along trails of workers travelling between the nests within the colony (Ellis & Robinson 2014). An important resource transported along these internest trails is honeydew (Ellis et al. 2014). Analysis of the structure of the network formed by the nests and internest trails in polydomous wood ant colonies has suggested that they are used to transport honeydew locally, between pairs of nests, rather than through the entire colony (Ellis et al. 2014). This suggests that individual workers are travelling, and transporting resources, along a particular trail between two nests, rather than travelling from nest to nest throughout the colony, but it is unknown how this is organised and how this pattern relates to the behaviour of individual ants.

Two mechanisms have been suggested for how individual workers could transport resources between nests in polydomous colonies. The first mechanism is based on the idea of a class of internest-transport workers. Transporters are workers specialized at moving resources along a particular internest trail. These workers would transport resources in both directions along the trail, dependent on the abundance of resources in the nests at each end of the trail. These transporters are associated with a particular trail, rather than a particular 'home' nest (i.e. the nest a particular worker is attempting to benefit). Social insect colonies generally contain many specialized classes of workers (such as foragers and nurses); and so a specialized class of internest transporters might be expected. Indeed workers specialized at transporting resources between polydomous nests have been found in some ant species (*Cataglyphis iberica* Dahbi et al. 1997; *Camponotus gigas* Pfeiffer and Linsenmair 1998) and suggested in red wood ants (Rosengren 1971).

The second possible mechanism is based on internest transport using the same behaviour as foraging (McIver 1991). Wood ants foraging for honeydew show a very high degree of route and site fidelity; marked ants have been observed following the same foraging trail to the same foraging site for entire foraging seasons, even after the reward at the end of the trail was no longer present (Rosengren & Sundström 1987; Gordon et al. 1992) or after an artificially extended winter (Rosengren & Fortelius 1986). Indeed, route and site fidelity is a common resource acquisition behaviour in a variety of ant species (Tilles & Wood 1986; McIver 1991; Quinet & Pasteels 1996; Gordon 2012). This method of foraging is possible because honeydew is a spatially and temporally stable food source for a red wood ant colony: the aphid colonies providing the honeydew appear to persist within and between years (Rosengren 1977; SE *pers obs.*). Route and site fidelity could also be used to transport resources between nests in a polydomous colony, with workers based in a particular nest treating other nests in the colony as food sources. In this case workers would travel from a particular ‘home’ nest to neighbouring nests, take the resources they need and return to their home nest, in the same way they visit honeydew-producing aphids in the canopy. Under the transporter mechanism, workers on internest trails are balancing resources between two nests, which has the effect of them working to redistribute resources over the whole colony. In contrast, the foraging hypothesis workers are only working to increase the resources in their home nest.

In this study we marked workers travelling between nests in polydomous *F. lugubris* colonies, to distinguish between these two alternative mechanisms. Specifically, we asked (1) Is there a class of internest workers travelling along the trails carrying honeydew in one direction and empty of honeydew in the other direction?, (2) Is this behaviour consistent (i.e. do workers maintain the same resource carrying over several days)? and (3) Is this based on ants from a particular nest giving to their neighbours, or ants from a particular nests taking food from their neighbours (table 3; figure 4)? A specialized transporter class would be expected to show workers redistributing resources in response to local demand, or local excesses. If a particular nest has a demand, a transporter may visit nearby nests to take food, or conversely if a particular nest has an excess, workers may take resources from that nest to neighbouring nests. This would result in workers transporting resources in both directions along trails in response to local conditions at the nests, or rather, perceived local conditions (individual workers are unlikely to have perfect information). Transport of resources in both directions along a particular trail would manifest itself either as workers constantly carrying resources both ways along a trail, or changing the direction in which they transport resources over short timescales. This inconsistency in transport direction is likely to be particularly obvious on trails between nests with relatively even resource levels. In contrast, a worker treating other nests as food sources will consistently transport resources towards her 'home' nest regardless of relative local demands.

We predict, therefore, that a transporter class will either travel laden with honeydew in both directions, or be inconsistent in the direction in which they do carry resources, along internest trails making it unclear if individual workers are giving food to neighbouring nests or taking from them. A foraging worker would, however, be expected to only travel laden in one direction, and for that direction of travel to be consistent, as they take food from neighbouring nests.

4.2 Methods

Study species and field site

This study was conducted at the Longshaw Estate in the Peak District National Park, UK. There is a large population of *F. lugubris* at the site, with over 900 nests within the 0.95ha area (f). The habitat at the site contains a mix of deciduous woodland, mixed woodland, open sparsely planted grasslands and the remains of historic scots pine plantations. There are no other members of the *F. rufa* group at the site.

Colonies

Five polydomous *F. lugubris* colonies with the appropriate trail types for this study (see below) were chosen from the results of a preliminary site survey undertaken in May 2013 (details: table 4.1). Before the experiment began, we accurately mapped the colonies, recording the locations of: nests; inter-nest trails; foraging trails; and the trees being foraged to (example: figure 4.1). By examining the colony maps, we classified each of the nests within the colonies as 'foraging' or 'non-foraging', based on the presence or absence of trails from the nest to a tree (Ellis et al. 2014). It is important to note that, as the definition of foraging is based only on connections to aphid bearing trees, it does not preclude non-foraging nests from performing other foraging activities such as scavenging and hunting. Internest trails were then classified as between two foraging nests (F-F), between two non-foraging nests (nF-nF) or between a foraging nest and a non-foraging nest (nF-F). Each experimental colony had two trails chosen for trials; one nF-F trail and one either F-F trail (three colonies) or nF-nF trail (two colonies). Some colonies had more than one trail in a particular category (for example, several nF-F trails). In these cases, the experimental trail was selected at random from the appropriate trails.

Colony	Number of nests	Experimental trail types	Relative position of trails.	Total number of twice marked ants
I	4	nF-F and F-F	Linked	189 and 118
II	12	nF-F and F-F	Separate	79 and 57
III	11	nF-F and F-F	Separate	104 and 104
IV	9	nF-F and nF-nF	Linked	184 and 147
V	3	nF-F and nF-nF	Linked	94 and 97

Table 4.1 Details of the five colonies used in this study. ‘Linked’ trails are those where the two experimental trails share a nest, whereas ‘separate’ trails are those where the two experimental trails are in different parts of the colony and do not share a nest. Number of marked ants refers to the total number ants painted on the nF-F trail and other trail respectively.

Assessing Load

Honeydew is transported in the crops of ant workers, and in *F. lugubris* storage causes a visible swelling of the gaster (SE *pers. obs.*). Applying gentle pressure to the gaster of a full worker will cause the honeydew stored in the crop to be regurgitated (e.g. Cherix 1987). We use both the visible swelling of the gaster and application of gentle pressure, to assess the presence or absence of honeydew in the gaster of the workers. During preliminary experiments, before the trials, our accuracy at assessing presence or absence of honeydew from visual cues alone was 96% (*SE unpublished data*).

Marking

To investigate how the movement of individual workers between nests facilitates transport of resources through polydomous *F. lugubris* colonies we individually marked workers as they travelled between nests along the chosen trails. Workers were marked with ‘Uni-Paint’ marker pens (Mitsubishi Pencil Co. UK Ltd). Paint marking has been used extensively in previous studies of ants with no disruption to their behaviour (Beverly et al. 2009; Franklin et al. 2010; Chen & Robinson 2013); we observed no overt changes in behaviour or increased mortality in the marked ants. We used a unique pattern of colours for each experimental trail within the colony.

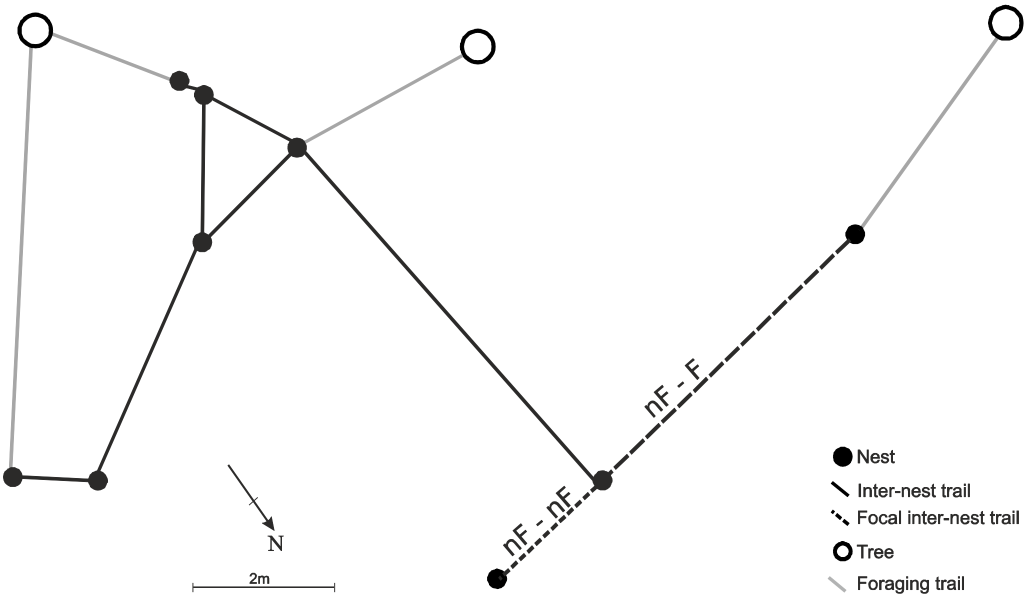


Figure 4.1 Example of a colony used in this study (colony IV, table 1). Closed circles are nest and black lines are internest trails. Open circles are trees, grey lines are foraging trails. The dashed trails are the experimental trails. nF-F indicates the trail between a foraging nest and a non-foraging nest, nF-nF indicates the trail between two non-foraging nests.

On the first day of a trial, a direction along the trail was randomly chosen. Five-hundred workers travelling in the selected direction were painted as they passed a defined point approximately halfway along the trail. The painting was done in batches of 100 ants over the course of one or two days to minimise time of day effects. Workers were painted one of two colours on the thorax; one colour if an ant was laden with honeydew (full) and another if the ant carried no honeydew (empty). Load was assessed by applying pressure to the gaster of the ant to observe for regurgitation of honeydew: this ensured a high level of accuracy for the marking. The following day, all painted ants passing in the opposite direction (to that painted the day before) were assessed for load, and then painted on the head as either full or empty. This second painting session was conducted in seven 30 minute intervals over the course of one day, to control for time of day effects (painting totals: table 4.1). The result after both marking sessions was that each trail had a cohort of workers marked as full or empty on both the thorax (representing load in one direction) and the head (representing load in the other direction).

Observation

For five days immediately following the completion of painting, the trails were observed for 30 minutes per day. The time of day for observation was chosen randomly from the times of day with amenable weather conditions. During the observations, for all marked ants passing a particular point we recorded the: direction of travel, painted pattern and presence or absence of honeydew load. Load was assessed by visual examination of the workers, rather than by applying pressure, to minimise further disruption to the workers. These sessions of trail observation are hereafter referred to as the ‘observation period’.

Straying

Previous work has suggested that ants workers in polydomous *F. lugubris* colonies are only travelling locally, between pairs of nests, rather than moving freely through the whole colony (Ellis et al. 2014). To confirm that workers are only moving locally, we estimated the rate at which ants stray from a given internest trail onto other trails in the same colony. Straying frequency was estimated by randomly selecting a trail sharing a nest with the experimental trail each day and observing that trail for 10 minutes, noting the direction of travel, paint pattern and honeydew-load of any doubly-painted ants on that trail. The type of trail workers stray onto may help reveal the role they are playing in the resource redistribution mechanism. Workers travelling through the colony to find food are expected to stray preferentially onto foraging trails, as the trees are the food sources for the colony.

Statistical analysis

Analysis was performed using generalised linear mixed effect models (GLMMs) in the ‘lme4’ package in R (R Development Core Team 2011). The response variables and fixed effects changed based on the question being investigated. Unless noted otherwise only, a single fixed effect was used. Additionally, colony of origin and day of the experiment were used as nested random effects to control for repeated observations of the same trail. Further details of the statistical models used are found in the appendices: the superscript in the text refers to the row of the table which contains the details (appendix F).

All GLMMs used a binomial error structure with a logit link function. Tests of significance were performed by a chi-squared analysis of deviance (AoD); the results of these tests are reported in the text. The analysis of deviance compares the full GLMM to the same GLMM but with the variable of interest removed. A significant difference between these two models indicates a significant effect of the variable in explaining the data. Where the significance of a particular variable is reported, the values are taken directly from the model.

4.3 Results

Our results revealed that a significant majority of workers on internest trails travel in one direction full and the other empty, and that these workers consistently carry resources in this single direction over the course of the five days of the experiment. In addition, the results suggest that the internest resource exchange mechanism is based on workers taking resources from, rather than giving resources to, neighbouring nests.

Directionality

Our results show that there is a class of workers travelling full in one direction and empty in the other (table 4.2). Overall, 742 of 1173 (63%) of marked workers had different loads in each direction, while only 80 (7%) were full in both directions and 351 (30%) were empty in both directions. The workers traveling full in one direction and empty in the other can be considered to be directional workers, carrying honeydew in one direction but not the other. There is a significant negative association between load in each direction (AoD$^{4.1}$, $\chi^2=48.1$, $df=1$, $p<0.001$), meaning that there are significantly more directional ants than ants travelling with the same load in both directions. The different colonies used in the experiment do have significantly different proportions of directional ants (AoD$^{4.2}$, $\chi^2=34.2$, $df=4$, $p<0.001$). This is driven by a significantly higher proportion of directional ants in colony I (GLMM$^{4.2}$, $z=-2.981$, $n=1173$, $p<0.01$). Proportion of directional workers also varies significantly depending on trail type (AoD$^{4.3}$, $\chi^2=24.3$, $n=1173$, $df=2$, $p<0.001$). There are a significantly lower proportion of directional workers on nF-nF trails than on other trail types (GLMM$^{4.3}$, $z=2.994$, $n=1173$, $p<0.01$). This is likely to be particularly influenced by the observation that one of the two tested nF-nF trails (on colony V) has a very low proportion of directional workers. The other tested nF-nF trail (on colony IV) shows a similar proportion of directional workers to other trails.

		AB Direction	
		Full	Empty
BA Direction	Full	80	516
	Empty	226	351

Table 4.2 Total number of ants painted in each category during the course of 10 trials. The trails are between nests arbitrarily named ‘A’ and ‘B’. The AB direction indicates ants travelling from nest ‘A’ towards nest ‘B’, and BA direction is ants travelling in the opposite direction (from nest ‘B’ to nest ‘A’).

Consistency

All journeys in the observation period were classified as either ‘consistent’ or ‘inconsistent’. A journey is consistent if the honeydew-load of an observed ant matches that which it was initially marked transporting. For example, an ant initially marked as full when travelling in a particular direction is acting consistently if, when later observed travelling in that same direction (during the observation period), it is transporting honeydew; conversely it is acting inconsistently if it is empty of honeydew.

Over the course of all ten trials, 693 of 927 (75%) of observed journeys were consistent; this is significantly higher than the number of inconsistent journeys (AoD$^{4.4}$: χ^2=11.7, *df*=1, *p*<0.001). Consistency of behaviour varies significantly based on painted pattern of the ant (AoD$^{4.5}$, χ^2=34.1, *df*=2, *p*<0.001; figure 4.2); ants painted as travelling full in one direction only are significantly more consistent than both those painted as full in both directions (GLMM$^{4.4}$, *z*=-5.9, *n*=927, *p*<0.001) and those painted as empty in both directions (GLMM$^{4.4}$, *z*=-2.1, *n*=927, *p*=0.04).

This consistency does not vary either with trail type (AoD$^{4.6}$, χ^2=3.5, *df*=2, *p*=0.17) or over the course of the five days of the experiments (AoD$^{4.7}$, χ^2=4.8, *df*=4, *p*=0.31). Interestingly, however, if ant painted type is introduced as an interacting fixed effect then there is a significant effect of day on the proportion of consistent ants (AoD$^{4.8}$, χ^2=43.0, *df*=5, *p*<0.001). The interaction between day and ant painted pattern is significant (AoD$^{4.9}$, χ^2=4.99, *df*=2, *p*=0.03); this is driven by a significant negative interaction between ants which were marked as travelling empty in both directions and day of the experiment (GLMM$^{4.9}$, *z*=-0.34, *n*=927, *p*<0.001). This suggests that, over the course of the experiment, ants initially travelling empty in both directions became significantly less consistent in their behaviour.

Straying

Ants strayed from their painted trail at an average rate of 1.16 ±0.27 (mean ± SE) per 10 minute observation (which is 8.1 ±13.4 % of the number of marked ants observed on the focal trails). Ants with differing marked patterns (e.g. full in both directions, full in one direction or full in neither direction) were equally likely to stray from the focal trail (AoD$^{4.10}$, χ^2=1.7, *df*=2, *p*=0.44). Ants were equally likely to stray onto other internest trails as onto foraging trails (AoD$^{4.11}$, χ^2=0.78, *df*=1, *p*=0.38).

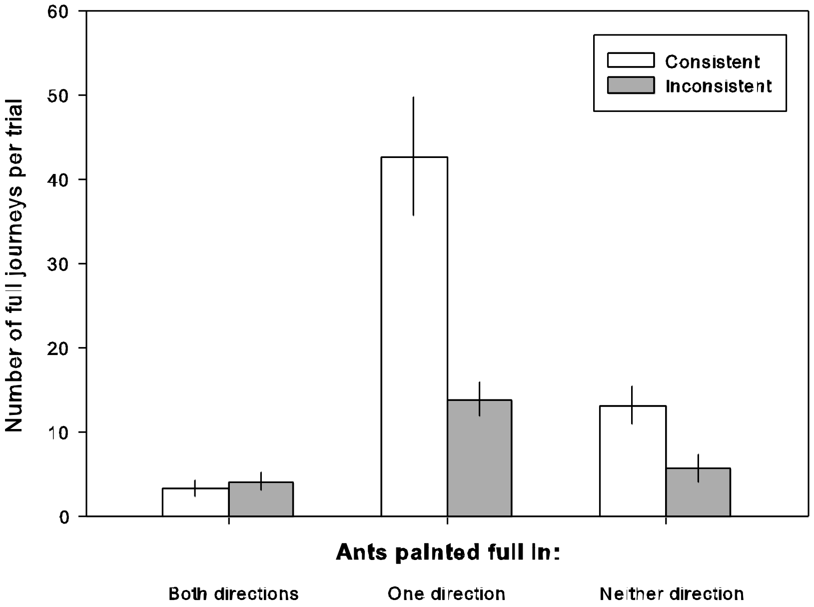


Figure 4.2 Mean (± SE) number of ants behaving consistently and inconsistently over the course of five days of observation based on their originally painted pattern. Workers are considered consistent if their observed behaviour matched their painted behaviour. Ants originally painted as laden with honey dew ('full') in one direction (and empty in the other) were significantly more consistent in their behaviour than either the ants travelling painted as laden in both directions or those painted as empty in both directions (AoD$^{4.5}$, $\chi^2=34.1$, df=2, $p<0.001$).

Give or take?

The results above suggest that there is a class of workers consistently travelling one direction along an internest trail full and in the other direction empty. However what is not clear from these results is whether resource exchange is based on workers from one nest carrying food to their neighbours (a give mechanism) or workers from a particular nest taking food from their neighbours (a take mechanism).

Given that workers are acting consistently (see above), some predictions of the behaviour can be made (summarised in table 4.3). For example, it would be maladaptive for an ant to leave its home nest to visit a neighbouring nest with an incorrect load. This applies in both the give and the take mechanism; an ant giving food to another nest is unlikely to leave its home nest empty, in the same way an ant going to take food from a neighbouring nest is not expected to leave its home nest full. However, there is a chance that an ant could reach its destination nest and not be able to deposit or collect its load, and then have to return to its home nest with the same load as when it left. Under a give mechanism, for example, an ant may reach the destination nest and not be able to find a worker willing to accept its food and have to return still full. Similarly, under the take mechanism, a worker may be unable to find honeydew at the destination nest and have to return to its home nest still empty. These assumptions therefore lead to differing expected patterns of inconsistency along a trail depending on the mechanism in place (table 4.3). By comparing ants painted pattern to the proportion of inconsistent journeys in either direction it is possible to differentiate between the two mechanisms. We found that that 75.6% (99/131) of inconsistent journeys made by ants marked carrying resources in only one direction (i.e. full-empty or empty-full ants) were inconsistent in the direction in which they were originally marked as laden with honeydew (the 'full' direction). The proportion of inconsistent journeys is significantly higher in this 'full' direction than in the direction they were marked as empty (full-empty marked ants; AoD$^{4.12}$, χ^2=19.8, *df*=1, *p*<0.001: empty-full marked ants; AoD$^{4.13}$, χ^2=14.0, *df*=1, *p*<0.001). A greater proportion of inconsistent journeys in the full direction is expected under the take mechanism (table 4.3).

	Give Mechanism	**Take Mechanism**
Mechanism	Workers leave their 'home' nest **full** travel to a neighbouring nest, **give** workers from that nest food and return to the 'home' nest **empty**.	Workers leave their home nest **empty**, travel to neighbouring nest, **take** food from workers in that nest, and return **full** to their home nest.
Prediction	Few workers will leave the home nest **empty**. However they may fail to find workers to give their food to and have to return to the home nest **still full**.	Few workers will leave the home nest **full**. However they may fail to find and food to take at the other nest and return to the home nest **still empty**.
Expected Result	More inconsistent journeys in the direction which the ants were marked as 'empty' than the direction which they were marked as 'full'.	More inconsistent journeys in the direction which the ants were marked as 'full' that in the direction which they were marked as 'empty'.

Table 4.3 Comparison of the expectations and predictions of the give and take mechanisms of resource redistribution between nests.

Resource movement

With one exception, resource movement is towards the non-foraging nest along nF-F trails. However it is interesting to note that, even though net-resource movement was towards the non-foraging nests, 31.9% (180/564) observed journeys on the nF-F trails were still transporting resources from the non-foraging towards the foraging nest. The net movement of resources along trails is significantly lower on F-F trails than on other trail types (AoD$^{4.14}$, χ^2=18.5, *df*=2, *p*<0.001; figure 4.3), suggesting that resource flow is significantly less uneven on F-F trails than other trail types. On both the nF-nF trails net-resource flow is from, a nest which has another trail to a foraging nest, towards a non-foraging nest which had no trails to foraging nests. The nF-F trail exception is colony II, and in this colony, when re-examined later in the season, the non-foraging nest had begun foraging, which may explain its unusual behaviour.

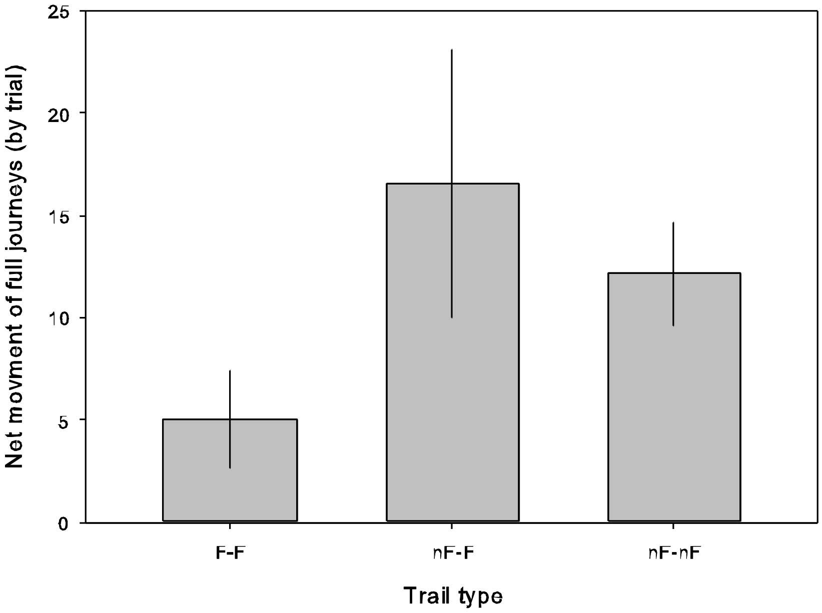


Figure 4.3 Mean (± SE) number of honeydew-carrying ('full') journeys along internest trails of different types. The net movement of resources is significantly lower on trails between two foraging trails (F-F) than on either trails from a non-foraging nest to a foraging nest (nF-F) or trails between two non-foraging nests (nF-nF), suggesting a less uneven exchange of resources on F-F trails (AoD$^{4.14}$, $\chi^2=18.5$, df=2, $p<0.001$).

4.4 Discussion

Our study shows that mechanism of honeydew-exchange between nests of polydomous *Formica lugubris* colonies is based on individual workers treating other nests of the colony as food sources. This is supported by the results showing: (1) a cohort of workers travel along internest trails in one direction full and the other empty, (2) they do this consistently, and do not change role or direction of transport over a short timescale and (3) workers are taking food from neighbouring nests, rather than giving food to their neighbours.

In general, when foraging, ant workers leave the nest, collect food and return to the nest transporting this resource. This process necessarily results in the majority of foragers travelling in one direction without carrying food, and the other direction carrying food. If workers are treating other nests in a polydomous colony as food sources, a similar pattern of transport would be expected. This is the pattern we found in the internest workers of the *F. lugubris* colonies, with significantly more workers observed travelling in one direction full and the other empty than travelling with the same load in both directions. In this study we have investigated only the movement of honeydew between nests; a system based on foraging to other nests would also work as a method to redistribute other resources such as arthropod prey and nesting material, but further investigation is necessary to determine if this is the case.

Foraging wood ants show high levels of site allegiance and route-fidelity; they consistently travel from the same nest along the same foraging route, in many cases independently of the presence or absence of a resource at the end of the route (Rosengren 1971; Gordon et al. 1992). If a similar pattern is used to transport resources between polydomous *F. lugubris* nests, it is to be expected that the workers marked as transporting resources in one direction will continue to transport resources only in that direction, and not change their role, or direction of transport, over time. In contrast, if resource transfer is undertaken by a specialist internest-transporter class the direction of resource transfer is expected to vary, especially between nests where resource flow is relatively even. For example, on a trail between two foraging nests a transporter might reach one of the nests, find an excess and therefore transport honeydew to the other nest. At the second nest they might, again, find an excess and therefore transport honeydew back to the original nest resulting in a relatively even resource flow and a cohort of workers transporting in both directions along a trail. The reverse is also true, if transporters find a need at particular nest they will react by going to the neighbouring nest and collecting food to return to the original nest. In this study we found that workers, especially directional workers, show a high degree of consistency in their behaviour, indicating that they consistently transporting resources in a single direction. This applies even on trails where the net resource flow is relatively even (F-F trails), strongly suggesting that internest resource transfer is based on high route fidelity and site allegiance with workers treating other nests of the colony as food sources, rather than a specialised transporter class.

Our results agree with previous working suggesting that workers on internest trails in *F. lugubris* colonies travel locally, between pairs of nests, rather than travelling freely through the whole colony (Ellis et al. 2014). The low frequency of ants straying between trails suggests that workers are only travelling along a single internest trail, not through the whole colony. As the trees are the food sources, we expect that if workers are travelling freely through the network to find resources they would preferentially travel to foraging trails. The result that workers are equally likely to stray onto internest trails as onto foraging trails, suggests that workers are not preferentially travelling to foraging trails. This in turn suggests that the strays may simply be lost or mislabelled ants, rather than part of the resource redistribution system.

Details of the development of this resource-redistribution mechanism are suggested by the observation that workers marked travelling empty in both directions along trails become less consistent in their behaviour over the course of the experiment, meaning that they have begun transporting resources. It could be that these workers are inexperienced foragers who are in the process of being recruited to a source of honeydew. Over the course of the observation period some of these inexperienced workers may have become recruited to the trail under observation and therefore become directional transporters. In our study, this would manifest as the workers marked traveling empty in both directions beginning to transport resources and therefore becoming inconsistent with their painted pattern, which is what we have found. Inexperienced foragers being recruited to foraging trails have been observed in wood ants (Gordon et al. 1992) but not investigated in the context of internest trails. The recruitment of inexperienced or naive workers to a particular task is widely reported in social insects generally. In many cases the recruited individuals revert to being naïve again either when the task has been completed (e.g. Langridge et al. 2004) or after a variable amount of time (e.g. Seeley & Buhrman 1999), whereas, in the case of foraging wood ants this recruitment appears to be permanent, at least for a subset of the foragers (Gordon et al. 1992; Lamb & Ollason 1994; SE *unpublished data*). Our results suggest that recruitment of inexperienced foragers to particular trails (in this case internest trails) may also apply to internest *F. lugubris* workers. The high proportion of ants marked as empty in both directions may be due to the action of this recruitment mechanism. However, it is also likely that a proportion of these empty-empty workers are engaged in other transport tasks such as transport of brood, prey or other workers (chapter 5). Further work is needed to elucidate the role of these internest workers which are not transporting honeydew.

Our study has shown that this consistent behaviour is directed towards taking food from neighbouring nests. This is an interesting result because it suggests that nests are acting almost independently to collect honeydew. Although allowing other workers from other nests to take food is form of passive support, nests appear to offer very little active support to each other. This is highlighted by the observation that even on trails between nF-F nests there is some honeydew carried from the non-foraging nest towards the foraging nests. Division of labour is a defining feature of eusociality (Hölldobler & Wilson 1990): at the simplest level this involves transferring resources from a sterile worker caste to a reproductive caste. Within this basic eusocial framework there are many examples of more complex division of labour, between foragers and nest workers for example, or between different classes of foragers. Active transfer of resources between these classes and castes is regularly observed and is necessary for the proper functioning of the colony. It might be expected that a similar mechanism is present in polydomous colonies, perhaps with larger foraging nests supporting other smaller non-foraging nests in the colony by actively supplying other nests of the colony with excess honeydew. The fact that this is not the case indicates that nests of the colony act almost independently when collecting honeydew, treating the rest of the colony as food sources. The only concession nests in a polydomous colony make to the rest of the colony is to allow their honeydew to be taken. This does not preclude different nests in the network performing different roles (some forage and others do not, for example) but it would suggests that this division of labour is not due to a colony-level strategy (with some nests as, for example, foraging specialists), rather it is based on the only on the properties and environment of individual nests.

The advantage of this ‘foraging’ mechanism is likely to be its simplicity. It requires no colony-level organisation, and results from simple self-organisation in worker behaviour. Newly-foraging ants begin as inexperienced foragers and are recruited to a food source with an excess, either a foraging tree or another nest, and then transfer resources consistently from that food source back to their home nest. A similar system has been found in some monodomous species, where colonies treat the nests of other colonies as food sources; stealing resources in a form of intraspecific kleptoparasitism (Breed et al. 1990; Yamaguchi 1995). The main difference between the intraspecific kleptoparasitism system and the polydomous nest system is that, whereas in the monodomous kleptoparasitic colonies this causes an aggressive response from the workers in the nest being taken from, within a polydomous colony there is no aggressive response to the intruders. A mechanism of resource redistribution within polydomous colonies based on workers treating other nests in the colony as food sources has been previously suggested, and modelled, in polydomous ants (McIver 1991; Schmolke 2009; Cook et al. 2013), but to our knowledge this is the first example of such a mechanism being observed in natural populations. Agent-based models of polydomous ant colonies have shown that workers treating other nests of the colony as food sources can result in resource redistribution through the whole colony (Schmolke 2009; Cook et al. 2013).

The mechanisms of resource acquisition and redistribution for a species are likely to be closely linked to the type of resource which they are attempting to exploit (Lanan 2014). For species relying on a temporally and spatially stable food source, like honeydew is for *F. lugubris*, the mechanism of resource acquisition may not need to be flexible to short term changes in resources availability (if there are any). Instead, it is likely to be more important for the ants to adjust to longer term trends in resource availability (Gordon et al. 1992), which a mechanism based on internest foragers would be capable of doing. In contrast, a mechanism based on either a specialist transporter class, or a nest giving food to its neighbours, requires the ability of workers to; (1) recognise the difference between food sources and other nests, and (2) assess the relative need of other nests in the network for honeydew and transport resources according to this need. Such a system may be able to adjust more accurately to short term changes in demand, but is much more computationally complex. This highlights the importance of timescale when investigating a species' foraging ecology. For species with low resource reserves that are dependent on unpredictable food sources, all nutrient sources, when they appear, are likely to be critical to survival (e.g. Rogers & Smith 1993; Bonter et al. 2013). In contrast, for species foraging on large and predictable food sources, short term changes are probably less important than the long-term trends which, in the case of many social insects, can be adjusted to over the scale of generations of workers.

A simple resource redistribution mechanism, such as that described here, may help explain the plasticity shown by *F. lugubris* and other *F. rufa* group species in their nesting strategy. If nests forage to neighbouring nests in the same way they forage to food sources, then the workers are using pre-existing resource acquisition behaviours. This means that there is less behavioural innovation required for a colony to move from being monodomous to polydomous. Rather than a complete change in life-history, involving new behaviours, polydomy can in fact be viewed as a continuation of existing behaviours requiring few innovations. Polygynous wood ant colonies usually reproduce by budding (Rosengren & Pamilo 1983; Ellis & Robinson 2014). If, when a new daughter nest is budded from an existing 'mother' nest, that daughter nest uses the mother nest as a food source and the mother uses the daughter as food source, a polydomous system has developed out of existing foraging behaviours.

Beyond the *F. rufa* group, in ants generally, polydomy has probably evolved many times independently (Debout et al. 2007). A mechanism of resource exchange based on existing behaviours may help facilitate this repeated evolution of the same nesting strategy. Polydomy may, therefore, provide an interesting variation on the idea of behavioural convergence. Behavioural convergence is the repeated evolution of the same behavioural traits by species in similar environments (e.g. Blackledge & Gillespie 2004; Stoks et al. 2005; Johnson et al. 2010; Alejandrino et al. 2011). In the case of polydomy, rather than the behaviour evolving multiple times in response to similar ecological conditions, the same behaviour may have evolved multiple times in response to a variety of different ecological conditions, due to an inherent pre-disposition for the behaviour. If this is the case, it may help explain the variety of habitats, life-history strategies and diets with which polydomy is associated (Debout et al. 2007).

This study has found that the complex task of redistributing resources through a spatially separated wood ant colony is achieved by the same behaviours used for foraging. This result is important in understanding how a species using different life-history strategies in different locations can switch between the strategies simply, with few behavioural innovations, by using pre-existing behaviours.

Chapter 5: The role of non-foraging nests in polydomous wood ant colonies

Abstract

A colony of red wood ants can inhabit more than one spatially separated nest, in a strategy called polydomy. Some nests within these polydomous colonies do not form foraging trails to aphid colonies in the canopy. In this study we identify and investigate the possible roles of these non-foraging nests in polydomous colonies of the wood ant *Formica lugubris*. To investigate the role of these non-foraging nests we: (i) monitored colonies for three years; (ii) observed the resources being transported between non-foraging nests and the rest of the colony; (iii) measured the amount of extra-nest activity around non-foraging, compared to foraging, nests. We used these datasets to investigate the extent to which non-foraging nests within polydomous colonies are acting as: part of the colony expansion process, hunting and scavenging specialists, brood-development specialists, seasonal foragers or parasites on the foraging effort of the rest of the colony. We found that, rather than having a specialised role, non-foraging nests are part of the process colony expansion. Polydomous colonies expand by founding new nests in random locations in the area surrounding the existing nests. Nests founded near food sources begin foraging and become part of the colony; other nests are not founded near to food sources, and are therefore non-foraging. Some eventually begin foraging and become foraging nests, other nests do not begin foraging and are abandoned. This is a method of colony growth not available to colonies inhabiting a single nest, and may be an important advantage of the polydomous nesting strategy.

5.1 Introduction

Foraging is a fundamental part of the life-history strategy of animals. The foraging strategy employed by an animal is dictated by a variety of factors, such as: the type of food resource, the competition for food resources and the distribution of resources (Stephens et al. 2007). Ants show a particularly diverse range of foraging strategies, and have a remarkable number of behavioural and morphological adaptations to exploit a wide range of food sources (Lanan 2014). The diversity of foraging strategies reflects the importance that the ability to exploit a range of food sources is likely to have had on the success of the ants.

Given the importance of foraging to ants, it is surprising that some nests appear not to be foraging. Whereas most nests in polydomous (multi-nest) colonies of red wood ants have foraging trails to food sources in trees, other nests do not have any foraging trails, and appear not to be performing any foraging (Ellis et al. 2014). In this study we investigated the possible roles of these apparently 'non-foraging' nests.

Foraging to honeydew-producing aphids in the canopy provides the majority of the food for red wood ant colonies (Rosengren & Sundström 1991). Red wood ants travel along well-defined trails from their nests to aphid-bearing trees; these trails are composed of thousands of ants foraging to the aphid colonies and can be present in the same position over the course of several years (e.g. Rosengren 1977; Gordon et al. 1992; Buhl et al. 2009). However, 41% (range: 11%-70%) of the nests within a polydomous colony have no foraging trails (Ellis et al. 2014). As workers do not travel to foraging trails originating in other nests, these non-foraging nests have no direct access to honeydew sources, instead the rely on other nests in the colony to provide honeydew (Ellis et al. 2014). The role of these non-foraging nests in polydomous colonies is unknown.

Polydomous ant colonies inhabit several spatially separated, but socially connected nests. In the case of wood ants, the social connection between nests consists of trails of workers travelling back and forth between the nests of the colony, much like on the foraging trails. Polydomy is a common nesting strategy in ants; it is present in at least 166 ant species from 49 genera (Debout et al. 2007), including many ecologically dominant species (e.g. *Oecophylla smaragdina*; Hölldobler 1983) and many invasive species (e.g. *Linepithema humile*; Holway & Case 2000). There does not appear to be an ecological or functional niche shared by polydomous species; it may be that the benefits of a polydomous nesting strategy vary from species to species. (Debout et al. 2007; Robinson 2014). In red wood ants the main benefit of polydomy has been hypothesised to be to help them exploit dispersed, but spatially and temporally stable, honeydew sources (Ellis & Robinson 2014). This is supported by recent results showing that the number of ants on the trails between nests within the colony is related to the difference in the amount of foraging being done by the nests being connected (Ellis et al. 2014). Specifically, on trails between a non-foraging and a foraging nest the number of ants on the trail (controlled for the size of the nest) is related to the amount of foraging being performed by the foraging nest (Ellis et al. 2014). This suggests that the structure of the trail network in polydomous colonies is driven by the local redistribution of honeydew between nests. The importance of honeydew exchange in structuring the polydomous colony makes it particularly unexpected that such a high proportion of nests within polydomous colonies appear not to be foraging.

We identified five possible, non-exclusive, roles that these non-foraging nests may perform in polydomous colonies. (1) Non-foraging nests could be part of the process of *colony expansion*. The foundation of new nests is fundamental to the life history of all social insects. Polydomous wood ant colonies often found new nests by budding: during budding a section of workers and queens leave the natal nest on foot and build a new nest nearby. If this nest remains connected to the natal nest it will become another nest in the polydomous colony. It could be that non-foraging nests appear as part of the process of nest foundation, acting as an intermediate between stage between nest foundation and the beginning of foraging. In general, non-foraging nests are smaller than foraging nests (mean size of worker population: foraging 72 630 ± 23 900 vs non-foraging 22 760± 4 923, Ellis et al. 2014); this may mean that they are more recently founded and that given time they will grow and begin foraging.

(2) Nests which are apparently non-foraging could act as *arthropod hunting and scavenging specialists*. We define a non-foraging nest as nest without foraging trails to aphid colonies in the trees. However, although honeydew provides the majority of food for nests, it not the only resource collected by red wood ants. Wood ant colonies collect both carbohydrate and protein; honeydew is a source of carbohydrate, protein is provided by hunting and scavenging for invertebrate prey. Red wood ants are the dominant invertebrate predators in woodland ecosystems in much of northern Eurasia (Savolainen & Vepsäläinen 1988). They hunt and scavenge for variety of invertebrate prey, including other ant species (Mabelis 1984; Cherix 1987; Robinson et al. 2008). In many ants, protein is important for the growth and development of brood (Hölldobler & Wilson 1990), and therefore of vital importance for the colonies long-term fitness. It may be the non-foraging nests, defined as nests not foraging for honeydew in trees, are actually important as scavengers and hunters of other invertebrate prey.

(3) Non-foraging nests could act as *specialised brood development chambers*. In insects, brood temperature is closely linked to development speed, and therefore fitness (Huey & Berrigan 2001). Although large wood ant nests can control the their internal temperature to a certain extent by various behavioural and metabolic mechanisms, external temperature remains important, especially in smaller nests (Rosengren et al. 1987; Chen & Robinson 2014). It may be that non-foraging nests are in areas with different temperature regimes than foraging nests and can therefore act as specialised brood development chambers for other nests in the colony.

(4) Apparently non-foraging nests could appear as a result of *seasonal foraging behaviours*. Seasonality is commonly observed in many aspects of ant behaviour (e.g. Heller & Gordon 2006; Stroeymeyt et al. 2014), including wood ant foraging (Sudd & Sudd 1985). Wood ants live in northern temperate latitudes and therefore show strong seasonality in their behaviour: they are quiescent during the winter and begin foraging in late-spring and through the summer (Rosengren & Sundström 1987). Aphids may bloom in different species of trees at different times of year (Sudd 1983), and it may therefore be beneficial for a colony to have nests near to trees which will be good for foraging at a different times of year. Nests which appear to be non-foraging at a particular time could, therefore, simply be foraging at other times of year.

(5) Finally, non-foraging nests could be simply be *parasites* on the foraging effort of the rest of the colony. There are many examples of cheating and selfish strategies within colonies of social insects (Bourke & Franks 1995). In a multi-nest colony there are likely to be many more opportunities for selfish strategies to evolve due to the colony-members being spatially dispersed (Helanterä 2009; Ellis & Robinson 2014). It may be that non-foraging nests are an example of a cheating strategy; taking food and resources from the rest of the colony to raise a distinct lineage of queens and workers. A parasitic strategy would mean that non-foraging nests are not providing a direct fitness benefit to the rest of the colony.

This study aims to differentiate between these hypothesised roles of non-foraging nests in polydomous wood ant colonies. Several predictions can be made based on each hypothesis (table 5.1) and we collected three empirical datasets to test these predictions: network remapping, trail observations and extra-nest activity counts.

5.2 Methods

Study Species and Site

Formica lugubris is a member of the ecologically important red wood ant (*F. rufa* group) species group. The red wood ant group consist of at least six closely related and ecologically similar species (Bernasconi et al. 2011; Goropashnaya et al. 2012). The red wood ants are characterised by their large nests constructed of pine needles and leaf litter over subterranean chambers; these nests can be large and contain over a million individuals (Ellis et al. 2014; Chen & Robinson 2014). Members of the group show diversity in nesting strategy both within and between species. This diversity in nesting strategy is associated with a matching flexibility in number of queens in each nest. Polydomous colonies are polygynous (multiple queens per nest) whereas monodomous colonies usually have only one queen per nest (Ellis & Robinson 2014).

This study took place on a large population (over 900 nests in 0.95 ha^{-1}) of *F. lugubris* at the Longshaw Estate, Peak District, England (53º 18.55 N, -1º 36.16 W); this is the same population as studied by Ellis et al. (2014). *Formica lugubris* is the only member of the *F. rufa* group at this site. The site is a mixture of historic scots pine plantations, deciduous and mixed woodlands and open grassland areas.

To assess the role of non-foraging nests in polydomous wood ant colonies it is first necessary to define a 'non-foraging nest'. We define a non-foraging nest as: a nest which is part of a polydomous colony, but has no foraging trails leading to trees (e.g. figure 5.1). We follow Ellis et al. (2014) and define a trail as a route between two points with more than 10 ants within 40 cm. This gives us a functional definition of a polydomous colony based on resource exchange along the trails between nests, rather than based on aggression or relatedness between nests. Preliminary surveys of the site in early-summer 2012 and early summer 2013 identified a range of polydomous colonies with non-foraging nests which could be used for the investigations.

We tested the predictions of the hypothesised roles of non-foraging nests by collecting three empirical datasets from: (1) mapping and remapping of colonies, (2) trail observations and (3) assessing the level of extra-nest activity (table 5.1). These three datasets are described in detail below.

Hypothesis	Predictions		Relevant dataset	
Non-foraging nests as part of the colony expansion process *Non-foraging nests are newly founded nests which will begin foraging in the future or be abandoned.*	i)	Newly founded non-foraging nests are more likely to be abandoned than newly founded foraging nests.	i)	Mapping and Remapping of polydomous colonies.
	ii)	Non-foraging nests will change to become foraging nests more often than the reverse.	ii)	Mapping and Remapping of polydomous colonies.
	iii)	Non-foraging nests which change to become foraging nests are less likely to be abandoned than those which remain as foraging nests.	iii)	Mapping and Remapping of polydomous colonies.
Non-foraging nests as invertebrate hunting and scavenging specialists *Non-foraging nests are specialised to hunt and scavenge for invertebrate prey and providing this prey to the rest of the colony*	i)	Non-foraging nests will have greater non-trail extra-nest activity than foraging nests.	i)	Extra-nest activity
	ii)	More prey will be carried along internest trails away from non-foraging nests than towards non-foraging nests.	ii)	Trail Observation
	iii)	There will be more prey bearing journeys on internest trails between non-foraging nests to foraging nests than between pairs of foraging nests.	iii)	Trail Observation
Non-foraging nests as specialist brood development chambers *Non-foraging nests are positioned to have a better temperature for faster brood development.*	i)	Non-foraging nests will have lower canopy cover (and therefore higher insolation) than foraging nests.	i)	Mapping and Remapping of polydomous colonies.
	ii)	Brood will be carried in a preferentially in one direction along internest trails between foraging and non-foraging nests.	ii)	Trail Observation
	iii)	There will be more brood carrying journeys on internest trails between non-foraging nests to foraging nests than between pairs of foraging nests.	iii)	Trail Observation
Non-foraging nests as seasonal foragers *Non-foraging nests are foraging nests at different time of year. Due to differing nutritional requirements at different times of year, or due to aphid blooms occurring in different tree species at different times of year.*	i)	Non-foraging nests will consistently change between being foraging and non-foraging nests at different times of year.	i)	Mapping and Remapping of polydomous colonies.
	ii)	The species of tree closest to non-foraging nests will be a different to the species of tree to the closest to foraging nests.	ii)	Mapping and Remapping of polydomous colonies
Non-foraging nests as colony parasites *Non-foraging nests are acting as parasites on the foraging effort of the rest of the colony.*	i)	There will be very little brood exchange between non-foraging nests and the rest of the colony.	i)	Trail Observation
	ii)	There will be very little worker exchange between non-foraging nests and the rest of the colony.	ii)	Trail Observation

Table 5.1 Hypothesised roles of non-foraging nests within polydomous *F. lugubris* colonies. The predictions, and datasets used to test these predications, resulting from the hypothesis (described in italics) are also listed.

Dataset 1: Mapping and Remapping colonies

To assess the foundation and survival of nests in polydomous colonies we remapped the same colonies five times over three years. The thirteen largest polydomous colonies from the early-summer 2012 preliminary survey were selected for use in this investigation. These thirteen colonies were then mapped in detail (discussed below) in August 2012, and then remapped in May and late-August 2013, and then again in May and late-August 2014. This gives five time-points for the 13 colonies, three from late-August (hereafter late-summer) and two in May (hereafter spring). The precise timing of the spring remapping depended on when weather became warm enough for the foraging and internest trails to become active.

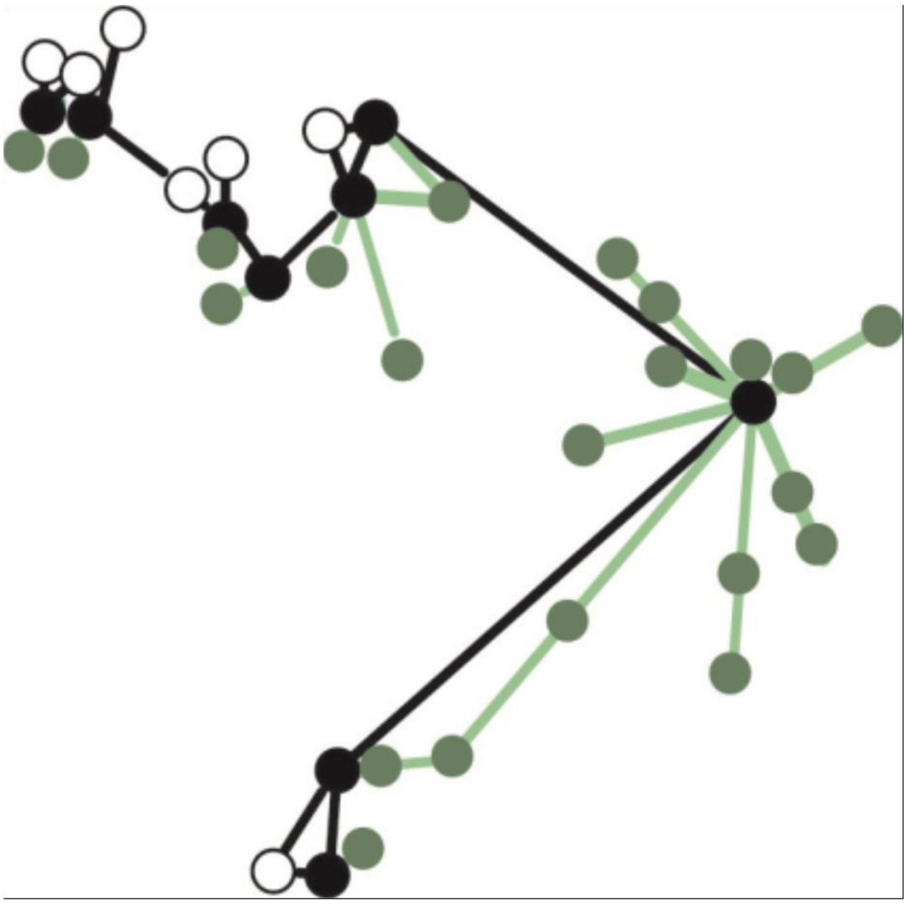


Figure 5.1 A polydomous *Formica lugubris* colony. Black circles represent foraging nests; open circles show non-foraging nests. Green circles are trees. Black lines are internest trails and green lines are foraging trails. Any nest without foraging trails leading to a tree is defined as non-foraging (appendix A: colony I).

In this study, we have defined a polydomous colony as two or more nests connected by trails of ants travelling between them. The trails are above ground and, usually, easy to observe. Similar trails are formed from the nests to the aphid colonies in the trees. During mapping, we recorded the layout of these nests and the trails between them (figure 5.1). In addition, we assessed both the populations of the nests in the network and the canopy cover over the nests. Nest population (hereafter nest size) was assessed based on the nest-mound volume, calibrated with a mark-release recapture method (Chen & Robinson 2013; Ellis et al. 2014). We estimated canopy cover from digital photos taken vertically 30cm above the highest point of the nest. We then used image analysis software (Image J: Rasband 2012) to calculate the proportion of the area above the nest shaded by the canopy (Ellis et al. 2014).

We used these maps to test the predictions associated with the hypothesised roles of non-foraging nests (table 5.1). For example, the foundation of new nests can be inferred by comparing a colony to its previous time-point and observing which nests have appeared. Similarly, nest abandonment can be inferred by examining the colony map from the next time-point and assessing the presence or absence of the nest. As the maps also include foraging trails the foraging or non-foraging status of a nest can be determined by the presence or absence of foraging trails at a particular time-point. This information can also be used to infer changes in foraging status by comparing the same nest at different time-points. We also used the maps to assess the canopy cover over particular nests, and the linear distance (rather than trail distance) to the nearest tree.

Dataset 2: Trail Observation

To understand the role of non-foraging nests it is important to know what resources are being exchanged between non-foraging nests and other nests in the polydomous network. We use ‘resources’ to refer to items being carried by workers between the nests; the resources being carried over the course of the observation periods could be categorised as either: prey, vegetation (nesting material), workers, pupae, larvae, empty pupal casings and queens. It is important to note that this list does not include honeydew, which is transported within the crop of the workers (i.e. internally and therefore not carried); the transport of honeydew between nests is discussed in more detail in Chapter 4. As our definition of non-foraging nests presupposes workers collecting honeydew at these nests, the movement of honeydew was not a useful measure for examining the role of the non-foraging nests.

These observations were conducted in July and August 2013. Eight colonies containing both foraging and non-foraging nests were randomly selected from the colonies surveyed in early-summer 2013. Before the beginning of observations the colonies were mapped in detail (see above). One trail between a non-foraging nest and a foraging nest, and one trail between two foraging nests, were randomly selected per colony.

Observations took place approximately mid-way between the two nests. At the mid-point we designated a 5cm section of trail as the observation window; if an ant carrying a resource traversed the length of this observation window it was considered to be travelling in that direction. Each trail was observed for 30 minutes on 3 consecutive days. The two trails per colony were observed in immediate succession in a random order. All observations were done between 10:00 and 17:00 in warm dry weather.

Along a trail between a non-foraging nest and a foraging nest, resources could either be carried towards the non-foraging nest or away from the non-foraging nest. We could then compare the resources being carried towards and away from non-foraging nests. We could also compare the resources being carried between non-foraging nests and foraging nests to those being transported between two foraging nests.

We compared the resources being transported in each direction along trails between non-foraging and foraging nests. On these trails, resources can either be carried towards the non-foraging nest, or away from the non-foraging nest. We compared the resources being carried along trails between non-foraging and foraging nests to those being carried between two foraging nests.

Dataset 3: Extra-nest activity

The aim of this investigation was to study whether there is a difference in extra-nest activity in the area around non-foraging nests compared to the area around foraging nests. The investigation was conducted at the same time, and used the same eight colonies, as the trail observations (dataset 2, above).

We used counts of activity in a defined area surrounding the nest to assess the extra-nest activity. This method takes into account both the activity-level of extra-nest workers and number of extra-nest workers. Similar methods have been used previously to assess abundance and activity of ants in the area surrounding nests (Hoffmann 2014). We used 15x15 cm squares of cardboard (hereafter: quadrants) placed 40cm from the edge of the focal nest at a randomly selected cardinal direction, to assess the activity in the area surrounding the nests. The quadrants were always placed 15cm or further from internest and foraging trails. Preliminary observation had shown that these distances were far enough away to avoid the confounding effects of ants joining, leaving and straying from trail and nests. A second quadrant was placed on the opposite side (180°) of the nest from the first. Quadrants were placed at least 60 minutes before the beginning of the observation to allow the extra-nest workers to acclimatise to them. Each quadrant was observed continuously for 15 minutes and the number of ants passing across the quadrant was recorded. Observations were repeated on three consecutive days. On each day the first quadrant for observation was chosen randomly, and subsequent observations alternated between the two nests being observed in the colony. For analysis the activity in both quadrants were summed and then divided by the worker population of the nest to give a metric for extra-nest activity, given the size of the nest.

Temperature is an important determinant of activity in ants. To be able to take account of temperature in our analysis we used a digital thermometer placed 50cm from the nest at the beginning of each observation to take the local temperature accurately, on a short time scale. Another important determinant of extra-nest activity is the number of ants present in the nest. To assess the nest population we used volume of the nests calibrated with a mark-release recapture method based on nest disturbance (Chen & Robinson 2013; Ellis et al. 2014).

Statistical analysis

Statistical analysis of this data was undertaken using Generalised Linear Mixed Effect Models (GLMMs). Using GLMMs allowed us to account for the nested nature of the system (repeated days and repeated colonies). GLMMs associated with dataset 1 used colony, year and, when appropriate, nest ID as random effects. GLMMs associated with datasets 2 and 3 used colony and day as nested random effects. Additionally, for dataset 3, temperature was also included as a random effect. The fixed effects(s) and response variable were chosen based on the question being asked. Further details of all reported tests are found in appendix G; the superscript number by each reported test refers to the row of the table. All results are the based on an Analysis of Deviance (AoD) between the GLMM in question and a null model based on the same variables but without the fixed effect; using this method allows a quantative assessment of the significance of a particular variable in explaining the modelled data. All analysis was performed in R (R Development Core Team 2011) using the ‘lmer’ and ‘languageR’ packages.

5.3 Results and Discussion

We used the three datasets described above to assess the extent to which non-foraging nests in polydomous *Formica lugubris* colonies are: (1) part of the colony expansion process, (2) arthropod hunting and scavenging specialists, (3) specialist brood development chambers, (4) seasonal foragers or (5) parasites on the foraging effort of the rest of the colony. These hypotheses are not mutually exclusive, as the non-foraging nests in polydomous colonies do not necessarily all have the same role.

(1) Non-foraging nests as part of a colony expansion process

a) Non-foraging nests as part of the colony expansion process

Predictions

Non-foraging nests could be part of the process of colony expansion in polydomous wood ants, acting as an intermediate phase between nest foundation and the beginning of foraging. The mechanism of colony expansion may be based on established nests budding new nests, some of which happen to be non-foraging. Those which do happen to be non-foraging are retained if they begin foraging and abandoned if they do not. Under this hypothesis rather than non-foraging nests being non-foraging *per se* they have simply not begun foraging.

The hypothesis that new nests are part of the process of colony expansion leads to several predictions (table 5.1). Firstly, we predict that newly founded non-foraging nests are more likely to be abandoned than newly founded foraging nests. Secondly, we expect that non-foraging nests will become foraging nests more often than foraging nests stop foraging and become non-foraging nests. We predict this asymmetrical change because, under the hypothesis that non-foraging nests are an intermediate phase between nest foundation and the beginning of foraging, non-foraging nests at a particular time-point will become foraging nests in the future, if they survive. Thirdly, we predict that non-foraging nests that do change to become foraging nests are less likely to be abandoned than non-foraging nests that have remained as non-foraging nests. This is expected because those which have changed are now fulfilling a role as foraging nests, and are therefore more likely to be retained.

Results

Over the course of the three years of observation we detected the foundation of 91 new nests. Of these newly founded nests 55 were foraging and 36 were non-foraging. Newly founded non-foraging nests were significantly more likely to be abandoned than newly founded foraging nests (non-foraging: 60% vs. foraging: 36%; AoD$^{5.1}$, χ^2=5.63, *df*=1, p=0.01; figure 5.2). In general, larger nests are significantly more likely to survive than smaller nests (AoD$^{5.2}$, χ^2=56.1, *df*=1, p<0.001). However, when size is included in the model, foraging status is still a significant determinant of the survival of newly founded nests (AoD$^{5.3}$, χ^2=5.64, *df*=1, p=0.03), whereas size is not (AoD$^{5.4}$, χ^2=0.01, *df*=1, p=0.34).

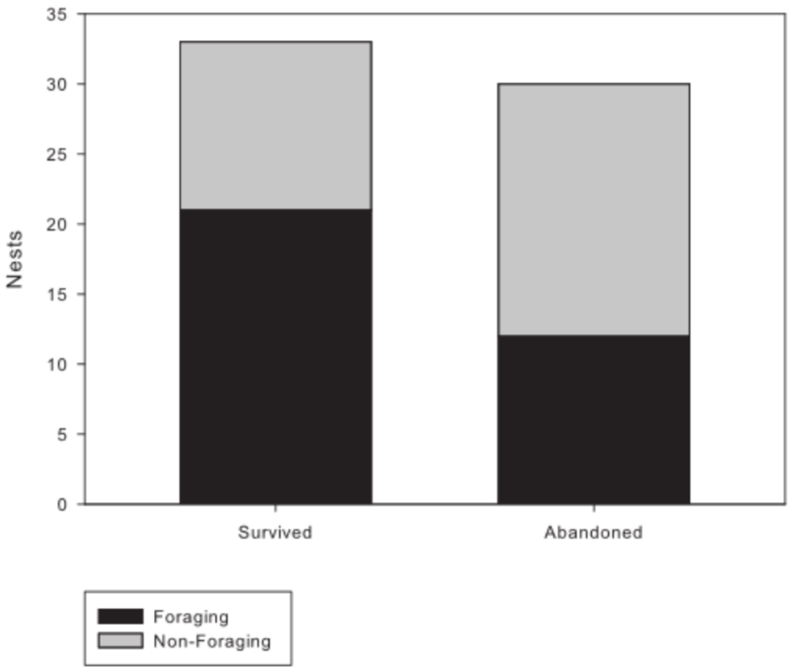


Figure 5.2 Comparing the survival of newly founded foraging nests and the survival of newly founded non-foraging nests. Significantly more newly-founded non-foraging nests are abandoned before the next observation than newly founded foraging nests (AoD[5.2], χ^1=5.63, *df*=1, p=0.01). As survival can only be ascertained at the next time-point only data up until spring 2014 is included in this analysis.

Of the non-foraging nests which survive between two time-points (e.g. between summer 2012 and spring 2013, or between spring and summer 2014) 36% (36/100) become foraging nests. In contrast, only 14% (32/256) of foraging nests change to become non-foraging nests. There are significantly more changes from non-foraging to foraging than from foraging to non-foraging (AoD[5.5], χ^2=13.7, *df*=1, p<0.001). The non-foraging nests which change to become foraging nests are significantly closer to trees, relative to other nests in the colony, than newly founded nests which remain as non-foraging nests (AoD[5.6], χ^2=4.21, *df*=1, p=0.04). Of the non-foraging nests that survive between two time-points, those which change to become foraging nests are significantly less likely to be abandoned by the subsequent time-point than those which remain as non-foraging nests (AoD[5.7], χ^2=9.5, *df*=1, p=0.002; figure 5.3).

Discussion

Examination of the changes in the polydomous colonies over time shows that non-foraging nests may simply appear as part of the process of colony expansion, rather than having a specific role. Overall, as predicted under the colony expansion hypothesis, newly founded non-foraging nests are more likely to be abandoned than newly founded foraging nests. In addition, non-foraging nests are more likely to change foraging role than foraging nests. Those non-foraging nests which do change foraging role to become foraging nests are both closer to trees and more likely to survive than those which remain as non-foraging nests. This supports the hypothesis that non-foraging nests are part of the process of colony expansion in *F. lugubris* because it suggests a mechanism by which polydomous colonies expand: new nests are founded some of which happen to be non-foraging, of these non-foraging nests those which are near to trees become foraging nests in time and those which are further away from trees do not. Those non-foraging nests which have begun foraging are retained whereas those that have not are abandoned. This will result in the observed pattern of a large number of non-foraging nests founded, but only a few that are retained. This process of colony expansion resembles the pruning-based growth patterns found in a variety of biological systems, where a system expands rapidly and then parts in unproductive areas are progressively abandoned (e.g. Nakagaki et al. 2004; Perna et al. 2008; Udan et al. 2013).

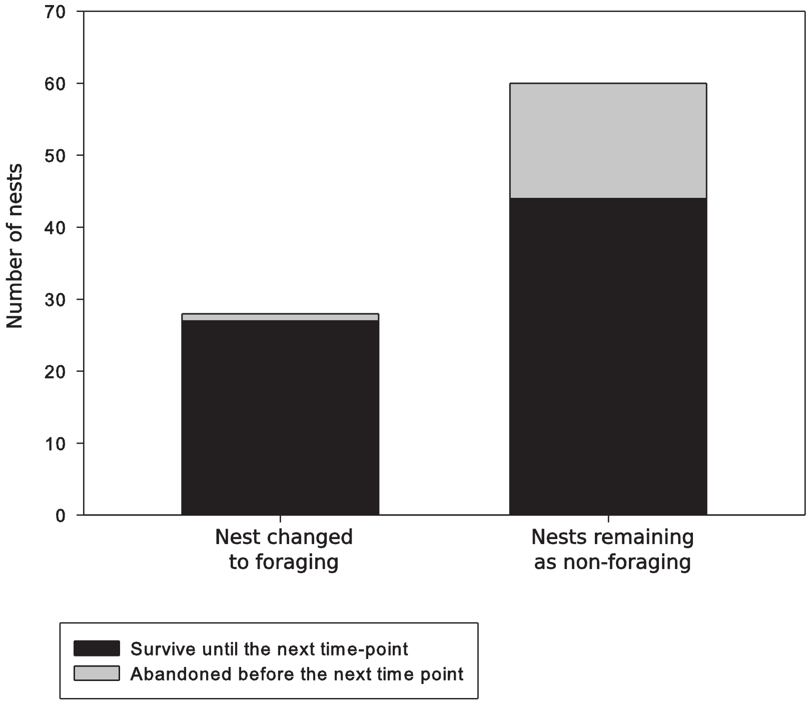


Figure 5.3 Comparing the survival of nests which change from non-foraging to foraging to those which remain as non-foraging nests. Nests which change role are significantly more likely to survive than those that do not (AoD$^{5.7}$, χ^2=9.5, *df*=1, p=0.002).

b) The mechanism of nest foundation in polydomous wood ant colonies

Predictions

A mechanism of colony expansion based on nests being founded and then abandoned if they are not profitable could function in one of two ways. New nests could be founded in random locations in the area surrounding the colony (random foundation). Conversely, nests could be preferentially founded in areas which may be profitable to the colony, for example close to existing food sources (directed foundation). Both of these mechanisms would result in a similar pattern of non-foraging nests being founded and then either changing to become foraging nests, or being abandoned. Even under the ‘directed’ mechanism just because a nest is founded in an area close to a food sources does not necessarily mean that it will be profitable.

Under a directed process of nest foundation a location that is very likely to be profitable to found a new nest is on a foraging trail. Founding on a foraging trail will allow the new nest easy access to an already exploited food source. Foundation on existing trails has been previously suggested as a mechanism of nest foundation for wood ant colonies (e.g. Mabelis 1979). Even if nests are not founded directly on foraging trails it would be beneficial for them to be founded nearer to trees than their natal nest. We therefore expect that, under directed nest foundation, new nests will be founded closer to trees than the existing nests in the colony. In contrast, nests founded at random are expected to be founded, on average, at the same distance from foraging trees as other nests in the colony.

Results

We observed the foundation of 91 nests over the course of the three years of observation. Of these, 16 (17.6%) were founded on foraging trails and 19 (20.8%) were founded on internest trails. In total, therefore, 60% of nests were founded in new locations, compared to 40% on existing trails. The newly-founded nests are not significantly closer to foraging trees than other nests in the colony (newly founded 8.48 ± 1.22 m vs. not newly founded 5.95 ± 0.3 m; AoD$^{5.8}$, χ^2=0.19, df=1, p=0.66).

Discussion

New nests to do not appear to be founded preferentially in areas that may be beneficial for foraging, rather they appear to be founded in random locations with respect to food sources. This may help explain why the proportion of non-foraging nests in many colonies is so high. Rather than being founded close to food sources, and therefore standing a high chance of beginning to forage, new nests appear to be founded in random locations (with respect to resources), which is likely to lead to a high proportion of nests which are founded far from food sources, and are therefore non-foraging.

(2) Non-foraging nests as hunting and scavenging specialists

Predictions

Red wood ants rely on two major sources of nutriment: honeydew from aphids, and protein from hunted and scavenged prey. Although our definition of non-foraging, based on trails to trees, precludes substantial honeydew gathering by these nests, the definition does not preclude non-foraging nests hunting and scavenging for prey. It may be that the non-foraging nests are protein sources to the rest of the colony, while relying on the foraging nests for honeydew.

We predict that, under the hypothesis that non-foraging nests are hunting and scavenging specialists, non-foraging nests will show greater extra-nest activity than expected for their size, as they have more foraging effort invested in hunting and scavenging the area around the nest. If non-foraging nests are providing protein to the rest of the colony it is expected that the net movement of transported prey will be away from the non-foraging nest on trails between a non-foraging nests and a foraging nest. Similarly, we predict that, if non-foraging nests are involved in hunting and scavenging, the amount of prey being carried along trails between non-foraging and foraging nests will be higher than on trails between pairs of foraging nests.

Results

Non-foraging nests have significantly higher extra-nest activity than foraging nests (AoD$^{5.9}$, χ^2=19.2, *df*=1, $p<0.001$). However, this relationship between extra-nest activity and foraging status is significantly different between colonies (AoD$^{5.10}$, χ^2=54.7, *df*=14, $p<0.001$), suggesting that this difference in extra-nest activity is not a consistent effect.

There is no significant difference in the direction which prey is carried along trails between non-foraging and foraging nests (AoD$^{5.11}$, χ^2=0.04, *df*=1, p=0.84). Similarly, and contrary to prediction of the prey-specialist hypothesis, a significantly higher proportion of ants travelling between foraging nests are carrying prey than those travelling between non-foraging nests and foraging nests (AoD$^{5.12}$, χ^2=128, *df*=15, $p<0.001$).

Discussion

Non-foraging nests do not appear to act as sources of hunted and scavenged prey to the rest of the colony. There is higher extra-nest activity in the area surrounding non-foraging nests.

However, the low proportion, and lack of consistent direction, of prey bearing journeys on trails between non-foraging nests and foraging nests suggests that non-foraging nests are not hunting and scavenging specialists for the colony.

Our results do not rule out non-foraging nests performing a disproportionate amount of hunting and scavenging but, if they, they do not appear to supply this excess to the rest of the colony. The higher levels of extra-nest activity around non-foraging nests does not necessarily suggest higher scavenging and hunting effort, it could also be due to searching for other resources such as nest material, or a be a defensive measure. It should also be noted, that our definition of foraging nest based on honeydew collection does not preclude foraging nests from also collecting prey. Indeed, several studies have found that a large proportion of the protein intake of wood ant colonies is provided by hunting and scavenging in the canopy, including on the aphids themselves (Cherix 1980; Cherix 1987; Rosengren & Sundström 1991; Robinson et al. 2008). In this study we found that more prey is carried along trails between two foraging nests. This could be due to the majority of prey being collected in the canopy.

Overall, it appears unlikely that non-foraging nests are acting as hunting and scavenging specialists, and providing protein to the rest of the colony. There does not appear to be any nest-level division of labour with respect to collection of protein.

(3) Non-foraging nests as brood development specialists

Predictions

Due to the important link between temperature and brood development speed in insects it may be beneficial for polydomous *F. lugubris* colonies to place non-foraging ‘brood development’ nests in areas of favourable temperatures (found in seasonally polydomous *Myrmica punctiventris*; Banschbach et al. 1997). Therefore, under the hypothesis that non-foraging nests are brood development specialists, we predict that non-foraging nests will be in areas with different insolation than foraging nests. In addition, if non-foraging nests are involved in brood rearing we predict greater brood-carrying activity on trails between non-foraging nests and foraging nests. We also predict that the movement of brood along trails between non-foraging and foraging nests will be directional. Depending on the precise brood development role non-foraging nests are fulfilling, this direction could be either towards or away from non-foraging nests. For example, it may be that, due to differing temperature requirements, brood are taken to the non-foraging nest as pupae and taken back to foraging nests as larvae, or *vice versa*. Brood could be also be moved to non-foraging nests in response to some weather conditions, but away from non-foraging nests in other weather conditions. In all these cases, for a specific trail on a specific day, the movement of brood is predicted to be directional. For social insects the main consumers of protein are brood (e.g. Lange 1967). Non-foraging nests acting as brood development specialists might, therefore, be expected to receive a disproportionately higher amount of protein prey than expected for their size, and/or have higher extra-nest activity than foraging nests (table 5.1).

Results

We used canopy cover as a proxy for insolation (see methods). We found no significant difference in canopy cover over non-foraging nests compared to foraging nests (AoD$^{5.13}$, χ^2=0.9, *df*=14, p=0.34).   We found no difference in the direction of brood movement along trails between non-foraging and foraging nests (AoD$^{5.14}$, χ^2=0.09, *df*=1, p=0.79). There is also significantly less movement of brood on trails between non-foraging and foraging nests than between pairs of foraging nests (AoD$^{5.15}$, χ^2=372, *df*=15, p<0.001). If larvae are considered separately there is still no significant difference in direction of movement along trails between non-foraging and foraging nests (AoD$^{5.16}$, χ^2=1.81, *df*=1, p=0.178). There were not enough pupa carrying journeys observed to test separately.

Discussion

There is no evidence to suggest that non-foraging nests are used to speed the development of brood, they are neither found in more insolated areas, nor do they have more brood related activity associated with them, than foraging nests.

We found that there is no difference in canopy cover over non-foraging compared to foraging nests. This lack of difference may mean that non-foraging nests would not provide a different temperature regime to foraging nests and therefore not be useful as brood development chambers. It is, however, important to note that this result disagrees with other studies from the same site, both of which found significantly higher canopy cover over foraging nests than non-foraging nests (Ellis et al. 2014; Chen & Robinson 2014). Nest size is another confounding factor when investigating the potential advantages of non-foraging nests as brood development chambers: larger nests tend to be found in areas of higher canopy cover, and are likely to be better at metabolic heat production, than smaller nests (Chen & Robinson 2014). More studies, in different environments, may be necessary to find the complete relationship between foraging status and canopy cover. It is clear that the relationship is not consistent either within or between colonies, and there is some overlap between the canopy covers of non-foraging and foraging nests.

However, even if the non-foraging nests were in areas of different canopy cover it would not necessarily imply that they were involved in brood development. The absence of increased brood exchange along trails between non-foraging nests and foraging nests compared to those between pairs of foraging nests, and the absence of directional movement of brood along trails between non-foraging and foraging nests, strongly suggests that non-foraging nests are not being used as specialised brood development chambers, at least not on the short timescale examined in the study. The absence of greater-than-expected protein collection behaviours also suggest that non-foraging nests do not contain higher amounts of brood than foraging nests.

The fact that non-foraging nests are not brood development specialists may be unsurprising. Moving brood between nests is likely to be risky due to factors such as the risk of desiccation, predation or being damaged during the journey. Given the importance of brood to ant colonies, the risks to brood during transportation may mean that even if there were a marginally faster development time in another nest, the risks may still be too high to make mass brood transportation a beneficial strategy. Similarly, as non-foraging nests are smaller, and more likely to be abandoned, than foraging nests it may be better to maintain the brood in the larger, safer nests.

(4) Non-foraging nests as seasonal foragers

Predictions

Nests which appear to be non-foraging could simply be foraging at other times of the year: we used repeated remapping of the same colonies over the course of three years to assess if this is the case. The remapping of the colonies occurred yearly in late-spring and late-summer; this should cover the diversity in both the red wood ant and aphid life-cycles. For the red wood ants, these time-points are at the beginning of the foraging season and peak of the foraging season respectively (Rosengren & Sundström 1987). In addition, in spring the colony produces sexual offspring, whereas in late-summer they are producing exclusively worker brood (Pamilo & Rosengren 1983). We therefore expected any seasonal foraging effects to be observable in these two, very different, periods in the annual colony life-cycle. Different species of aphid are often present on different tree species. Aphids have complex life-cycles which can result in rapid population increases, blooms, at certain points in the annual cycle. These blooms may occur at different points in the life-cycle of different aphid species on different trees. Under the hypothesis that non-foraging nests forage to different tree species to take advantage of seasonal aphid blooms we predict that non-foraging nests will be closer to different species of tree than the species of tree closest to foraging nests.

Results

Of the 66 nests present for all three years of the study only two consistently changed role between spring and summer. One switched from non-foraging in spring to foraging in late-summer (and foraged to Scots pine, *Pinus sylvestris*), whereas the other switched from foraging in spring to non-foraging in late-summer (and foraged to larch, *Larix spp.*). The nearest tree to a particular nest can be inferred from the colony maps. The species of these nearest trees can then be assessed. The species of tree nearest to non-foraging nests are not significantly different from the species of tree nearest to foraging nests (AoD[5.17] χ^2=4.8, *df*=4, p=0.31).

Discussion

There are very few nests which show a seasonal switch between foraging and non-foraging. The low numbers of seasonally foraging nests suggests that seasonal foraging is not an important role of non-foraging nests in polydomous wood ant colonies. The lack of relationship between the species of the nearest tree and foraging status of the nests suggests that nests are unlikely to be specialising on aphids blooming on different species of tree at different times of year. Though, it is important to note we only had two time-points over the course of an entire foraging season, we could be missing more rapid changes in food availability on different trees. Previous studies have shown that red wood ants show a high degree of route and site fidelity when foraging, even to the extent of following the same routes after the winter quiescence (Rosengren 1971; Gordon et al. 1992). This consistency to a particular route may suggest that the same foraging trees are providing honeydew for long-periods of time. If there is little seasonal variation in food availability in different trees at this site it may explain why there seems to be little seasonal variation in where wood ant colonies forage.

(5) Non-foraging nests as a parasitic strategy

Predictions

Rather than having a specific role, non-foraging nests may simply be parasites on the foraging effort of the rest of the colony. For this parasitic strategy to be maintained, the non-foraging nests must have a high degree of reproductive isolation from the other nests of the colony, allowing a distinct lineage of queens and workers to develop. This distinct lineage of queens and workers could then increase their fitness at the expense of the rest of the colony. For a nest to have a distinct lineage of queens and workers there would need to be little brood exchange between non-foraging nests and the rest of the colony. Similarly, as carried workers may not be able to navigate back to their home nest (e.g. Pratt 2005), we predict that a parasitic non-foraging nest would exchange few workers with the rest of the colony.

Results

There is significantly less movement of brood along trails between non-foraging and foraging nests than between pairs of foraging nests ($\text{AoD}^{5.14}$, χ^2=0.09, *df*=1, p=0.79). However, there is still some movement of brood; on average 7.6 ± 3.1 brood items are transported within a 30 minute observation.

Workers were the most commonly observed item being carried between nests (figure 5.4). Along trails between a non-foraging and a foraging nest there were mean of 34 ± 8.6 workers carried in a 30 minute observation.

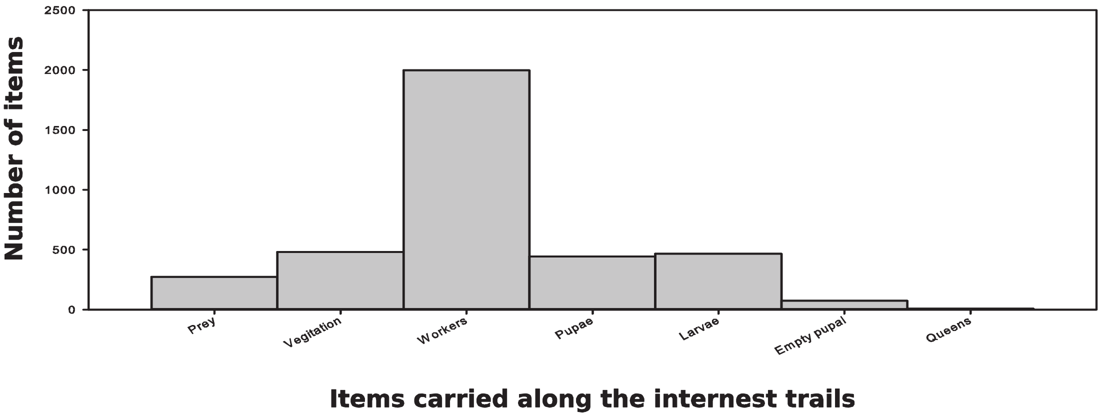


Figure 5.4 Items being carried between nests (dataset 2). The figure shows the total number of items, summed across all 10 trials (including trails between two foraging nests, trails between two non-foraging nests and trails between an non-foragin nest and a foraging nest).

Discussion

The impact of brood and worker movement between nests in polydomous *F. lugubris* colonies is difficult to assess. However the fact that there is any exchange at all indicates that the non-foraging nests are not completely reproductively isolated. Due to their high levels of polygyny, relatedness within polydomous red wood ant nests and colonies is general quite low (Sundström et al. 2005). This does not preclude more complex social and genetic structure within this generally low-relatedness social environment (e.g. Bargum & Sundström 2007; Schultner et al. 2013). The existence of brood and worker exchange between non-foraging nests and the rest of the colony suggests that if the non-foraging nests do have a distinct genetic lineage it is not a case of simple parasitism. This does not preclude non-foraging nests being part of a more complex intra-colony competition system, for example worker exchange could be a form of slave-making, but the relationship with the rest of the colony does not appear to be that of simple parasites stealing resources. To understand the impact of the observed movement of brood and workers, and to understand the effect this has on intra-colony and intra-nest relatedness, it will be necessary to collect detailed genetic information over a long timescale.

5.4 General Discussion

In this study we found that non-foraging nests in polydomous *Formica lugubris* colonies appear to be part of the process of colony expansion. We found little evidence to suggest a specialised role, such as hunting for arthropod prey or brood development, for non-foraging nests. The process of colony expansion in polydomous wood ant colonies appears to be based on new nests being founded in random (with respect to food) locations. Of these newly founded nests, those which begin foraging are more likely to be retained whereas those which do not are abandoned. This is a colony expansion strategy which is not available to monodomous colonies. For a monodomous colony, nest foundation must either be successful or the colony fails, whereas for a polydomous colony nest foundation can fail without long term fitness consequences for the colony.

We have found that, despite not appearing to be directly involved with foraging, non-foraging nests can act as an intermediate stage before a nest begins foraging. This highlights the importance of the spatially and temporally stable resource of honeydew to red wood ants: even nests which are not foraging to honeydew are part of a mechanism to more efficiently exploit honeydew sources in the environment. Our results also illustrate a possible link between foraging to honeydew and polydomous nesting in the red wood ants. An important benefit of polydomy for red wood ants may be to more efficiently exploit stable food sources in the environment (Ellis & Robinson 2014). Founding new nests as non-foraging nests may allow colonies to discover new food sources, or to more efficiently exploit already known food sources; by, for example, allowing multiple nests to be involved in the recruitment of workers to the resource, or by reducing the costs associated with long foraging trails (Robinson 2014). As this method of exploring the environment is only available to polydomous nests it may provide an important benefit of the polydomous nesting strategy.

The role of non-foraging nests as part of the colony expansion process also suggests an interesting dynamic within polydomous colonies between nest-level co-operation and nest-level selection. On one hand, non-foraging nests are founded and then, in effect, supported by the rest of the colony, providing very little benefit in return until they begin foraging: an example of nest-level co-operation. On the other hand, non-foraging nests which do not begin foraging are regularly abandoned, a strong form of nest-level selection. The dynamic between these two effects may be very important in determining a colony's foraging success and the extent to which colonies' expand. Both of these are likely to have important consequences for the long-term fitness of the colony.

It is also interesting that non-foraging nests do not appear to have a specialised role within the colony. Rather than having a specialised role and providing a benefit to the entire colony, we found that non-foraging nests actually survive, in the long term, based on their own ability to acquire food (by beginning foraging). This agrees with other studies of polydomous red wood ants which have suggested a lack colony-level organisation (chapter 3) and a simple worker behaviours facilitating honeydew redistribution between nests of the polydomous colony (chapter 4). Rather than a polydomous colony acting as cohesive whole, with shared survival and fitness prospects, the individual nests may survive based on their own ability to acquire resources, with little reference to the rest of the colony. Part of that acquisition of resources involves taking food from other nests in the colony (chapter 4) which is clearly a form of passive support so the nests are not entirely independent. However, nests within the polydomous colony appear to offer little active support to other nests.

In this study we have found that, rather than having a specific role, non-foraging nests in polydomous wood ant colonies are part of the process of colony growth by nest foundation. This has interesting implications for the evolution and maintenance of the polydomous nesting strategy in red wood ants.

Chapter 6: Survival, reproduction and growth: how position in a dynamic network impacts life-history

Abstract

Life history is shaped by both ecological and social environments. The social environment is known to be important to an animal's fitness. However, it is less well understood how an individual's position within the social environment relates its life-history and survival. Social environment can be represented as a network, using patterns of interactions to characterise social positions. The challenge when investigating life-history traits is that many are dynamic: survival, reproduction and growth, for example, are inherently time-dependent. Dynamic networks, where both the social structure and the individuals change over time, are therefore, the only context in which the association between life-history and social position can be fully assessed. Here we show, using a novel approach combining survival models with dynamic network analysis, that the social positions of nodes can affect their life-history. We use multi-nest colonies of the wood ant Formica lugubris as a model system to investigate the relationship between social position and life-history in a dynamic system. Multi-nest ant colonies inhabit a network of nests connected by trails; the social position of a nest is its location within the nest-network structure of the colony. We find that the flow of resources through a nest, which is based on its position in the nest-network, determines its likelihood of surviving, reproducing and growing. Flow through a particular nest can change with time, as other nests reproduce and disappear. The life-history of an individual is, therefore, not only affected by local social interactions, but by interactions throughout the dynamic network.

6.1 Introduction

Life-history is shaped by both an individual's ecological and social environment. The social environment of an individual can be represented as a network to capture how local social interactions relate to a broader, global-level, pattern of social structure. Social structure has, for example, been shown to be an important determinant of: information transfer between individuals (e.g. Blonder & Dornhaus 2011; Farine et al. 2015), disease spread through a population (e.g. Cross et al. 2004; Otterstatter & Thomson 2007), cooperation (e.g. Hemelrijk 1990; Croft et al. 2006) and gene flow (e.g.Wolf & Trillmich 2008; Godfrey et al. 2014). Similarly, social structure is likely to translate into consequences for many ecological and evolutionary processes such as social evolution, population stability, dispersal strategy and the success of ecological invasions (*reviewed in:* Kurvers et al. 2014). An important aspect of the relationship between social organisation and evolution is the relationship between an individual's position in the social structure and their life-history. Here, we investigate the relationship between network position and life-history traits.

Social structures are inherently dynamic, but examination of the relationship between life-history and social structure typically assumes a static network. In reality, most environments change with time and many evolutionarily important measures can only be assessed in a dynamic system (Blonder et al. 2012). Indeed many life-history traits are inherently time-dependent. For example, the survival, or rather the death, of an individual is an event that occurs at a point in time. To understand the factors influencing an individual's survival it is therefore necessary to examine survival within a dynamic framework. In the same way, the timing of reproduction is an inherently dynamic trait. When an individual choses to reproduces has a fundamental influence on an individual's reproductive success and ultimately fitness (Daan & Tinbergen 1997). As an inherently time-dependent trait the factors influencing the timing of reproduction can only be considered within a dynamic system. Growth is another time-dependent life-history trait. An individual's change in size often reflects foraging attainment and can be an important indicator of life-history success (Cuthill & Houston 1997). To understand the effects of social environment on an individual's survival, reproduction and growth, it is necessary to view the social environment in a dynamic context.

We use the nest-networks of polydomous wood ants as a model to investigate the relationship between dynamic social position and life-history traits. Polydomy is a widespread nesting strategy in ants: a polydomous colony inhabits several spatially separated, but socially connected, nests (Debout et al. 2007; Robinson 2014). The social environment of an individual nest is its connections to other nests, and its position in the nest-network system. Processes analogous to survival, reproduction and growth can all be observed within dynamic polydomous ant nest-networks. The survival of a given nest can be inferred by its continued inhabitation, or conversely its abandonment, at a later time. The survival probability of a nest will vary through time in much the same way as an individual animal's. Reproduction in individual animals is analogous to the process of budding in polydomous ant colonies. During budding, workers and queens leave a nest on foot to found a new nest (Bourke & Franks 1995). This process results in one nest acting as the founder of another nest, analogous to the parent and offspring relationship. Similarly, growth in individual animals is analogous to the changes in nest size observed with time within the polydomous colonies.

The advantage of using polydomous ant colonies is that nest-networks are, in many ways, simpler and easier to study than social networks based on interactions between individual animals. For example, inferring the strength, or even presence, of a connection between individuals in a social network presents many sampling and methodological challenges (Whitehead 2008a; Croft et al. 2008; Croft et al. 2011). In contrast, the connections between nests in a polydomous colony can be directly observed, and their strength measured (Ellis et al. 2014). Similarly, whereas the fission-fusion dynamics of many social networks means that the boundary between one network and another can be difficult to define (Whitehead 2008a), in nest-networks the functional boundaries can be clearly defined between those that are connected to the network and those which are not (e.g. Ellis et al. 2014).

We investigate how these life-history traits are affected by three levels of organisation in the nest network: attributes of the individual nest (such as nest size), position of the individual nest within the network, and properties common to the whole network. Differentiation between these levels allows us to investigate the importance of the nest's position within a network compared to other factors that may influence the survival of a nest. In a system in which social position is a determinant of life-history it is expected that the position of the individual within the network will have significant effects on its life-history, whereas properties shared by the whole system will not. In this study, we investigate the effects of inherent nest traits, nest-within-network traits and colony-level traits on the survival, foundation of new nests and growth of nests within polydomous wood ant colonies.

6.2 Methods

Study species and study site

We investigated the dynamics of the nest-networks of the polydomous red wood ant *Formica lugubris*. The red wood ants (*F. rufa* group) are a group of closely related, behaviourally and morphologically similar species found in woodland across much of Northern Eurasia (Bernasconi et al. 2011; Goropashnaya et al. 2012). Wood ants show plasticity in their nesting strategy, both within and between species. *Formica lugubris*, for example, is polydomous in England and parts of Switzerland but monodomous (inhabiting only one nest per colony) in Ireland and parts of Finland (Ellis & Robinson 2014). Wood ants are the dominant invertebrate predator in their environment, they hunt and scavenge for a variety of invertebrate prey, including other ant species (Mabelis 1984; Savolainen & Vepsäläinen 1988); however, honeydew collected from homopteran colonies in the canopy actually provides the majority of a red wood ants food (Rosengren & Sundström 1991). These homopteran colonies provide a spatially and temporally stable food source for the ants. This study was conducted at the Longshaw Estate in central England. *F. lugubris* is the only red wood ant species at this site. The site is a mixture of woodland pasture and historic plantations. The nests of wood ants are large, well-constructed and contain many queens (Ellis & Robinson 2014). Wood ants have no significant predators in England. Their large size, lack of predators and competitors and stable food sources means that nests are often present in the same location for a long period of time.

Network mapping

We examined how the nest-networks of thirteen polydomous wood ant colonies changed over time. Thirteen of the largest colonies at the site were studied, chosen from a preliminary survey in May 2012 (details, table 6.1). The colonies were first fully-mapped in late-August 2012. For the next two years (2013 and 2014) the colonies were mapped twice per year, once in late-spring and again in late-summer. Wood ants show seasonal activity patterns: they are quiescent over-winter, beginning foraging activity (and producing sexual offspring) in late-spring, and then continue foraging throughout the summer and early autumn. Remapping colonies in late-spring and again in late summer therefore, represents the beginning, and the height, of the foraging season respectively. The timing of the late-spring mapping was dependent on the timing of spring in each year, and was not performed until temperatures were high enough that both foraging and internest trail activity were being performed (Rosengren 1977). Late-summer mapping was always done in the second half of August. Wood ant activity can be dependent on temperature and weather conditions so all colonies were mapped when the colonies were fully active, in warm, dry conditions.

We represented the polydomous colonies as networks, with the nests and trees as nodes and the internest and foraging trails as edges (figure 6.1). Wood ants form clear above-ground trails between nests (internest trails) and between nests and trees (foraging trails). The trails consist of workers travelling between the nests transporting a variety of resources, predominantly honeydew, invertebrate prey and brood (chapter 4; chapter 5). We define a polydomous colony as two or more nests connected by internest trails (Ellis et al. 2014). Our definition of a colony is, therefore, based on functional resource exchange between nests, rather than on the basis of aggression or relatedness.

	Total number of nests					**Net Change in number of nests**	**Maximum number of Trees**
	2012-Summer	2013-Spring	2013-Summer	2014-Spring	2014-Summer		
I	21	16	15	11	14	-7 (-33.3%)	37
IIa	4	3	4	4	4	0 (0%)	2
IIb	6	6	6	9	9	+3 (+50%)	4
III	12	12	8	9	16	+4 (+33.3%)	7
IV	12	9	6	7	7	-5 (-41.6%)	9
V	14	11	10	8	2	-12 (-88%)	4
VI	14	12	12	13	11	-3 (-21%)	9
VII	7	7	4	5	8	+1 (+14%)	6
VIII	6	3	4	6	4	-2 (-33.3%)	10
IX	9	11	17	11	15	+6 (+66.6%)	10
X	13	8	10	9	8	-5 (-38%)	8
XI	20	15	10	10	17	-3 (-15%)	8
XII	6	6	3	8	3	-3 (-50%)	7

Table 6.1 Details of the colonies used in this study. Numbers refer to the nests present in the colony at that time-point. Spring refers to late-May at the beginning of the foraging season and summer in late-August, at the peak of the foraging season. Net change in nests describes the difference in number (and percentage) of nests in the colony between summer 2012 and summer 2014. Maximum trees is the largest number of trees foraged to by the colony in any particular time point.

For each colony, at each mapping, we recorded the spatial and topological layout of the nests, trees and trails. For the trails, we measured the length of the trail, compass direction of the trail and the traffic on the trail. The traffic on the trail was measured as the length of trail needed to find 10 workers, which can be converted into number of ants per cm of trail, and then number of ants on the entire length of the trail. The advantage of basing trail traffic on distance needed to find ants, rather than a rate based measure, is that it is not reliant on the speed at which the ants are moving, which is strongly affected by the ambient temperature (Rosengren 1977). Ant traffic is a measure of trail strength based only on the number of ants passing along the trail, however, this is likely to be affected by the number of workers available to travel along trails in the nests connected by the trail. Trail weight is a measure of trail importance to a particular nest relative to the populations of the nests being connected. We calculated each trail's weight by dividing the total number of ants on the trail by the mean population of the nests connected by the trail (Ellis et al. 2014). For foraging trails, the weight of the trails are relative the population of the foraging nest (Ellis et al. 2014). For each remapping of each colony we also estimated the populations of the nests (see section 3.2), measured the canopy cover over the nests and the recorded species of the trees used for

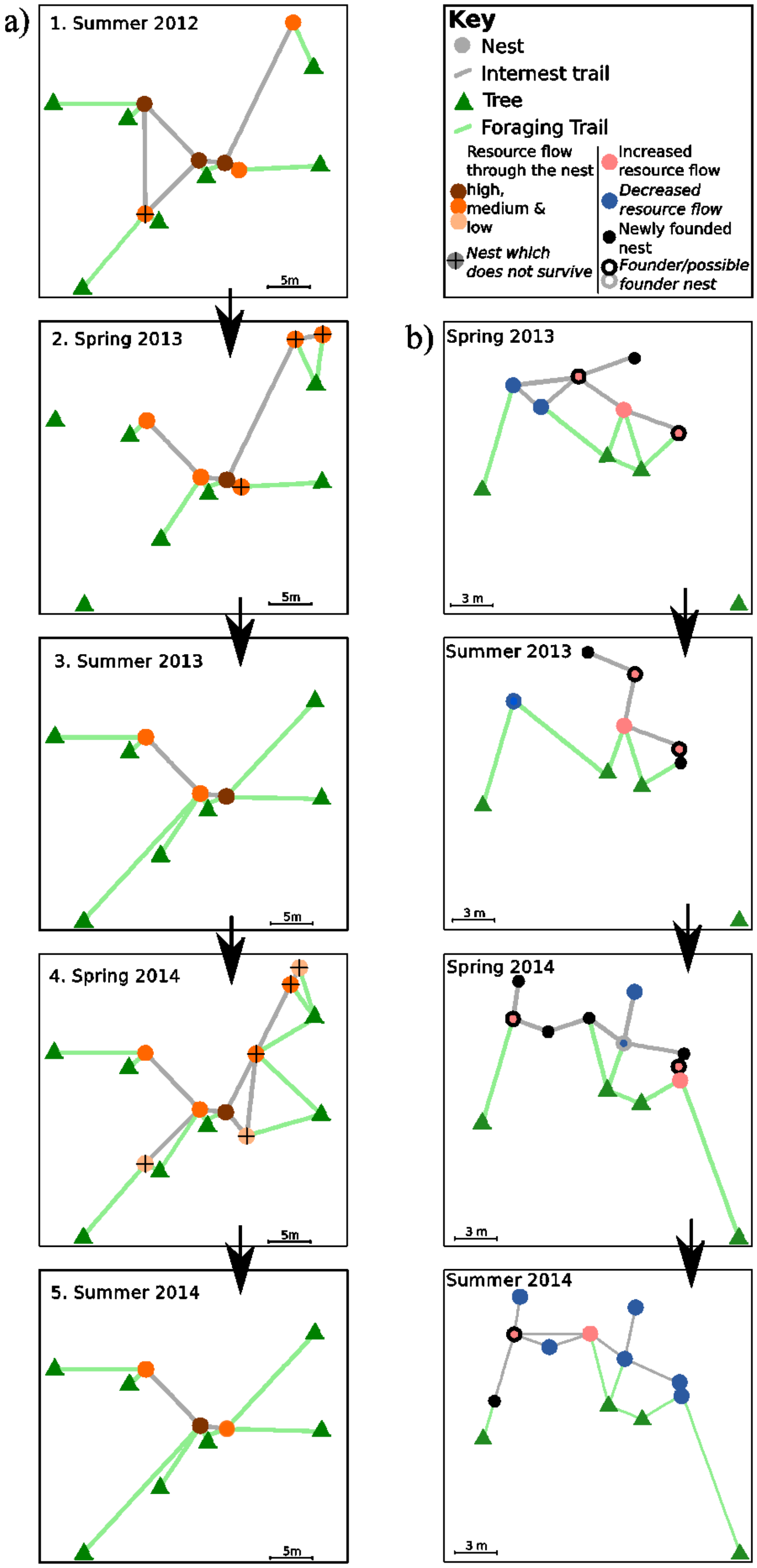


Figure 6.1 Time series of two networks used in this study to illustrate some of the effects of nest position on nest life history traits. Smaller networks were chosen for the purpose of simplicity.

a) Colony XII at the five time-points used in this study. Nests are represent as circles and categorised as having a low resource flow (normalised betweenness of less than 0.25: pale orange) a medium-level of resource flow (normalised betweenness of more than 0.25 and less than 0.75: orange) and a high-level of resource flow (normalised betweenness of greater than 0.75: deep orange/brown). Nests with a black cross are those which will not survive until the next time-point (no data for after summer 2014 so no nests are marked as being abandoned). Green triangles represent trees. The lines between points represent foraging (green) and internest trails (grey). Nests with a low or medium flow were abandoned more often than those with a high flow.

b) Colony IIb at 4 time-points in the study. As above, circles represent nests. Circle colour represent the change in flow through the nest since the last time-point, blue indicates a decrease or no change in flow of resources since the last time-point. Pink shows nests which have increased in resource flow since the last time point. Black circles are the newly founded nests. Black outlines represent nests from which a new nest(s) has been founded (founders), grey outlines represent possible founders. Nests with an increased resource flow were more likely to found new nests than those with a static or decreased resource flow.

foraging. Canopy cover was estimated on the basis of digital photographs taken from vertically above the nest (Ellis et al. 2014). Each map is used to construct a spatially embedded network of the colony, with edges weighted by trail strength, and the node properties of: nest population (hereafter: nest size), distance to the nearest tree and canopy cover.

Analysis

We are interested in how various temporal characteristics of nests within polydomous colonies are influenced by their inherent attributes (nest-attributes), their position within the nest-network (network-position) and attributes shared with the whole colony (colony-attributes). Nest-attributes are those based directly on inherent attributes of the nest. Nest attributes used in this study are: nest size, canopy cover over the nest and distance from the nest to the nearest tree. All analysis was performed in R (R Development Core Team 2011) using the ‘igraph’ package (Csardi & Nepusz 2006).

Network-position properties depend on a nests position in the colony nest-network. Resource exchange between the nests of a polydomous wood ant colony is based on workers from a given nest travelling along internest trails to neighbouring nests, collecting honeydew and then returning to their original, home, nest (chapter 4). This mechanism is based on local resource exchange, between neighbouring nests, without reference to the efficiency colony-level resource redistribution (Ellis et al. 2014). Pairs of nests exchanging resources can still result in the redistribution of food through the entire polydomous system. A resource exchange mechanism based on workers from a given nest treating other nests as food sources has the potential to result in resource exchange through the entire colony (Schmolke 2009; Cook et al. 2013). In a system based on local resource exchange the amount of resources available to a given nest can be thought of as the flow through that nest; in wood ant colonies resources flow from the trees, through the internest trail network, to the nests. Flow through a node in network can be measured as betweenness centrality. Betweenness is a measure of the total number of shortest paths between pairs of nodes in the network which pass through a particular node (e.g. Croft et al. 2008; Whitehead 2008b). In our polydomous networks, all trees are at the end of a network on their own branch, and therefore have a betweenness of zero. In contrast, the betweenness of a nest is based on the number of shortest paths passing through it, including those from trees to other nests in the network. Betweenness can therefore act as a measure of resource flow through a particular nest, dependent on its pattern of trails to other nests and trees in the network. We used a weighted measure of betweenness to account for the number of ants on a trail, given the size of the connected nests (trail weight). To allow comparison between networks for each colony, the betweenness was normalised within each network by dividing all betweenness values by the largest value for that colony (e.g. Lusseau & Newman 2004).

Colony-attributes are those which are shared by all the nests within a nest-network. At the colony-level we are interested in the how the amount of resources collected by the entire colony influences the life-history of the nests. We use the number of ants on foraging trails as a measure of a colonies foraging effort. The number of ants on foraging trails can be calculated by multiplying the ants per cm for every foraging trail by the length of that foraging trail, and the summing these values for the whole colony. This foraging metric is a measure of the resource acquisition effort of the entire colony, not a count of the number of foragers in the colony. The ratio of the total population of the colony (summed size of all the nests in the colony) to the foraging effort of the colony, hereafter worker:foraging ratio, gives an estimate of foraging effort per worker in the colony. A low worker:foraging ratio suggests a high foraging effort per worker, whereas a high worker:foraging ratio indicates a low foraging effort per worker. We use the worker:foraging ratio as a measure of colony-level resource acquisition. We investigate how worker:foraging ratio predicts various life-history traits of nests (see below) to see how this colony-level measure of resource acquisition compares to the network-position based resource acquisition measure: normalised betweenness.

Internest trails can also have inherent, within-network and colony attributes. An important inherent trait of an internest trail is the ant traffic on that trail. Ant traffic along a trail does not take into account the size of the nests connected by the trails. Trail weight takes into account the size of the nests being connected by the trails. The life-history traits of an internest trail are likely to be affected by the normalised betweenness of the nests which they join. Similarly, the number of internest trails and foraging trails associated with the nests an internest trail connects can also be considered a within-network attribute. The betweenness of a trail, unlike the betweenness of a nest, is a colony-level effect. Trail betweenness is a colony attribute because it represents the importance of a trail to colony-level resource flow, rather than the amount of resources passing through a particular nest.

Survival

We are interested in which factors (nest-attributes, network-position or colony-attributes) influence the survival of a nest in a polydomous wood ant colony. We used survival analysis, adapted for use with network data, to investigate the factors influencing nest survival. Survival analysis is used to describe the time until an event occurs; for our purposes the event in question is that a nest is abandoned (Kleinbaum & Klein 2012). The advantage of using survival analysis rather than more conventional statistical techniques is that censored data can be included. Censored data occurs when some information is known about an individual, for example when a nest is founded, but not other information, for example when is abandoned. This is useful for our data as many nests survive longer than our study period. Survival analysis allows us to investigate how the survival of a nest changes with time in relation to network dynamics.

We used a Cox Proportional-Hazard (Cox PH) model to investigate how explanatory variables (X_n) correlate with the hazard potential, *h(t)* (equation 2). The hazard potential is the instantaneous potential per unit time that a nest (or trail) is abandoned, given that the nest (or trail) has survived up to time *t* (Kleinbaum & Klein 2012). The survival function, *S(t)*, describes the probability that a nest survives longer than a given time *t*.

Equation 2.

$$h(t,\boldsymbol{X}) = h_0(t)e^{\sum_{i=1}^{p}\beta_i X_i}$$

$$\boldsymbol{X} = (X_1, X_2, \dots X_p)$$

In the Cox-PH models reported in this study, the explanatory variables (X_p) were the nest-attribute, network-position or colony-attribute variable(s) being investigated. Colony was also used as an explanatory variable in all models. When the model was used to describe the survival of a trail the survival of the nests associated with the trail was used as an additional explanatory variable. The presence of the nests which bound the trail is, clearly, vital to the survival presence of the trail itself and was always highly significant.

Survival models assume independence of data; an assumption that is violated by network data. Thus, our null model was based on the quadratic assignment procedure using 10,000 node attribute permutations (Croft et al. 2011). We then measured the experimental test statistic against this null distribution to derive statistical significance. Permutations were constrained within each map (i.e. within each colony map from a particular time-point). All reported statistics associated with survival were based on the quadratic assignment procedure. For some analyses the smallest colonies (IIa and VIII) were not included because the lack of variation prevented the Cox PH model defining the confidence intervals, this is indicated in the text by lowered values of *n*. Survival analysis was performed in R using the ‘survival’ package (Therneau 2012).

Nest Reproduction

New nests were often founded within the polydomous colonies used in this study. If a particular nest was not present at the previous time-point we considered it to be newly founded. We can use the colony maps to infer the nest from which the newly founded nest budded. We refer to a nest from which a new nest is budded as its natal nest. To infer which nests are the natal nests we assumed that (1) newly budded nests stay connected to their natal nest by a trail and (2) the natal nest is the nearest nest to which the newly budded nest is attached. We use these assumptions to categorise all the nests within a colony as either: newly founded, founders (those from which a new nest has been founded) or non-founders (those from which a new nest has not been budded). In some cases the nearest nest to a newly founded nest was another newly founded nest. As the order of foundation cannot be inferred, the nearest established (i.e. not newly founded) nest was characterised as a possible founder.

We used General Linear Mixed Effect Models (GLMMs) to analyse how budding relates to various nest, nest-within-network and colony attributes. In the GLMMs founder status (i.e. founder, non-founder or possible founder) was used as the response variable with the variable(s) of interest as the fixed effect. Colony, nest ID and season were included as random effects. Further details of the tests are given in the appendices; the superscript in the text refers to the row of the table (appendix H). All GLMMs used a binomial error structure and a logit link function. We tested significance using a chi-squared analysis of deviance (AoD) which compares the full model to a null model without the fixed effect. If the null models and full model are significantly different it suggests that the fixed effect has a significant impact in explaining the data. GLMMs were performed in R using the 'lme4' package (R Development Core Team 2011).

Change in nest size

In many species, growth is well- correlated with foraging and provisioning success (Stephens et al. 2007). We investigated the change in nest size (which can be positive or negative) over time by comparing the population change in a particular nest between two time-points. We used GLMMs to investigate how the change in size related to other nest, nest-within-network and colony factors. In these analyses proportional change in nest size was used as the response variable, the factor of interest as the fixed effect and colony, nest ID and season as random effects (further details in appendix H). GLMMs used a Gaussian error structure and an identity link function. Analysis of deviance was used to test the significance of variables.

6.3 Results

Survival Analysis

The position of a nest within the network is a key predictor of its survival. Nests with a higher normalised betweenness are significantly more likely to survive than nests with a lower normalised betweenness (Cox PH: z=-3.64, n=581, p=0.0001; figure 6.2). This relationship between normalised betweenness and survival is robust even when nest size is introduced into the survival model. Larger nests are significantly more likely to survive than smaller nests (Cox PH, z=-3.08, n=581, p=0.0001) but, when included in the same model as an additional fixed effect, nests with a higher normalised betweenness are still significantly more likely to survive than nests with a lower normalised betweenness (Cox PH: normalised betweenness, z=-1.64, n=581, p=0.0334; nest size, z=-2.02, n=581, p=0.0345).

The survival of internest trails is, similarly, predicted by the position within the nest-network of the nests they connect. Internest trails connect two nests; each nest has a value of normalised betweenness. The lower of these two normalised betweenness values is significantly related to the survival of the internest trail (Cox PH: z=-1.30, n=476, p=0.0373). The relationship is negative: a trail associated with a nest with a low normalised betweenness is less likely to survive than a trail associated with a nest with a high normalised betweenness. Nests with a high normalised betweenness are more likely to survive than nests with a low normalised betweenness (above) which may explain the negative relationship between trail survival and nest normalised betweenness. Trails associated with a nest with low normalised betweenness are less likely to survive because the nest is less likely to survive, rather than due to the position of the trail within the network.

We found no relationships between survival and colony-level effects. Nest survival is not significantly related to colony worker:foraging ratio (Cox PH: z=0.24, n=558, p=0.3739), even when nest size is also included in the survival model (Cox PH: z=0.38, n=558, p=0.3485). Similarly, trail survival is not significantly related to either trail betweenness (Cox PH: z= -1.68, n=476, p=0.0646) or colony worker:foraging ratio (Cox PH: z= -1.37, n=476, p=0.1016).

Attributes of the nests and trails can also influence their survival. Larger nests are significantly more likely to survive than smaller nests (Cox PH: z= -2.75, n=581, p=0.0028). However, other nest-attributes do not affect survival. The survival of a given nest is not significantly affected by either: the distance from the nest to the nearest tree (Cox PH: z= -

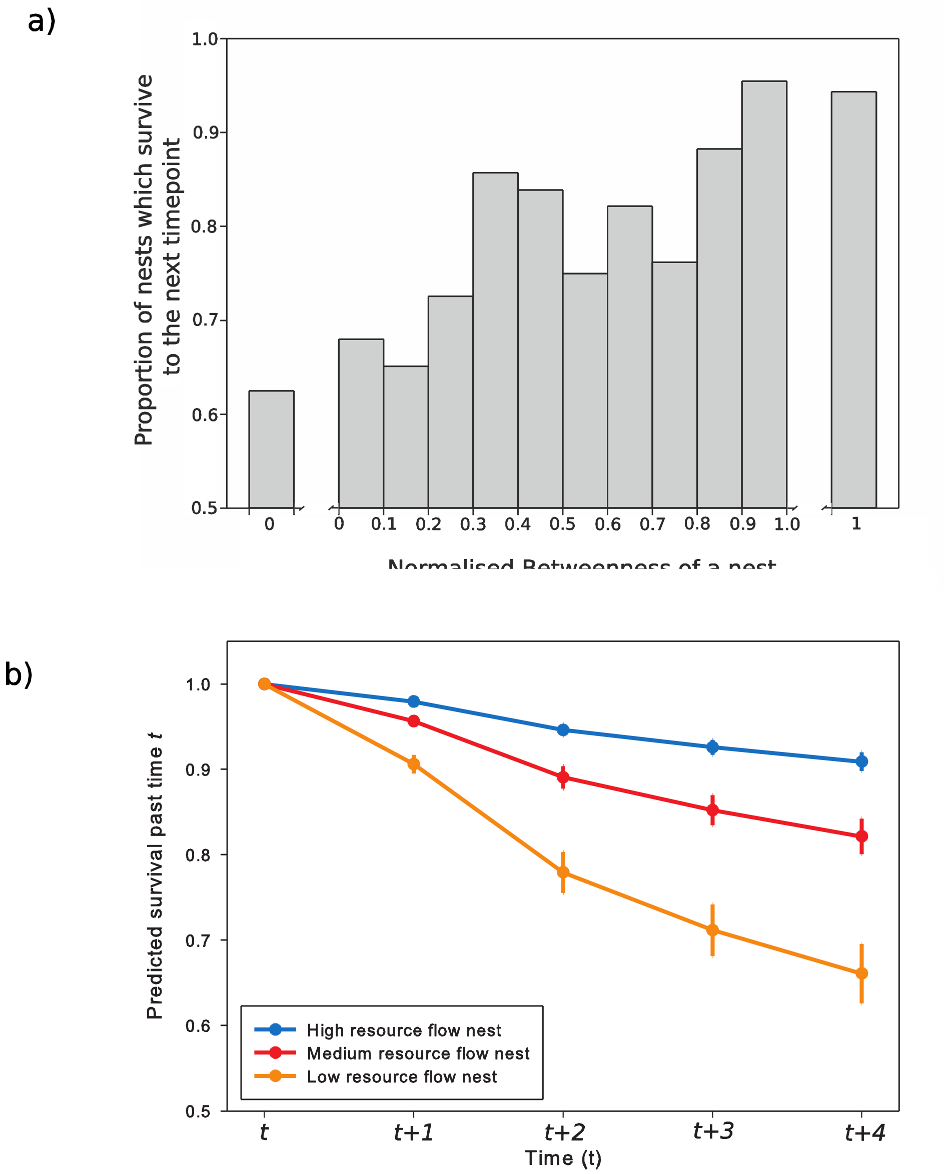


Figure 6.2 Differential survival of nests dependent on flow (the normalised betweenness) of resources.

a) The proportion of nests which survive until the next time-point categorised by normalised betweenness. As the data is normalised 0 and 1 are treated separately as they are present (by definition) in all colonies.

b) Survival of nests predicted by the Cox-PH survival model. Curves represent how the survival of nests with a defined (and unchanging) resource flow are predicted to change with time. A high resource flow is a nest with a normalised betweenness of 0.9, medium resource flow is a nest with a normalised betweenness of 0.5 and a low resource flow is a nest with a normalised betweenness of 0.1. Curves are calculated using the Kaplan-Meier method. The error (SE) is the difference between survival in different colonies. Each point represents the survival of a nest at *t*+*x* time-points after the nest is founded, four is the maximum time-points after foundation as our study only covered five time-points.

1.24, n=581, p=0.1017), or the canopy cover over the nest (Cox PH: z=-0.17, n=563, p=0.3798). Internest trails with a high ant traffic were significantly more likely to survive than trails with low ant traffic (Cox PH: z= -2.4, n=476, p=0.0042). However, there is no significant relationship between trail weight (which is adjusted for the sizes of the connected nests) and trail survival (Cox PH: z= -0.59, n=476, p=0.2699).

Nest reproduction

Nests which reproduce, i.e. nests from which new nests have been founded (founders), have a significantly higher normalised betweenness than those from which no new nests have been founded (non-founders) (AoD$^{6.1}$: χ^2=9.7, df=2, p=0.008; figure 6.3). Nests often change in normalised betweenness between two time-points. Founder nests have a significantly greater increase in normalised betweenness over the period which the new nest was founded than non-founder nests (AoD$^{6.2}$: χ^2=8.6, df=1, p=0.002).

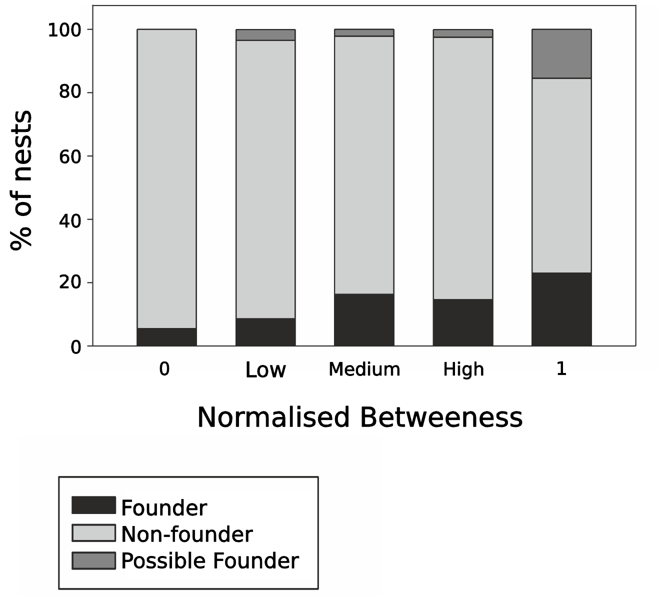


Figure 6.3 The percentage of nests with differing normalised betweenness which will act as founders, not act as founders (non-founders) and possible founders of new nests. Betweenness is categorised as 0, 1, low (<0.25), medium (0.25-0.75) or high (>0.75). There are a higher proportion of nests which act as founder with higher normalised betweenness.

Nests in colonies with a low worker:foraging ratio (i.e. a high foraging effort per worker) are not significantly more likely to be founders than nests in colonies with a high worker:foraging ratio (AoD$^{6.3}$: χ^2=0.15, df=1, p=0.70). Similarly, nests in colonies which have a lowered worker:foraging ratio (i.e. an increase in foraging effort per worker) are not significantly more likely to be founders than nest in colonies with a raised worker:foraging ratio (AoD$^{6.4}$: χ^2=0.04, df=1, p=0.80).

Nest-attributes do not predict if they have acted as a founder. Founder nests are not significantly larger than non-founder nests (AoD$^{6.5}$: χ^2=0.20, df=1, p=0.65). Similarly, nests which had a greater increase in size are not significantly more likely to be founders than nests which have had a lower increase or a decrease in size (AoD$^{.6.6}$: χ^2=0.03, df=2, p=0.87). Founder nests are neither significantly closer to trees (AoD$^{6.7}$: χ^2=0.19, df=1, p=0.66), nor have a significantly lower canopy cover nest (AoD$^{6.8}$: χ^2=0.78, df=1, p=0.38) than non-founder nests. Nests are not significantly more likely to act as founders in spring than in summer (AoD$^{6.9}$: χ^2=0.16, df=1, p=0.69).

Size change

Nests often changed considerably in size between time-points, ranging from an increase of over 6000% to a decrease of 99%. The median change in size was a decrease of 17% and the mean change in size was an increase of 91%. Change in nest size is significantly positively related to change in normalised betweenness (AoD$^{6.10}$: χ^2=19.9, df=1, p<0.001; figure 6.4). Nests with an increase normalised betweenness usually grew size, whereas nests with a decreased in normalised betweenness usually showed a reduction in size.

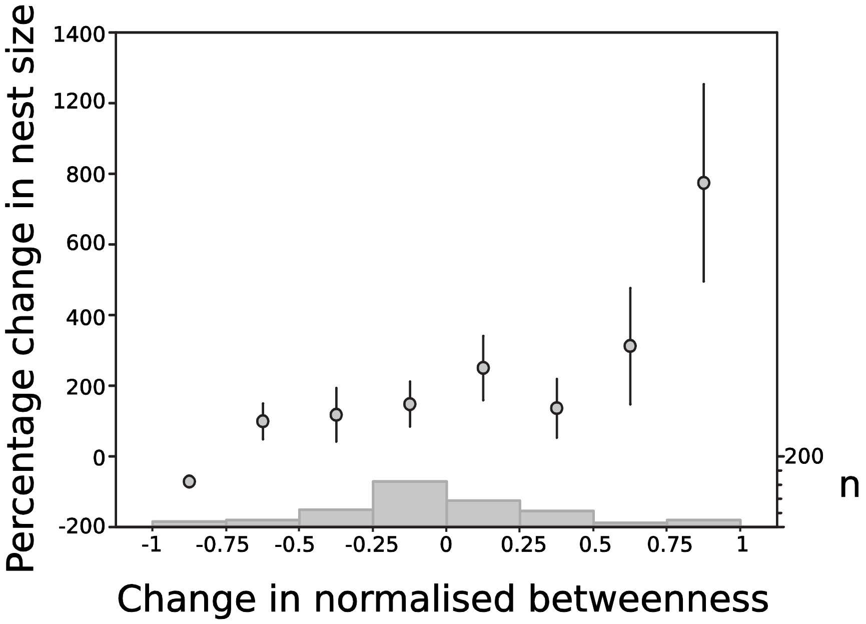


Figure 6.4 The mean (± SE) percentage change in size of nests with different levels of change in resource flow (normalised betweenness). Change in flow is categorised in intervals of 0.25 from -1 to 1. The bar chart shows the number of nests (n) in each category.

Worker:foraging ratio is calculated by dividing the total sizes of all the nests in the colony by the foraging effort of the colony. Due to the potentially confounding effect of using nest size to calculate both worker:foraging ratio and the change in nest size we use an alternative measure of colony foraging effort. We use the mean number of foragers per nest as a substitute colony-level attribute. There is no significant relationship between the change in nest size and the change in mean number of foragers per nest (AoD$^{6.11}$: χ^2=0.14, df=1, p=0.70).

Nests which are nearer to trees do not show a significantly different change in size than those which are further from trees (AoD$^{6.12}$: χ^2=0.08, df=1, p=0.7748). Similarly, change in nest size is not significantly related to the canopy cover over the nest (AoD$^{6.13}$: χ^2=0.85, df=1, p=0.36).

6.4 Discussion

We found that the position of a nest within the nest-network of polydomous *Formica lugubris* colonies had significant effects on life-history traits. Specifically, a nest with a higher flow of resources has an increased chance of surviving, reproducing and growing than a nest with a lower flow of resources. In contrast, the amount of food collected by the whole colony does not influence these nest life-history traits. These results show that position in a network can influence fundamental life-history traits of individuals in a dynamic network.

Our results show that important life-history traits of nests within a polydomous colony are predicted by a nest’s ability to acquire resources, rather than the amount of resources collected by the colony. An individual nest within a polydomous colony, therefore, resembles a monodomous colony (a colony inhabiting a single nest), which is also likely to survive, grow and reproduce based on its own ability to acquire resources. Crucially, however, a polydomous nest’s ability to acquire resources depends on its position within the nest-network structure. The life-history of the nest is dependent on its social, as well as its physical, environment. The importance of the social environment shows that the nests within the colony show a high degree of interdependence, at least in terms of resource acquisition. The extent to which nests can be considered as part of the same colony, super-colony or super-organism is an important consideration when assessing, for example, the level at which selection acts in a colony (Helanterä et al. 2009; Moffett 2012; Kennedy et al. 2014). Our results show that in an ecological sense the interconnected nests of a polydomous colony can be considered a single functional unit.

The importance of resource flow to the life history of nests highlights the role of the polydomous nest-network as a transportation network. Resources are often distributed heterogeneously in the environment; polydomy may be a way to more efficiently exploit these dispersed resources (Holway & Case 2000; Schmolke 2009; Lanan et al. 2011; Cook et al. 2013). The nest and foraging network of polydomous colonies can be viewed as a transportation network to move resources from food sources to the nests and then between nests (Latty et al. 2011; Cook et al. 2014). Transport efficiency refers to the ease with which resources can flow through a network. In the polydomous nest system, nests with a high betweenness, and therefore a high resource flow, are at points in the network important for colony-level resource redistribution, and therefore colony-level transport efficiency (e.g. Croft et al. 2008; Perna & Latty 2014). If network transport efficiency is being retained we predict that within a colony nests and trails with a higher betweenness, and therefore greater importance for efficiency, will be more likely to survive than those with a lower betweenness. Although nests with a higher betweenness do stand a greater chance of surviving that those with a lower betweenness, trails with a higher betweenness are not more likely to survive than trails with a lower betweenness. These results suggest that transportation efficiency is not being preserved within polydomous colonies. It may be that transport efficiency is not under strong selective pressure in this system.

Nests are gained and lost as the network changes over time. A consequence of this dynamic is that the flow through a particular nest can change. The integrated nature of the system means that a given nest could keep all the keep the same connections to neighbouring nests and trees but still undergo a change in the amount of resources available to it (and therefore its chances of surviving, reproducing and growing), due to nests being abandoned or founded elsewhere in the colony. Nests in unprofitable areas, and therefore a low resource flow, are more likely to be abandoned than nests in profitable areas. These dynamics will result in the colony moving towards resources and away from unprofitable areas. For a spatially-embedded network, such as a polydomous network, this movement is physical movement of nodes. In networks which are not spatially embedded, such as social networks for example, this process could result in a network clustering around a certain node, for example individuals with information. The reverse could also occur; a network could cluster away from specific nodes, for example diseased individuals in a social network. These changes in the network structure are self-organised, resulting from selective pressure based on an individual's position in the network. Networks are often studied as static cross-sections of a period of time; our results demonstrate that dynamic changes in a network can result in important life-history consequences.

The network dynamics illustrate the potential feedback between the individual-level and the system-level in biological networks. The position of an individual within a biological system can affect that individual's exposure to, for example, food, mates, information and disease (e.g. Christley et al. 2005; Oh & Badyaev 2010; Aplin et al. 2012). The structure of the network is, in turn, affected by the nodes within the network. For example, the overall pattern of interactions between individuals in a system can be influenced by a variety of biotic and abiotic factors such as: food availability, sex demographics and season (Brent et al. 2013; Darden et al. 2009; Foster et al. 2012).The nests within polydomous colonies highlight how these effects can be reciprocal in a dynamic system. Differential survival and reproduction of individuals in a system will change the structure of the network as new nodes appear and others disappear. This will, in turn, change an individual's relative position within the network, altering its chances of surviving and reproducing. The network, therefore, will be continually restructuring, resulting in a dynamic system which is not stable through time. A dynamic system is likely to react very differently to ecological and environmental changes to a static system (Kurvers et al. 2014).

In conclusion, we found that the survival, budding and change in size of nests within polydomous *Formica lugubris* colonies is related to their position in the trail network. These results highlight how important life-history traits such as survival, reproduction and growth of individuals within a dynamic biological network can be affected by the position of an individual within the system.

Chapter 7: Discussion

7.1 Thesis Overview

In this thesis I have presented a series of studies investigating the relationships between nests within polydomous *Formica lugubris* colonies. In chapter 2 we reviewed the literature relating to polydomy and applied it to the red wood ant group. We suggested that polydomy is likely to be closely linked to polygyny in the red wood ants, and that an important benefit of polydomy for the group is likely to allow a colony to more efficiently exploit dispersed, but spatially and temporally stable, food sources. In chapter 3 we show that resource redistribution within polydomous *F. lugubris* colonies is based on local exchange between pairs of nests, rather than in reference to a colony-level strategy. Chapter 4 shows that this local resource exchange is based on ants from a particular nest treating other nests of the polydomous colony as food sources, consistently travelling to that nest, taking food, and then returning to their home nest. In chapter 5 we show that those nests not providing food to the rest of the colony are part of the process of colony expansion, new nests are founded at random locations in the environment, and then survive if they begin foraging, and otherwise tend to be abandoned. Finally, in chapter 6, we show that the position of the nest within the polydomous nest-network is an important determinant of that nest's likelihood of surviving, reproducing and growing.

7.2 Polydomy in red wood ants

Our results have shown that, in terms of resource acquisition, a nest within a polydomous colony is very similar to the nest of a monodomous colony. Nests within polydomous colonies: survive, reproduce and grow based on their own ability to: acquire resources (chapter 6) and expand based on the same processes used for budding in monodomous colonies (chapter 5). Nests also: show no specialised colony-level effects, either in terms of colony-wide resource redistribution (chapter 3) or division of labour between nests (chapter 5); and require no specialised worker behaviours to facilitate resource redistribution between nests (chapter 4). This similarity between nests within polydomous colonies and the nests of monodomous colonies suggests a mechanism by which a polydomous red wood ant population may develop from a monodomous, monogynous, ancestral population. Rather than a specialised adaptation to provide a particular benefit in particular niche, polydomy may simply be a consequence of population development.

As a monodomous, monogynous wood ant population expands through a habitat it is likely to rapidly become nest-site limited. Nest-site limitation will occur due to both biotic and abiotic factors. Insolation, for example, is an important abiotic factor affecting the nesting of wood ants (Chen & Robinson 2014). Dispersing queens rely on finding a colony of a host species (usually a member of the *Serviformica* or *Coptoformica* groups) to parasitise (e.g. Rosengren et al. 1993); the presence or absence of these hosts is an important biotic factor influencing wood ant nesting. As wood ants tend to exclude their host species a large wood ant population may cause nest-site limitation, even in a habitat with many appropriate nest sites (Mabelis 1984; Seppä et al. 1995; Vepsäläinen et al. 2000). In a nest-site limited environment philopatry becomes a beneficial strategy for newly mated gynes (Rosengren & Pamilo 1983; Pamilo 1990; Rosengren et al. 1993; Sundström et al. 2005). If gynes are philopatric, and then reproduce in their natal nest, the colony has become polygynous.

Reproduction by budding is a safer method of reproduction for queens in polygynous colonies (section 2.2: The relationship between polydomy and polygyny). If a newly budded nest remains connected to the natal nest the colony has become polydomous. The studies presented in this thesis show that this process of staying connected to other nests is based on foraging behaviours already present in a monodomous colony. In addition, resource redistribution occurs on a pair-wise basis between nests, as expected of a process of development based on remaining connected to a natal nest. It appears that mechanistically polydomy is not particularly different from monodomy for red wood ant colonies.

The high nest turnover within the polydomous colonies, and the sometimes quite dramatic changes in nest-network structure, illustrate how new colonies may form within a polydomous population. The destruction of a particular nest can mean that two parts of the colony become physically separated. If these parts of the colony fail to reconnect the separate entities are their own functional units and therefore, by our definition of polydomy, have become separate colonies.

Our results also suggest advantages that remaining connected to other nests may provide a nest within a polydomous colony. If remaining connected to other nests did not provide a benefit to the nests involved then a polydomous population might be expected to be invaded by the nests with a polygynous, but monodomous strategy. However, polygynous, monodomous colonies appear to be rare in wood ants (but have been reported in *F. rufa*: Collingwood 1979; Skinner 1980b), suggesting that remaining connected to other nests does provide a benefit. The mechanism by which new nests are founded in polydomous *Formica lugubris* populations (chapter 5) suggests a possible advantage of polydomy to a colony. The foundation of a new nest (even by budding) is likely to have a high chance of failure (48% of new nests founded in the colonies studied in chapters 5 and 6 were abandoned). The failure of new nest founded by a monodomous colony, will result in the death of the workers and queens of the new nest/colony, and the loss of the reproductive investment of the founding colony. In contrast, in a polydomous system, workers and queens abandoning a nest can return to their natal nest, which clearly provides a fitness benefit to the workers and queens involved, and also provides a benefit to the natal nest in terms of rescued reproductive effort. Less-risky reproduction may be an important advantage of the polydomous nesting strategy.

The dynamic changes we observed in the nest-networks also highlight an advantage of polydomous nesting for exploiting honeydew sources. The changes in the structure of the colony nest-networks are driven by the ability of nests to acquire resources, which depends on their position within the nest and foraging system of the colony. Similarly, newly founded nests survive based on their ability to acquire resources. Individual wood ant queens may live for 15-20 years (Otto 2005). Given the high nest turnover observed within the colonies at Longshaw it is likely that a particular queen may have to move nests over her lifetime. The effect of this will be that queens do not remain in unproductive or struggling nests but are moved, within the dynamic nest-network, to more productive areas. This is likely to allow the queens to maintain a high level of resource intake, which is likely to translate into reproductive success. Although, monodomous colonies have been observed as temporarily bi-domous, which may allow the colonies to move short-distances through the environment (Breen 1979; Robinson & Robinson 2008), this is likely to be much more costly and risky for a monodomous colony than the simple colony expansion of polydomous colonies. Due to the high costs of moving for monodomous colonies they will not be able to move far, even if the area becomes unproductive (due to, for example, long-term changes in aphid populations, or competition from other colonies), or if resources could be exploited more efficiently from a different location. In this situation the lifetime reproductive success of a queen in a polydomous colony will be higher than that of a queen in a monodomous colony.

Our results suggest a simple mechanism by which a monodomous monogynous wood ant population could become a polydomous polygynous population. The studies presented here also highlight some advantages that the polydomous nesting strategy may provide wood ant colonies. The existence of a simple ecological pathway to an advantageous strategy may explain the repeated evolution of polydomy in red wood ants (figure 7.1). Monodomy and monogyny are likely to have been the basal strategy for the red wood ant group, but polygyny and polydomy have evolved at least three times in the group (Goropashnaya, Fedorov & Pamilo 2004). Given the simplicity of the switch from monodomy to polydomy suggested by the results presented here, this may not be particularly surprising. It may be that the polydomous phenotype will develop in a wood ant population with little or no evolutionary adaptation needed. The polydomous nesting strategy is then maintained within a population by the advantages that it provides the colonies relative to alternative nesting strategies, and therefore selecting against the evolution of internest barriers.

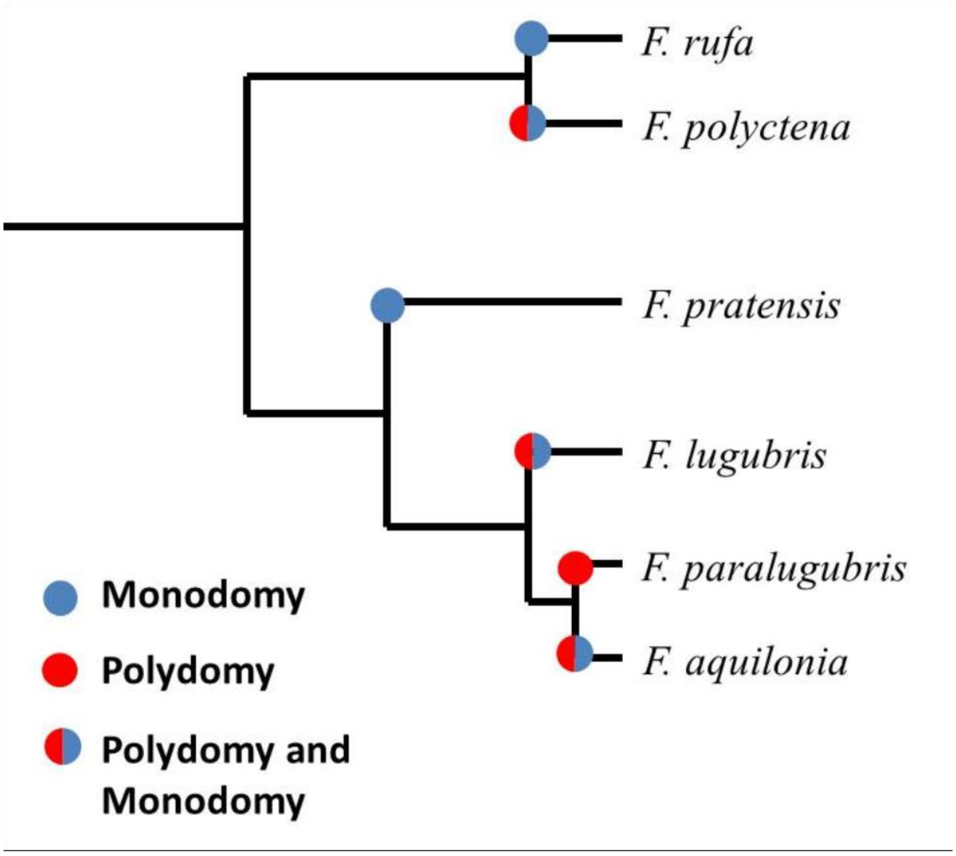


Figure 7.1 Phylogeny of the *Formica rufa* group showing the distribution of polydomous and monodomous nesting strategies. Phylogeny based on Goropashnaya et al. (2012). Branching points are correct but branch length does not represent divergence.

7.3 Limitations and future work

A clear limitation of the studies presented in this thesis is that they are all based on a single population at a single site. Broad conclusions are reliant on the assumption that other wood ant populations, at other sites, function in the same way. Although the members of the *Formica rufa* group share a very similar morphology and ecological niche there are some differences between species. For example, several studies have found that , whereas *F. lugubris* tends to inhabit the edge of forests, *F. aquilonia* is more often found in deeper woodland (Punttila 1996; Punttila & Kilpeläinen 2009). The physiological differences underlying these differing habitat preferences are largely unknown. Within species, it is also unclear the extent that the British *F. lugubris* population is representative of the populations in the rest of Europe. Although the British and Irish population do have some genetic differences from continental populations, they do not appear to be phylogenetically distinct (figure 7.2). The conclusions drawn above assume that the behaviours underlying polydomy are the same in different wood ant populations and species. Further work is necessary to assess the extent to which the Longshaw *F. lugubris* population is representative of other populations of the same species, and then the extent to which polydomy in *F. lugubris* is representative of polydomy in other members of *F. rufa* group.

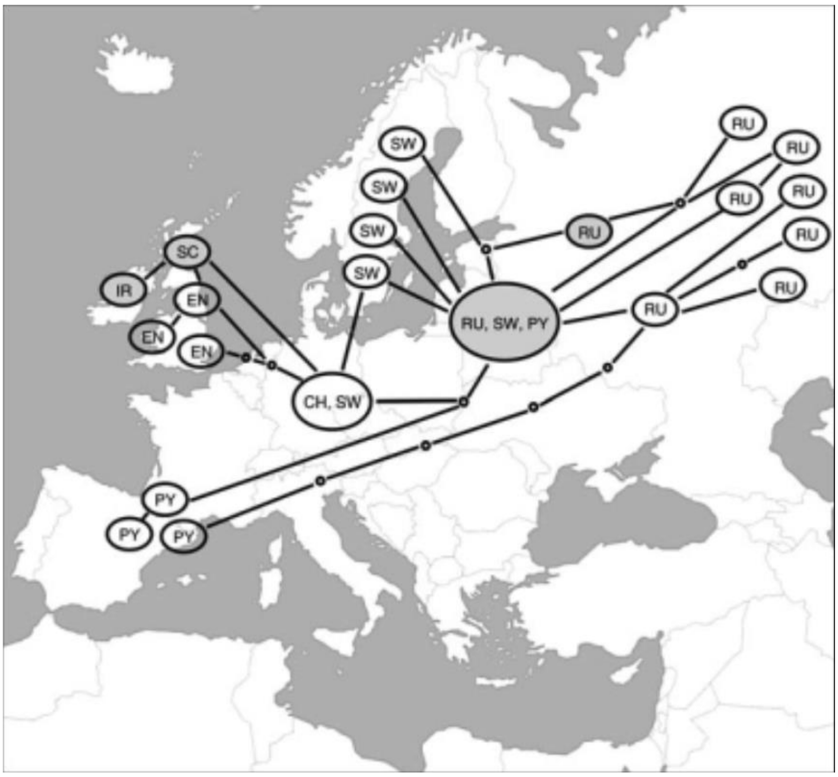


Figure 7.2 Mitochondrial-haplotype network of *Formica lugubris* in Europe. Taken from Maki-Petays & Breen (2007). Ovals represent *F. lugubris* populations: CH are from Switzerland, EN are from England, IR are from Ireland, PY are from the Pyrennes, RU are from Russia and SW are from Sweden. Each connecting line represents a single mutation. Small circles indicate unobserved intermidiates. Oval fill represents sample origin.

Formica lugubris is the only member of the *F. rufa* group present at the Longshaw Estate. This differs from many other *F. lugubris* populations in other countries where more than one species are often found in the same area (Finland e.g. Punttila & Kilpeläinen 2009: Switzerland e.g. Bernasconi et al. 2011). The presence of interspecific competitors may have profound effects on the behaviour and life-history of *F. lugubris*. The nesting behaviour of *F. lugubris* in areas with interspecific competition is sometimes different from areas without interspecific competition. For example, whereas *F. lugubris* is usually monodomous in

Finland (Rosengren & Pamilo 1983; but not not always see Rosengren 1971), where there are other wood ant species; they have only been ever reported as polydomous in England, where they are the only species present at the sites studied (Sudd et al. 1977; Gyllenstrand & Seppä 2003). There are however exceptions, in Ireland *F. lugubris* is the only wood ant species but has only been reported as monodomous and monogynous (Breen 1979; Maki-Petays & Breen 2007). The extent, and form, of interspecific competition between wood ants is unknown, but it may have profound effects on many aspects of a species life-history, including nesting behaviours (e.g. Droual 1984; Dahbi et al. 2008). The hypothesised development of polydomy in a wood ant population is based on competition for nest sites. Interspecific competition could affect this in a variety of ways, for example, species could compete directly for nest sites. A thorough understanding of the extent to which different red wood ant species compete for the same nest-sites would give predictions of which species are expected to be polydomous within a particular area. These predictions could then be tested against the known patterns of monodomous and polydomous populations.

In our studies we have no information about relatedness either between nests within the colony or between colonies. High genetic viscosity has been found in many polygynous wood ant populations (e.g. *F. paralugubris*: Chapuisat et al. 1997; Chapuisat and Keller 1999; *F. lugubris*: Gyllenstrand and Seppä 2003; *F. aqulionia*: Mäki-Petäys et al. 2005; Pamilo et al. 2005). Based on our results showing that colony expansion is based on budding from existing nests, a similar pattern might be expected at Longshaw; with nests within the colony showing a closer relatedness than unconnected nests. It would also be interesting to investigate how the intracolony relatedness patterns match the dynamic changes we have observed in the nest-network structure. For example, ants in a newly budded nest would be expected to be more closely related to ants in their founder nest than to ants in the rest of the colony. Examining the relatedness between polydomous colonies would also reveal interesting information about the way that new colonies are formed. Under the hypothesis that new colonies are founded by separating from existing polydomous colonies, nearby unconnected nests are expected to be more closely related than more distant unconnected nests.

Understanding the relatedness within and between nests is also important for understanding how selection may act in these populations. In general, relatedness within polygynous wood ant nests is very low, for example in a Finnish *F. aquilonia* population within nest relatedness was close to zero for both queens (r=0.02 -0.13) and workers (r=0.01-0.22) (Pamilo et al. 2005). However, in the unicolonial *F. paralugubris* there does appear to be genetic differentiation between nests within a population, even though effective within-nest relatedness is very low (Holzer et al. 2006). In the studies presented in this thesis we had no information about the relatedness within or between nests; this is a limitation of our work, and an important avenue for further study. Investigation of relatedness between nests and colonies is important to understand how selection is acting in these populations, and how differential success of colonies translates into changing gene frequencies in a population.

More generally, in these studies we have not investigated reproduction (i.e. production of gynes and males). We have equated colony expansion, with colony success, whereas fitness is actually decided on the basis of production of sexual offspring. Our results show that, in many aspects of their life-history, nests within polydomous wood ant colonies act like monodomous nests. It would be very interesting to investigate if this similarity to monodomous nests extends to the production of sexual offspring. This could be achieved by intensive observations of polydomous colonies in early-spring, when sexuals are being produced. This data could then be applied to, for example, network position the previous autumn, to see if nests which had a higher flow of resources produced more sexual offspring, or a different sex-ratio of sexual offspring. If this were the case it would mean that network position has very clear fitness consequences for the queens (and perhaps the workers depending on intranest relatedness) within that nest.

Another interesting question that we have not investigated as part of this thesis is the relationship between nest-mate recognition and polydomy. Ants use cuticular hydrocarbons to differentiate between nestmates and non-nestmates. The extent to which genetic and environmental factors influence hydrocarbon profiles appears to vary between ant species (e.g. Buczkowski & Silverman 2006; Sorvari et al. 2008; Zweden et al. 2009). Broadly, in a monodomous colony, all members of the colony will share the same cuticular hydrocarbon profile, which facilitates recognition of nestmates, and aggression towards non-nestmates. In a polydomous wood ant colony nest-mate recognition is complicated by the spatial separation (and therefore potentially differing environmental conditions) of parts of the colony, and the very low intra- and inter- nest relatedness. Above we discuss how colony separation within a population may be based on parts of the colony physically separating from their parent colony, and therefore effectively becoming separate functional unit. We did not examine the role that loss of nest-mate recognition may have in this process. Physically separated parts of the colony may diverge in cuticular hydrocarbons (Sorvari & Hakkarainen 2004), which is is likely to play an important role in colonies reforming internest trails in the spring. Investigating how the cuticular hydrocarbons vary between nests in a colony, and how this varies with time and the structure of the nest-network, is important to fully understand how colonies are separated within red wood ant populations.

7.4 General Discussion

Polydomy is not confined to the red wood ant group. The nesting strategy has been recorded in 166 ant species filling many different ecological niches (figure 1.1; Debout et al. 2007). Although the ecology and evolutionary history of these different species is very varied, all polydomous colonies are likely to need a mechanism to redistribute resources between nests. In this thesis we have found a simple mechanism of resource redistribution between nests within polydomous wood ant colonies. If simple resource redistribution mechanisms are common in polydomous species, it may help explain the repeated evolution of the nesting strategy. A simple resource redistribution mechanism may mean that in other polydomous ant species, like in wood ants, there is minimal evolutionary innovation needed to switch between monodomous and polydomous nesting strategies. If the development of polydomy in a population is evolutionarily simple then polydomy would be expected to evolve in ant populations when it provides a fitness benefit. This fitness benefit does not have to be the same in different circumstances; indeed it is likely to be different, as polydomy has been suggested to provide many different advantages in different species (e.g. dispersed central place foraging, Cerdá et al. 2002; territorial defence, Hölldobler & Lumsden 1980; evading competitors, Dahbi et al. 2008) . This may help explain the wide phylogenetic spread, and lack of common ecological niche, of polydomy in the ants.

Dispersed nesting strategies are particularly common in invasive ant species, indeed all five of the ant species in the IUCN list of the world’s worst invasive species are polydomous (Lowe et al. 2004; Robinson 2014). Although the life-histories of these invasive species tend to be very different from those of red wood ants, our results may offer some insights into how the dispersed supercolonies of these species operate. For example, an important determinant of the success of many invasive species may be their ability to rapidly discover and exploit new food sources (Heller & Gordon 2006). A dynamic nesting system, such as that described in this thesis, would be an efficient and self-organising way to rapidly discover and exploit, new food sources. The difference between the wood ant system and a similar system operating in an invasive species, is likely to be the timescale over which it operates; whereas wood ant nests survive, grow and reproduce over the scale of months, for invasive species nests are likely to survive, grow and reproduce over the timescale of days. Understanding the dynamics of how the polydomous system changes with time, and how this relates to the ability of individual nests to acquire resources is likely to be important to understanding the spread of an invasive species through a habitat.

Understanding how polydomous species spread through a habitat is also important for the wood ants themselves. As a keystone species it is important to better understand how wood ant colonies function to help preserve natural woodland ecosystems. Our intensive study at the Longshaw estate has highlighted, how even a comparatively small area can maintain a very high wood ant population, if the conditions are favourable. The results presented here also show that, despite the high investment in nests, the interactions between nests allow the colonies to continue even if some nests are lost. The dynamic nature of the colony structure also illustrates how, on a long enough timescale, wood ant colonies are likely to be able to react to changes in environmental food availability caused by, for example, the felling of a tree. However, they also highlight how this change will be slow; colonies are unlikely to be able to restructure their nest-network quickly enough to react to rapid changes in food distribution. A related point is that, as the direction of colony expansion is dictated by the presence of honeydew resources, colonies are less likely to expand across any large areas without trees. This means they are likely to be particularly vulnerable to habitat fragmentation.

In this thesis we have shown how resource redistribution through a complex system can be achieved by simple self-organised rules. Understanding a transportation network in several dimensions (local, global and temporal) gives important insights into how the system as whole functions. For example, an interesting observation is the lack of efficiency at all levels of the system: on all trails there are ants carrying resources in both directions, even on trails between non-foraging and foraging nests. Similarly, at the colony-level, trails which would be important for colony-level resource redistribution are neither stronger nor more likely to survive than those with less colony-level importance. Within transportation networks the most usually discussed trade-offs are those between: network efficiency, network robustness and the costs of network infrastructure (Perna & Latty 2014). The results presented here may represent another trade-off between efficiency and simplicity. Although the red wood ant polydomous network is not an efficient transportation network it is governed by simple rules. Resources are redistributed through the network by individual workers treating other nests as food sources, and the network itself changes in response to the ability of a particular nest to acquire resources. This system requires no colony-level organisation or structure, which is likely to be important for it repeated evolution, and can operate on the basis of very simple rules given to the agents involved.

The studies presented in this thesis have focused on understanding the proximate organisation of polydomy in *F. lugubris*. Within Tinbergen's 'four questions' framework (Tinbergen 1963): we have focused on the 'causation' question by investigating the mechanism of resource redistribution between the nests of the colony. We also investigated the factors influencing how the colony structure changes through time via the foundation and abandoning of nests: a form of colony development (Tinbergen's 'Ontogeny'). However understanding these proximate causes has given insights into the ultimate functions of the strategy. The simple resource redistribution mechanisms we found may facilitate the evolution of the nesting strategy (Tinbergen's 'Evolution'). Similarly, the importance of resource redistribution to the structure of the nest-network suggests that more efficient foraging may be the main benefit of the polydomous nesting strategy (Tinbergen's 'Survival value'). Our results highlight how the proximate causes of a behaviour are intimately intertwined with the ultimate function of that behaviour, and that only by understanding both can we fully explain natural phenomena.

Appendices

Notes on the Appendices

Appendix A

All the colony nest-network maps used in chapter 3 have been published as ‘supplementary materials 1’ with Ellis et al. (2014). These same maps (with the addition of three others) were used as the first time-point in the dynamic network analysis presented in chapter 6. All the time-points of all the colony nest-networks sued in chapter 6 are presented in this appendix. Those also used in chapter 3 are those labelled as summer 2012. The names given to these colonies in chapter 3 are noted and the foot of each page. Those not included are labelled ‘not included’.

All nest-networks are labelled with the year in which they were mapped. ‘a’ indicates a late-spring map, ‘b’ indicates a late-summer’ map. All maps from 2012 were collected in late-summer.

Appendix B

Published as ‘Supplementary materials 2’ with Ellis et al. (2014)

Appendix C

Published as ‘Supplementary materials 3’ with Ellis et al. (2014)

Appendix D

Published as ‘Supplementary materials 4’ with Ellis et al. (2014)

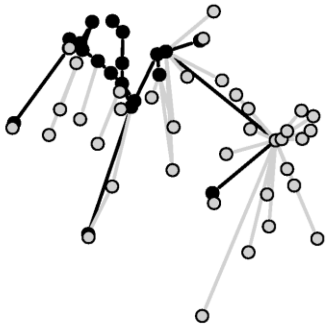
Appendix E

Published as ‘Supplementary materials 5’ with Ellis et al. (2014)

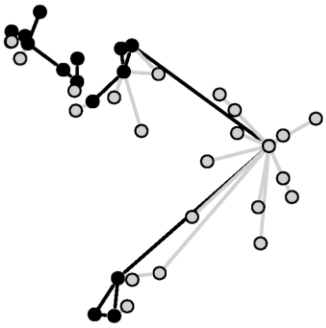
Appendix A: Colony Maps

I

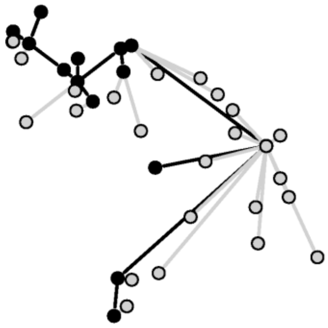
2012



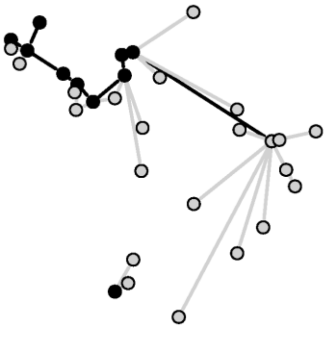
2013a



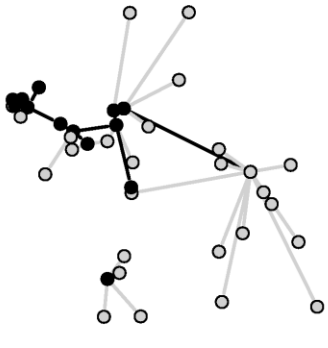
2013b



2014a



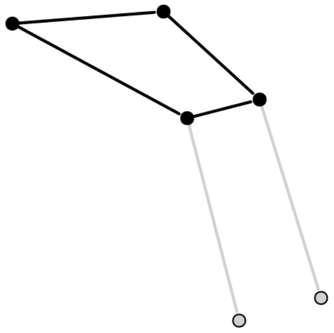
2014b



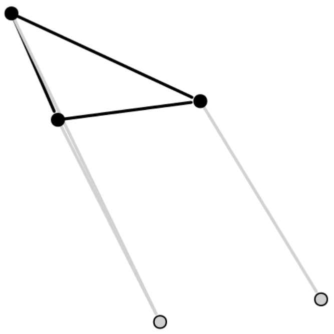
‘2012’ included in chapter 3 as colony 1.

IIa

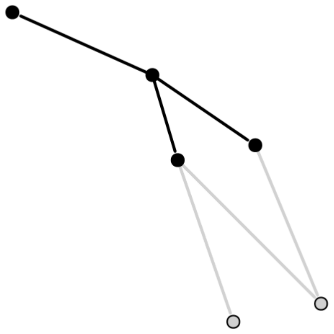
2012



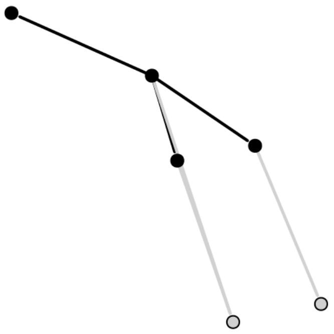
2013a



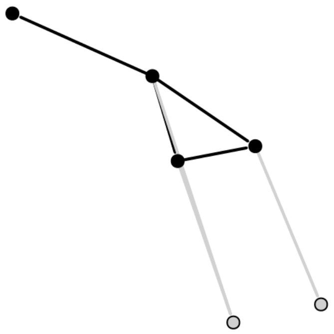
2013b



2014a

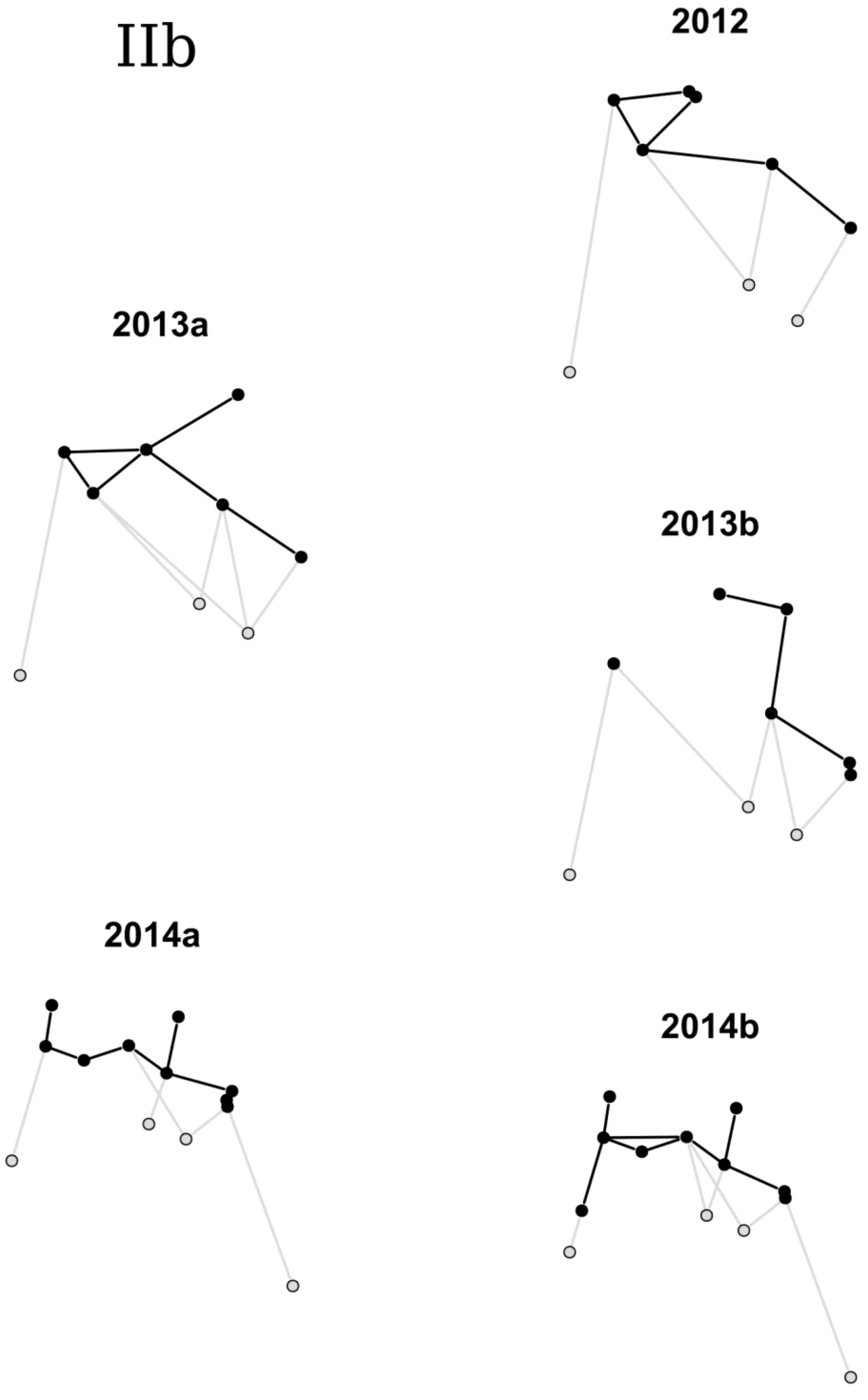


2014b



‘2012’ not included in chapter 3.

IIb



2012

2013a

2013b

2014a

2014b

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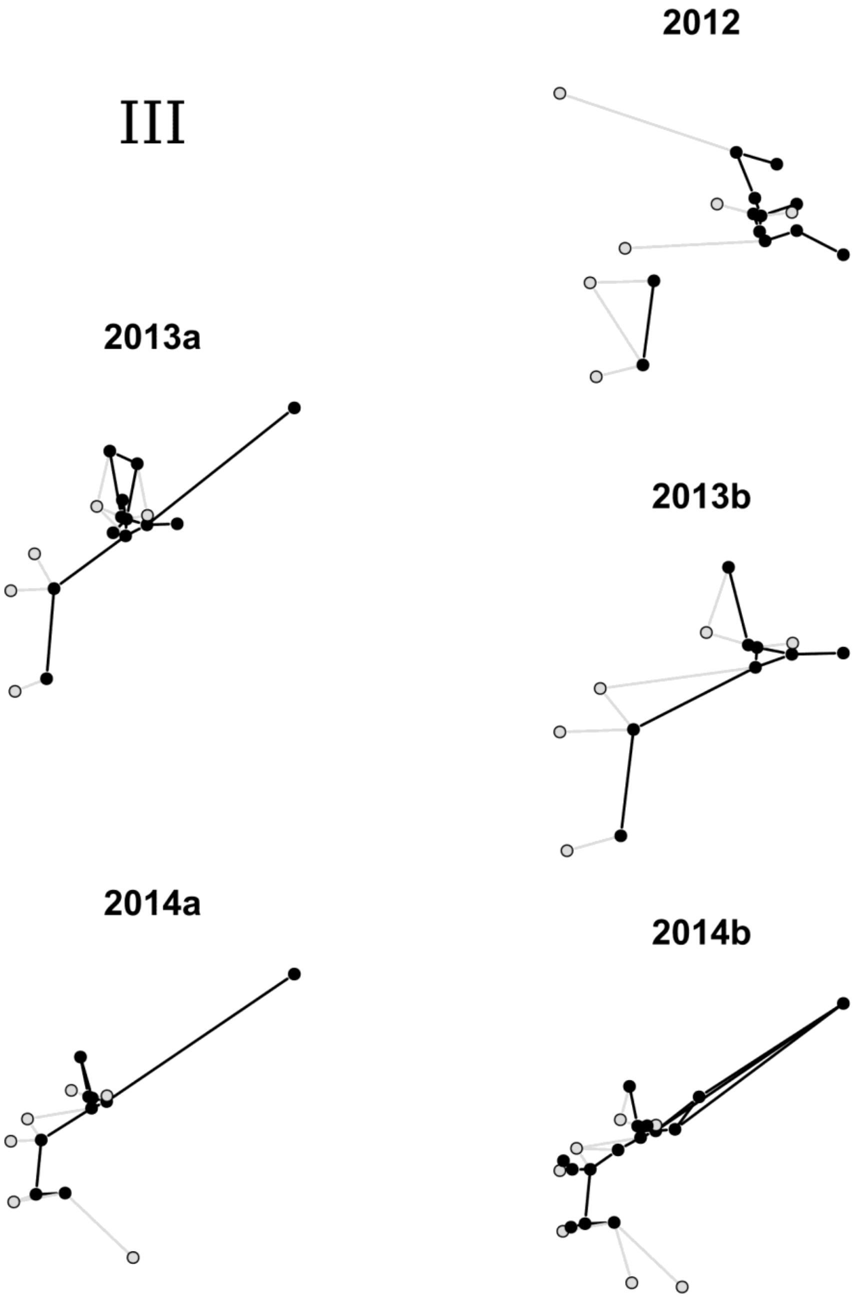
III



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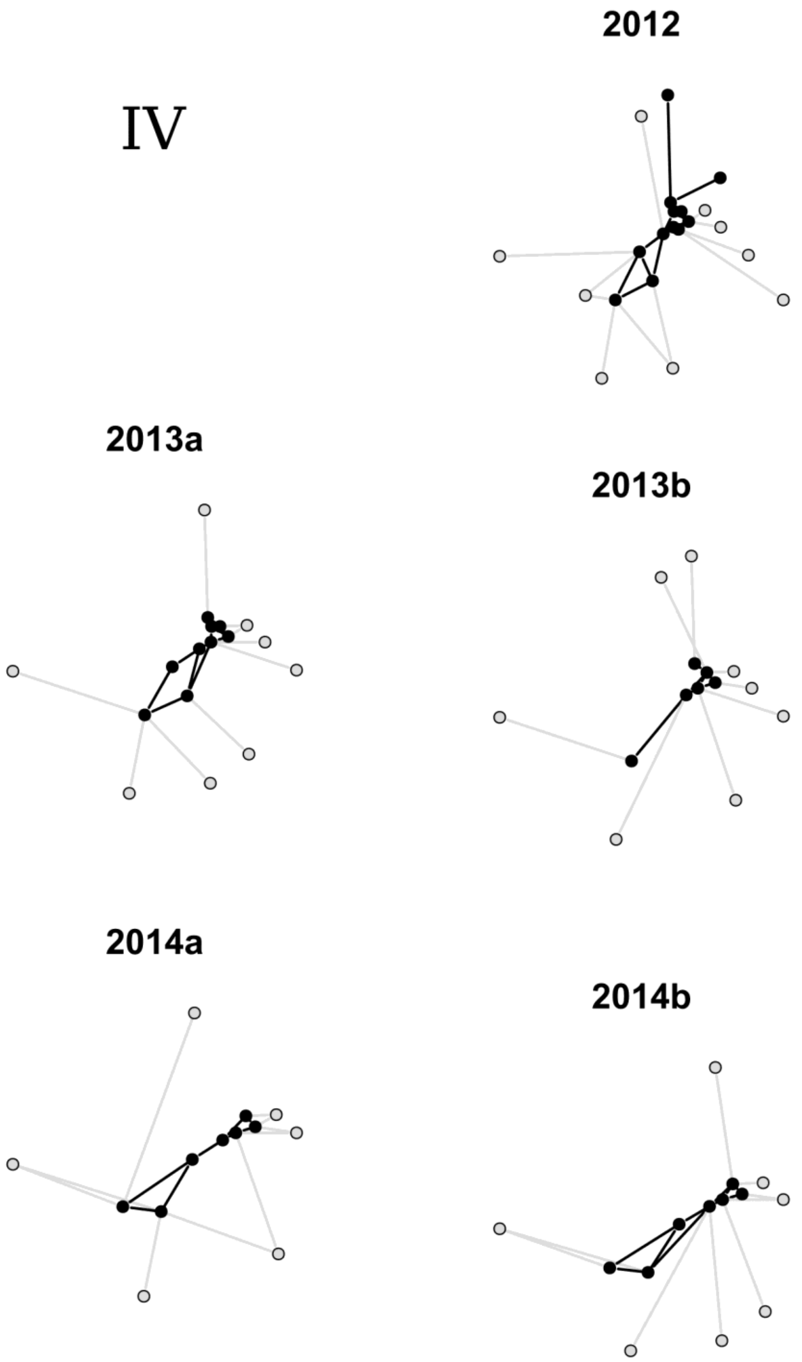
IV



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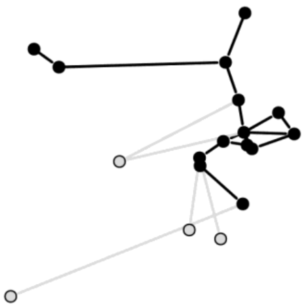

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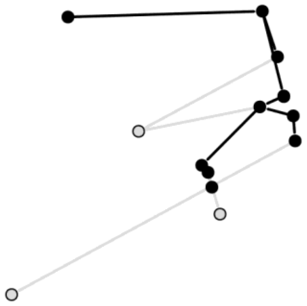

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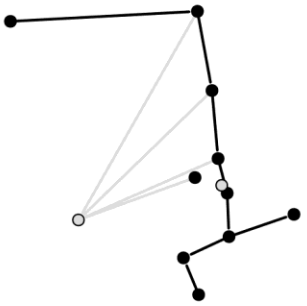

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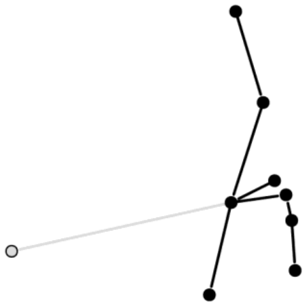

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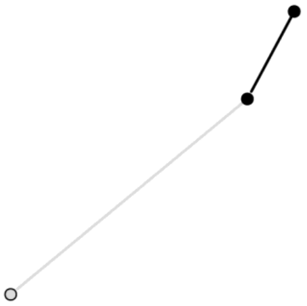

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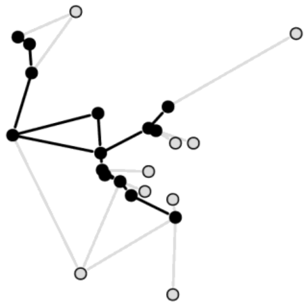
VI

2012



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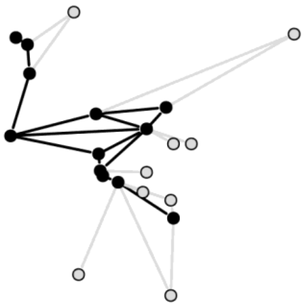

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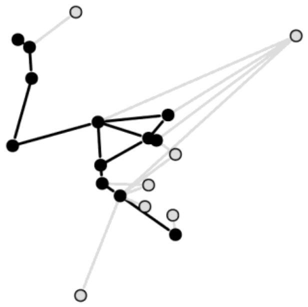

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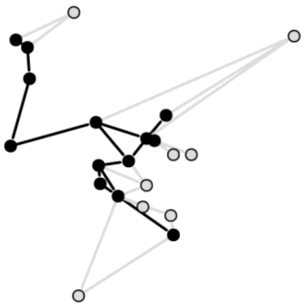

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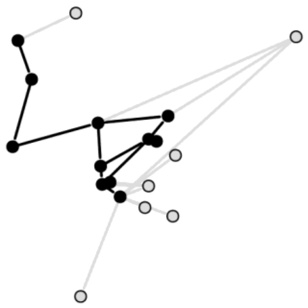

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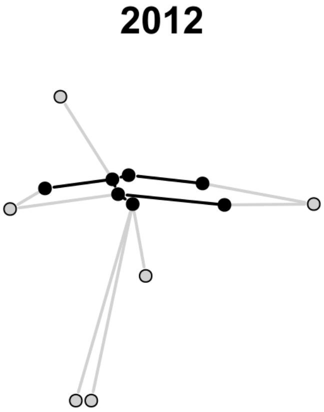
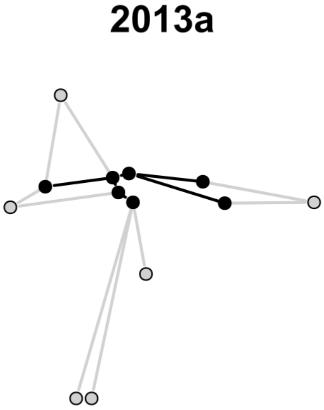
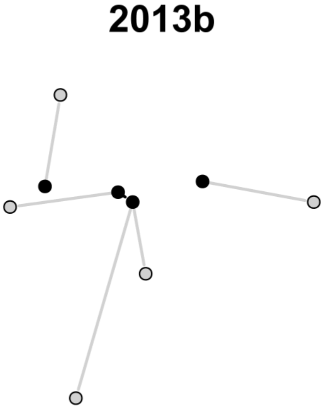
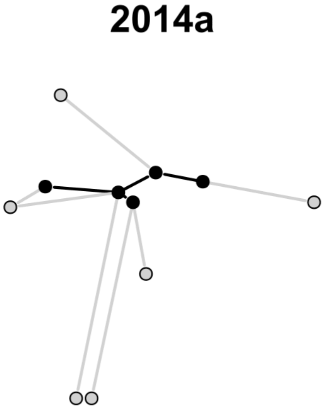
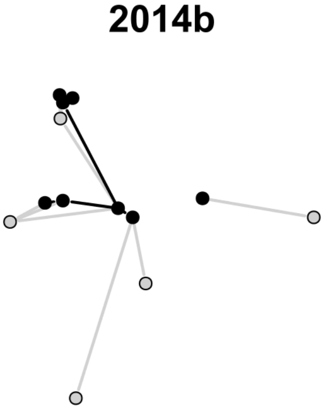
VII











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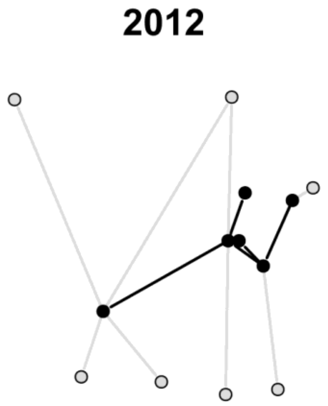
VIII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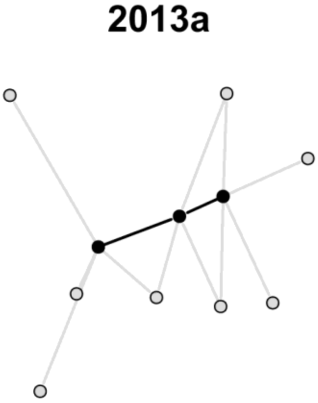
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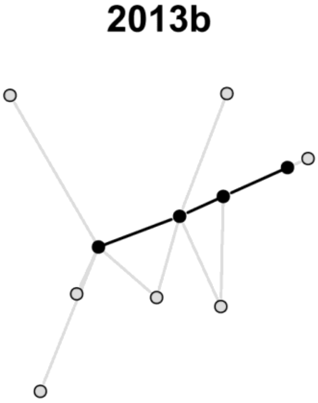
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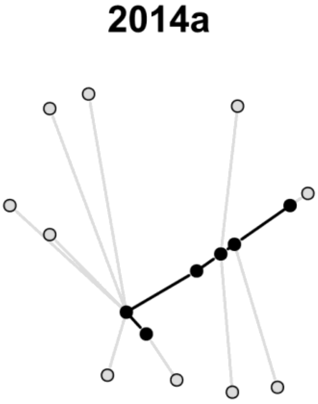
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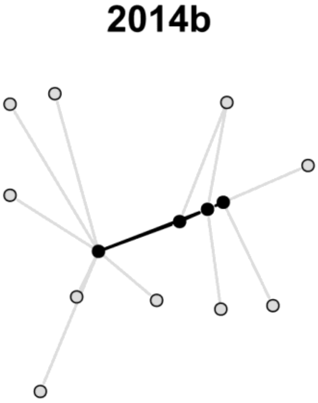
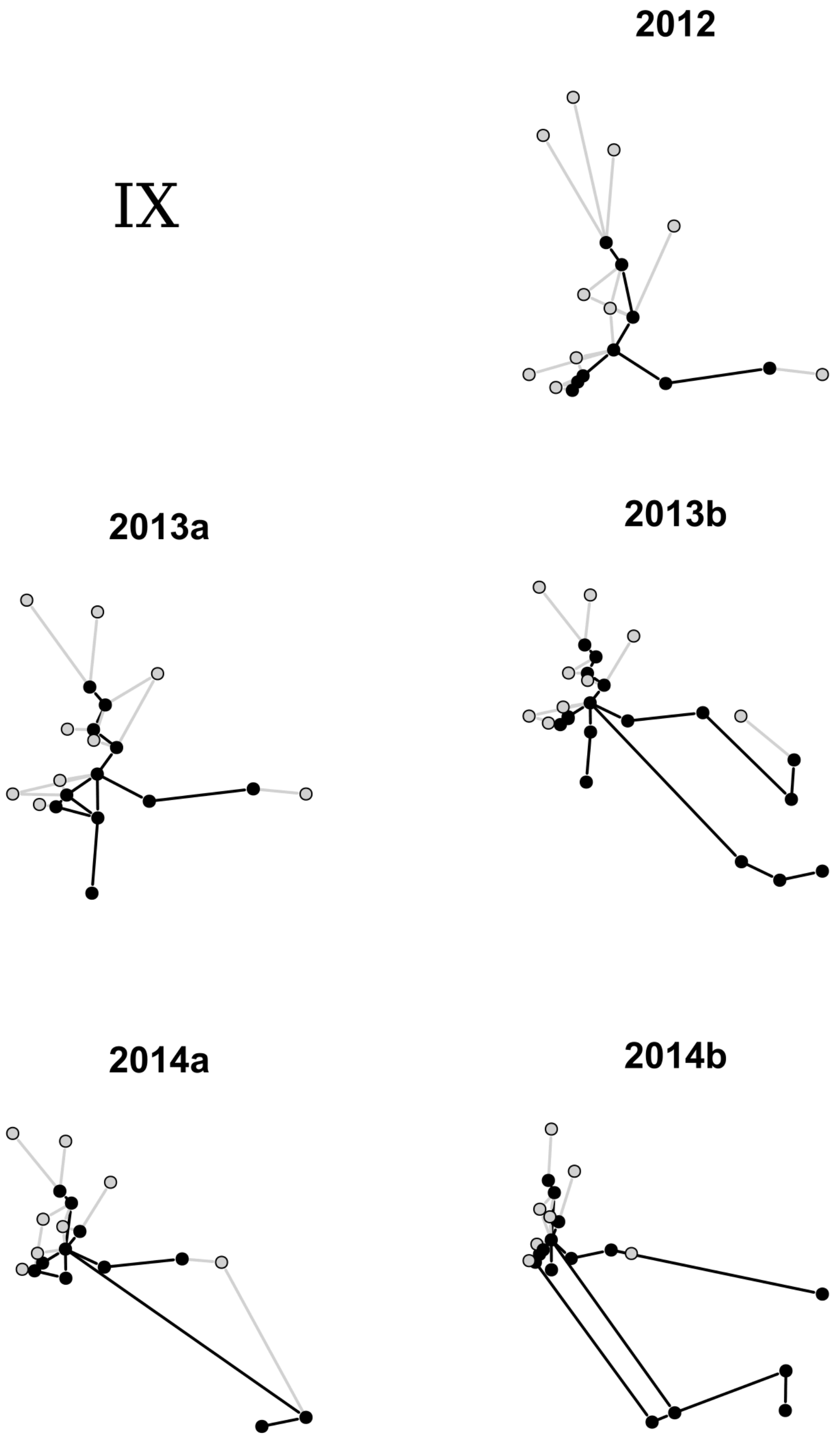

2014b

‘2012’ included in chapter 3 as colony 7.

IX



2012

2013a

2013b

2014a

2014b

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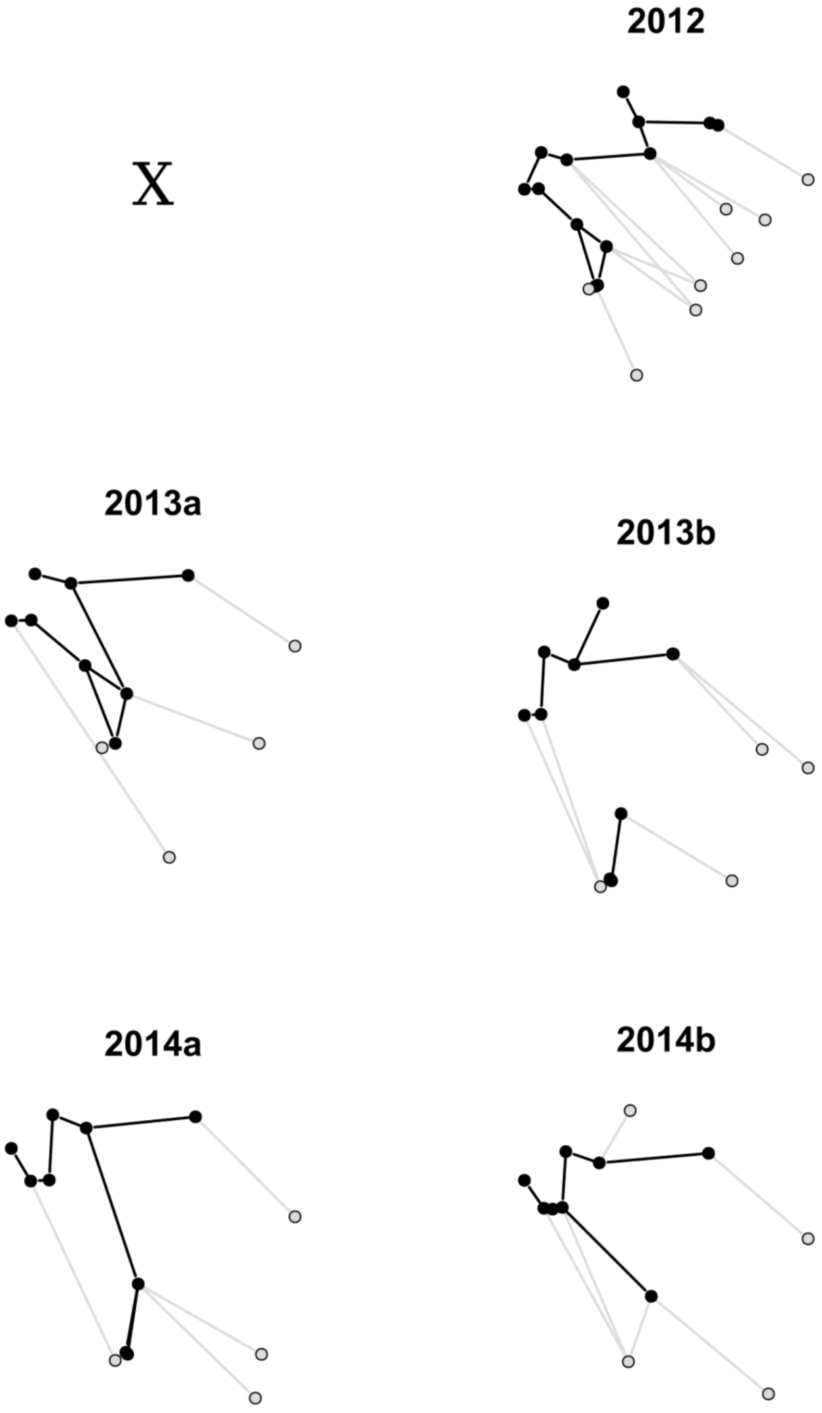

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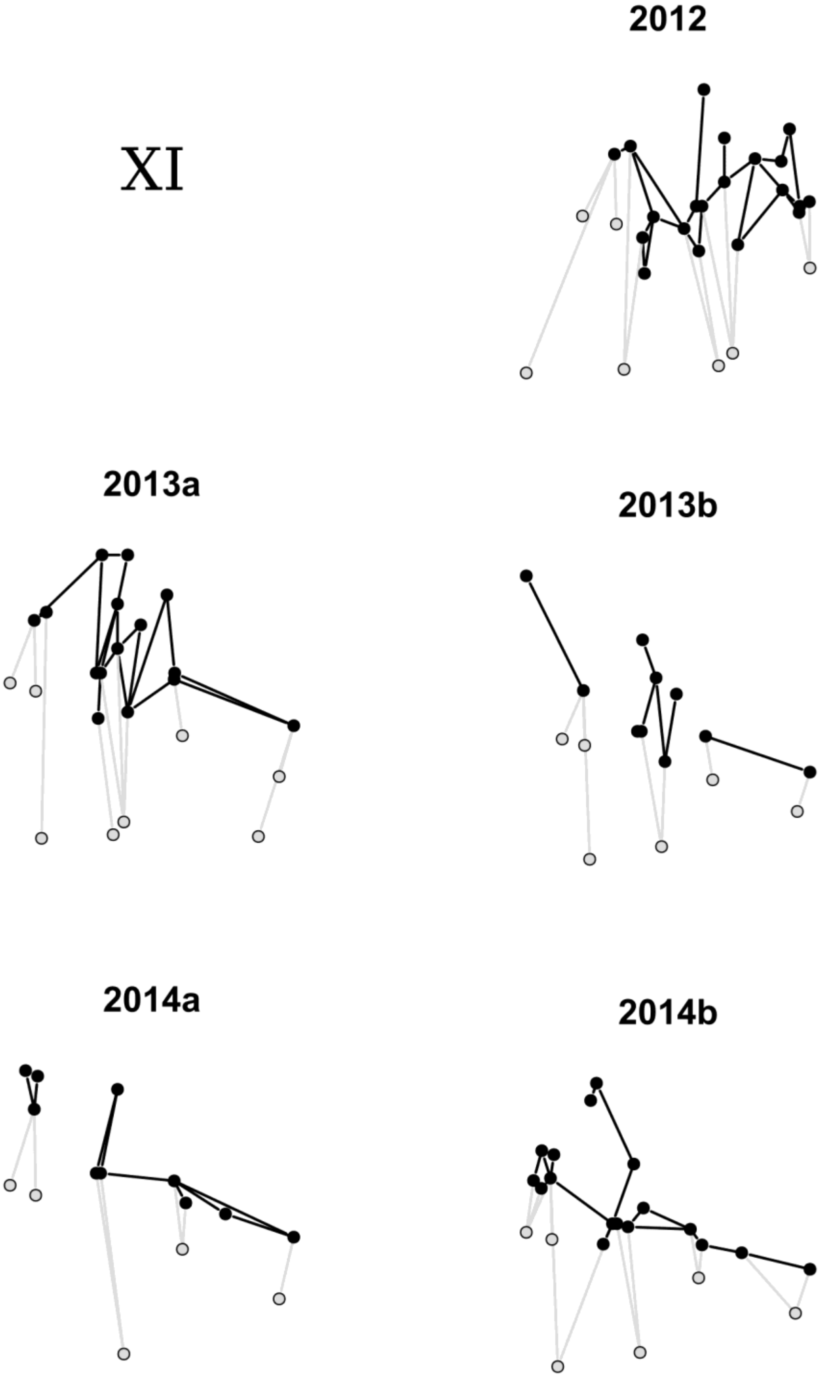
XI



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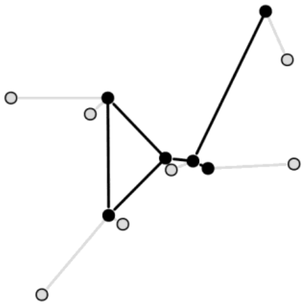
XII

2012



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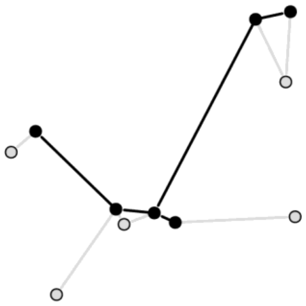

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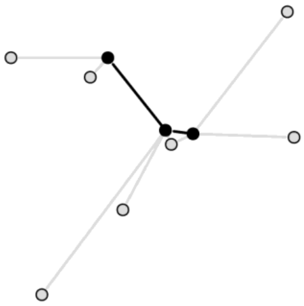

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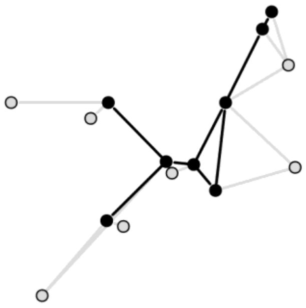

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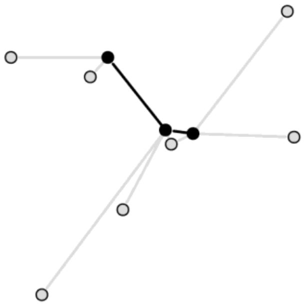

‘2012’ not included in chapter 3.

Appendix B: Nest properties vs. Network Properties

Summary of tests of the relationships between nest properties (rows) and network structural properties (columns) in *F. lugubris* polydomous colonies. ρ values refer to the Spearman's rank correlation coefficient, χ^2 to Kruskall Wallis statistic. Centrality tests were performed individually for each network as the values are reliant on the number of nodes and therefore cannot be directly compared. If n<10 statistics were considered unreliable and are not included. We performed the Bonferroni correction to control for repeated testing on the same data.

	Connectedness	**Centrality**	
	Degree (n=140)	Closeness	Betweenness
Size	*Unweighted* (χ^2=8.45,p=0.952) *Weighted* (ρ =-208,p=0.152)	*Unweighted* p<0.05: 0/10 Networks *Weighted* p<0.05: 1/10 Networks	*Unweighted* p<0.05: 1/10 Networks *Weighted* p<0.05:0/10 Networks
Foraging vs. Non-Foraging	*Unweighted* (χ^2=0.921,p=1.000) *Weighted* (χ^2=0.214,p=1.000)	*Unweighted* p<0.05: 0/10 Networks *Weighted* p<0.05: 0/7 Networks	*Unweighted* p<0.05:0/10 Networks *Weighted* p<0.05:0/7 Networks
Amount of Foraging	*Unweighted* (χ^2=4.14,p=1.000) *Weighted* (ρ =0.14,p=0.856)	*Unweighted* p<0.05: 0/10 Networks *Weighted* p<0.05:0/7 Networks	*Unweighted* p<0.05: 0/10 Networks *Weighted* p<0.05: 0/7 Networks
Canopy cover	*Unweighted* (χ^2=1.06,p=1.000) *Weighted* (ρ =0.02,p=1.000)	*Unweighted* p<0.05: 0/10 Networks *Weighted* p<0.05: 0/10	*Unweighted* p<0.05: 0/10 *Weighted* p<0.05: 0/10(1)

Appendix C: Assortativity by colony

Assortativity of nest size and amount of foraging within polydomous *F. lugubris* colonies, *r* is Newman's Assortativity coefficient; a positive value shows positive assortment. An asterisk indicates p<0.05 and a dot indicates p<0.1) All p-values have been adjusted with a Bonferroni correction to control for repeated assortativity tests on the same colony (table 3.2).

Colony	**Size**		**Amount of Foraging**	
	r	*p*	*r*	*p*
1	-0.05	1.00	-0.10	1.00
2	-0.42	0.273	-0.49	0.115
3	0.00	1.00	-0.12	0.993
4	-0.37	0.039*	-0.04	1.00
5	-0.32	0.252	-0.18	1.00
6	0.28	0.225	-0.09	1.00
7	0.18	0.249	0.51	0.033
8	-0.05	1.00	0.07	0.477
9	-0.25	0.798	-0.12	1.00
10	0.10	0.234	0.15	0.345

Appendix D: Trail Strength vs. Trail Betweenness

The relationship between the strength of a trail and the trail betweenness. All statistics are Spearman’s Rank Correlation tests with a p value constructed from constrained randomisation (see methods). If nest number was less than 10 the statistics were considered unreliable and not included.

Colony	**ρ**	**p**
1	-0.30	0.09
2	-0.19	0.262
3	0.009	0.466
4	-0.006	0.478
5	-0.13	0.313
6	-	-
7	-0.05	0.418
8	-	-
9	0.11	0.632
10	-0.02	0.468

Appendix E: Trail Type vs. Trail Betweenness

Differences in trail betweenness for different types of trail for weighted (F-F, F-nF, nF-nF) and unweighted networks. All statistics are Kruskall-Wallis tests with a p value constructed form constrained randomisation (see methods). If nest number was less than 10 the statistics were considered unreliable and not included.

Colony (n)	**Unweighted**		**Weighted**	
	Kruskall -Wallis χ^2	*p*	*Kruskall -Wallis* χ^2	*p*
1 (22)	2.64	0.282	1.95	0.416
2 (10)	1.99	0.552	1.18	0.674
3 (21)	0.57	0.755	1.19	0.564
4 (14)	2.69	0.258	4.67	0.069
5 (14)	1.44	0.541	1.00	0.631
6 (7)	-	-	-	-
7 (10)	0.01	0.844	0.01	0.838
8 (9)	-	-	-	-
9 (13)	2.39	0.309	1.89	0.480
10 (20)	3.39	0.148	0.25	0.893

Appendix F: Chapter 4 GLMM details

Details of the statistics used in the study. # refers to the superscript number in the text. The Dependent variable, fixed effects and random effects describe the GLMM used, all used a binomial error structure. In all tests errors were heteroscedastic and were not overdispersed. χ^2, *df* and *P* describe the results of an analysis of deviance, which compares the model to a null model which lacks the variable of interest.

#	**Response Variable**	**Fixed Effect(s)**	**Random Effect(s)**	χ^2	***df***	***P***
4.1	Direction 1 load	Direction 2 load	Colony/trail type	48.1	1	<0.001
4.2	Direction 1 load	Direction 2 load , colony	trail type	34.2	4	<0.001
4.3	Direction 1 load	Direction 2 load , trail type	Colony	24	2	<0.001
4.4	Consistent journeys	Inconsistent journeys	Colony/day, trail type	11.7	2	<0.001
4.5	Consistent vs. Inconsistent journeys	Paint pattern	colony/trail type/ day	34	3	<0.001
4.6	Consistent vs. Inconsistent journeys	Paint pattern, trail type	Colony/day	35.5	2	<0.001
4.7	Consistent vs. Inconsistent journeys	Day	Ant type| Colony/trail type	4.8	4	0.31
4.8	Consistent vs. Inconsistent journeys	Day x Ant type	Colony/ trail type	43.0	5	<0.001
4.9	Consistent vs. Inconsistent journeys	Day x Ant type	Colony/ trail type	5.0	2	0.03
4.10	Stray journeys	Ant type	Colony/ trail type/ day	1.7	2	0.44
4.11	Stray journeys	Trail type	Colony/ trail type	1.8	1	0.18
4.12	Consistent vs. Inconsistent journeys	Direction	Colony/ trail type/ day	19.8	1	<0.001
4.13	Consistent vs. Inconsistent journeys	Direction	Colony/ trail type/ day	14.0	1	<0.001
4.14	Flow AB direction vs BA direction	Trail type	Colony/day	18.5	2	<0.001

Appendix G: Chapter 5 GLMM details

Details of the statistics used in the study. # refers to the superscript number in the text. The Dependent variable, fixed effects and random effects describe the GLMM used, all used a binomial error structure. In all tests errors were heteroscedastic and were not overdispersed. χ^2, *df* and *P* describe the results of an analysis of deviance, which compares the model to a null model which lacks the variable of interest.

#	Dependent Variable	Fixed Effect(s)	Random Effect(s)	χ^2	*df*	*P*
5.1	Survival	F/nF	Colony Year	5.63	1	0.01
5.2	Survival	Nest size	Colony Year	56.1	1	<0.001
5.3	Survival	F/nF Nest size	Colony Year	5.64	1	0.03
5.4	Survival	F/nF Nest size	Colony Year			
5.5	Changed/Unchanged	F/nF	Colony Nest ID	13.7	2	<0.001
5.6	Changed/Unchanged	Relative distance to the nearest	Colony Nest ID	4.21	1	0.04
5.7	Changed/Unchanged	Survival	Colony Nest ID	9.5	1	0.002
5.8	New/Established	Linear distance to nearest tree	Colony Year	0.19	1	0.66
5.9	Relative number of extra-nest workers	F/nF	Colony|day Temperature	19.19	1	<0.001
5.10	Relative number of extra-nest workers	F/nF Colony	Colony|day Temperature	54.7	14	<0.001
5.11	Prey towards nF nest	Prey towards F nest	Colony Temperature	0.043	1	0.84
5.12	Prey bearing journeys (prop.)	Trail type Colony	Colony|day	128	15	<0.001
5.13	F/nF	Canopy Cover	Colony	0.9	1	0.34
5.14	Brood towards nF nest	Brood towards F nest	Colony Temperature	0.09	1	0.80
5.15	Brood bearing journeys (prop.)	Trail type Colony	Colony|day	371	15	<0.001
5.16	Larvae towards nF nest	Larvae towards F nest	Colony Temperature	1.81	1	0.18
5.17	F/nF	Species of the nearest tree	Colony	4.76	4	0.31

Appendix H: Chapter 6 GLMM details

Details of the statistics used in the study. # refers to the superscript number in the text. The Dependent variable, fixed effects and random effects describe the GLMM used, all used a binomial error structure. In all tests errors were heteroscedastic and were not overdispersed. χ^2, *df* and *P* describe the results of an analysis of deviance, which compares the model to a null model which lacks the variable of interest.

#	**Dependent Variable**	**Fixed Effect(s)**	**Random Effect(s)**	χ^2	*df*	*P*
6.1	Founder or non-founder	Normalised Betweenness	Colony Nest ID Season	8.5	1	0.004
6.2	Founder or non-founder	Change in normalised betweenness	Colony Nest ID	8.9	1	0.003
6.3	Founder or non-founder	Worker:foraging ratio	Colony Nest ID Season	0.15	1	0.70
6.4	Founder or non-founder	Proportional change in worker:foraging ratio	Colony Nest ID	0.04	1	0.8
6.5	Founder or non-founder	Nest Size	Colony Nest ID Season	0.2	1	0.65
6.6	Founder or non-founder	Proportional change in nest size	Colony Nest ID	0.027	1	0.87
6.7	Founder or non-founder	Linear distance to the nearest tree	Colony Nest ID	0.019	1	0.66
6.8	Founder or non-founder	Percentage canopy cover	Colony Nest ID	0.78	1	0.38
6.9	Founder or non-founder	Season	Colony Nest ID	0.16	1	0.69
6.10	Proportional Change in nest size	Change in normalised betweenness	Colony Nest ID Season	19.9	1	<0.0001
6.11	Proportional Change in nest size	Proportional change in mean number of foragers per nest	Colony Nest ID	0.14	1	0.7
6.12	Proportional Change in Nest size	Linear distance to the nearest tree	Colony Nest ID	0.0818	1	0.77
6.13	Proportional change in nest size	Percentage canopy cover	Colony Nest ID	0.85	1	0.38

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