An analysis of the dynamics of resource sharing networks in ant colonies

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Abstract

Cooperation ties animals together into social groups that often demonstrate complex emergent behaviours. One striking example of this are social insect societies that emerge from extreme cooperative behaviour and have an important impact in many ecosystems. Colonies of most social insects construct and inhabit a single nest. However, colonies of some species have been found to spread across many different nests – known as polydomy. This strategy is thought to have evolved in response to several different drivers, such as increased foraging efficiency, avoiding nest-size limitations and territory defence. In this thesis, I present studies that investigated how polydomous colonies function and the reasons that some, but not all, ant species use polydomy as a strategy. I used a mathematical model to demonstrate that decentralization of the nest population can be advantageous under a variety of different conditions, which explains why polydomous species are so behaviourally and phylogenetically diverse. Using a longitudinal study of several years of data on a set of polydomous colonies I found that resource sharing networks become more static over a season and that while nest foundation occurs throughout the season, nest abandonment occurs at a much faster rate in the latter part of the season. Through sampling several polydomous colonies, I found that there is a correlation between relatedness of the inhabitants of different nests and the rate of resource sharing between the nests. The most likely mechanism for this is that nests that share resources become more closely related due to migration and brood transfer. Finally, using a resource manipulation study, I found that resource-sharing networks can adapt in response to change in the availability of food. Together these results demonstrate how ant colonies can use polydomy to their advantage and the various factors that are important in determining the dynamics of these complex societies.

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Author Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

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Chapter 1 - Introduction

General Introduction

Many animals cooperate with others through social interactions such as food sharing and grooming. These repeated acts of cooperation bind large numbers of animals together into groups. Group-living can convey important benefits to individuals, such as increased defence against predators and higher foraging efficiency. However, group-living also incurs costs, such as an increased rate of disease transmission and a higher demand for resources. Groups are also vulnerable to cheating, whereby individuals access the benefits of group-living without suffering the costs. Together this means that group-living may be difficult to maintain without significant benefits to individuals.

The importance of cooperation to groups is most obvious in eusocial societies, such as is found in many social insects. In a eusocial insect colony, non-reproductive workers can attain fitness benefits indirectly through promoting the success of reproductive individuals that they are related to. However, recent work has demonstrated that relatedness in colonies of many species is very low due to multiple-mating by queens and the presence of several queens. This is taken to an extreme by ant colonies that divide into sub-colonies that inhabit distinct nests that are connected through resource sharing on trails. This nesting strategy, known as polydomy, is found in a taxonomically and behaviourally diverse set of species, which indicates that it must provide some important benefits in a wide range of niches (Debout, 2007; Robinson 2014). In this thesis, I present models, experiments and observational studies using a polydomous ant as a study species, to investigate the way that polydomy functions and the important factors in driving its evolution.

Animal social networks

Introduction

Until recently animal social groups were studied by recording interactions between pairs of individuals. However, this approach cannot account for indirect interactions between individuals, which are often important (Brent 2015). An approach that deals with this issue is social networks, where groups of animals are considered as networks with the individuals or groups being represented by nodes and the interactions between those individuals being represented by edges (Croft et al. 2008; Farine and Whitehead 2015). This approach has been increasingly used in research on animal social groups in recent years and has led to many important discoveries about how such groups function. Social networks are now seen as an important framework for understanding the interactions in animal social groups and explaining how these interactions shape aspects of life history. For example, recent work has demonstrated that an individual's network position can influence survival (Lehmann et al. 2015), risk of infection (VanderWaal et al. 2014) and access to information (Farine et al. 2015).

Change in animal social networks

Until recently, animal social network analysis was almost exclusively limited to time-aggregated social networks that treated networks as static structures. This can be appropriate when networks change at a slow rate that doesn't result in drastic differences in network structure over the study period (Farine 2018). However, social networks are all dynamic to some degree - individuals change the direction and the frequency of their interactions (Croft et al. 2016). As a result of this, there have been significant efforts to develop dynamic network analysis techniques (Blonder et al. 2012; Fisher et al. 2017; Hobson et al. 2013; Silk and Fisher 2017).

One area of research that has benefited significantly from the use of dynamic network techniques is the study of robustness in networks. Robustness is a property of networks that describes how well a network can tolerate disturbance. Until recently, studies of network robustness involved simulated manipulations of networks such as removal of nodes or edges, where the potential effect of removing a node or edge from a static network was studied (e.g. Silvis et al. 2014). Simulated manipulations can be useful when studying networks that are static or not capable of rapid change, because in those cases simulations are likely to reflect how removals will affect network structure. However, simulated removals are not useful for predicting how networks that are capable of rapid change - as is the case for most animal social networks - will be affected by loss of nodes or edges. Instead, recent work has focused on experimental manipulations of networks (e.g. Firth et al. 2017) or observations of network adaptation in response to naturally changing conditions (Post et al. 1999). This work has demonstrated that, in certain cases, animal groups can alter social network structure to adapt to changes to environmental conditions (Firth et al. 2017; Post et al. 1999; Stroeymeyt et al. 2018). For example, Stroeymeyt et al. (2018) showed that ant colonies that are infected by contagious diseases change their social structure to limit the spread of disease. Adaptation of social network structure likely emerges from simple changes to individuals' behaviour in response to changing environmental conditions, but the way that this functions is still not well understood.

Analysis of social networks

Introduction and examples

Methods for analysis of networks are used to investigate systems composed of interconnected units, known as nodes, for example a molecule in a network of molecular interactions, a network of railway stations (Chopra et al. 2016) or a social network of friendships (Fowler and Christakis 2008). Network analysis is quickly becoming more common in Ecology for answering a variety of questions, and techniques developed for analysis of other types of networks are now being applied to a variety of topics within ecology including animal movement (Jacoby and Freeman 2016), species interactions (Meise et al. 2019; Pascual and Dunne 2006) and structures built by organisms (Perna and Latty 2014). The most common use of network analysis in Ecology is for the study of social interactions in animal social groups, which is known as Social Network Analysis (SNA) (Croft et al. 2008). Network analysis is well-suited for the study of animal social groups as it considers groups holistically, which is important as: 1) Analyses can account for lack of independence between individual data points; and 2) Analyses can control for or investigate indirect effects, which are often influential in animal social groups (Brent 2015). For example, information or disease spread through population can be studied as connections between all individuals are known, not just dyads (Silk et al. 2019).

What methods are used in SNA?

There are a variety of different methods in SNA that are used to analyse social networks data, which can be separated into two types: descriptive and statistical.

Until recently, almost all analyses used in SNA were descriptive. Descriptive statistics, such as centrality, a measure of a node's importance in a network, and density, a measure of how connected networks are, allow researchers to describe the structure of different social networks. Descriptive social network techniques are a powerful tool for identifying the social structure of different animal groups and the importance of this social structure in determining factors such as survival and disease transmission. For example, Stroeymeyt et al. (2018) show how ant colonies manipulate the modularity, clustering and assortativity of their social networks at the expense of efficiency in response to infection.

More recently, statistical techniques have become more common in SNA (e.g. Fisher et al. 2017; Silk and Fisher 2017). Statistical techniques are important as they allow investigation of the factors that are important in causing networks to form, many of which are not possible to model using the randomization techniques that are normally used in SNA (Silk and Fisher 2017). For example, Rouco et al. (2018) used an Exponential Random Graph Model (ERGM) to show that there was no difference in the connectivity (number of social interactions) of male and female brushtail possums in their social networks, which indicated that sex was not an important factor in the spread of bovine tuberculosis in the population. However, despite the increasing popularity of techniques for statistical network analysis, their use is limited to unweighted networks that are a certain size and where all interactions and nodes in the network are known (Silk and Fisher 2017). Furthermore, although there are extensions of statistical models to incorporate temporal information, spatial information, node attributes and trail strengths (Fisher et al. 2017; Silk and Fisher 2017), it is not currently possible to incorporate all this additional information in a single model.

Statistical methods can also be useful for investigating dynamic networks. For example, Hunt et al. (2018) used Stochastic Actor-Oriented Models (SAOMs) to demonstrate that although the boldness of individual spiders does not influence whether they form social connections, their social connections influence their boldness. This kind of analysis is only possible using dynamic network analysis as the order of events is important.

Polydomy

What is a colony?

Until recently, social insect colonies were generally believed to contain a single singly-mated queen and many highly related workers cooperating to produce the next generation of offspring. However, this view is no longer widely accepted to be true (Heinze 2008). Instead, colonies are now understood to be much more complex, often containing several multiply mated queens spread across several nests that share resources (Ellis et al. 2017b; Heinze 2008). As a result, defining what a colony is and determining the boundaries of a single colony are much more complex than previously believed (Ellis et al. 2017b).

There are several possible methods that can be used to define where a colony's boundaries are, but the outcome of these different tests do not always agree (Ellis et al. 2017b). For example, groups of ants inhabiting different nests may be genetically distinct, but still share resources with each other. If a functional definition of colony boundaries is used these two groups belong to the same colony. In contrast, if a genetic definition of colony boundaries is used the two groups belong to different colonies. Consequently, there is currently not a single definition of what a colony is. Ellis et al. (2017b) outline four such methods that can be used to establish where a colony's boundaries lie: (1) resource exchange; (2) spatial clustering; (3) mutual non-aggression; and (4) genetic similarity. In Chapter 3, I describe an observational study of several polydomous ant colonies that was performed over eight years that investigated resource exchange and spatial clustering of groups of polydomous nests. We then used this dataset to investigate the role of genetic similarity in determining colony boundaries, which I present in Chapter 4.

What is polydomy?

Social insects live in colonies that generally inhabit nests. The number of nests that a single colony inhabits can vary between a single nest, which is called monodomy, or multiple nests, which is called polydomy. In polydomy, the colony is spread over several nests that share resources with each other such as brood, food and workers to form a network of nests (Debout et al. 2007; Robinson 2014). This network of nests can be considered as a network where the individual nests are represented by nodes and trails represented by edges.

Polydomy has been identified in several ant species and a few termite species (Debout et al. 2007; Robinson and Robinson 2008). In ants, polydomy is spread widely throughout the phylogenetic tree of species and appears to have arisen independently several times (Debout et al. 2007) (Figure 1.1).

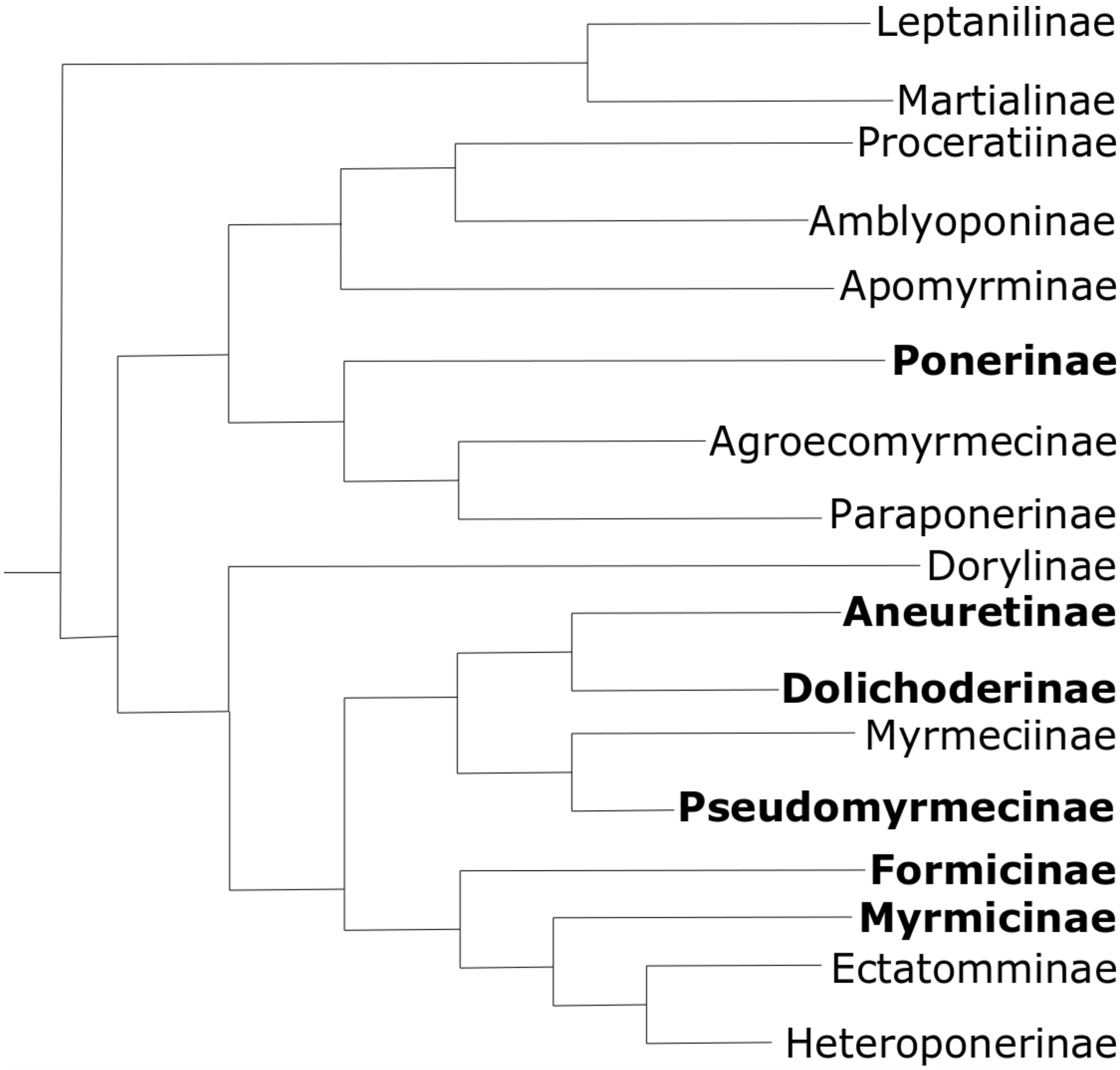


Figure 1.1 Phylogeny of ants, with families that are known to have polydomous species shown in bold. Phylogeny adapted from Borowiec et al. (2019). Species of polydomous ants are highlighted in bold as detailed in Debout (2007). Although there are many species that are known to be polydomous spread throughout the ant phylogeny, there are undoubtedly many more whose behaviour has not yet been described. Consequently, this is a non-exhaustive list of the ant families that contain polydomous populations.

The diversity of polydomy

Polydomous colonies of different species of ant are extremely diverse in size, behaviour, nest type, diet, dominance, and queen number (Table 1.1). For example, colonies of *Temnothorax* spp. that demonstrate polydomy are generally small (<500 individuals), only polydomous at certain times of the year, inhabit existing crevices, are not dominant and generally have a single queen. In contrast, colonies of *Formica* spp. that demonstrate polydomy are often very large (>10^5 individuals), polydomous throughout the year, build large over-ground nests, are very dominant and have several queens in each nest. This diversity in niche occupance means that it is difficult to identify the key evolutionary drivers of polydomy, probably because there are many situations where polydomy becomes advantageous.

Table 1.1. Case studies of polydomous species. Polydomy is found in a broad range of taxa that are behaviourally and functionally diverse.

Trait	***Formica rufa group spp.***	***Cataglyphis spp.***	***Temnothorax spp.***	***Oecophylla spp.***
Colony size	>10^5	<1000	<500	>10^5
Nest type	Overground mounds of plant material	Dug underground	Natural cavities	Leaves weaved together in trees
Number of queens per colony	Species dependent (1 or 1<)	1	Species dependent (1 or 1<)	1
Resource distribution	Large fixed resources	Small and ephemeral	Small and ephemeral	Small and ephemeral
Dominance	Dominant	Subordinate	Subordinate	Dominant
Habitat	Woodland	Desert	Species-dependent	Tropical

An important difference between colonies of polydomous species is the number of queens in the colony. Colonies can either have a single queen, called monogyny, or multiple queens, called polygyny. In monogynous polydomous colonies the queen produces brood which may then be transported to other nests. In some monogynous polydomous species, brood can be reared in a different nest than the queen, allowing workers to avoid queen control (Snyder and Herbers 1991). In contrast, in polygynous polydomous colonies queens in each nest produce brood which can then be reared in that nest, although brood transfer between nests may still occur (Debout et al. 2007; Robinson 2014). Although polygynous and monogynous polydomous colonies appear to function in similar ways, there are some important differences. For example, polygynous colonies generally produce new colonies through queens and a group of workers budding from the maternal colony. In contrast, monogynous colonies reproduce by independent foundation of nests by queens that are unaided by workers.

What are the hypothesised costs and benefits of polydomy?

Polydomy is hypothesised to convey several benefits to polydomous colonies, such as increased foraging efficiency, minimisation of damage to colonies from stochastic processes and competitive dominance (Debout et al. 2007; Robinson 2014). Polydomy is also hypothesised to incur several costs to polydomous colonies such as reduced communication (Cook et al. 2013), poorer defence against predators (Robinson 2014) and high costs of resource movement (Snyder and Herbers 1991). I describe the various benefits and costs of polydomy and investigate them further using a model in Chapter 2.

Polydomous colonies as networks

Polydomous colonies can be considered as networks with nests represented by nodes and the trails between nests represented by edges. Additionally, if food sources are easy to identify, foraging activity can also be considered as networks with food sources as nodes and foraging trails as edges (e.g. Buhl et al. 2009). Analysing polydomous colonies as networks allows researchers to identify biologically important information such as the robustness of nest networks and the effect of network position on survival and reproduction (Ellis et al. 2017a).

When analysing networks of nests, it is necessary to incorporate important information about the nodes and edges such as spatial information (e.g. coordinates of nests), edge attributes (e.g. edge length) and node attributes (e.g. nest population size). This complexity means that at present statistical social network methods are not suitable for analysis of these networks as they have not yet been adapted for multiple extra attributes in a dynamic context. However, descriptive network methods are useful for analysis of polydomous networks as they are able to account for inter-dependencies that other analyses are not able to.

Formica rufa group wood ants

Introduction

Wood ants are a dominant species group found in many forests across the northern hemisphere with colonies controlling territories that can be several km^2 (Stockan and Robinson 2016). This dominance is possible due to populous colonies and high levels of organisation, underpinned by a division of labour.

Wood ants are generally found in woodlands where they farm aphids for sugary excretions that form a large part of their diet but are also important predators of many other species, leading them to be considered as keystone species (Robinson 2016). Wood ants are also considered to be ecosystem engineers as they promote decomposition on the forest floor (Frouz 2016).

Wood ant colonies are known for the large mounds they inhabit that can reach several metres in height and house millions of ants. These thatched mounds are known to be inhabited for up to 30 years, although it is likely that they are inhabited for considerably longer (Robinson and Robinson 2008).

Distribution

Wood ants are widely distributed in the northern hemisphere, being present in many locations in North Europe, Asia and North America. Generally they are found in woodlands, but some species can be found in other habitats such as bogs or open pasture (Punttila and Kilpeläinen 2009). The distribution of wood ant nests appears to be influenced by a number of factors including climate, aspect, shading and availability of food (Risch et al. 2016). However, different species of wood ant differ significantly in their habitat preference with some preferring forest interiors and others preferring forest edges (Risch et al. 2016).

Although most wood ant populations are naturally occurring, there have been some experimental introductions of wood ants in North America for control of pests (Trager 2016). Some of these introductions have been successful with populations surviving for decades (Storer et al. 2008), but others were not successful due to predation by native ant species (Trager 2016).

Ecological importance

Wood ants are considered to have a critical role in shaping the woodland ecosystem habitats where they live. They contribute to many important ecosystem services such as decomposition and mineralization of nutrients (Frouz 2016) and play an important role in determining the structure of invertebrate species communities through predation (Robinson 2016). Wood ants also have mutualistic interactions with several other invertebrate groups, with the most important mutualism being with the aphids that they farm for sugary secretions.

The farming of aphids by wood ants provides the majority of the carbohydrates colonies need, with estimates ranging from four to 500kg of honeydew being harvested every year by a single nest, which can cause significant damage to tree growth (Domisch et al. 2016). However, wood ants can also provide some benefits to the trees that they forage on through protection from herbivores and parasites (Robinson 2016). As a result of the combination of cost and benefits, the effect of ant colonies on trees that they forage on is mixed, with some trees being negatively affected by ant presence and others being positively affected (Domisch et al. 2016).

Conservation considerations

Considering the ecological importance of wood ants as keystone species their conservation is important for both the ants and the ecosystems that they inhabit. The largest threat to wood ant populations appear to be habitat degradation and fragmentation, although climate change and pollution are also likely to be significant threats (Sorvari 2016).

Woodland management can have a significant impact on wood ant populations. For example, clear-felling can cause temporary fragmentation and degradation of woodlands (Sorvari and Hakkarainen 2007) and results in wood ants being unable to get the food that they need to survive (Sorvari and Hakkarainen 2009). Consequently, woodland managers aiming to maintain a healthy population of wood ants should avoid clear-felling and choose trees to fell carefully.

Climate change is likely to influence the distribution of wood ants as wood ants are sensitive to both temperature and rainfall (Sorvari 2016). However, whether wood ants will be negatively or positively affected by climate change is unclear as colonies seem to benefit from warmer summer temperatures (Lappalainen et al. 2008), but suffer higher mortality due to higher overwintering temperatures (Sorvari et al. 2011).

Colony organisation

Wood ant colonies can establish a new nest through either social parasitism or budding (Maeder et al. 2016). Nest foundation through social parasitism occurs by a queen flying to a nest of a different ant species and attempting to usurp the current queen. If successful, the queen will then slowly replace the worker population with her own offspring (Buschinger 1986). Nest foundation through budding occurs by a group of workers and a queen or multiple queens leaving their natal nest to form a new nest (Hölldobler and Wilson 1990). There seems to be some relationship between queen number and method of foundation, with monogynous colonies only founding through social parasitism and budding only occurring in polygynous colonies (Maeder et al. 2016).

Table 1.2. Colony organisation of different *Formica rufa* group species. X indicates that the trait has been identified in a population of this species. Adapted from Ellis & Robinson (2014).

Species	Polydomy	Monodomy	Polygyny	Monogyny
F. aquilonia	X	X	X	
F. lugubris	X	X	X	X
F. paralugubris	X		X	
F. pratensis		X		X
F. rufa	X	X	X	X

Polydomy in wood ants

Wood ant colonies of different species and even populations can either inhabit single nests, monodomy, or multiple nests, polydomy, (Ellis and Robinson 2014). The reasons that some populations of wood ants are polydomous while others are not are still not clear. However, several adaptive benefits of polydomy have been suggested including: 1) Increased foraging success; 2) Reduced risk of nest predation; 3) Improved defence; and 4) Higher competitive dominance (Ellis and Robinson 2014).

The importance of each of these proposed benefits has not been tested due to the challenges of performing experiments in the field on polydomous colonies of wood ants.

Polydomous colonies of wood ants tend to consist of fewer than 100 cooperating nests, although there are some examples of colonies with several thousand nests (Marko et al. 2012). There are also records of unicoloniality in wood ants (Chapuisat and Keller 1999). Unicoloniality is essentially polydomy on a larger scale and is common in invasive ant species. In unicolonial colonies there is no aggression between individuals of a large population spanning many hectares.

Polydomous wood ants produce new nests by budding, which allows colonies to expand into new areas for foraging, while remaining competitively dominant (Ellis and Robinson 2015). In general, polydomous wood ant colonies are polygynous, whereas monodomous wood ant colonies tend to be monogynous. However, this could be because similar conditions favour both polydomy and polygyny (Debout et al. 2007; Ellis and Robinson 2014; Robinson 2014).

In polydomous species there is confusion regarding the terms used to refer to the colony, the nest and the population inhabiting the nest. In this thesis, I use colony to refer to a single group of socially connected nests (but see Ellis et al. 2017b), nest to refer to the physical nest structure and sub-colony to refer to the population of ants inhabiting a nest structure.

Field site

Description

The field site used for work in this thesis is Longshaw Estate which is in the Peak District, UK (53°18'55"N, 1°36'18"W) and is managed by National Trust. The estate is roughly 650 hectares and is composed of a mixture of open grazed land, sparse woodland and old forestry plantation. The old plantation at the site is dominated by Scots pine (*Pinus sylvestris*) and larch (*Larix decidua.*) trees, while trees in the open woodland at the site are mostly oak (*Quercus spp.*), silver birch (*Betula spp.*), Scots pine (*Pinus sylvestris*), larch (*Larix decidua*) and sycamore (*Acer pseudoplatanus*).

Distribution of wood ants at the site

There is only one species of wood ant at Longshaw Estate, the Northern Hairy Wood Ant (*Formica lugubris*). Wood ant nests can be found in most areas of the site, but the highest density is found in the sparse woodland and on the edges of the old plantation. I have observed wood ant nests foraging on all the common tree species at the site, with no obvious difference in preference.

How is the site managed?

In the past 5 years, woodland managers at the site have been thinning the old plantation and planting more trees in the open grazed land with the aim of creating more sparse woodland. One reason for doing this is to create more habitat for the wood ants at the site. Although we did not survey all nests at the site before and after thinning or planting, I have observed nests foraging on newly planted trees and there are also now several nests in a newly thinned area where there were previously not any nests. Consequently, it seems likely that the management of the site is leading to a growing population of wood ants.

The site is grazed by both sheep and cattle at a low rate throughout the year. The impact of grazing on the ant colonies is unclear, but as a result of the grazing it is easy to identify and measure foraging and inter-nest trails in the short grass.

What past work has been done on this site?

Previous work at the site has included significant research on the function of polydomous nest networks. Cook et al. (2014) investigated the robustness and efficiency of the nest networks and found that trail networks do not form minimum spanning trees, indicating that they are robust to disruption, and that inter-nests connections are influenced by factors other than distance. Ellis et al. (2014) extended this work to find that the relative difference in the amount of foraging done by a pair of nests is a key determinant of whether the nests share food – nests that forage very little are likely to be connected by inter-nest trails to nests that forage a lot. Ellis and Robinson (2016) investigated how inter-nest sharing occurs and found that ants from one nest "forage" on an inter-nest trail to the other nest, essentially treating the other nests as food sources. However, the ability of nests to survive on food gained from inter-nest trails was investigated by Ellis and Robinson (2015), who found that nests that do not forage at all are eventually abandoned.

One significant dataset that has been generated at the site because of this work contains network maps of 10 colonies that were mapped at least once per year between 2012 and 2016. In Chapter 3, I present an extension of this work which adds three years to this dataset including maps of four nests that were collected 16 times over two years.

Thesis outline

In this thesis I present four data chapters that detail models, experiments and observational studies that were designed to investigate polydomy in red wood ants and ants more generally. In Chapter 2, I describe a model that was designed to test the important costs and benefits of polydomy and monodomy. In Chapter 3, I describe an observational study of several polydomous ant colonies that was performed over eight years. In Chapter 4, I describe an experiment that investigated the influence of changing resource distribution on the inter-nest and foraging networks of several ant colonies. Finally, in Chapter 5, I present an observational study of the effect of relatedness on cooperation between wood ant nests.

Chapter 2 - The costs and benefits of decentralization and centralization of ant colonies

Abstract

A challenge faced by individuals and groups of many species is determining how resources and activities should be spatially distributed: centralized or decentralized. This distribution problem is hard to understand due to the many costs and benefits of each strategy in different settings. Ant colonies are faced by this problem and demonstrate two solutions: (1) Centralizing resources in a single nest (monodomy); and (2) Decentralizing by spreading resources across many nests (polydomy). Despite the possibilities for using this system to study the centralization/decentralization problem, the trade-offs associated with using either polydomy or monodomy are poorly understood due to a lack of empirical data and cohesive theory. Here, we present a dynamic network model of a population of ant nests which is based on observations of a facultatively polydomous ant species (*Formica lugubris*). We use the model to test several key hypotheses for costs and benefits of polydomy and monodomy and show that decentralization is advantageous when resource acquisition costs are high, nest size is limited, resources are clustered and there is a risk of nest destruction, but centralization prevails when resource availability fluctuates and nest size is limited. Our model explains the phylogenetic and ecological diversity of polydomous ants, demonstrates several trade-offs of decentralization and

centralization, and provides testable predictions for empirical work on ants and in other systems.

Introduction

Centralization and decentralization are opposing strategies for the spatial organisation of resources or workers. In centralized systems the resources are located in a single site, while in decentralized systems resources are dispersed across multiple sites. Decentralization and centralization have many interacting costs and benefits, making it difficult to identify which should be adopted in a given context (Bernstein and Turban, 2018; Ireland and Garnier, 2018; Robinson, 2014). One potential source of insights for the trade-offs between centralization and decentralization are ant colonies (Hölldobler and Lumsden, 1980; Ireland and Garnier, 2018).

The workforce and resources of most ant colonies are centralized in a single nest, which is known as monodomy. However, some ant species decentralize their colonies by dividing into several semi-autonomous sub-groups that inhabit multiple nests, known as polydomy (Debout et al., 2007) (e.g. Figure 2.1). Decentralization through polydomy is hypothesised to confer several benefits on colonies including: (1) Reducing the risk of colony extinction, through spreading risk (Le Breton et al., 2007; Robinson, 2014; Van Wilgenburg, 2007); (2) Enabling colonies to overcome population limits imposed by structural or organisational limitations on nest size (Robinson, 2014; Van Wilgenburg and Elgar, 2007); (3) improves colony foraging and defence through nests being well-distributed through the foraging area (Cook et al., 2013; Davidson, 1997; Hölldobler and Lumsden, 1980; Holway and Case, 2001; Lanan et al., 2011; Schmolke, 2009; Stroeymeyt et al., 2017); (4) Buffering the effects of local environmental variability (Cook et al. 2013; Robinson, 2014; Schmolke, 2009); and (5) Enabling colonies to benefit from a large colony size, without the associated reductions in productivity (Kramer et al., 2014; Stroeymeyt et al., 2017). Despite these benefits, most ant colonies are monodomous. The reason for the relative abundance of monodomy may be due to some key costs of spreading across multiple nests, including: (1) Reduced defensive abilities, as defenders may be spread too thinly (Robinson, 2014); (2) Impaired information transfer between individuals when they are spread across multiple nests (Cook et al., 2013); and (3) Costs of moving resources between nests due to predation or resource theft (Robinson, 2014; Snyder and Herbers, 1991).

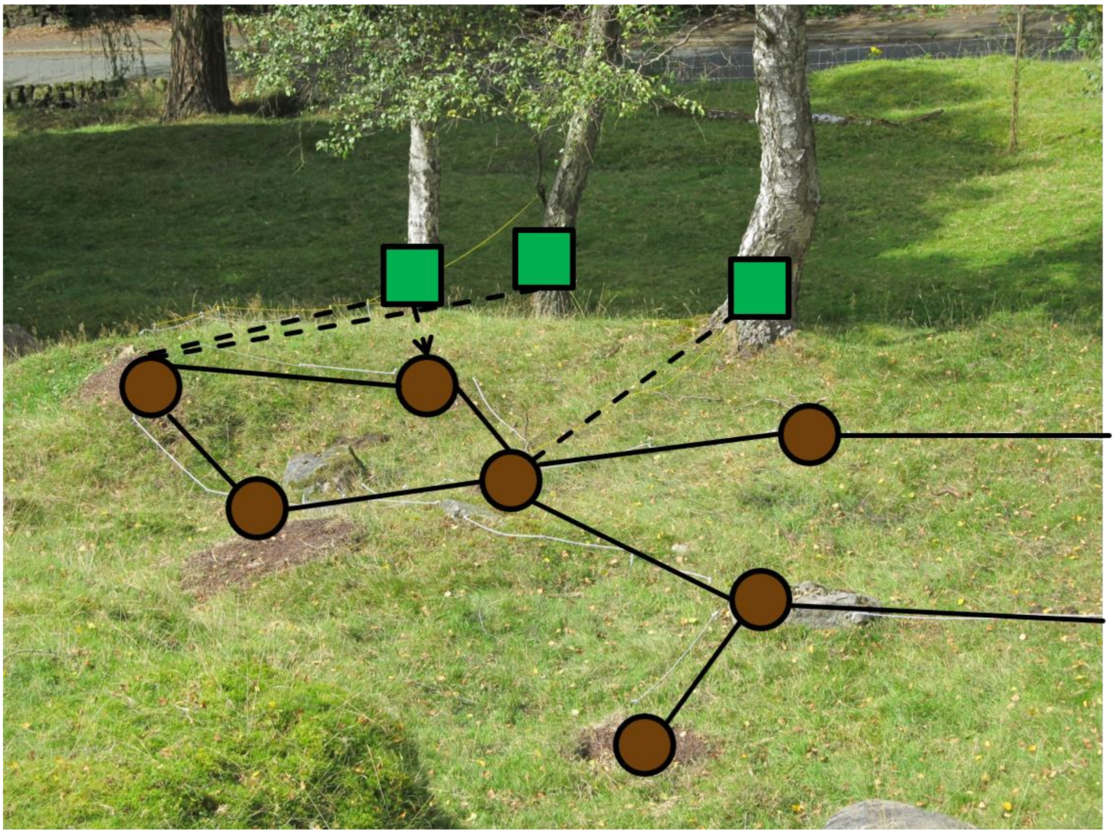


Figure 2.1 A polydomous colony of wood ants foraging on aphids that live in trees. For clarity nests are marked with brown circles, food sources (trees) are marked with green squares, foraging trails are marked with dashed lines and inter-nest trails are marked with solid lines.

The many inter-related costs and benefits of decentralization of an ant colony's workforce make understanding the reasons that various ant species have evolved polydomy challenging to test in the field or laboratory. Furthermore, the species of ants that demonstrate polydomy are phylogenetically and ecologically diverse, as polydomy has evolved multiple times, seemingly in response to different selective pressures (Debout et al., 2007). Consequently, no single hypothesis for the adaptive benefits of polydomy fits with the evidence from every case of polydomy (Robinson, 2014). One key difficulty is that we lack clear predictions of how colony spatial structure should respond to different environmental pressures. One method that has been used, with some success, is mathematical models which compare the success of polydomy and monodomy in different situations; however, current models of nesting organisation have been designed to test a single hypothesis and cannot be generalised to most cases of polydomy (Bottinelli et al., 2015; Cook et al., 2013; Höfener et al., 1996; Schmolke, 2009). Consequently, models that provide proof-of-concept tests and verifiable predictions for experimental and observational research are a necessary and relatively unexplored method for research into the trade-offs between centralization and decentralization of ant nests.

Here, using a dynamic network model we take a novel approach which investigates multiple hypotheses for the ecological benefits of spatial decentralization of ant colonies.

Methods

Model overview

We model the dynamics of a population of ant colonies, some of which have a polydomous colony organisation and others a monodomous colony organisation. The model considers ant colonies as networks with nodes representing nests and food sources, and connections representing resource exchange between those nodes (e.g.

Figure 2.2). Over time the strategies compete against each other, allowing us to test various hypotheses for ecological benefits of decentralization and centralization in ant colonies by manipulating parameters of the model. As the model is stochastic, we run multiple replicates under each set of conditions; every replicate is a unique realisation of the model. We use this model to formalise five existing hypotheses (Table 2.1) for the adaptive benefits of polydomy and identify ecological conditions where each is likely to be important in driving the evolution of polydomy.

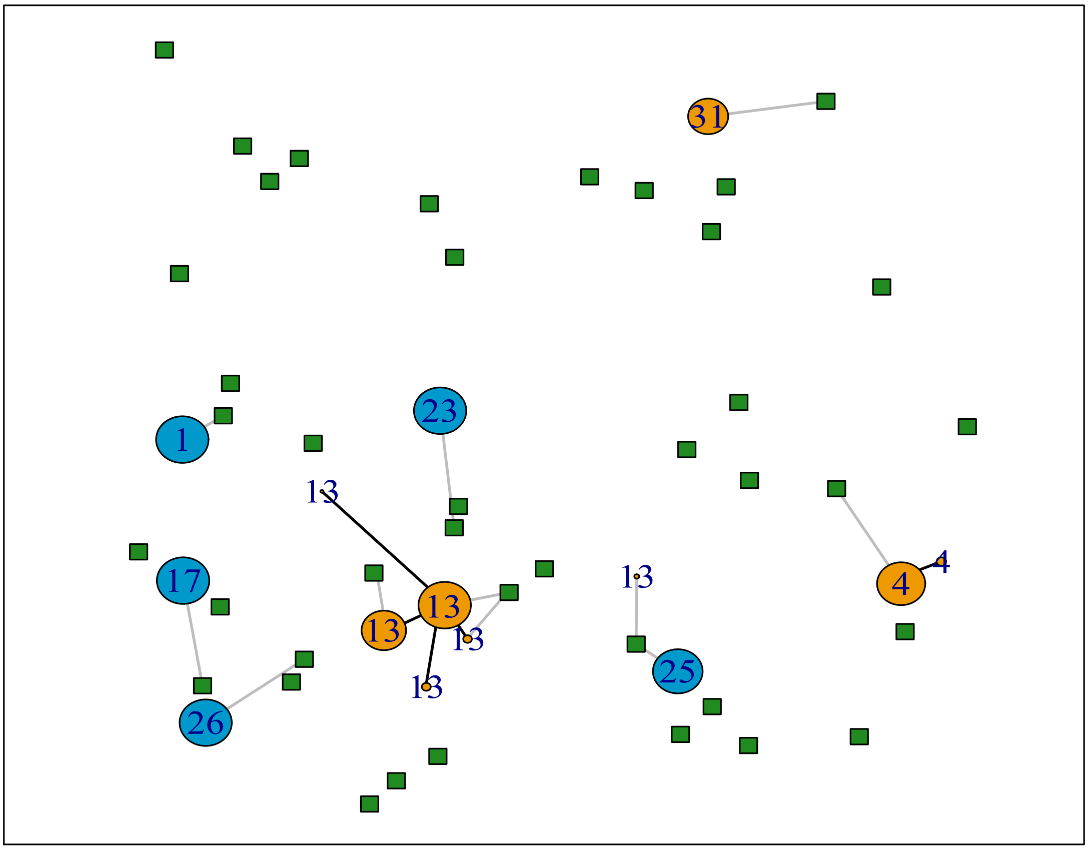


Figure 2.2 Graphical representation of the model. Circles represent nests and squares represent food sources. Nest colour indicates whether the nest belongs to a polydomous (orange) or a monodomous (blue) colony, and nest number is the colony identity of that nest. Grey lines indicate foraging connections and black lines indicate inter-nest connections.

Table 2.1 Hypotheses and factors changed in the model to test logic of these hypotheses

Hypothesis	Change in model	Levels
Polydomy is favoured when:		
(1) the costs of foraging are high	Foraging cost	Low, high
(2) nests are limited in size	Nest-level carrying capacity	Low, high
(3) food sources are clustered	Food source distribution	Clustered, Random
(4) there is a risk of stochastic nest destruction	Probability of stochastic nest destruction per season	None, 1%
(5) food sources vary in availability	Food source productivity	Constant, Fluctuating

Here we present an overview of how the model works; a fully detailed model description can be found in Appendix A. The model processes are based on empirical observations of the facultatively polydomous wood ant *Formica lugubris* (Ellis and Robinson, 2014). Empirically measured values for several of the model parameters are unavailable, but the model results are not highly sensitive to these parameters (Appendix I). The model is implemented in R Version 3.4.1 (R Core Team, 2013). Although implemented to investigate polydomous wood ants, our general modelling framework describes interactions between dynamically varying networks (nests and food sources in our case) and is readily adapted; the modular nature of the processes involved (as detailed below) facilitates such adaptation.

Environment and agents

In the model there are nests and food sources, each of which has a fixed location (e.g. Figure 2.2). At the start of each replicate of the model, nests are randomly distributed in space, whilst food sources can be either randomly distributed or clustered depending on the condition (see Table 2.1 – Hypotheses and Appendix B). Nests contain resources that are implicitly assumed to be ants capable of foraging. Each nest also has a colony organisation, which can be either polydomous or monodomous. At the start of each replicate, half of the nests are polydomous and half are monodomous. Food sources have a fixed location throughout each replicate of the model. Food sources produce food at either a constant rate, or at a rate that fluctuates over time (Table 2.1).

Foraging connections

Nests make foraging connections to food sources. These are representative of foraging trails commonly found in wood ants (Ellis and Robinson, 2014). Nests use food received from foraging connections to increase the quantity of resources (equal to ants) in the nest. However, not all foraging connections are profitable. The profitability of a connection is determined by the availability of resources at the food source, the number of ants foraging on it, and its length, with longer connections being costlier. Such costs may include factors such as energy used by foragers, maintenance costs and time costs.

Inter-nest connections

Nests that belong to the same polydomous colony can form connections to each other, allowing them to take food. As with foraging connections, each connection can be costly if it is long or if there are few resources available.

Competition

If two nests from different colonies make a connection to the same food source they compete through interference competition for the food. A sensitivity analysis of the effect of competition on the model results is detailed in Appendix C.

Nest foundation, growth and death

Nests are capable of ‘parenting’ new nests using their resources. If a new nest is created by a nest with a polydomous colony organisation, then it will belong to the same colony as the ‘parent’ nest and have a connection allowing it to take resources from the ‘parent’ nest. In contrast, nests parented by nests with a monodomous colony organisation will become a new, independent colony. We assume that cooperative nests can occasionally found non-cooperative nests and *vice versa*. This prevents either strategy from becoming extinct. Polydomy appears to be a fairly flexible strategy in many ant species, which are facultatively polydomous (e.g. Ellis and Robinson, 2014). Consequently, cooperative strategies among groups may often arise in response to local environmental conditions, rather than being inflexible.

Nests grow in size at a rate that depends on the quantity of resources that they receive from their connections. They also suffer a constant death rate and are limited in size by a ‘nest-level carrying capacity’ (Table 2.1).

Nests can ‘die’ if the nest population (resources contained in the nest) reaches below a certain threshold. Nests can also ‘die’ randomly, which is included to represent processes such as predation, parasitism or other stochastic causes of nest ‘death’ (Table 2.1).

Timescales

The model cycles over a set number of seasons (Table AA1). Seasons in our model are simply used to index time and, as such, there is no variation in conditions between seasons. In each seasonal cycle nests grow at a rate determined by the connections that they have to food sources and other nests. At the end of each season nests can change their connections, depending on profitability, and produce new nests. We run the simulations for multiple independent replicates, each represented by a single complete run of the model for a fixed number of seasons.

Experimental design and hypotheses

At the end of every season the locations, sizes, foraging connections and internest connections of every nest that is currently active are recorded. We use these data to test five different hypotheses (Table 2.1) by changing factors in the model in a full factorial design (i.e. all 2, 3, 4 and 5-way interactions between factors are also tested). We ran 30 replicates of each unique condition (30 replicates x 32 conditions = 960 total replicates). The hypotheses and changes to the model are detailed in Table 2.1.

Statistical analyses

We used a general linear model (GLM) with a binomial error distribution and a logit link as a framework to quantify how each of the factors that we change in the model affects the proportion of ants that belong to polydomous nests. Details of model selection are described in Appendix E. We do not report p-values as they do not represent “statistical significance” (James et al., 2013; Wasserstein and Lazar, 2016). Rather, for the fixed number of replicates under consideration, the odds ratio confidence intervals are used to indicate factors with a clear effect. We calculate odds ratios for each of the covariates of the model to show the effect of changing them on the frequency of polydomy. The odds ratio values for each effect indicate the increase in probability that a randomly selected ant in a replicate under a certain condition belongs to a polydomous nest by changing the factor of interest (e.g. an odds ratio of 4 indicates that there is four times the probability that a randomly selected ant is from a polydomous nest when compared to replicates in which the factor of interest is set to the alternative value). As such, the odds ratio values indicate whether differences between conditions are expected to be biologically meaningful but should not be used to compare relative importance of each effect as an adaptive benefit of polydomy or monodomy, because factor levels (Table 2.1) cannot be standardized across factors.

Results

Of the 960 model replicates, the population survived until the final season in 91.5% of replicates. Replicates in which the population died before the final season were excluded from further analyses. Population survival under different conditions is shown in Figure AF1. We present multiple sensitivity analyses for model parameters in Appendices 3 and 9.

Foraging costs

Our results support the hypothesis that polydomy is favoured when foraging is costly. There is a clear effect of foraging cost on the frequency of polydomy (Table 2.2), with polydomy being more frequent when foraging costs are high, compared with when they are low (Figure 2.3; effect sizes are detailed in Table 2.2). There are no important interaction terms between foraging cost and any of the other factors in the model.

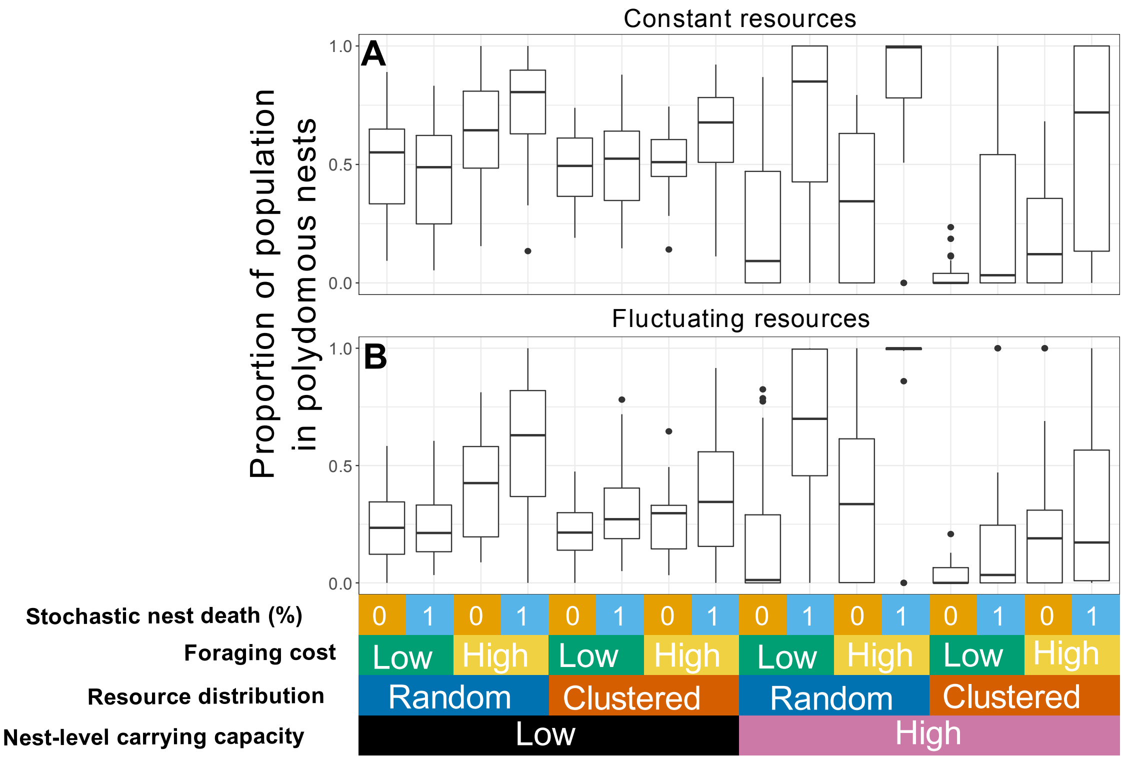


Figure 2.3. The proportion of the population in polydomous nests at the end of 500 seasons in each condition when food sources are either constant (A) or fluctuating (B) in availability. Middle lines represent median values, lower and upper hinges represent 25th and 75th percentiles respectively and whiskers reach to the lowest (lower whisker) or highest (higher whisker) value, with a maximum reach of 1.5 x IQR from the hinge. Values outside of this range are plotted as outliers.

Table 2.2. Factors included in the final GLM. Odds ratio indicates the increase in probability of randomly selecting an ant from a polydomous nest when the factor (or factors) is set to the value given in parentheses in Factor. 2.5% and 97.5% C.I.s indicate 95% confidence intervals for each effect size. Factors with odds ratio confidence intervals that do not overlap 1.00 (i.e. no effect) are highlighted in bold.

Factor	z	Odds ratio	2.5% C.I.	97.5% C.I.
Intercept	-9.08	N/A	N/A	N/A
Foraging trail cost (high)	**4.99**	**2.15**	**1.59**	**2.91**
Nest level carrying capacity (low)	**3.87**	**3.75**	**1.94**	**7.43**
Food source distribution (clustered)	**7.20**	**5.78**	**3.61**	**9.41**
Stochastic nest destruction (high)	**5.20**	**4.61**	**2.62**	**8.31**
Food source stochasticity (constant)	1.12	1.39	0.78	2.48
Food source stochasticity (constant) and nest-level carrying capacity (low)	**2.43**	**2.11**	**1.16**	**3.87**
Food source stochasticity (constant) and food source distribution (clustered)	-0.66	0.82	0.45	1.48
Nest-level carrying capacity (low) and stochastic nest destruction (high)	**-4.68**	**0.23**	**0.13**	**0.43**
Nest-level carrying capacity (low) and food source distribution (clustered)	**-3.71**	**0.31**	**0.17**	**0.58**

Nest size limitations

Our results support the hypothesis that polydomy is favoured when nest populations are limited in size. There is a clear effect of nest-level carrying capacity on the frequency of polydomy (Table 2.2), with polydomy being found more frequently when nest-level carrying capacity is low, compared to when it is high (Figure 2.3). We also find two-way interactions between nest-level carrying capacity and stochastic nest destruction, food source distribution and food source stochasticity on the frequency of polydomy (details are in Table 2.2; descriptions of each interaction are in the below sections of Food source distribution, Stochastic nest destruction, and Fluctuating food source availability).

Food source distribution

Our results support the hypothesis that polydomy is favoured when food sources are clustered. In the final GLM, we find an effect of food source distribution on the frequency of polydomy (Table 2.2), with clustered food sources resulting in higher frequencies of polydomy than when food sources are randomly distributed (Figure 2.3). However, there is also an interaction between food source distribution and nest-level carrying capacity (Table 2.2), with clustered food sources promoting polydomy less strongly when nest-level carrying capacity is low than when nest-level carrying capacity is high.

Stochastic nest destruction

Our data support the hypothesis that polydomy is favoured when nests are at risk of stochastic nest destruction. In the final model, the frequency of polydomy is affected by stochastic nest destruction (Table 2.2), with stochastic nest destruction resulting in higher frequencies of polydomy (Figure 2.3). However, there is also an interaction between stochastic nest destruction and nest-level carrying capacity (Table 2.2): the increase in polydomy caused by stochastic nest destruction is smaller when nest-level carrying capacity is low than when nest-level carrying capacity is high.

Fluctuating food source availability

Our model results support the hypothesis that polydomy is influenced by fluctuations in food source availability, but only when nest-level carrying capacity is low (Table 2.2). However, the direction of the effect is opposite to that hypothesised, with fluctuating food sources resulting in a lower frequency of polydomy (Figure 2.3). The overall effect (without interactions) of fluctuations in food source availability is not found to be important.

Discussion

Our results show that polydomous colonies perform better when resource acquisition costs are high, nest size is limited, resources are clustered and there is a risk of nest destruction, but monodomy performs better when resource availability fluctuates temporally. Taken together these results explain why species of ants that have polydomous colonies are phylogenetically and ecologically diverse.

Our model supports the hypothesis that decentralising across multiple nests may be favoured when acquisition of resources from the environment is costlier than resource sharing, and there is a benefit to groups that donate resources to others, for example through inclusive fitness or reciprocation. Foraging costs in our model represent energetic costs, time costs and forager loss through predation costs. Polydomy appears to allow colonies to reduce these costs, for example by limiting the time it takes for each individual to travel to a food source. Reducing foraging costs is likely to be important in many species, including the polydomous desert ant *Cataglyphis iberica*, whose workers forage in the middle of the day when temperatures are highest and longer foraging trips may result in death (Cerda et al., 2002).

Another important determinant of the cost of foraging is recruitment, which allows colonies to quickly exploit food sources that are far from the nest without each forager having to find the food source independently. When recruitment is possible and food sources are large, then being spread across multiple nests may actually be costly, because mobilisation of enough workers to exploit a food source may be not be possible from small nests (Cook et al., 2013). Here, we model the behavior of the population of individual nests, rather than individual ants. Consequently, we do not model recruitment explicitly, but instead assume that ants are able to effectively recruit to form trails to food sources.

Nest size limitation is one of the clearest causes for a colony spreading across multiple nests and is supported by the model. It is most apparent in cavity-dwelling species, and is commonly associated with seasonal polydomy, when colonies temporarily outgrow their nest (Cao, 2013). Although the pressure of nest size limitations on colony size is most clear in cavity-dwelling ants, similar processes may be at work in species with high nest-size dependent mortality or organisational constraints on nest size (Kramer et al., 2014; Le Breton et al., 2007; Van Wilgenburg and Elgar, 2007).

The effect of resource distribution on success of polydomy in our model may be because polydomous colonies can monopolise clusters of permanent food sources and become difficult to displace. In contrast, monodomous colonies may be unable to monopolise large clusters of food sources due to the foraging range of individual nests being limited and may be easily invaded. Monopolisation of food sources and subsequent absence of interference competition appears to be an important factor in the success of many invasive species, such as the Argentine ant (Holway and Case, 2001), and of species that forage on large stable food sources, such as ant species that have mutualistic relationships with trophobionts (Ellis and Robinson, 2014; Lanan, 2014). This interpretation is also supported, as when competition is removed from our model the frequency of polydomy decreases (Appendix C). The effect of food source distribution on the frequency of polydomy found by our model may be because when nest size is limited, being close to several food sources is less beneficial, as nests are restricted in the number of foraging and inter-nest trails they can form. Consequently, food source distribution may be less influential in determining social organisation strategy when nests are only able to forage on a few food sources, or in a small area.

Our findings contrast with Cook et al. (2013) who found that monodomy performs better than polydomy when food sources are clustered. We suggest this is because Cook et al. model a single colony and does not consider inter-colony competition. In our model, polydomous colonies are often able to be more numerically dominant than monodomous colonies as they can grow larger. Consequently, when food sources are clustered, polydomous colonies in our model can monopolise clusters more easily making it difficult for competitors to invade, which may be an important mechanism in ant species that forage on large, consistent food sources, such as populations of aphids (Ellis and Robinson, 2014). When interference competition is removed from our model then, like Cook et al, we find that polydomy is less prevalent when food is clustered (Appendix C).

Our model supports the hypothesis that polydomy allows colonies to spread the risk of nest destruction. Decentralization may allow the wider group to spread the risk of damage from external processes across multiple nests: if one nest is destroyed, the colony can still persist, provided there are multiple queens in the colony (Robinson, 2014; Van Wilgenburg and Elgar, 2007). Pressure from stochastic nest destruction is likely to be important in species where nests are targeted by predators (Le Breton et al., 2007; Van Wilgenburg and Elgar, 2007), but could also represent other processes through which random nest destruction occurs, for example social parasitism, which may be important in many ant species (Czechowski and Godzińska, 2015), or environmental damage. Some of the effect of stochastic nest destruction on the success of polydomy is lost when nest size is very limited. When nests are limited to being small there are likely to be more of them, because individual nests use less food than when nests can grow very large, meaning that the maximum number of nests that an environment can support is higher. When this is the case, stochastic destruction of a single nest is likely to be a less detrimental event for a polydomous colony.

In our model, stochastic nest destruction is a completely random process that occurs independently for each nest. However, ecological and environmental processes that lead to nest destruction are often non-random: factors such as nest size, physical location and network location are likely to be important in determining the likelihood that a nest is predated (e.g. Van Wilgenburg and Elgar, 2007).

In polydomous ants, there is good evidence that sharing between nests occurs when one nest has excess resources and another nest has a resource deficit (Ellis et al., 2014). Consequently, polydomous colonies may have an advantage over monodomous colonies through being able to cover a larger area and absorb stochasticity in local environmental conditions (Holway and Case, 2001), for example food production. However, we do not find support for this hypothesis. Instead we find that polydomy is actually costly when food sources fluctuate in quality. The difference between the hypothesised result and the outcome of our model may be a consequence of small nests, common in polydomous colonies in our model, being more vulnerable to local reduction in food availability. Small nests may also be unable to grow quickly enough to fully exploit food sources that become very productive, an effect that has previously been found in models of polydomy (Cook et al., 2013). We demonstrate the logic of this hypothesised explanation using a simple model in Appendix G. Furthermore, there may be other situations not covered by our model, in which resource fluctuations result in higher frequencies of polydomy. For example, polydomous colonies may be better suited to surviving seasonal changes or spatial variation in resource availability than monodomous colonies, due to the ability of nests to share resources.

While our model shows increased foraging costs are associated with higher frequency of polydomy, we have not investigated the effect of altering resource sharing costs. Costs of resource sharing are likely to comprise a combination of time and energetic costs, and increased risk of predation and theft of transported resources whilst outside of the nest (Robinson, 2014). We expect the relative costs of foraging and sharing are likely to be important to the profitability of polydomy in many different ant species. However, future work is necessary to determine the direction and effect of this interaction.

In this study, we focus on the adaptive benefits of either centralization of decentralization in ant colonies but have not considered the effects of genetic structure. Future work could adapt the model to include relatedness, which may provide important insights into the emergence of polydomous colonies. This is likely to be particularly important with respect to invasive supercolonies where population bottlenecks appear to be important (Giraud et al., 2002; Van Wilgenburg et al., 2010). We also do not look at how differences in the numbers of queens in each colony influence each of the hypotheses. The number of queens in polydomous colonies varies, with colonies of some species having many queens (polygynous) and colonies of other species having a single queen (monogynous) (Robinson 2014). This dichotomy is likely to have consequence for the risk spreading benefit of polydomy: if a nest in a monogynous colony containing the queen is predated, the colony will not survive if it is not able to rear a queen from existing brood. However, the influence of different numbers of queens per colony on the benefits of polydomy are likely to be less important for the other hypotheses because processes such as foraging are unlikely to be influenced by queen distribution. Future work could adapt our model to look at how each of the hypotheses we investigated are influenced by the number of queens in each colony.

Our research adds to a growing collection of studies that have used generative approaches - that is, process-based predictive models - to model biological networks (Cantor et al., 2015; Ilany and Akçay, 2016; Pinter-Wollman, 2015; Seyfarth, 1977); the predictions of such models can then be tested empirically (Ilany and Akçay, 2016). The advantage of using such models is that it is possible to test the logic of existing hypotheses and generate novel predictions about the way that different biological networks behave. The findings can then be used to inform the design of experiments. Our model highlights several key adaptive benefits to both centralization and decentralization in ecological systems, demonstrating how there may be multiple drivers of this trait and also helping to form testable hypotheses.

Chapter 3 - A longitudinal study of nest occupancy, trail networks and foraging in a polydomous wood ant population

Abstract

Most ant colonies live in a single nest (monodomy) or a group of nests (polydomy). However, the length of time for which nests are inhabited varies significantly between different species. Although colonies of some species frequently move nest sites, in others, colonies inhabit the same nest or group of nests for many years. Similarly, in some species foraging and resource-sharing trails are highly dynamic, while in other species trails are used for years. Wood ants are a group of keystone species that inhabit many northern hemisphere woodlands, where they are important predators of invertebrates and indirectly act as herbivores through the farming of aphids. Wood ant colonies exhibit both monodomy and polydomy, and can inhabit nests for many years. Trails in wood ant colonies are also thought to be relatively stable. However, information about colony dynamics is mostly anecdotal as, until now, no longitudinal datasets have been collected. In this study, we collected data from ten polydomous wood ant colonies annually for eight years and a subset of four colonies 16 times over two years. We found that most polydomous wood ant nests are abandoned in the first two years after being constructed and are more likely to be abandoned in the latter part of the active season. However, the rate of nest abandonment decreases after two years and is lower in larger nests. We also found that wood ant trails are relatively static

within an active season and become more static later in the season as trails become established.

Introduction

Colonies of most ant species inhabit nests that provide shelter from weather, defence from predators and parasites, and a place to store resources (Hölldobler and Wilson 1990). However, the duration of nest site occupancy differs dramatically across ant species. In many ant species, colonies move between nesting locations frequently in response to changes in local conditions, availability of food, current nest quality, parasitic load, disease, predation, seasonality, competition and to allow colony growth (McGlynn 2012). For example, colonies of cavity-dwelling *Temnothorax* spp. ants will move to new nest sites if they are better quality than their current nest site (Dornhaus et al. 2004). In contrast in other species, established colonies may remain in the same nest for decades (Breen 1979; Klimetzek 1981; Ingram et al. 2013; Robinson and Robinson 2008); however, information on nest occupancy in natural populations is limited to a few studies that have monitored individual ant nests for long periods (Klimetzek 1981; Ingram et al. 2013; Robinson and Robinson 2008) and anecdotal information.

Ant colonies can inhabit either a single nest, termed monodomy, or multiple nests, known as polydomy. In monodomous colonies, a colony occupies a single nest where all resources (e.g. workers, food, brood) are stored. In contrast, in polydomous colonies a colony is divided into several sub-colonies that inhabit different nests (Debout et al. 2007; Robinson 2014). In this study, where it is important to distinguish between the physical nest structure and the population of ants inhabiting the nest structure, we refer to the physical nest structure as the “nest” and the population of ants inhabiting the nest structure as the “sub-colony”. Across ant species, both monodomous and polydomous nesting strategies can be associated with either multiple queens (polygyny), or a single queen, (monogyny) (Debout et al. 2007; Robinson 2014).

Ant species use a wide range of foraging strategies that are influenced by the characteristics of the resource they are foraging on (Lanan 2014). For example, species that forage on resources that are small and ephemeral in the environment (e.g. small prey) generally forage solitarily, while species that exploit resources that are clumpy and stable (e.g. aphid colonies) generally forage on long-term trail networks (Lanan 2014). In polydomous colonies, there is an extra layer of complexity, because resource distribution influences the ability of different sub-colonies to access food, which in turn influences the topology of internest networks (Ellis & Robinson 2015; Burns et al. 2020). Therefore, it is likely that the spatiotemporal distribution of resources causes different rates of change in the inter-nest networks of different polydomous species.

Wood ants (*Formica rufa* group) are found in many northern hemisphere woodlands, where they are important predators of many invertebrates and, therefore, are considered to be a keystone species (Robinson et al. 2016). Wood ants are also considered to be ecosystem engineers as they construct large nest mounds of plant material that are kept at high temperatures throughout the year, meaning that they are important sites for decomposition (Frouz et al. 2016). Colonies of wood ants can be either polydomous or monodomous, depending on species and population (Ellis and Robinson 2014). In general, polydomous wood ants form a small number of sub-colonies that inhabit distinct nests and share resources with each other. However, there are some populations of unicolonial wood ants that have formed colonies of several thousand nests (e.g. Higashi & Yamauchi 1979; Marko et al. 2012).

Wood ants forage predominantly on aphid colonies, which they farm for sugary secretions. Consequently, they mainly forage and share food on relatively static foraging and inter-nest trails (Lanan 2014). Recent work identified that wood ant inter-nest and foraging networks can be altered in response to experimental manipulation of resource distribution (Burns et al., 2020). However due to a lack of longitudinal data, relatively little is known about how these networks change under natural conditions.

Although ants are a commonly used study system, few studies have observed the activities of colonies over a long period. Consequently, there is a lack of basic information regarding colony dynamics. The study of colony dynamics is important for conservation as it provides necessary information on how colonies interact with their habitat and can be used to track population health. Polydomous wood ants are an ideal system for studying nest and trail usage over long timescales as: (1) It is possible to identify nests and estimate their population without disturbance (Chen and Robinson 2013); (2) Most of the foraging occurs on trails so the food available to each sub-colony can be quantified (e.g. Ellis et al. 2014); (3) Trails are above-ground so the destination of trails from each nest is easy to identify and strength of trails can be measured (e.g. Ellis et al. 2014); and (4) Nest and trail use change relatively slowly meaning that it is possible to identify the majority of changes and the order that they happened (e.g. Ellis et al. 2017a).

In this study, we present a dataset of ten polydomous *Formica lugubris* colonies that we observed annually over eight years. We also present data on a subset of four of those ten colonies that were observed 16 times over a two-year period. We investigate the rate at which nests and trails are established and abandoned in natural ant colonies and compare colony behaviour at different times in the active season.

Methods

Study site

The population of wood ants (*Formica lugubris)* used for this study is found at National Trust's Longshaw Estate in the Peak District, UK (53°18'55"N, 1°36'18"W). The site is ideal for the study of wood ant trail networks as vegetation is relatively sparse, meaning that it is easy to identify trails and quantify trail strength, and ants at the site forage mostly on aphids found in trees, meaning that food sources are easy to identify. Wood ants at the site are predated at a low level by green woodpeckers (*Picus viridus*) and European badgers (*Meles meles*), although the frequency of predation events seems to be rare and does not generally result in nest abandonment. The field site has been used for the study of wood ants for many years, including work on nest networks (e.g. Cook et al. 2014; Ellis et al. 2014; Ellis and Robinson 2016).

Mapping

We collected data only on warm, dry and sunny days, when the colonies are most active (Burns *personal observation*). To map the colonies, we identified foraging and inter-nest trails, which are visible due to short grass at the site. For each trail we recorded length and strength. We estimated the length of each trail by measuring the distance between the two nests or the nest and food source that the trail connected. We estimated trail strength by measuring the length of the trail it took to find 10 ants, with a minimum detectable strength of 10 ants/4m of trail and a maximum detectable strength of 10 ants/10mm of trail. There is usually no difference in the strength of the trail depending on how close it is to the mound as trails generally do not split (Burns *personal observation*). In the rare cases where a trail did split, we always measured the strength after the split. We estimated number of ants active on a trail (trail activity) by multiplying the length and the strength of the trail (Trail length x ants m^{-1}). Because we did not collect directional data, it is not possible to estimate the proportion of active ants on an inter-nest trail that came from each nest. Therefore, for inter-nest trails, we assign half of the ants from a trail to each of the nests connected by it. The proportion of a colony active on trails was estimated through dividing the nest population by the total number of ants active on trails. We recorded new trails as being “added” by a colony when they were detectable using this method and recorded old trails that were no longer detectable using this method as being “removed”.

We recorded the size and spatial location of all inhabited nests that were connected through the inter-nest trail network. Measurements of each nest’s volume was then used to estimate the population size of the sub-colony inhabiting it, using a formula calibrated previously at this site (Ellis et al. 2014). Finally, we recorded the location and species of trees that were connected to nests by foraging trails. Using the data, we produced network maps of the inter-nest connections and the foraging connections between nests and trees (e.g. Figure 3.1). This method of mapping is the same as has previously been used to map this population (e.g. Ellis and Robinson 2015).

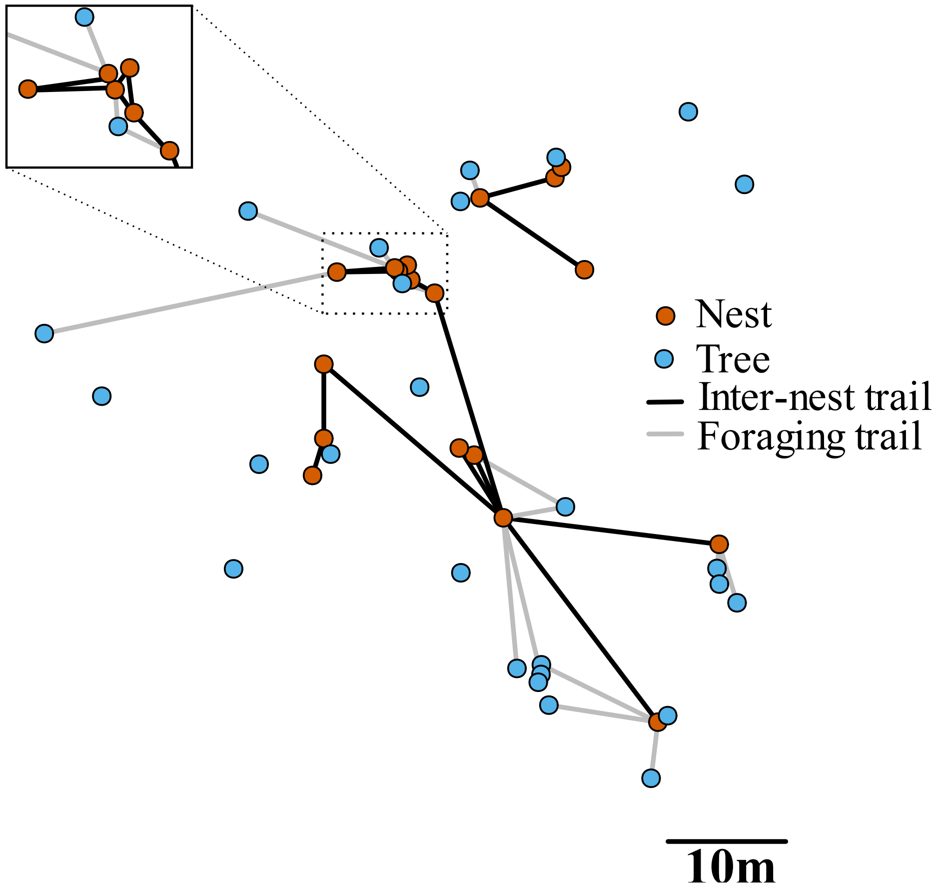


Figure 3.1. An example network map. Nodes are positioned relative to their locations in space. Red nodes indicate nests and yellow nodes indicate trees. Each node is labelled with the unique ID of the node. Foraging trails are indicated by grey edges and inter-nest trails are indicated by black edges. Trails are drawn as topological straight lines as the actual shape of each trail was not measured. Trail strength is not illustrated in this diagram.

Estimating time of nest foundation and abandonment

We collected network maps for ten colonies annually for eight years (2012-2019). Colonies were defined as being any group of nests that were connected through inter-nest trails. Nests that were at some point connected to the network but were not connected at a later time point were also considered to be part of the same colony. Although there are over 900 nests at the study site, not all nests are incorporated into large polydomous colonies. For this study, ten of the largest polydomous colonies at the site were selected for observation. At the first time point, colonies occupied a mean of 10 nests (range: 4-20). Throughout this study, we continued to record maps for each colony annually. In addition, for four of these colonies, we recorded maps every 2-4 weeks in the active season (roughly April-September) for 2017 and 2018, resulting in eight time points per colony per year. Five of the colonies that are included here were subject to manipulation of food sources in 2017 as part of an experiment (Burns et al., 2020). However, because we did not find any effect of the experimental manipulation on nest abandonment or foundation (Burns et al., 2020), we have not excluded these colonies from the study of nest inhabitancy. The subset of four colonies that were mapped regularly in 2017 and 2018 were used only as controls for the manipulation experiment and, therefore, were unaffected by manipulation and are used for the study of changes to trail networks. The details of mapping dates for each colony are detailed in Appendix J.

We estimated the foundation and abandonment dates for each nest that was inhabited in any of the timepoints where the full set of colonies were observed. We did not include data from timepoints where only a subset of the colonies were observed in this analysis, because this would have led to differing data quality between colonies. To approximate the date of nest foundation we use the midpoint between the first time-point that the nest was observed and the previously recorded time-point. Similarly, to estimate the date of nest abandonment we use the midpoint between the last time-point the nest was observed and the next time point. This method can result in nest foundation dates being outside of the active season (i.e. a nest is abandoned or founded after the last time-point of one year and before the first time-point of the next year). However, this method minimises error between our estimations and the likely actual nest foundation and abandonment dates. If a nest was abandoned and then recolonised we counted the abandonment and recolonization as founding and abandonment events.

Assignment of nest founders

We used inter-nest trails and distances to assign the nests inhabited by founding sub-colonies (Ellis et al. 2017a). In most cases (150/273, 54.9%), a new nest was connected by a single inter-nest trail which led to an old nest, which we assigned as the founder. If there was more than one inter-nest trail to previously existing nests (53/273, 19.4%) then we assigned the nest with the shortest connection as the founder. Finally, if the new nest was not connected to any old nests (70/273, 25.6%) then we assigned the closest old nest as the founder. If a new nest is only connected to another new nest we do not consider this to be a potential founding connection because it is not possible to ascertain which nest was founded first.

Assessment of seasonal differences

We used the subset of four colonies that were measured 16 times over two years to assess seasonal differences in foundation, abandonment and activity. We divided the active season into two parts to assess differences between total trail activity in the early part of the active season and total trail activity in the late part of the active season. We considered any time-point in the first half of the active season (May or June) to be ‘early season’ and any time-point in the second half of the active season (July or August) as ‘late season’.

Statistical analysis

We used Linear Mixed Models (LMMs) and Generalized Linear Mixed Models (GLMMs) to test different hypotheses using the data. We used Levene’s test to check each model for equal variances. The descriptions and results of each statistical model are detailed in Appendix K.

To describe the distribution of the number of new nests founded by each sub-colony we tested the observed distribution against a selection of different distributions to identify if there were any distributions that fit the data well. Full results of the model fits are detailed in Appendix L.

All data manipulation and analysis was performed in R version 3.5.2 (R Core Team 2013), statistical models were fitted using the lme4 package (Bates et al. 2015) , distributions were fit using the MASS package (Venables and Ripley 2013), network diagrams were made using igraph (Csardi & Nepusz 2016), and data manipulation and graphs were produced using the Tidyverse suite of packages (Wickham et al. 2019).

Results

Age structure, survival and nest foundation

In the dataset of 10 colonies that were mapped annually over 8 years we found that 30 of the 107 (28.04%) nests occupied at the most recent time-point (June 2019) were established before the study period began (July 2012). Consequently, although we do not know exactly how long these nests have been inhabited, we do know that they have been continuously inhabited for at least 7 years. Of the remaining 77 nests, 37 (48.05%) were inhabited for less than a year (Figure 3.2). Across the years that we observed the colonies, 32.2 ± 6.8% (mean ± SD) nests were abandoned per year, on average.

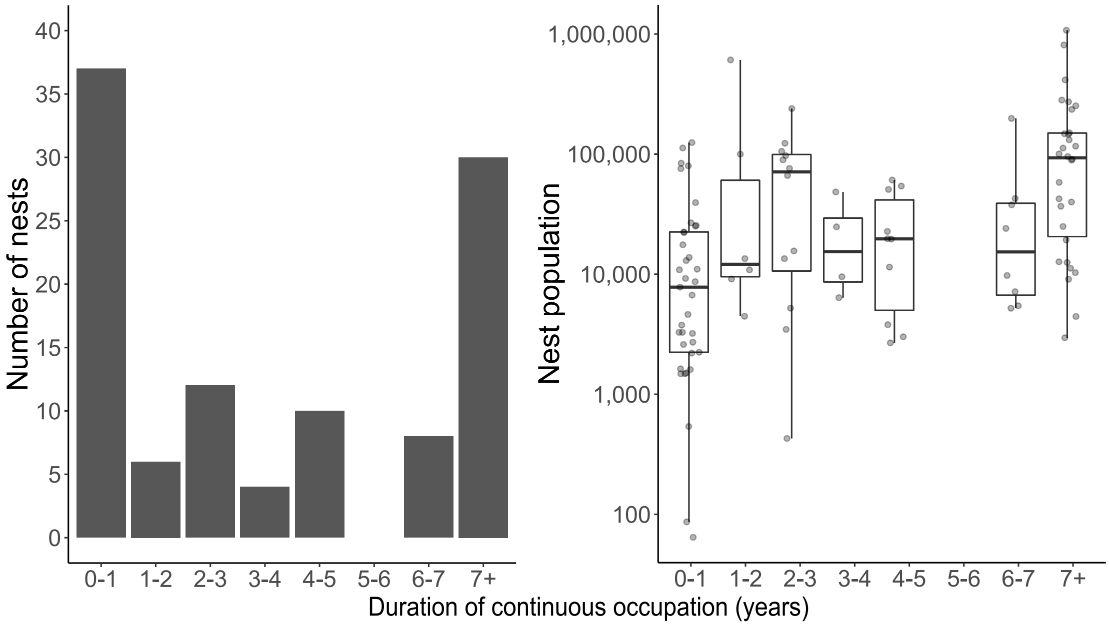


Figure 3.2. Distribution of the time that nests that were inhabited at the end of the study (2019) had been continuously inhabited (i.e. age structure of the population of nests) and the size of nests in each group. In the panel on the right each data point represents a single data point from an individual nest. For nests inhabited for the duration of the study, only the minimum age is known, indicated as 7+. Most nests are either abandoned in the first year or inhabited for many years.

During the first 4 years of the study period 89 new nests were established in our 10 study colonies. We found that 52 (58.4%) of these nests were inhabited for less than 2 years before being abandoned, while 25 (28.1%) were inhabited for at least 3 years (Figure 3.3).

The number of nests founded by a single sub-colony fits a negative-binomial distribution very closely with a mean of 0.52 and a size of 0.61 ($\chi^2 = 4.24$, $p = 0.64$, full fitting details in Appendix L), with the majority of sub-colonies (105 of 157, 66.9%) that founded new nests founding a single nest in the season (Figure 3.4). We find an extreme outlier of 10 new nests founded by a single sub-colony but it is possible that the new nests were not actually newly founded and, instead, may have been part of a separate previously unconnected network. Foundation of new nests during the study period was mostly performed by a minority of sub-colonies, with 33.5

± 24.5% (mean ± SD) of sub-colonies in a colony founding new nests in any given year. Larger nests were more likely to survive than smaller nests (Figure 3.5; Table AB1: Model 9; GLMM: Z = 7.33, p < 0.001) and were also more likely to found another nest (Figure 3.5; Table AB1: Model 8; GLMM: Z = 4.94, p < 0.001).

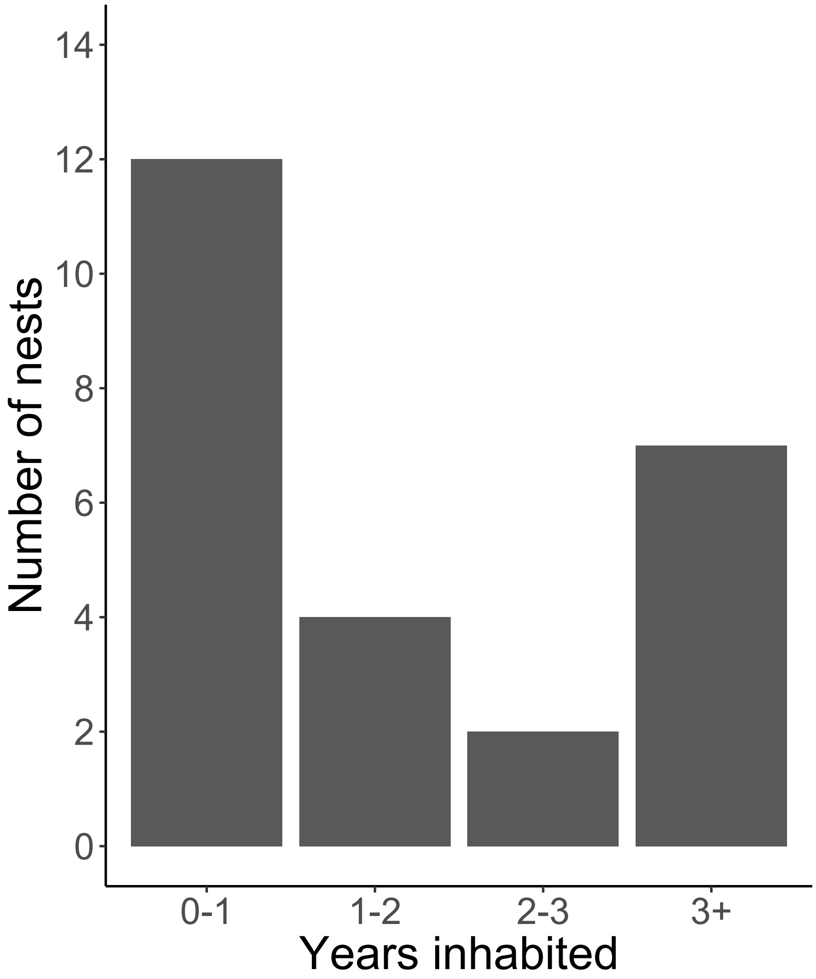


Figure 3.3. Distribution of the time that nests founded in the first four years of the study were inhabited before being abandoned. Due to continued occupancy of nests at the end of the study period, we only know the minimum occupancy for some nests. Most nests are either abandoned quickly or inhabited for many years.

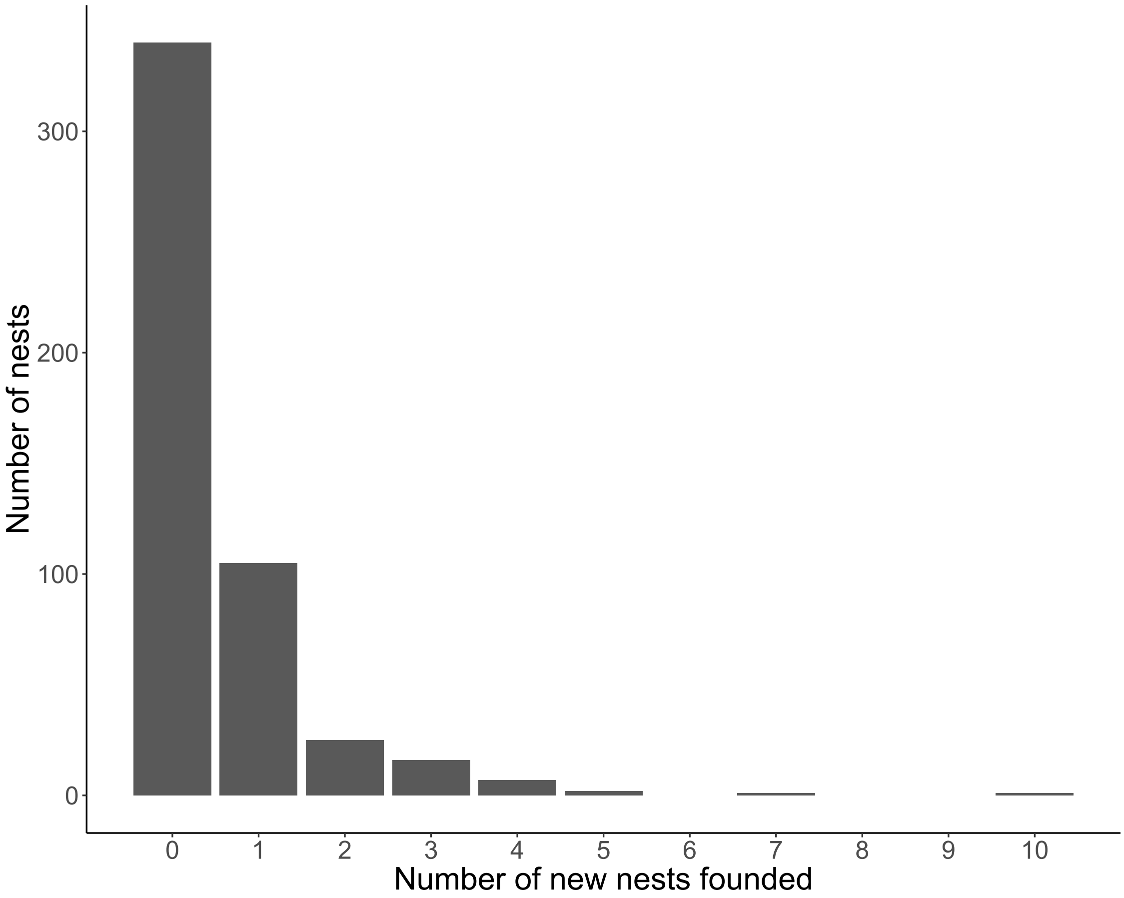


Figure 3.4. Distribution of the number of new nests founded by each nest in a season. All data points represent the activity of a single nest in a season. Most nests do not found any other new nests, but those that do generally produce a single new nest.

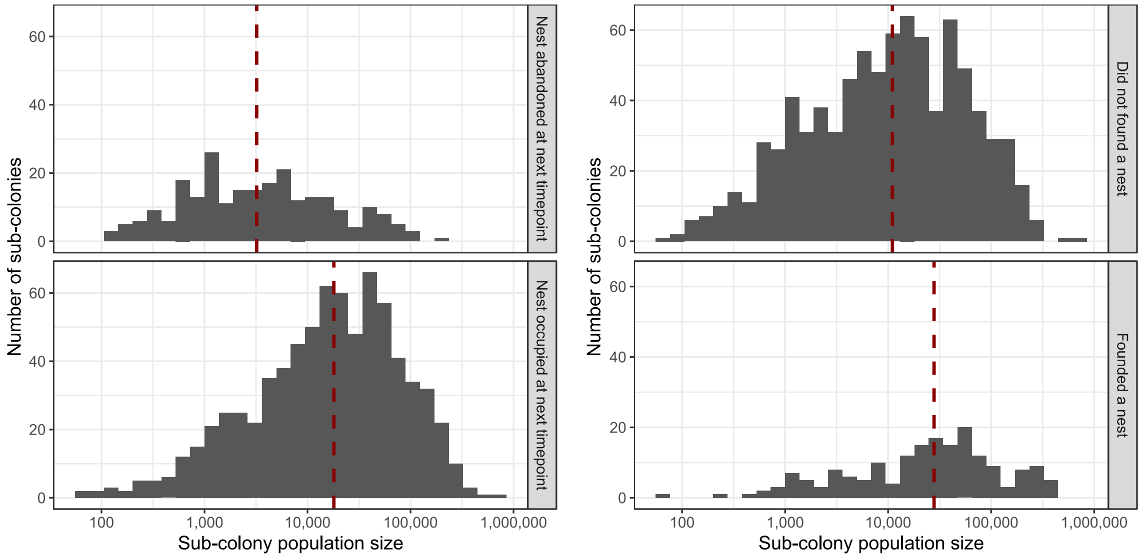


Figure 3.5. The distribution of population sizes of sub-colonies in different groups. Red dashed lines indicate median values. The left panel shows the distribution of the population of sub-colonies that were occupied at the next timepoint and those that were not. Sub-colonies that were still occupying their nest at the next timepoint were larger than those that abandoned their nest before the next timepoint. The right panel shows the distribution of the population of sub-colonies that founded a new nest and those that did not. Sub-colonies that founded new nests were larger than those that did not.

Seasonal differences in sub-colony activity

In sub-colonies inhabiting nests from the four colonies that were monitored at high frequency in 2017 and 2018, we did not find any difference in the total strength of foraging (Table AB1: Model 1; LMM: T = -0.92, p = 0.36), inter-nest (Table AB1: Model 2; LMM: T = 1.02, p = 0.31) or both foraging and inter-nest (Table AB1: Model 3; LMM: T = 0.17, p = 0.86) trails between maps recorded early and those recorded late in the season (Figure 3.6). There was also no significant seasonal difference in the rate of nest foundation (Figure 3.7A; Table AB1: Model 4; GLMM: Z = -1.3, p = 0.20). However, there were more nests per colony abandoned in the late season compared with the early season (Figure 3.7B; Table AB1: Model 5; GLMM: Z = 1.99, p = 0.047), with, on average, 1.57 more nests per colony (mean = 1.57, S.D. = 3.10) being abandoned in the late season compared to the early season. Furthermore, nests that were founded in the later part of the season were more likely to still be inhabited the following season (Figure 3.8; Table AB1: Model 10; GLMM: Z = 2.55, p = 0.011). Additionally, the rates at which trails were added (Table AB1: Model 6; GLMM: Z = -3.94, p < 0.001) and removed (Table AB1: Model 7; GLMM: Z = -2.21, p = 0.027) were higher in the early season compared to the late season (Figure 3.9).

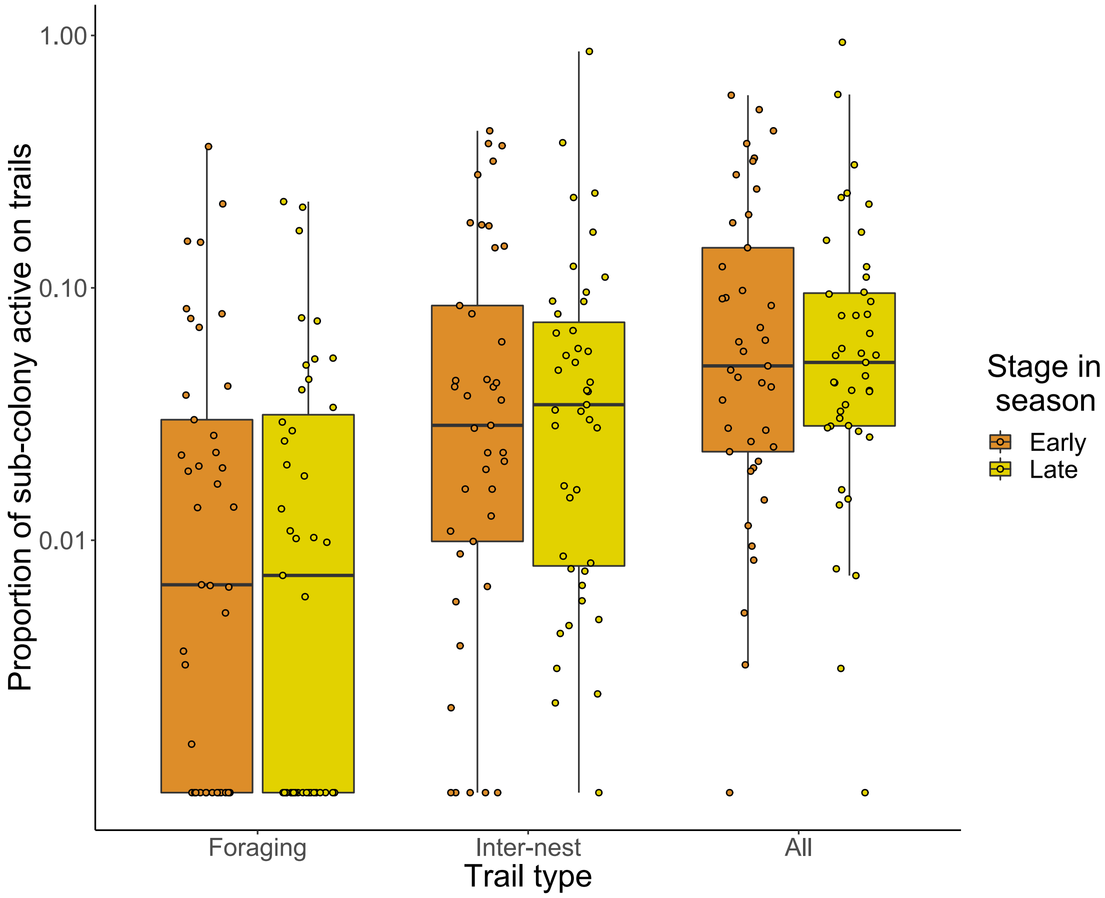


Figure 3.6. Boxplot demonstrating trail activity of sub-colonies early and late in the season. Each point represents a single sub-colony. All data are plotted as individual points. There is no difference in the amount of activity of sub-colonies on foraging or inter-nest trails depending on stage of season.

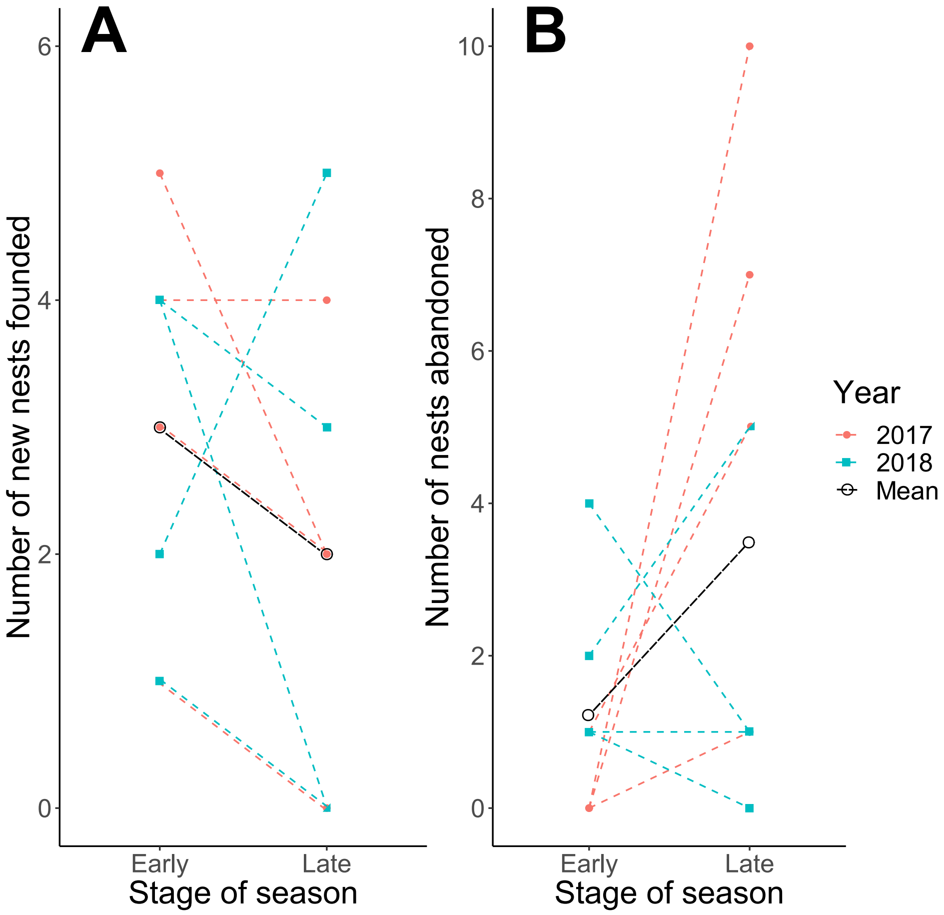


Figure 3.7. Seasonal differences in nest foundation (A) and abandonment (B) for nests in four regularly mapped colonies in 2017 and 2018. Each point represents a colony and dotted lines indicate data from the same colony in the same year. Means for each season are represented by open black circles. There is no difference in nest foundation between early and late season, but more nests are abandoned in the latter part of the season.

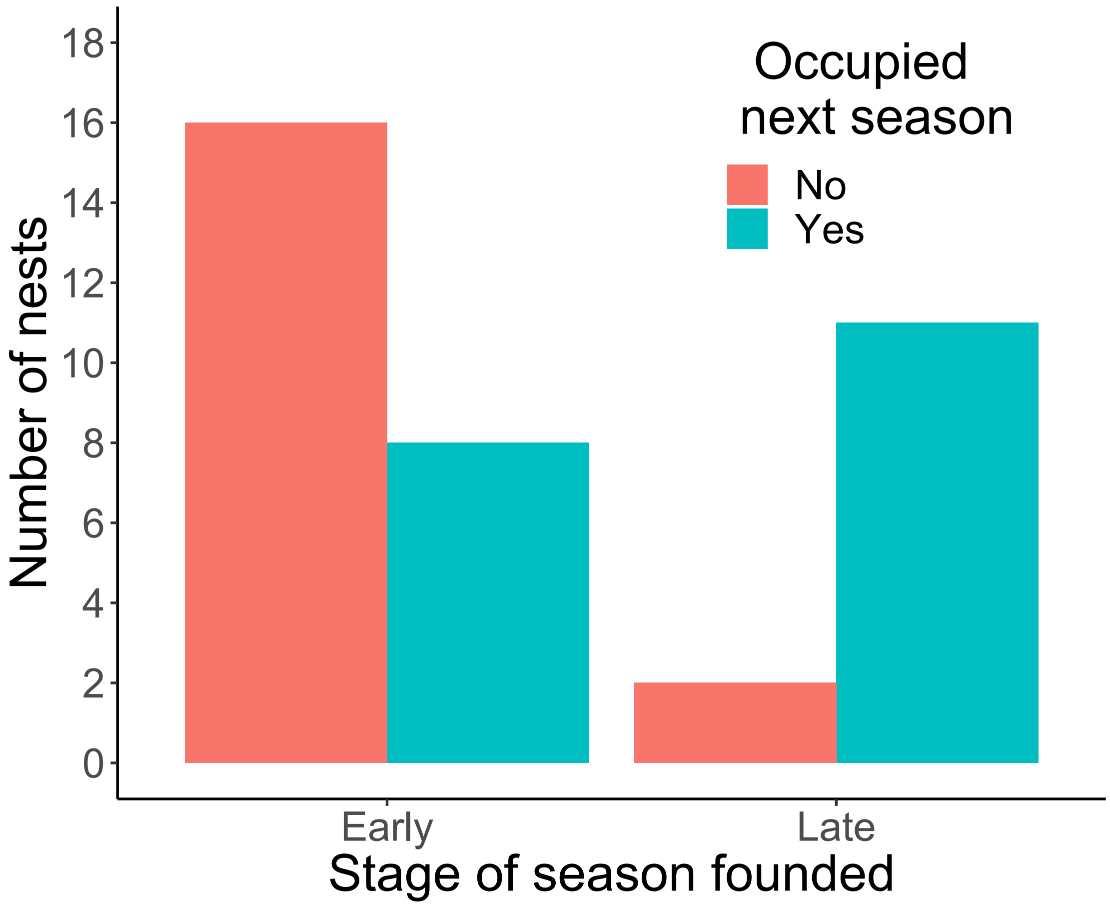


Figure 3.8. The number of nests that were occupied the following active season and the part of the season that they were founded in. Nests that were founded in the early stage of the active season were less likely to still be occupied the following season than nests that were founded in the late stage of the active season.

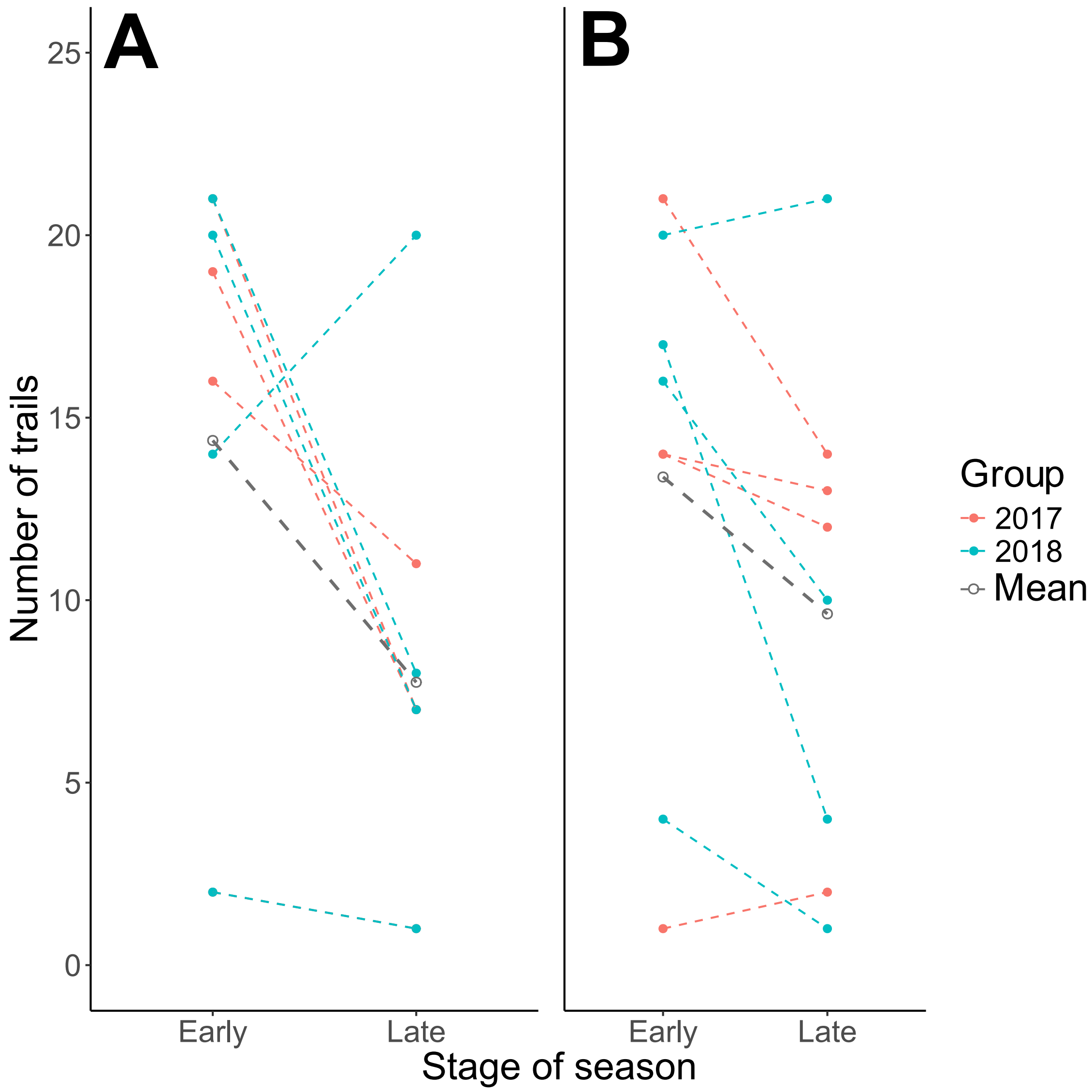


Figure 3.9. Number of trails added (A) or removed (B) early and late in the season. Each point within the season stage represents a colony and dotted lines indicate paired data from the same colony in the same year. Means for each season are represented by open black circles. There are more trail additions and trail removals in the early season compared to the late season.

Rate of trail usage and colony change

In the four colonies that were mapped frequently in 2017 and 2018 (Table AA1), trail usage changed (new trails added, or existing trails removed) at a mean rate of 0.36 ± 0.33 (mean ± S.D.) trails per nest per week. Consequently, the rate of change for trail networks was slow, with 59.8% of foraging trails and 64.5% of inter-nest trails that were active at the first time-point of the season also being active at the last time-point of the season. Of the nests that were inhabited at the first time-point of the season, 82.7% were still inhabited at the last time point of the season.

Discussion

We found that most nests were inhabited for less than a year before being abandoned. However, the nests that were inhabited for longer than a year grew larger and were often inhabited for more than three years, and many of these were still occupied at the end of our sampling period. This finding supports previous evidence showing that newly founded nests in polydomous colonies have a much higher rate of abandonment than established nests (Sorvari and Hakkarainen 2007). These findings also support the hypothesis that nest foundation in polydomous colonies is an important way for colonies to optimise the position of their nests (Ellis et al. 2017; Ellis and Robinson 2015). Sub-colonies that build nests in favourable locations begin foraging, grow and inhabit the nest for many years. In contrast, sub-colonies that build nests in unfavourable locations are unable to forage effectively and abandon the nest after a short time (Ellis and Robinson 2015). We also found that nest foundation events are relatively rare, with only around a third of nests in a colony budding a new nest in a given year, and with larger nests being more likely to bud a new nest than smaller nests.

A previous study found that most new polydomous *Formica yessensis* nests were constructed early in the season and most abandonments occur late in the season (Higashi 1976). Nest foundation early in the season is thought to be important for allowing nests the maximum amount of time to grow and collect resources for the winter months (Higashi 1976; Risch et al. 2016). However, we find a similar number of foundation events occurring in both the early and the late part of the season. Interestingly, our results show that nests that were founded in the late part of the season were more likely to still be inhabited the following season. This may be because sub-colonies that separate in the early part of the season have time to move or be absorbed into another sub-colony before the end of the season, while nests founded later in the season have less time to respond to local foraging conditions. This finding contrasts with previous hypotheses that nest foundation occurs in the early part of the season to maximise survival into the next season (Higashi 1976; Risch et al. 2016). In concordance with previous work (Higashi 1976), we found that more nests were abandoned late in the season. This might be because sub-colonies that found nests early in the season move the nest to a more favourable location or move into another existing nest before the season is finished. We also found that larger nests are less likely to be abandoned than smaller nests and more likely to found new nests. This is perhaps unsurprising as larger nests are likely to have become large as a result of being in a good location and are more likely to survive winters due to being larger and, therefore, easier to heat (Frouz et al. 2016).

Previous observations of polydomous wood ants indicates that inter-nest trails are established after foraging trails (Rosengren 1983). In this study we find that inter-nest and foraging activity are similar in the early and the late season. One likely explanation for this difference is that both inter-nest and foraging trails were established by the time we produced the first maps in May. Interestingly, we find that the rate of change for trails was reduced in the later part of the season with fewer trails being added or removed late in the season compared to early in the season. As the distribution of food in the environment is relatively static, trail networks may become more stable as the network becomes better matched to the resource environment.

In general, we find that foraging and inter-nest networks in the wood ant colonies change at a relatively slow rate, with most trails that are active at the start of a season also being active at the end of the season. This is not surprising as wood ants forage on static resources (Lanan 2014) and the configuration of inter-nest networks is influenced by the configuration of foraging networks (Ellis and Robinson 2016; Chapter 5). It is likely that foraging and inter-nest networks are much more dynamic in polydomous ant species that forage on more transient food sources.

Previous work on another polydomous wood ant species, *Formica aquilonia*, found annual nest abandonment rates of <2% in undisturbed forest interiors, but >50% in recently clear-cut areas (Sorvari & Hakkarainen 2007). In this study we found an annual nest abandonment rate of 32.2 ± 6.8% (mean ± SD), which is considerably higher than was found in undisturbed habitat by Sorvari & Hakkarainen (2007), but lower than the abandonment rate that they found in clear-cut areas. One possible reason for this difference is that *Formica lugubris* is an edge specialist, so the nesting locations available in the habitats where *F. lugubris* is present are much more variable in quality due to resources being sparser. As a result, there may be a higher rate of nest abandonment due to nests being abandoned and rebuilt elsewhere.

It seems likely that rates of nest foundation and abandonment would be quite different in populations that are monodomous or monogynous compared to the polydomous and polygynous species studied here. First, the survival of monogynous colonies is finite due to the dependence on a single queen, while polygynous colonies can, in theory, survive indefinitely. Second, it is possible that there is less nest relocation in monogynous colonies as the cost of losing the queen during moving is higher. Third, in monodomous colonies, nest foundation is riskier than in polydomous colonies, as sub-colonies that fail to establish a nest cannot return to their previous nest. Finally, monodomous colonies are less likely to succeed in founding a new nest as, generally, nests are founded by a single queen, whereas many workers are involved in founding a new polydomous nest. In contrast to this supposition, one previous study on several different, mostly monodomous, wood ant species found that nests were abandoned at a rate of 21-33% and roughly 31-51% of nests were involved in nest foundation every year (Klimetzek 1981). This is very similar to what we found in this study and indicates that different nesting strategies may not actually be so important in determining the duration of nest occupancy and rates of nest foundation. However, a more direct comparison of different strategies in similar locations would be necessary to test this hypothesis more conclusively.

Although we found that some nests were inhabited for a long time, we also found that many of the nests were inhabited very briefly. This may be due to a high level of resource utilisation, where new nests cannot grow due to a lack of available resources. If this is the case, when older nests are abandoned they may be replaced quickly by new nests that are able to take over food sources. However, there is little information available on the factors that cause nest abandonment in new nests. Future work to establish what factors are important in determining the continued inhabitation of new nests would likely provide useful information regarding the vulnerability of polydomous wood ants populations and guide conservation efforts.

In this study we mapped colonies annually for eight years, but many nests were present throughout, meaning we have no data on their foundation or abandonment. Continued monitoring of this and other comparable populations would increase the quality of the available data and help to further develop an understanding of how ant colonies use nests and how their activities change over time. Future work in this area would likely provide useful insights into ant ecology and behaviour. For example, it would be interesting to compare what we have found in polydomous wood ants to similar data for monodomous wood ants and polydomous ants of different species, particularly those that forage on resources with very different characteristics. The evolutionary and ecological drivers of polydomy and monodomy are still not clear (Burns et al. 2019a; Ellis and Robinson 2014; Robinson 2014). Consequently, comparative work on closely related species may help improve understanding of the evolution of colony organisation and of cooperation more generally.

Chapter 4 - Genetic distance is correlated with trail investment in polydomous wood ants

Abstract

Cooperative behaviour between kin allows individuals and groups to increase their direct or indirect fitness. Despite this, recognition of kin is difficult, so it is generally necessary to use proxies for relatedness to determine whether to cooperate. In this study, we aimed to identify the role of relatedness in resource sharing between distinct groups in ant colonies. Colonies of most ant species inhabit a single nest. However, in polydomous species the colonies divide into sub-colonies that inhabit distinct nests, often connected by resource sharing. Previous work on polydomous ants has demonstrated that the topology of resource sharing networks is driven by both spatial factors and disparities in food availability to sub-colonies. However, there are also differences in the relatedness of sub-colonies in polydomous colonies. If sub-colonies can cooperate by resource sharing preferentially with closely related sub-colonies then they may be able to increase their indirect fitness. To test this hypothesis, we used a long-term dataset of cooperative trail networks in polydomous wood ants and measured the relatedness among sub-colonies within those networks. We found that presence of a trail connecting two nests was best predicted by proximity, but both relatedness and differences in food availability were positively correlated with the strength of resource sharing between sub-colonies; i.e. cooperative trails are strongest when they link sub-colonies that are closely related and when there is a resource

imbalance between the sub-colonies. We hypothesise that sub-colonies are not able to detect relatedness to direct cooperation to more closely related sub-colonies. Instead, we suggest that relatedness between sub-colonies increases as a result of sharing a stronger connection, due to higher rates of brood transfer and migration. These findings highlight how, between groups, cooperation can become more common between relatives without the need for kin discrimination.

Introduction

The evolution of cooperative behaviour has long been a challenge for biologists to explain because it results in direct fitness benefits to another individual and does not always result in direct fitness benefits for the actor (Hamilton 1964; Heinsohn and Legge 1999). Most cooperative behaviours are thought to be advantageous either through direct fitness mechanisms, where the behaviour allows individuals to maximise the number of offspring they successfully rear (Cant 2011; Clutton-Brock 2009; Clutton-Brock and Parker 1995), or through indirect fitness mechanisms, where any direct fitness costs are ameliorated through an increase in the fitness of relatives (Gardner et al. 2011; Hamilton 1964).

Cooperation between non-kin can evolve where cooperation is the optimal strategy for maximising direct fitness. This can be a result of all co-operators increasing their direct fitness, because of either mutual benefit or reciprocity (Clutton-Brock 2009). However, it is also possible for cooperation to evolve where some co-operators suffer a loss in direct fitness. Such behaviours can evolve when there is policing of cooperation, whereby non-cooperation results in a high cost to direct fitness through punishment (Cant 2011; Clutton-Brock and Parker 1995). As a result, the optimal strategy may be to suffer a small fitness loss to avoid a higher fitness loss (Wenseleers et al. 2004).

Cooperation with kin is advantageous when it causes an increase to inclusive fitness (Hamilton 1964). However, recognising relatives is difficult as information about relatedness is difficult or impossible to access. Instead, it is necessary to use proxies for relatedness. These proxies can include acquired cues such as scent, recognisable characteristics, proximity or a combination of these factors (Leedale et al. 2018). Proximity is a commonly used proxy of relatedness because dispersal is often limited, meaning that relatedness is highly correlated with proximity (Hatchwell 2010). When this is the case cooperation can be directed towards nearby conspecifics as, on average, they are likely to be relatives.

In many cases, proxies for relatedness may be useful for directing cooperation; however, proxies can also become inaccurate, leading them to be maladaptive. For example, social parasites hijack proxies for relatedness, such as chemical signals, to elicit cooperative behaviour from their hosts (e.g. Akino et al. 1999; Buschinger 2009). Similarly, very large ant colonies that are spread across thousands of nests, known as ‘unicolonial’, are thought to have emerged as a result of the widespread use of proxies for relatedness that are optimal in small and highly related colonies (Helanterä et al. 2009). However, as these colonies grow, these proxies may become maladaptive (Helanterä et al. 2009).

Social insects have often been used as models for investigating the factors that drive cooperation. In many social insect species, most individuals do not reproduce, meaning that their fitness is entirely captured by indirect fitness. Therefore, cooperation with relatives is essential to maximise fitness and, as a result, social insect colonies can maintain large complex societies. However, relatedness in social insect colonies is often quite low due to the presence of several multiply mated queens (Heinze 2008). Furthermore, in some cases colonies are spread across multiple nests, known as polydomy, often with many queens inhabiting each of these nests (Debout et al. 2007; Robinson 2014). This means the idea that a colony is a well-defined group inhabiting a single nest with cooperation between all individuals within that group, is being challenged (Ellis et al. 2017b; Heinze 2008). Instead, cooperation and conflict within social insect colonies may be more complex, with both relatives and non-relatives cooperating.

In polydomous colonies, cooperation occurs not only between individuals, but also between spatially distinct sub-groups or ‘sub-colonies’ (Robinson and Barker 2017). These sub-colonies comprise workers, brood and (often) reproductives, inhabiting different nests in a polydomous colony and sharing resources, such as food, workers and brood, with each other (Ellis et al. 2017b; Robinson 2014). In polydomous wood ant colonies, the availability of food to each sub-colony is important in determining the resource sharing network (Ellis et al. 2014; Ellis and Robinson 2015).

In some cases, polydomous colonies have a single queen that produces brood that is distributed to the queen-less sub-colonies as brood or adult workers (Robinson 2014). In these cases, there is little variation between the relatedness of different sub-colonies and, therefore, relative relatedness of sub-colonies is unlikely to be an important factor in determining which sub-colonies cooperate. However, in many polydomous species each sub-colony contains at least one queen (Robinson 2014). As a result, there is variation in the average relatedness among pairs of sub-colonies, and sub-colonies that could direct cooperation towards more related sub-colonies might be able to increase their indirect fitness (Procter et al. 2016). Whether ants are actually able to detect the relatedness of other sub-colonies is unclear: in honeybees, nepotism is not found in multiply-mated colonies (Châline et al. 2005) nor colonies that have been fused (Rangel et al. 2009).

There are several different proxies for relatedness that sub-colonies might be able to use to help them to determine which sub-colonies they should share resources with. The simplest proxy that might be used is proximity. New sub-colonies in polydomous colonies are produced through budding, where a queen and a group of workers leave the nest and build a new nest nearby (Ellis & Robinson 2014). Therefore, there is a correlation between proximity and relatedness in polydomous wood ant sub-colonies (Holzer et al. 2006; Pedersen and Boomsma 1999; Procter et al. 2016). If there are mechanisms that lead to high intra-group relatedness then group identity may be a useful indicator of relatedness (Dyble and Clutton-Brock 2020). In ant colonies, individuals generally use olfactory signals as an indication of group identity. However, in ants chemical signals used for recognition are phenotypically plastic (Martin et al. 2019) and, therefore, are not useful direct proxies for relatedness.

At present, the only study to have directly assessed how relatedness influences cooperation between sub-colonies of polydomous ant colonies found no significant effect of relatedness on cooperation (Procter et al. 2016). However, they did not have access to historic colony trail data or information about the quantity of food available to each sub-colony through foraging. Consequently, they were unable to account for important factors such as the stability of cooperative relationship between sub-colonies, and the relative value of resource transfer (Ellis et al. 2014; Ellis and Robinson 2015).

In this study, we investigate the relationship between relatedness and co-operation among sub-colonies in polydomous wood ant (*Formica lugubris*) colonies, considering the resource environment and colony life-history. We hypothesise that the level of cooperation between sub-colonies will be predicted by the relatedness of their members.

Methods

Field site

The study site was National Trust's Longshaw Estate, Peak District, UK (53°18'55"N, 1°36'18"W). At the site there are over 900 wood ant nests belonging to a single species (*Formica lugubris*), spread across an area of roughly 300 ha (Ellis et al. 2014). The wood ants at the site are polydomous and include 10 polydomous colonies that have been mapped at least once per year for eight years (Burns et al. *Accepted*). The site is ideal for the study of wood ants as it is mostly composed of sparse woodland that is grazed by cattle and sheep, meaning that it is easy to identify trails of ants in the thin vegetation.

Sample collection

We collected samples of workers from 195 nests across the site, including all nests that were known to belong to one of the 10 colonies that had been regularly mapped for eight years. In total, we collected samples from: (1) all 106 nests that belonged to the 10 colonies that had previously been mapped; and (2) 89 nests that were spread across the site and had never been connected to any of the study colonies in the previous seven years. For nests that belonged to a mapped colony, we took samples after mapping was completed so sample collection did not affect observations. We collected 20 worker ants from each nest and stored them in 95% ethanol at -18ºC. As nests generally contain several thousand worker ants, the loss of 20 individuals is unlikely to be important and disturbance was otherwise kept to a minimum by removing ants only from the surface of the nest.

Due to unforeseen circumstances, we were only able to perform genetic analyses on the 105 nests that belonged to 10 colonies that had previously been mapped. The other 90 nests will be analysed in the future but are not available for inclusion in this thesis chapter.

Mapping

We mapped all ten colonies of wood ants at the time of sample collection (June 2019). Each colony had also been mapped at least once per year for seven years before sample collection. We considered nests to be part of the same colony if they had been connected by a social trail to the same network of nests at any point in any of the network maps collected. Each colony was mapped by identifying all nests in the colony and any foraging trails or inter-nest trails connected to each nest. We estimated the population of each nest by measuring the nest’s volume using a technique developed by Chen and Robinson (2013) and calibrated for use at this site by Ellis et al. (2014). We also estimated the strength of each trail by measuring the distance it took to count ten ants on the trail. The latitude and longitude coordinates of each nest were recorded using a GPS and used to calculate the distance between all pairs of nests.

Estimating foraging income and trail investment

We estimated the quantity of resources available through foraging to each sub-colony by summing the strengths of all foraging trails going to the nest and dividing this number by the nest population. We calculated the difference in food availability between all pairs of nests as this has previously been found to be important in determining resource-sharing in polydomous ant colonies (Ellis et al. 2014). To estimate trail investment in individual trails we estimated the number of ants on each trail by multiplying the strength of the trail (ants/m) and the length of the trail (m) and dividing this value by the mean population inhabiting the two connected nests, as was done in Ellis et al. (2014).

Estimating relatedness

Eight individual worker ants from each nest were genotyped at 11 polymorphic microsatellite loci. We identified microsatellites for eight individual worker ants from each nest. We extracted DNA from each sample by crushing two legs per individual while in liquid nitrogen and placing the product in 200µl 5% Chelex solution. We then incubated this at 95℃ for 30 minutes and vortexed for 15 minutes. We then amplified the DNA using a reaction mixture comprising: 1.5µl DNA template; 5µl QIAGEN Multiplex PCR-mastermix and 0.05µl forward and reverse primers of each primer pair. We adjusted the reaction mixture volume to 10µl using MilliQ water. We then identified microsatellites at the following loci using primers developed by Gyllenstrand et al. (2002): FE-7, FE-11, FE-13, FE-16, FE-19, FE-21, FE-37 and FE-38. We also used identified microsatellites at the following loci using primers developed by Chapuisat and Keller (1999): FL-12, FL-20 and FL-21. These primers were the same as used in a study on a nearby population by Procter et al. (2016). Each of the forward primers were labelled with fluorescent dyes for ABI and were of high purification quality (HPLC), while reverse primers were lower quality (de-salted). Genotyping was done using ABI3100 and genotypes were scored visually using Genemapper. Further details on the PCR conditions and labels can be found in Appendix M.

We calculated fixation index (F_{ST}) scores between all pairs of sub-colonies using the genet.dist function from the hierfstat package (Goudet 2005). In some cases, F_{ST} scores between pairs of sub-colonies were slightly below zero indicating that intra-group relatedness was lower than inter-group relatedness. This can happen when a pair of sub-colonies are essentially the same population, but randomness from sampling results in slight deviations from zero. In these cases, we set the genetic distance to zero.

Statistical analysis

To test for correlations between genetic distance, difference in the availability of food and Euclidean distance and resource sharing between sub-colonies we fitted a two-part multivariate generalized linear mixed model (GLMM) to the data using the MCMCglmm package (Hadfield 2010). We used a two-part model as we wanted to look at effects on both the presence of trails (binary) and the investment in trails (log-transformed to gaussian). The first part of the model used connection as the response variable and had a binomial error structure. The second part of the model used the log of investment in connections as a response variable and used a gaussian error structure. Each model had several fixed predictors and random effects of colony ID, dyad ID and multi-membership random effect of sub-colony ID (Equation 1). As the data used represent dyads of sub-colonies within a colony, it was necessary to add dyad ID and a multi-membership random effect of sub-colony ID as random effects to control for interdependence (Franks et al. *Accepted*; Kenny 1995; Kenny 2018).

$$\begin{aligned}(\text{trail, no-trail})_{ij} \sim \beta_0 + \beta_1\,\text{euclidean distance}_{ij} + \beta_2\,\text{genetic distance}_{ij} + \beta_3\,\text{food income distance}_{ij} \\ + \text{colonyID}_{ij} + \text{dyadID}_{ij} + \text{sub-colony ID}_i + \text{sub-colonyID}_j + \varepsilon_{ij} \qquad (1)\end{aligned}$$

To test whether Euclidean distance was correlated with genetic distance, we performed a Mantel test on the two distance matrices representing all pairwise Euclidean and genetic distances, with 9999 permutations.

All analyses were performed in R (R Core Team 2013). Data manipulation and plotting was performed using the Tidyverse suite of packages (Wickham et al. 2019), measurements of distance between coordinates were performed using the geosphere package (Hijmans et al. 2017), models were fitted using the MCMCglmm package (Hadfield 2010) and genetic distances were calculated using the "WC84" method in the genet.dist function from hierfstat package (Goudet 2005).

Results

Genetic diversity

We found that the genetic distance between pairs of sub-colonies at the site varied between 0 and 0.61 (median = 0.077, I.Q.R. = 0.094), indicating that there is significant genetic diversity at the site (Figure 4.1). Additionally, we found a weakly positive correlation between Euclidean distance and genetic distance in pairs of sub-colonies both at the population level (Mantel test: $Z = 134599$, $p < 0.001$) and within functional colonies (Mantel test: $Z = 721$, $p < 0.001$, Figure 4.2).

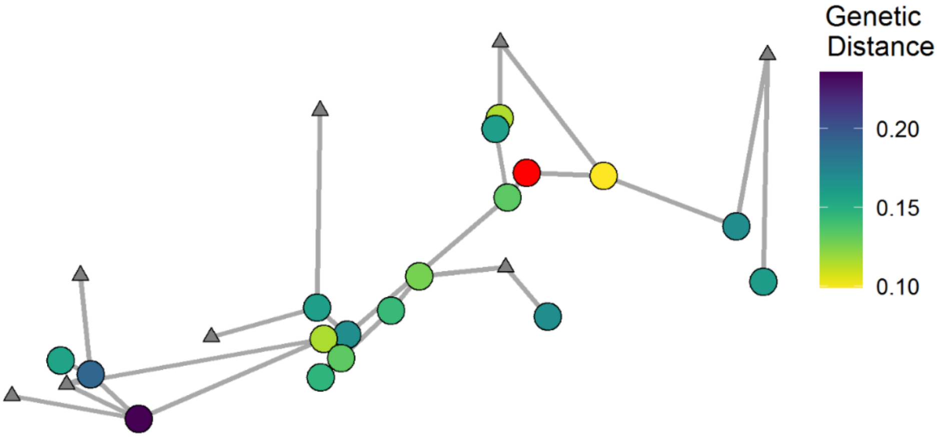


Figure 4.1. A polydomous foraging and inter-nest network. Circular nodes indicate nests and triangular nodes indicate trees. The colour of nest nodes indicates the relatedness of each nest to a selected node (highlighted in red).

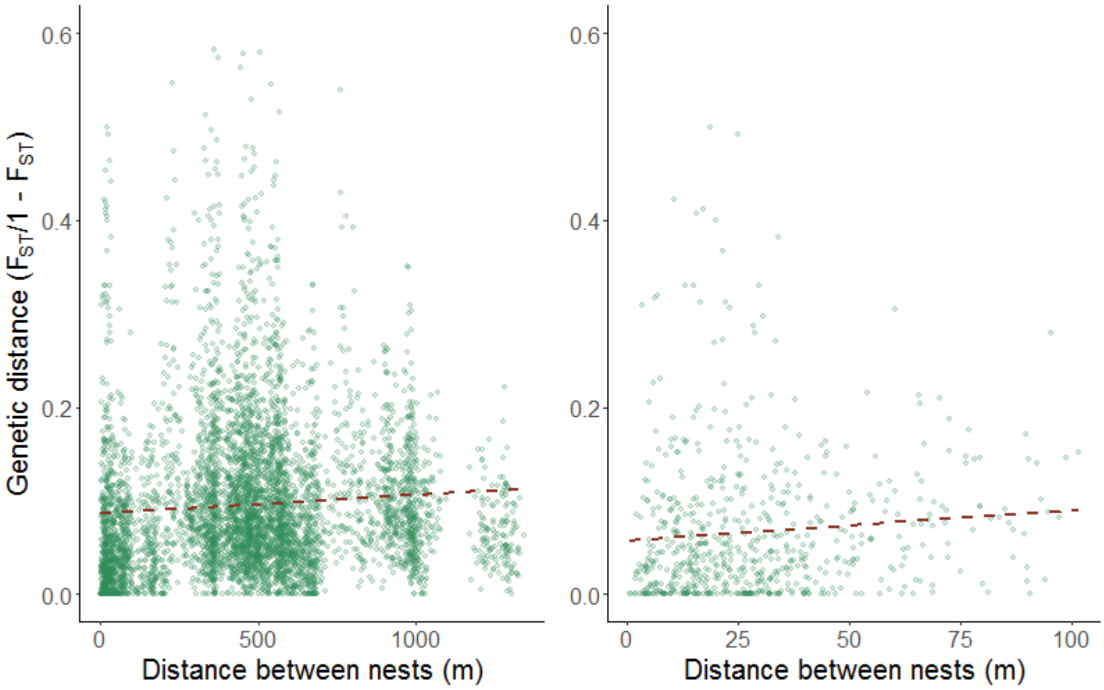


Figure 4.2. Euclidean distance and genetic distance of pairs of sub-colonies are positively correlated both at: (A) Population level; and (B) Within functional colonies. Each point represents a pair of nests and the dashed line represents the fit of a linear model.

Effect of distance, relatedness and the food environment on presence and investment in inter-nest trails

At the time of mapping there were 87 inter-nest trails between nests observed in the study. Our two-part model tested whether each factor influenced the presence of inter-nest trails between a pair of nests and, if there was a trail, the investment in that inter-nest trail. We found that Euclidean distance between nests was a significant factor in predicting whether there was a connection between two nests (GLMM: post.mean = -0.16, 95% credible interval: -0.21 - -0.13, pMCMC < 0.001, Figure 4.3A), but not on the investment in trails between sub-colonies (GLMM: post.mean = 0.22, credible interval: -0.13 - 0.61, pMCMC = 0.26, Figure 4.4A). We did not find a significant effect of genetic relatedness on the presence of inter-nest trails (GLMM: post.mean = -2.53, credible interval: -7.87 - 1.39, pMCMC = 0.20, Figure 4.3B), but there was a significant effect of genetic relatedness on the investment in trails between sub-colonies, with more closely related sub-colonies investing more in trails between them (GLMM: post.mean = -0.38, credible interval: -0.71 - -0.053, pMCMC = 0.020, Figure 4.4B). Similarly, while we did not find a significant effect of difference in food income between sub-colonies on the presence of inter-nest trails (GLMM: post.mean = -1361.97, credible interval: -4538.64 - 1899.54, pMCMC = 0.28, Figure 4.3C), there was an effect of difference in food income on the investment in trails between sub-colonies, with higher investment in trails between sub-colonies with a higher difference in food income (GLMM: post.mean = 0.35, credible interval: 0.061 - 0.67, pMCMC = 0.028, Figure 4.4C).

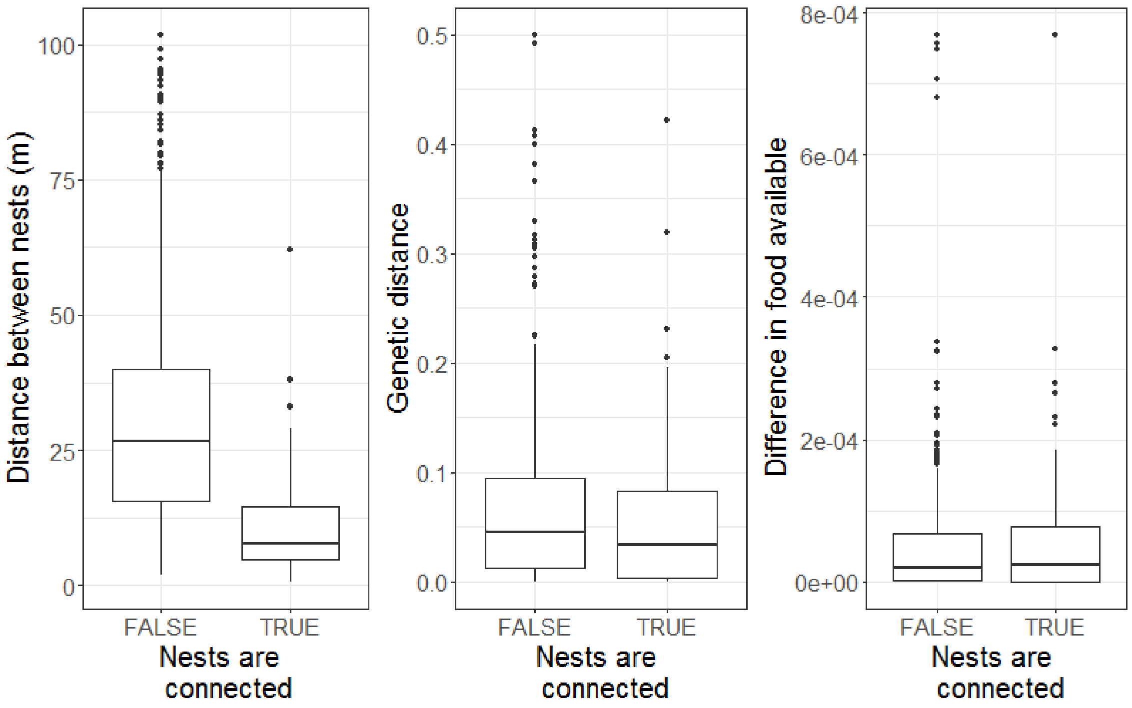


Figure 4.3. Differences between connected and unconnected nests for: (A) Euclidean distance; (B) Differences in food available; and (C) Genetic distance.

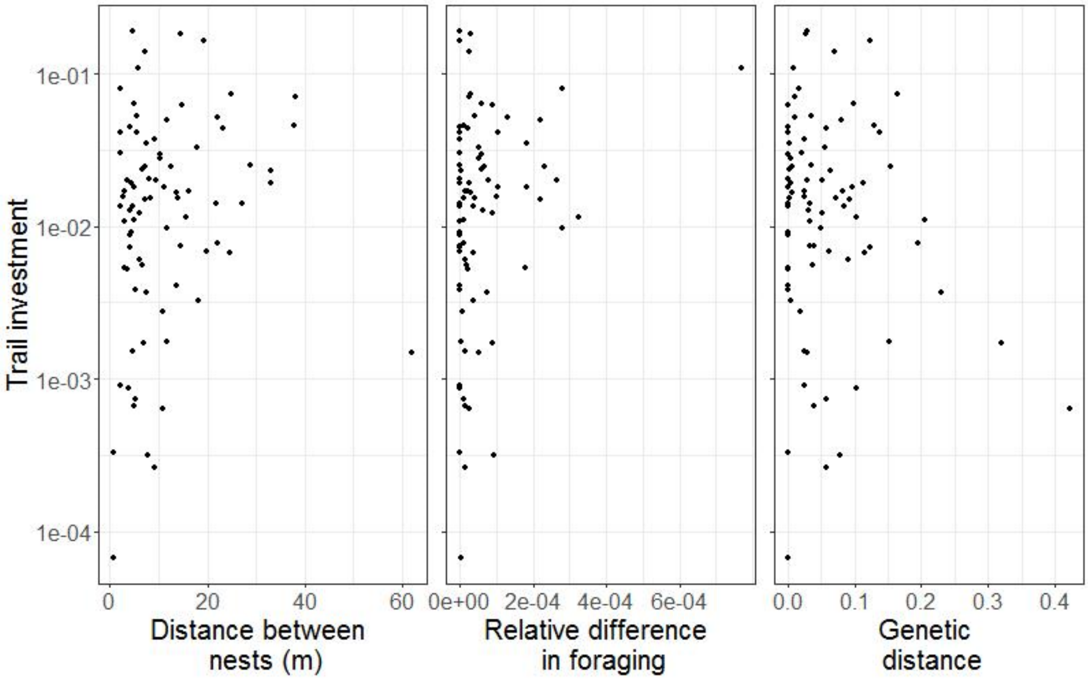


Figure 4.4. Correlations between trail investment and dyad-level measures: (A) Euclidean distance; (B) Differences in food available; and (C) Genetic distance.

Discussion

Our results clearly show that distance between nests is important in determining the presence of inter-nest trails, while relatedness and the difference in availability of foraging resources are correlated with the relative investment of sub-colonies in these connections. We found that although genetic distance is not correlated with the *likelihood* that two sub-colonies are connected, it is correlated with the *strength* of connections between connected sub-colonies. While the mechanism for the interaction between genetic distance and resource sharing is not clear, it highlights the possibility that sub-colonies are able to access indirect fitness benefits through investing more heavily in sharing resources with other related sub-colonies. Together these results demonstrate the interplay between spatial factors, the kin context and the food environment in shaping cooperative resource-sharing networks in polydomous colonies.

We found that relatedness was not a predictor of whether two sub-colonies were likely to share resources with each other, which is the same result as Procter et al. (2016). However, when a trail did exist between two sub-colonies, we found that relatedness was positively correlated with investment in that trail. One possible mechanism for this is that sub-colonies direct cooperation towards sub-colonies to which they are more closely related. However, this would require ants to be able to detect their relatedness to members of other sub-colonies. The most likely way that sub-colonies could do this is through olfactory cues. However, while polydomous wood ants can distinguish between nestmates and non-nestmates using olfactory cues (Holzer et al. 2006), these cues do not seem to be useful as direct signals of relatedness (Martin et al. 2019). Instead, olfactory cues are probably useful only as indicators of the nest that an individual inhabits. One alternative explanation for our results is that strong connections between sub-colonies result in higher rates of migration and brood transfer, which subsequently lead to a higher level of relatedness. The relatedness of two groups can change over time due to changing group membership, while relatedness between individuals is fixed. Therefore, changes to relatedness as a result of cooperation are only possible in groups.

Previous work on the same colonies of polydomous wood ants found a significant relationship between both distance and differences in foraged food on the investment in connections between connected nests (Ellis et al. 2014). Interestingly, we find that the distance between a pair of nests influences the likelihood that the sub-colonies inhabiting the nests will connect through a trail, while the difference in food available to sub-colonies influences the investment in existing trails. The likely mechanism for this discrepancy is that trails between sub-colonies are formed mostly in response to distance, but only useful trails (e.g. between a sub-colony with excess resources and another with a lack of resources) are strengthened by heavy recruitment. This would match previous findings that inter-nest networks are driven by the distribution of resources across sub-colonies (Burns et al. 2020; Ellis and Robinson 2015).

One important limitation of this work is that we are not able to draw conclusions about the direction of causation between relatedness and resource sharing. There are a few possible study designs that could be used to investigate this further. One option would be to take samples from nests at several timepoints and look at how relatedness between these nests changed over time. Through this method it would be possible to see whether sub-colonies that are connected by strong trails become more closely related. Another option would be to use a transplantation experiment. For example, it would be possible to transplant sub-colonies into distantly related colonies and observe the changes in the resource sharing network. This type of experiment could be possible in wood ant species as transplantation of wood ant nests have been successfully performed (Nielsen et al. 2018). However, the success rate of nest transplantation in wood ant colonies is generally low and often the nest will be moved after transplantation (Nielsen et al. 2018). Therefore, other polydomous species may be better suited to this kind of experiment.

This work is the first to find that there is a correlation between the strength of social connection between groups in polydomous colonies and the relatedness of those groups. An interesting extension of this work would be to investigate this relationship in other polydomous ant colonies or even in other taxa where there is cooperation between groups (Robinson and Barker 2017).

In conclusion, we found that while distance is the only important determinant of the existence of resource sharing between the sub-colonies that we measured, both relatedness and difference in food availability correlate with the quantity of resource sharing between sub-colonies. This result demonstrates that sub-colonies could access indirect fitness benefits from resource sharing. However, indirect fitness benefits may not be a result of sub-colonies sharing resources preferentially with more closely related sub-colonies. Instead, we hypothesise that higher relatedness between strongly cooperating sub-colonies is a consequence of a higher rate of migration and brood transfer between strongly connected sub-colonies. More broadly our study has highlighted the connections between resource distribution, relatedness and cooperative interactions in groups.

Chapter 5 - Ant colony nest networks adapt to resource disruption

Abstract

Animal social structure is shaped by environmental conditions, such as food availability. This is important as conditions are likely to change in the future and changes to social structure can have cascading ecological effects. Wood ants are a useful taxon for the study of the relationship between social structure and environmental conditions, as some populations form large nest networks and they are ecologically dominant in many northern hemisphere woodlands. Nest networks are formed when a colony inhabits more than one nest, known as polydomy. Polydomous colonies are composed of distinct sub-colonies that inhabit spatially distinct nests and that share resources with each other. In this study, we performed a controlled experiment on ten polydomous wood ant (*Formica lugubris*) colonies to test how changing the resource environment affects the social structure of a polydomous colony. We took network maps of all colonies for five years before the experiment to assess how the networks change under natural conditions. After this period, we prevented ants from accessing an important food source for a year in five colonies and left the other five colonies undisturbed. We found that preventing access to an important food source causes polydomous wood ant colony networks to fragment into smaller components and begin foraging on previously unused food sources. These changes were not associated with a reduction in the growth of populations inhabiting individual nests (sub-colonies), foundation of new nests or survival, when compared with control colonies. Colony splitting likely occurred as the availability of food in

each nest changed causing sub-colonies to change their inter-nest connections. Consequently, our results demonstrate that polydomous colonies can adjust to environmental changes by altering their social network.

Introduction

Changes to environmental conditions influence not only the composition of assemblages in different habitats, but also affect the social structure of many animal societies (Sueur *et al.* 2019). Social structure is the organization of units (e.g. individuals, groups) that emerges from their social behaviour. Understanding how social structure responds to environmental conditions is important because changes to social structure can have cascading effects within an ecosystem, for example changes to wolf pack size, caused by an altered climate, influences plant growth in certain areas (Post *et al.* 1999). Furthermore, in many places environmental change is occurring at an increased rate due to human activity and, consequently, understanding the effect that this may have on animal social structure is important for conservation (Snijders *et al.* 2017).

Social network analysis is a tool for studying social behaviour, where systems of socially connected units are modelled as networks with nodes representing units and connections representing the flow of resources, such as food or information (Croft *et al.* 2011; Brent 2015). Most research on animal social networks has focused on the structure and function of static networks. However, animal social networks are dynamic: the identities of the nodes present, their characteristics and their connections change over time (Blonder *et al.* 2012). Changes in animal social networks can occur in response to an internal network change, such as nodes strengthening their connections when another node is removed (Firth *et al.* 2017); however, in many cases, social network structure changes in response to changing environmental conditions (Sueur *et al.* 2019).

There are a variety of environmental factors, including habitat complexity, availability of food and predation, that can influence social structure, and spatial variation of these factors can cause social structure to vary spatially (Lattanzio & Miles 2014; Lantz & Karubian 2017; Tavares, Samarra & Miller 2017). One important environmental factor in influencing changes to social structure in many animal social groups is resource distribution. For example, in years when food is scarce, killer whales (*Orcinus orca*) form less connected networks than in years when food is abundant, possibly as a consequence of having more time available for social interactions, rather than searching for prey (Foster *et al.* 2012). Although there is some evidence from observational studies for the effect of resource distribution on social structure (Ansmann *et al.* 2012; Silk *et al.* 2014; Foster et al. 2012; (Henzi *et al.* 2009), few empirical tests exist (Sendova-Franks *et al.* 2010; Firth & Sheldon 2015; Bles, Deneubourg & Nicolis 2018). This is because resource distribution is often difficult to manipulate in the field, and networks are time consuming to map, meaning that multiple time points or replication at the network level are often infeasible. One system that overcomes these limitations is colony nest networks of ants (Robinson 2014). Colony nest networks are found in certain species of ants where colonies divide into several sub-colonies that inhabit spatially discrete nests, a strategy known as polydomy. Polydomous nest networks are a useful system for studying dynamic social networks because: (1) Complete networks can be mapped quickly without disturbing the animals; (2) Local environmental factors, such as the distribution of food, can be manipulated; and (3) Multiple networks can be mapped allowing replication at the network level.

Polydomy may benefit colonies by increasing foraging efficiency, reducing vulnerability to predators, and increasing competitive dominance (Robinson 2014; Burns *et al.* 2019a). Polydomy can also carry some costs for colonies such as limitations to communication and costs incurred by moving resources between nests (Robinson 2014). This trade-off between multiple costs and benefits has led to polydomy evolving independently several times in a diverse set of taxa (Debout *et al.* 2007).

An important taxon group with polydomous colonies are wood ants, considered keystone species in many northern hemisphere woodlands (Stockan & Robinson 2016). Many populations of wood ants have polydomous colonies, with nests that share resources almost exclusively on foraging trails (Ellis & Robinson 2014; Procter *et al.* 2016). Although wood ants do predate many invertebrates, they rely heavily on carbohydrates from exudates secreted by aphid colonies that they farm on a variety of tree species (Rosengren 1991; Stockan & Robinson 2016). Because of their dependence on aphids, wood ants are vulnerable to tree loss: clear-felling of forest plantations has consequences for nest survival, the presence of ant-associated species and rates of decomposition on the forest floor (Sorvari & Hakkarainen 2007; Sorvari, Elo & Härkönen 2016; Elo, Penttinen & Sorvari 2018; Härkönen & Sorvari 2018). However, due to the difficulties of manipulating resource distribution and producing accurate network maps, the effect of changes to resource distribution on the social network structure of polydomous ant colonies has not yet been studied.

Social network structure has been shown to be important for buffering the effects of environmental variability in some species (Henzi *et al.* 2009; Silk *et al.* 2014), but consequences may also be undesirable, such as facilitating the spread of disease through a system (e.g. Hamede *et al.* 2009). However, social network structures can be modified to limit the spread of negative effects, such as disease, by changing the interactions between certain groups (Stroeymeyt *et al.* 2018). As a result, the way that networks adapt to environmental changes and the consequences of changes to social structure on the growth, survival and reproduction of individual nodes are difficult to predict.

In this study, we aimed to investigate how environmental factors influence colony topology. Nest locations in polydomous wood ant networks are heavily influenced by the distribution of food sources and trail networks are formed in response to the relative distribution of food in these nests (Ellis, Franks & Robinson 2014; Ellis & Robinson 2016). Consequently, changes in resource distribution may cause significant disruption to trail networks. To investigate this hypothesis, we experimentally manipulated resource availability in multiple polydomous ant colonies by removing a key food source. We predicted that manipulation of resource distribution in polydomous colonies would cause changes to foraging networks and, subsequently, to inter-nest networks, as resource flow would be disrupted.

Materials and methods

Study system

This study was conducted at Longshaw Estate in the Peak District, UK (N53° 18’ 33’’, E-1° 36’ 96’’). At the site there are over 900 ant nests from a number of polydomous colonies (Ellis, Franks & Robinson 2014). Most of the site is grazed by sheep and cattle, which means that trails from each nest are easily identifiable. The wood ant colonies at the site are all *Formica lugubris*, which forage predominantly by farming aphids that feed on a variety of tree species, including Scot’s Pine (*Pinus sylvestris*), Birch (*Betula* spp.), Oak (*Quercus* spp.) and Larch (*Larix decidua*) through the active season (roughly April-September) and are inactive for the rest of the year, when it is too cold to forage. Foraging and inter-nest trails that are used by the ants are established at the start of the foraging season; new trails are often added, and old trails may be abandoned. Both the difference in foraged food available to two sub-colonies and the distance between the nests are important factors in determining the presence and strength of an inter-nest trail between the nests (Ellis, Franks & Robinson 2014). Thus, inter-nest networks are neither minimal spanning nor highly connected. Instead they are formed in a way that trades off efficiency and robustness (Cook, Franks & Robinson 2014). Similarly, foraging networks are connected in a way that trades off trail length and the distance between the nest and foraging sites (Buhl *et al.* 2009).

This study used ten polydomous colonies which had been mapped (see *Mapping of colonies*) at least once every year for four years (2012-2015) before the beginning of this study during previous observational work on this system (see e.g. Ellis & Robinson 2015). Between-colony differences are likely to exist due to factors such as varying environmental conditions and different network sizes. At the start of this study, colonies had a median of 11 nests (range: 4-20) and a median estimated colony population size of 658,059 ants (range: 78,798 – 1,218,878). Sub-colonies inhabiting nests in each of the 10 colonies foraged on an average of 1 (median = 1, I.Q.R. = 1) foraging trail to trees and an average of 1 (median = 1, I.Q.R. = 1) inter-nest trails to other nests at the start of the study. In total colonies foraged on an average of 6 (median = 6, I.Q.R. = 3) trees at the start of the study. Ten colonies were used to provide replication at the network level, which was important as we were interested in colony-level effects (James, Croft & Krause 2009).

Mapping of colonies

Colonies were mapped only on warm and sunny days between 10.00 and 18.00, when the ants are most active. Each time we mapped a colony we recorded the spatial position of nodes: inhabited nests and food sources. We also recorded the nodes connected by inter-nest and foraging trails. We estimated the strength of each trail by recording the length of trail required to count 10 foragers; the minimum and maximum lengths it was possible to do this accurately were 10 mm and 4 m respectively. We estimated the number of ants active on a trail by multiplying the strength of the trail by the length of the trail. Finally, we used the volume of each nest to estimate the population inhabiting the nest using the mound-volume technique described in Chen and Robinson (2013) Although not as accurate as mark-recapture methods, the mound-volume method for estimating colony size has been found to be a useful measure of colony size when avoiding disturbance is important, (Chen and Robinson 2013) and has been calibrated against mark-recapture data at this site in a previous study (Ellis, Franks & Robinson 2014) This network mapping protocol follows previous work on this population (Ellis and Robinson, 2015).

Experimental design

We split the ten colonies being used in the experiment into pairs with a similar number of nests and then randomly allocated one colony into the control group and the other into the treatment group. We then selected a focal tree which was to be excluded in the treatment colonies or undisturbed in control colonies. Focal trees were selected by two criteria: (1) The tree must have been foraged on at every previous time point; and (2) The tree must be suitable for fitting with an exclusion collar to prevent ants accessing it (i.e. no branches touching the ground and a single trunk, e.g. Figure 5.1). If more than one tree in a colony matched these criteria, we chose the tree that had the strongest total trail strength going to it at the previous time point (August 2016). We used this method so that we selected only trees that were important food sources for the colony. At the start of this study, the focal trees were foraged on by an average of 3 ± 1 (median ± IQR) nests and accounted for 33.3 ± 22.9 % (median ± IQR) of the foraging trails in the colonies.

Figure 5.1. Example of tree fitted with exclusion collar. Collar is fixed to tree using silicone sealant and underside is painted with Fluon® to prevent ants from accessing the canopy of the tree.

In each of the excluded colonies we limited ant access to the focal tree before foraging began in early 2017 by fitting an exclusion collar (Figure 5.1). Each exclusion collar consisted of a plastic cone wrapped around the tree with any gaps between the cone and the tree being filled with clear aquarium grade silicone sealant (Everbuild AquaMate Sealant®). The underside of the plastic cone was painted with Fluon (Whitford®), to prevent ants from accessing the tree’s canopy. We found that these exclusion collars were effective for four out of the five trees they were fitted to, with a mean reduction of 98.3% in foraging trail strength to the trees. However, in one colony ants accessed the canopy of the focal tree through the canopy of a neighbouring tree (Burns DDR, *personal observation*). This colony was removed from all analyses.

We left the collars in place for one year, after which we removed them, before foraging began in Spring 2018. Before the experiment began these colonies had been mapped at least once every year for 4 years. For this study we mapped each colony at four time points: (1) Before we installed the collars, in August 2016; (2) After we installed the collars, in June 2017; (3) Late in the season, in August 2017; and (4) After the first overwinter since the collars were installed, in June 2018. Here, we use the August 2016 and August 2017 time points to assess changes to colonies and nests. These time points were selected as it allows comparison between colonies a year before the installation of the exclusion collars (August 2015 – August 2016) and a year after the installation of the exclusion collars (August 2016 – August 2017). All data, including maps not used in the analysis here, are available from the OSF Data Repository (Burns *et al.* 2019b).

Statistical analyses

To test whether there was an effect of the exclusion on the number of independent groups of connected nests (hereafter referred to as *network components*), number of inter-nest trails, survival of nest populations, change in growth of nest population compared to previous year (2015-2016) and new nest foundation we used Brunner-Langer f1-ld-f1 models to compare the effect of time on both excluded and control colonies using the ANOVA-type test within that model (Brunner *et al.* 2002). Brunner-Langer f1-ld-f1 models are an extension of the Wilcoxon-Mann-Whitney test designed specifically for use for non-parametric analysis of repeated-measures of samples divided into different treatments, as was performed in our experiment. Brunner-Langer f1-ld-f1 models have similar assumptions to the Wilcoxon-Mann-Whitney test, such as that samples and treatments are independent of each other, the independent variables are distinct groups and that the dependent variable is either continuous or ordinal (Brunner *et al.* 2002). The models were fitted using the nparLD package in R (Noguchi *et al.* 2012) in R version 3.5.0 (R Core Team 2013).

Results

Change in number of network components

Before the installation of the exclusion collars, most colonies were composed of a single network component (median = 1, IQR = 0). However, after a year, colonies in which a single important food source was removed had split into several network components (Figure 5.2; median = 5, IQR = 3.25), which was a significant change from the previous year (f1-ld-f1: F = 13.36, $p < 0.0005$). In contrast, control colonies were not composed of significantly more network components (Figure 5.2; median = 1, IQR = 2; f1-ld-f1: F = 2.61, $p = 0.11$).

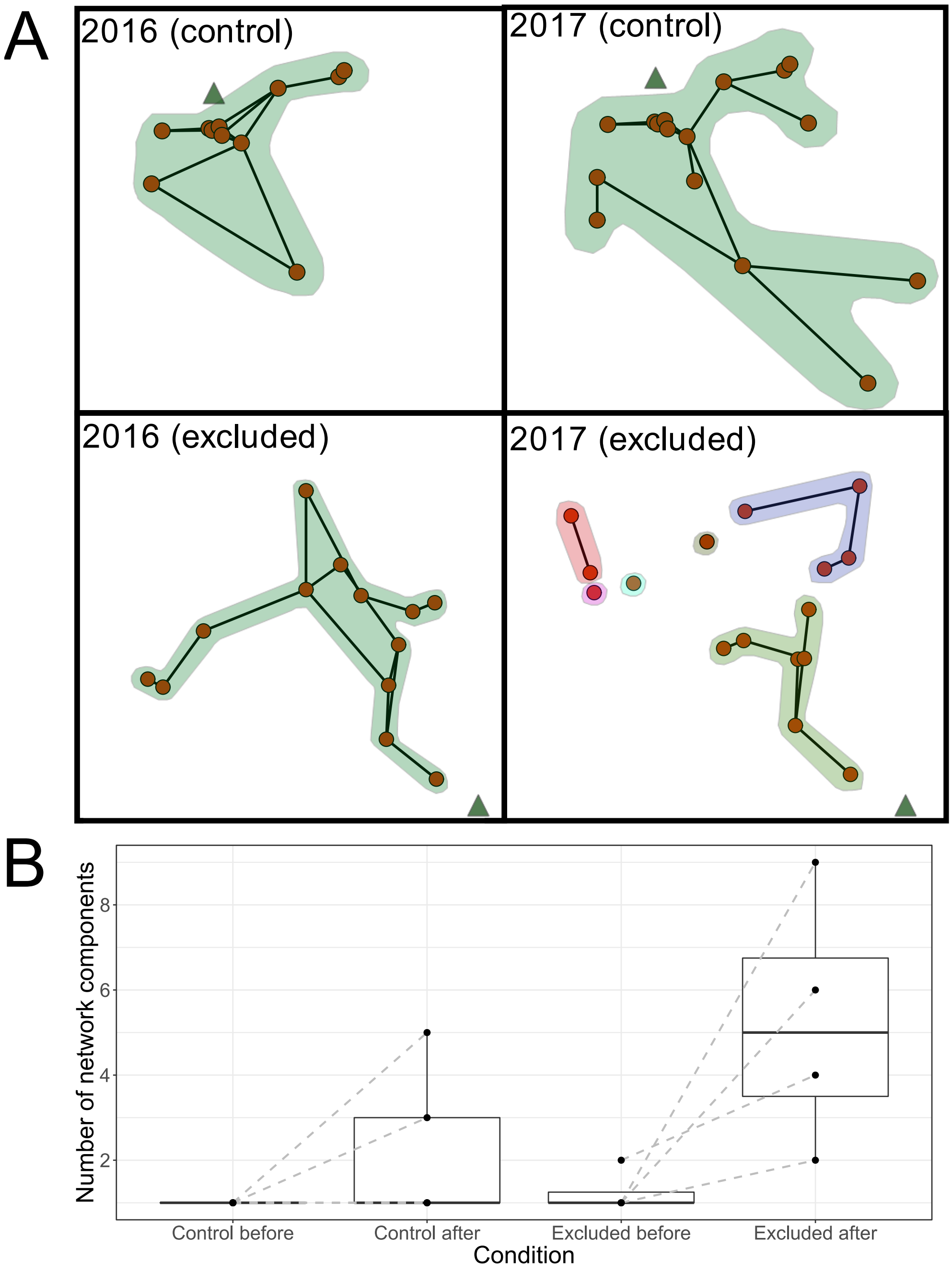


Figure 5.2. Control networks showed no change, while networks with exclusion showed an increase in network components. A: Examples of inter-nest networks of colonies before (2016) and after (2017) a single key food source was either unchanged (control condition) or excluded (excluded condition). Nodes indicate nests, black connections indicate inter-nest trails and coloured polygons indicate independent network components. Green triangles indicate the location of the focal food source. B: Comparison of number of network components of colonies before (August 2016) and after (August 2017) in the control and "excluded" conditions. All data are plotted as individual points. Lines between points indicate colony identity.

Changes to inter-nest trails

Before the excluders were installed, exclusion colonies had a median of 12 and an IQR of 6.5 inter-nest trails, while control colonies had a median of 13 and an IQR of 4 inter-nest trails (see network maps in Appendix N). Following the installation of the excluders the number of inter-nest trails in the excluded colonies reduced by a non-significant amount (median = 9.5, IQR = 3) trails (f1-ld-f1: F = 1.51, p = 0.22). Control colonies had roughly the same number of inter-nest trails as the previous timepoint (median = 15, IQR = 14), which was also not a significant change (f1-ld-f1: F = 0.098, p = 0.75). However, there was a significant reduction in number of internest trails to nests that were previously foraging to the focal tree in exclusion colonies (Figure 5.3; f1-ld-f1: F = 4.65, p < 0.05), but not control colonies (Figure 5.3; f1-ld-f1: F = 0.91, p = 0.34).

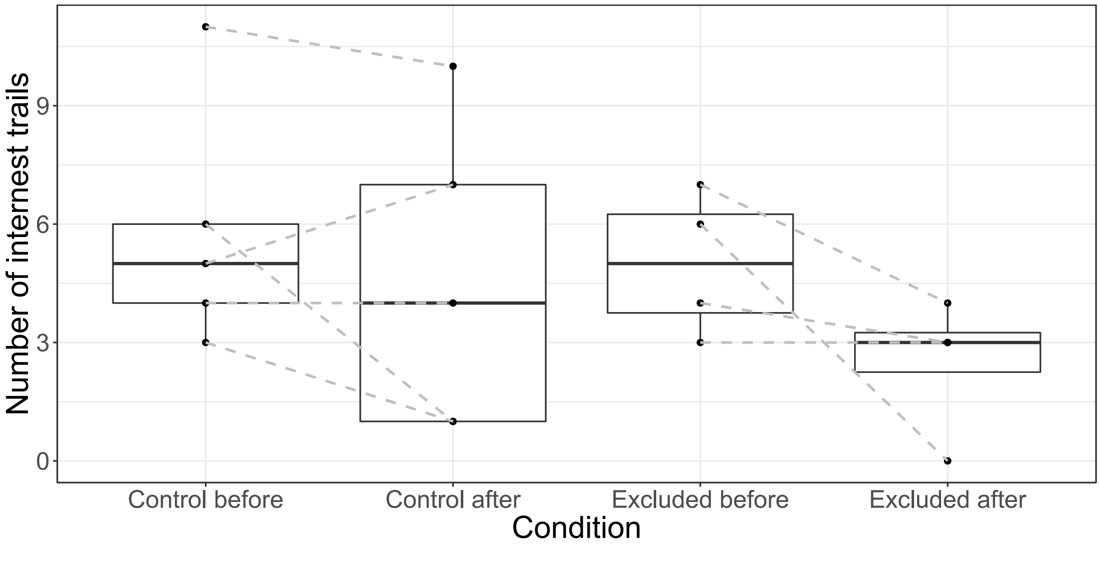


Figure 5.3. Comparison of number of inter-nest trails to nests that foraged on focal trees in colonies before (August 2016) and after (August 2017) in the control and "excluded" conditions. All data are plotted as individual points. Lines between points indicate colony identity.

Total inter-nest trail lengths for excluded networks did not change significantly after the exclusion (f1-ld-f1: F = 2.21, p = 0.14). Control colonies had roughly the same length of inter-nest trails as the previous timepoint, which was also not a significant change (f1-ld-f1: F = 0.013, p = 0.91). Similarly to the number of inter-nest trails, there was a significant reduction in the length of inter-nest trails to nests that previously foraged on the focal tree in exclusion colonies (f1-ld-f1: F = 6.95, $p < 0.01$), but not control colonies (f1-ld-f1: F = 0.0066, p = 0.94).

Nest abandonment and growth

Across all colonies, 78 of the 109 nests (71.6 %) that were inhabited in August 2016 were still inhabited in August 2017. There was no difference in the number of nests that were abandoned in the excluded condition (f1-ld-f1: F = 0.13, p = 0.72) or the control condition (f1-ld-f1: F = 0.0027, p = 0.96), when compared to nest abandonment in the same colonies from August 2015 to August 2016.

Between August 2016 and August 2017, growth in excluded colonies was not significantly different to the previous year (f1-ld-f1: F = 1.83, p = 0.18; Figure 5.4). In contrast, control colonies grew less between August 2016 and August 2017 than in the previous year (f1-ld-f1: F = 28.93, $p < 0.0001$; Figure 5.4). However, the difference in growth between control and excluded colonies appears to be due to higher variance in colony growth of excluded colonies than in control colonies. Before the experiment began, control colonies grew at a median rate of 60 % with an IQR of 0.5%, while excluded colonies grew at a median rate of 31 % with an IQR of 80%. This high initial variance in excluded colonies means that we have little power to detect changes in growth. Furthermore, the difference in growth between control and excluded colonies were not different before (Kruskal-Wallis: $\chi^2 = 0.96$, p = 0.33) or after (Kruskal-Wallis: $\chi^2 = 0.06$, p = 0.81) the excluders were installed (Figure 5.4).

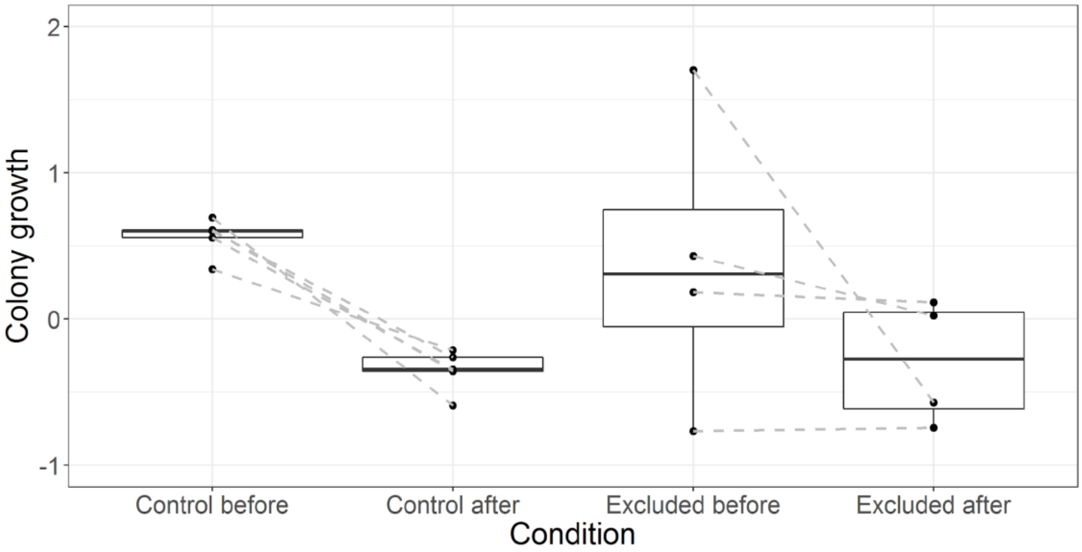


Figure 5.4. Comparison of growth in colony population between August 2015 and August 2016 (before) and between August 2016 and August 2017 (after) in the control and "excluded" conditions. All data are plotted as individual points. Lines between points indicate colony identity.

Nest foundation

Across all colonies 44 new nests were founded between August 2016 and August 2017. We standardized nest foundation for the number of nests in each colony by dividing the number of new nests by the number of nests in the colony at the previous time point. We found no difference in the number of nests founded in excluded (f1-ld-f1: F = 0.62, p = 0.43) or control colonies (f1-ld-f1: F = 1.15, p = 0.28) compared with the number of nests founded in the previous year (Figure 5.5).

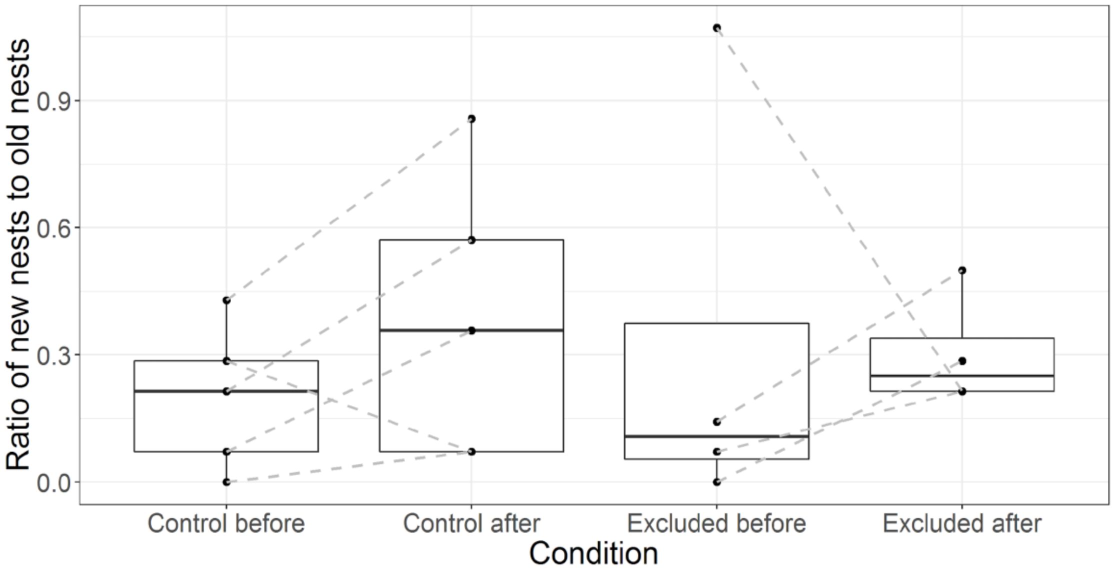


Figure 5.5. Comparison of number of new nests founded between August 2015 and August 2016 (before) and between August 2016 and August 2017 (after) in the control and "excluded" conditions, standardized by the number of nests in the colony at the previous timepoint. All data are plotted as individual points. Lines between points indicate colony identity.

Change in foraging networks

After exclusion, exclusion treatment colonies started foraging to a median of 1.5 and IQR of 1.5 new trees (see network maps in Appendix O), which was significantly more than in the year before exclusion (f1-ld-f1: F = 8.40, $p < 0.005$; Figure 5.6). In contrast, control colonies started foraging to a median of 0 and IQR of 5 new trees, which was not significantly different compared to the previous year (f1-ld-f1: F = 1.48, p = 0.22; Figure 5.6).

The proportion of the colony population active on foraging trails decreased in treatment colonies after the exclusion (f1-ld-f1: F = 5.42, $p < 0.05$; Figure 5.7), while in control colonies the proportion of the colony population active on foraging trails remained the same (f1-ld-f1: F = 0.030, p = 0.86; Figure 5.7).

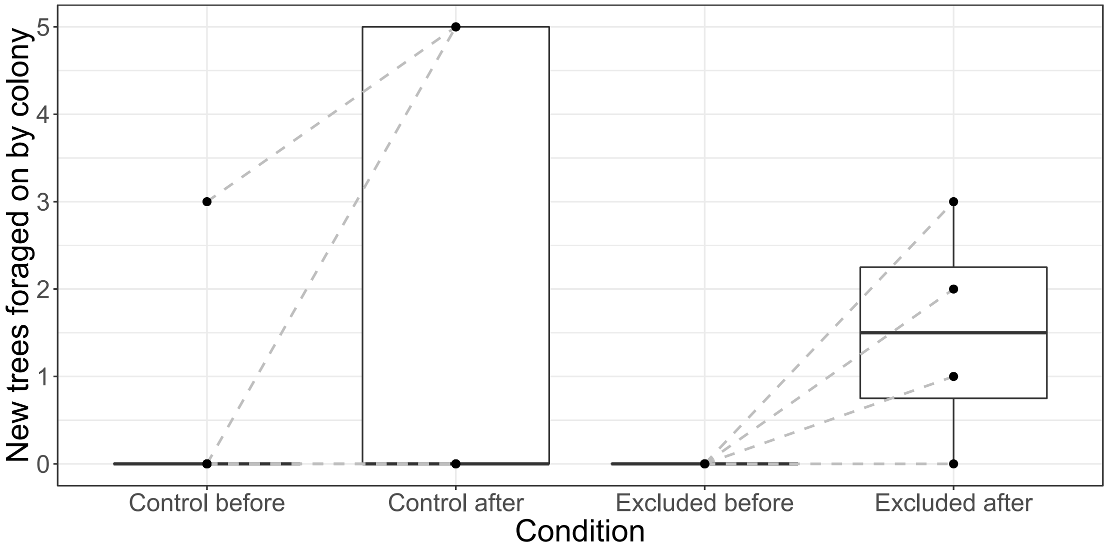


Figure 5.6. Comparison of number of new trees foraged on by colonies in August 2016 (before) and in August 2017 (after) in the control and "excluded" conditions. All data are plotted as individual points. Lines between points indicate colony identity.

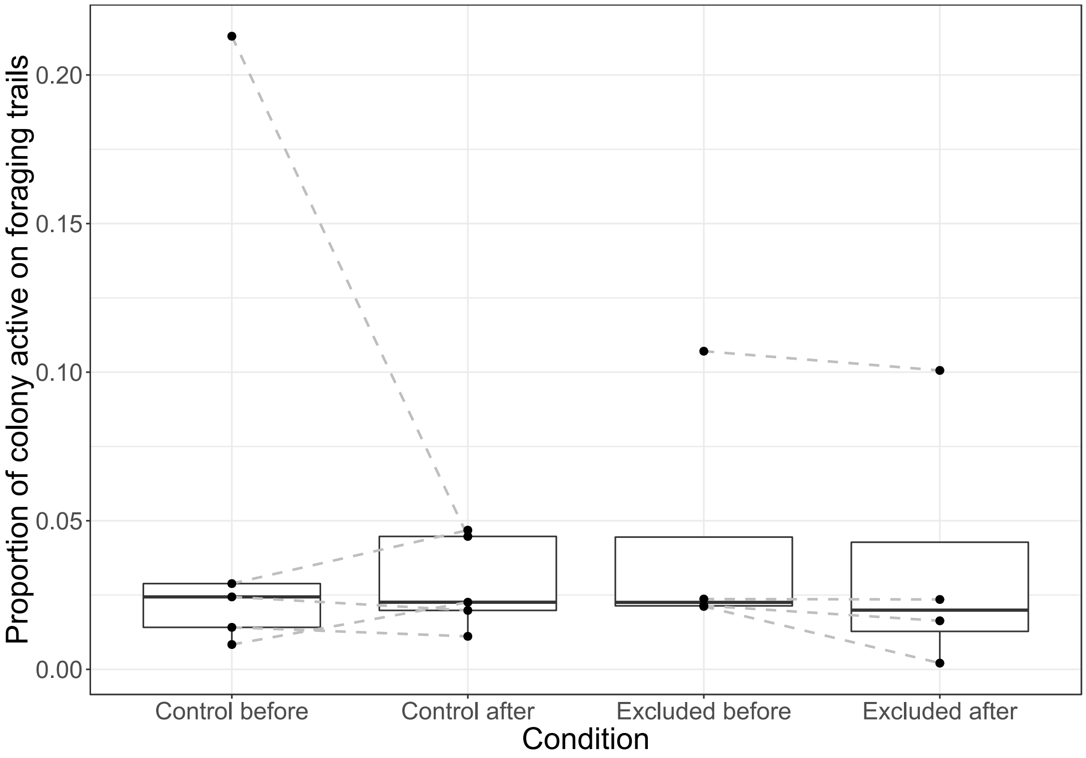


Figure 5.7. Comparison of proportion of colonies active on foraging trails in August 2016 (before) and in August 2017 (after) in the control and "excluded" conditions. All data are plotted as individual points. Lines between points indicate colony identity.

Discussion

Our study demonstrates that animal social networks adapt to changes in environmental conditions by changing their social network structure. We found that polydomous wood ants change their inter-nest networks in response to changes in resource distribution. Specifically, we found that nests that were previously foraging on an excluded food source lost inter-nest trails, contributing to fragmentation of inter-nest networks. We also found that colonies began foraging on new food sources in response to the loss of a key food source. The likely mechanism that ties these results together is that changes in resource availability causes sub-colonies to alter their foraging patterns, which results in changes to the distribution of resources across the colony. This change in resource distribution modifies the utility of inter-nest trails used in resource sharing, and thus leads to alterations in inter-nest network structure, which can result in formerly stable colonies splitting into many components.

Resource redistribution in polydomous nest networks occurs on a local scale: sub-colonies share resources with other nearby nests (Ellis, Franks & Robinson 2014; Procter *et al.* 2016). In our study, changes to inter-nest networks in excluded colonies appear to be due to a shift in the distribution of resources in the colony. Previous work has found that the relative abundance of resources entering each of a pair of nests is a key predictor of whether those two sub-colonies will connect via an inter-nest trail: sub-colonies with fewer resources connect to sub-colonies with abundant resources (Ellis & Robinson 2015). This previous work supports our hypothesis that the changes observed in inter-nest networks in our study are likely to have occurred because of changes in the relative abundance of resources across nests. The loss of inter-nest trails connecting nests that previously foraged on the removed food source also supports this hypothesis. Together these results demonstrate how disruption of the resource environment surrounding polydomous ant colonies can cause changes to the structure of resource sharing networks.

To test how colonies responded to change, we manipulated the environmental conditions surrounding polydomous colonies. This follows other recent studies of perturbations of animal social networks, where environmental conditions or networks have been experimentally manipulated (Lattanzio & Miles 2014; Firth & Sheldon 2015; Wilson *et al.* 2015; Formica *et al.* 2016; Leu *et al.* 2016; Firth *et al.* 2017; Lantz & Karubian 2017; Stroeymeyt *et al.* 2018). However, many previous studies have investigated the effect on networks of hypothetical removal of nodes or edges from static network maps (e.g. Lusseau 2003; Shimazaki *et al.* 2004; Manno 2008; Silvis *et al.* 2014). As our results highlight, networks are dynamic and adapt to changes by changing their connections. Consequently, hypothetical removal of nodes or edges from static network maps cannot give useful predictions about how networks are affected by change as it is not possible to predict how networks will adapt in response to removals. Instead, experimental manipulation is important for understanding how networks change over time and the effect that this has on network structure and function.

In networks where nodes are spatially fixed, such as nests in polydomous nest networks, the spatial distribution of resources is likely to be particularly important in determining the effect of local environmental conditions on social structure, as it may be cheaper in the short-term for nodes to change connections than to relocate. However, in other systems where nodes can move freely (e.g. mammalian social networks), we might expect that nodes may change their location, rather than changing their connections to other individuals. We may have observed movement of nests away from excluded resources if we had carried out exclusions for longer than a year.

However, nest movement is likely to be costly and, therefore, sub-colonies may avoid moving if survival in their current nest is possible.

In our study, we collected five years of network maps for each colony before the experiment began, and then mapped disrupted treatment colonies for a year and followed the colonies for a further year once the ants were given access to the resources again. The long-term data was vital in demonstrating that the food sources that we removed were important stable food sources for each colony and showing that the changes to the network were due to the manipulations. However, it is possible that if resources were removed for longer than a year that the changes that we observed could have been different. For example, over time colonies may have become centralised further away from excluded resources to be closer to profitable locations. Future work could investigate long-term changes in polydomous colonies caused by disruption to resource distribution and the speed at which networks are able to respond to changes to the resource distribution.

The loss of inter-nest trails caused colonies to split into multiple independent components. However, there was no associated change in nest growth, abandonment or foundation of new nests. This may be because the ability of networks to adapt allows them to limit the costs of environmental change.

We have used experimentation on several networks to show how networks of wood ants adapt to changes in resource distribution by fragmenting, which does not appear to have a negative effect on colonies' populations. These findings contribute to our understanding of how networks adapt in response to environmental change and highlight the importance of replication and experimentation in network studies.

Chapter 6 - Discussion

Overview

In this thesis, I have presented studies of the different factors that are important in determining how cooperation between sub-colonies in polydomous colonies functions. In Chapter 2, I used agent-based modelling to demonstrate that there are several costs and benefits to ant colonies from being polydomous. This provides an explanation for the high behavioural and phylogenetic diversity of polydomous ant species. In Chapter 3, I presented an observational study carried out over 8 years describing the nesting behaviour and the trail network dynamics of colonies of one of these polydomous species, *Formica lugubris*. This work demonstrates how resources sharing networks in polydomous colonies can vary between being static and dynamic over time, possibly as a result of optimisation. In Chapter 4, I presented a study of the interaction between relatedness and cooperation between sub-colonies in polydomous ant species. We found that sub-colonies that share a lot of resources with each other are more closely related than sub-colonies that do not share many resources with each other. A likely mechanism to explain this is that there is a higher rate of brood transfer and migration between sub-colonies that are strongly connected, which results in higher relatedness. This finding indicates that sub-colonies may be able to benefit through indirect fitness by cooperating with other sub-colonies. Finally, in Chapter 5, I presented an experimental study of the effect of changing resource distribution on polydomous nest networks. We found that changes to resource distribution causes foraging and inter-nest networks to change. A likely reason for this is that changes to resource distribution causes the distribution of resources in nests to change, which causes inter-nest networks to be altered.

Cooperation

The work presented in this thesis and other work in this field are revealing the interactions between the environment, social behaviour and group identity. In polydomous ants, it seems that the distribution of resources influences the shape of the foraging network, which shapes the resource sharing network and, consequently, the colony identity of each sub-colony (Figure 6.1). However, other factors may also be important. For example, in Chapter 4 I presented a study that investigated the role of relatedness in colony networks. We found that relatedness is higher between sub-colonies that cooperate more strongly. We hypothesised that high levels of cooperation resulted in higher levels of relatedness. However, it is also possible that sub-colonies direct cooperation to more closely related sub-colonies. Therefore, at present the relationship between relatedness, colony identity and the resource sharing network in polydomous ants is still quite unclear.

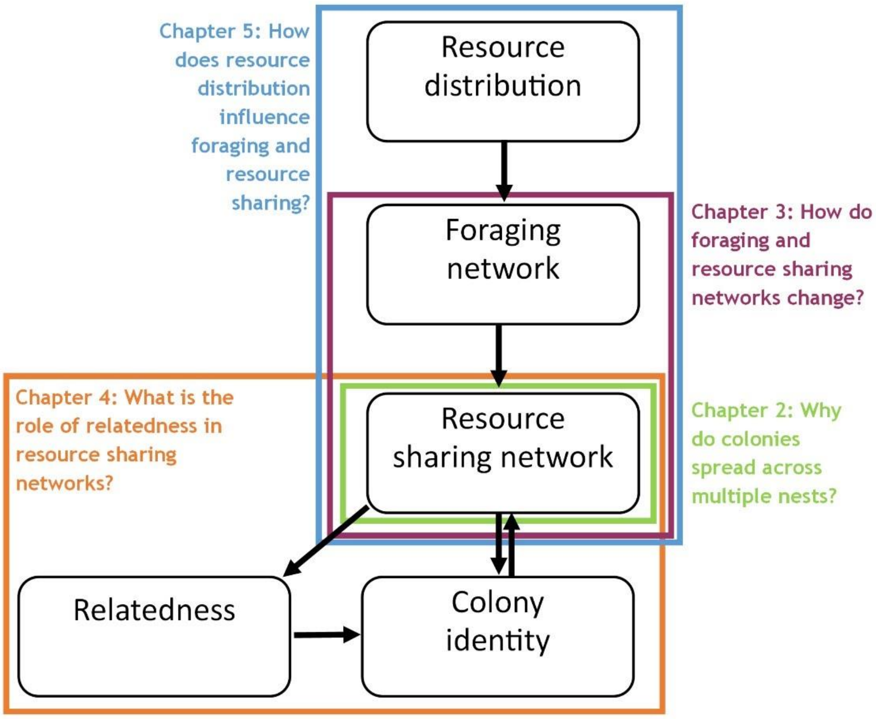


Figure 6.1. Overview of our understanding of the proximate and ultimate mechanisms of polydomy and the topic approached by each of the data chapters presented in this thesis.

Group identity, the local environment and social behaviour appear to be tied together in many different social animal groups. For example, bonobo groups are less aggressive to each other when competition for resources is low (Lucchesi et al. 2020) and killer whales form larger and more connected groups in years when resources are abundant (Foster et al. 2012). However, the way that these factors interact in different species appears to be different. For example, it seems that the key factor in determining group identity in polydomous ants is the need for resources, whereas in killer whales it seems that family ties are more important. Research of these interactions in a wider range of species is needed to fully understand the similarities and differences of each system.

An interesting distinction to draw between cooperation between groups and cooperation between individuals is the possibility for relatedness between groups to change as a result of migration. Therefore, the indirect fitness benefits of cooperating with different groups can change over time. This is particularly important if migration is correlated with cooperation. When this is the case groups will end up cooperating with related groups over time and receive a higher indirect fitness benefit as a result. In Chapter 4, we found evidence that this may be occurring in polydomous ant colonies as we found a correlation between the strength of connection and relatedness. It is possible that this is also the case in other examples of cooperation among groups, such as in humans (Robinson and Barker 2017).

Polydomy in *Formica rufa* group spp.

Polydomy is a strategy found in many populations of *Formica rufa* group spp. which appears to allow colonies to expand colony limits and optimise resource use (Ellis and Robinson 2014). Nests are budded from the colony in a random direction and only maintained if foraging from the nest is possible (Ellis and Robinson 2015). In Chapter 3, I presented a study demonstrating that nests are produced and abandoned at a high rate, with most nests being abandoned within a year of being built. This high turnover is not surprising given the results of previous work that indicated that nests that are not well-located are quickly abandoned, while those that are well-located are maintained for longer (Ellis and Robinson 2015). However, it does provide additional evidence for the hypothesis that polydomy in *Formica rufa* group spp. allows colonies to optimise nesting locations (Ellis and Robinson 2015).

One interesting topic that has arisen from previous work in this field and in my work is the interplay between cooperative behaviour and group identity in social insects (Ellis et al. 2017b). In polydomous ants, sub-colonies can be grouped into colonies using functional, spatial or genetic methods, which often yield different outcomes (Ellis et al. 2017b). This implies that colony identity may not be fixed – if the distribution of resources changes then colony identity may also change. In Chapter 5, I presented a study where we prevented colonies from accessing a key resource to investigate how this would influence colony identity. We found that foraging and inter-nest networks changed, resulting in inter-nest networks splitting into several parts. The consequence of this is that by the functional definition of a colony (Ellis et al. 2017b), the colony identity of different sub-colonies changed. Tied together with our finding that relatedness and strength of cooperation are correlated, it is possible that changes in functional colony boundaries later lead to genetic divergence and, eventually, changes to genetic colony identity. This is an interesting finding as it implies that colony boundaries of different types may be more flexible than previously believed.

Limitations and future directions

The studies presented in this thesis focus mainly on the behaviour of a single population of a single species, *Formica lugubris.* This is a significant limitation to drawing broad conclusions about polydomous behaviour as polydomous ants are both behaviourally and phylogenetically diverse. Therefore, it is quite likely that several of our findings may not be widely applicable to polydomous ants. Reliance on a single species is a consequence of the difficulty of performing studies, lack of study sites and limitations of time. However, with more resources it would be interesting to see how the results of these studies differ between species and populations. It seems likely that similar results would be found for other polydomous species that forage on relatively predictable and abundant resources, such as meat ants (*Iridomyrmex purpureus*). In contrast, polydomy may function in a very different way in species that forage on resources that vary significantly, both temporally and spatially. If resource sharing networks change more frequently, we wouldn't expect them to be associated with high levels of brood/worker transfer, and thus wouldn't expect them to have significant effects on relatedness. Therefore, we might expect relatedness patterns in these more dynamic species to be better predicted by spatial diffusion patterns than by cooperative networks

Working with polydomous wood ants in the field allowed us to investigate several important questions using experimental and observational study designs. Furthermore, as the study system is a wild population the behaviours that we observed are likely to be ecologically important. For this reason, study of wild populations is essential to improve our understanding of ecological processes. However, working on wild populations also presents several challenges that limit experimental design. For example, wild wood ant colonies at the site we used are difficult to manipulate, limited in number and subject to many external conditions that cannot be controlled. In this section, I will discuss some of the limitations presented by working on this study system, extensions of this work that we did not have time to do and some ideas for work in other systems that do not have these limitations.

In Chapter 5, I described a field experiment conducted on several colonies of wood ants where I manipulated the foraging environment by removing a key food source. The experiment was very difficult to implement and I was only able to achieve a sample size of nine. Furthermore, colonies in the field are subject to many external factors that are not possible to control and can be difficult to measure, for example changes in aphid abundance, weather conditions, disease, disturbance and predation. An interesting possibility for future research that would address these problems would be to use laboratory colonies of polydomous ants. This would require use of a different species as used in this thesis, as polydomous wood ant colonies can typically inhabit territories of 10,000 m^2 or more. Instead, species such as Argentine ants (*Linepithema humile*) or rock ants (*Temnothorax albipennis*) might be good candidates for such experiments.

Laboratory experiments on polydomous colony networks would allow investigation of many different important questions about network function, robustness and adaptation. For example, it would be interesting to see how limiting network structure by only allowing certain sub-colonies to connect affects colony function. Performing such experiments could provide useful information on the important factors in determining trail network topology in polydomous colonies. Another interesting possibility would be to look at how colony trail networks adapt to the removal of connections between sub-colonies. This would provide useful insights into the way that networks adapt to changes and how changes in one place in a network are transmitted through the network.

Despite the challenges of using field experiments our results provided some useful insights about how colonies adapt to changes in their environment and network. One interesting possibility to extend this work would be to look at the effect of transplanting polydomous sub-colonies to different locations in the trail network or even to different trail networks. In the observational study of the relationship between relatedness and cooperation presented in Chapter 4 we found that there was a correlation between relatedness and the strength of connection between sub-colonies. This is likely to be due to a higher rate of migration and brood transfer between strongly connected sub-colonies. However, it is also possible that this is a result of sub-colonies preferentially sharing resources with more closely related sub-colonies. To test the direction of this correlation it would be necessary to perform a transplantation experiment. It would be interesting to see if sub-colonies inhabiting transplanted nests formed new inter-nest trails to other nearby nests and if these inter-nest trails were stronger between closer relatives. If this happened, it would demonstrate that relatedness is not a key factor in influencing cooperation between sub-colonies in polydomous colonies, but that relatedness between sub-colonies can be a consequence of cooperation. In contrast, if inter-nest trails were not formed, this would demonstrate the importance of relatedness in influencing cooperation. This type of experiment could be quite challenging to implement in wood ants as transplantations are often unsuccessful and difficult to perform (Nielsen et al. 2018). One alternative would be to use a different species that is easier to transplant. For example, it might be possible to design a semi-natural experiment on a cavity-dwelling species using artificial nest sites that are likely to get inhabited and can be easily transplanted.

In most of their range, including our field site, polydomous wood ants only form small colonies of fewer than 50 nests (Ellis and Robinson 2014). This means that the statistical network methods that it is possible to use are quite limited. However, there are records of colonies with many thousands of nests (Marko et al. 2012). In a larger network, it would be possible to use statistical social network tools to identify the factors that are most important in determining network topology (Fisher et al. 2017; Silk and Fisher 2017). A useful extension of the work presented in this thesis would be to make network maps of these much larger colonies with information on their foraging activity, which could be used to identify key factors in network formation, that may also be applicable in smaller networks.

Conclusions

In this thesis, I have presented several studies that investigate the function and evolution of polydomy though modelling, observation and experiments. Through use of these different study types we have been able to demonstrate the costs and benefits that are likely to be key in the evolution of polydomy, to further describe the way that polydomous colonies of wood ants function and to investigate the interplay between environment, social behaviour and group identity in social animals. These studies demonstrate how systems like ant colonies can use both decentralization and centralization to improve their efficiency and effectiveness under certain sets of conditions.

Appendices

Appendix A - Model description

Purpose

We compare the success of polydomy and monodomy across a range of environments with varying resource qualities and spatial distributions. We model the population dynamics of a community of ant nests - some of which have a polydomous colony organisation and others a monodomous colony organisation. The model is implemented in R Version 3.4.1.

Environment and agents

The environment is a continuous 2-dimensional area, containing a set number (Table AA1) of food sources, each with fixed spatial coordinates. Each food source *i* produces food at constant rate F_i throughout the season (parameter values in Table AA2). This scenario is broadly based on our empirical systems where a 50x50m area contains many trees, each of which may act as an independent food source for ant nests.

The agents in the model represent nests, each of which belongs to one of several colonies. Nests contain ants, which are equivalent in the model to food (i.e. 1 food = 1 ant). Consequently, the model focuses on the behaviour of nests and does not model individual ants. Nests can either have a polydomous or monodomous colony organisation. If a nest's colony organisation is polydomous then it can form outgoing connections to nests of the same colony, and to food sources. A nest whose colony organisation is monodomous can only form connections to food sources (see Figure 2 for graphical representation). Each nest takes food from food sources and nests - to which it has an outgoing connection - at a rate that is determined by a combination of biologically-motivated factors captured by Equations 3-5. Each nest is described by a nest identification number, a colony identification number, fixed spatial coordinates, a population size (N_i for nest i), a colony organisation type (polydomous or monodomous), and a matrix of outgoing connections to food sources and nests.

Table AA1. Experimental method

Description	Value	Units
Starting number of nests	10	
Starting number of colonies	10	
Number of food sources	40	
Food source distribution	Random; Clustered	
Length of season	20	arbitrary units
Number of seasons	500	
Number of replicates per condition	30	
Arena length/width	50	m

Processes

Overview and schedule

After an initialisation stage, the model repeats over a set number of seasonal cycles. Within each season, there are six stages (2-7 in Figure AA1) which are explained in detail below. We run the simulations for multiple independent replicates (see Appendix 8), each represented by a single complete run of the model for a fixed number of seasons (Table AA1). For clarity of exposition, in the description of steps 2-6, the index of the season (i.e. the simulation "year") is dropped.

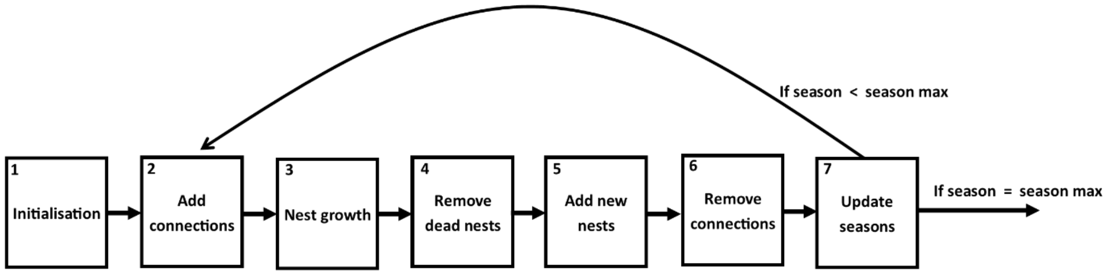


Figure AA1. Schedule of processes in model

Initialisation

During initialisation, the locations of food sources and nests are generated (Table AA1). Each food source is allocated a unique identification number and is assigned location coordinates. The method for assigning the location of food sources depends on the condition and can be either random or clustered (details in Appendix B). Nests are given unique nest and colony identification numbers, and their location coordinates are selected with a uniform random distribution over the arena. At initialisation all nests are assigned to belong to different colonies, with half of the initial nests having a polydomous colony organisation and the other half having a monodomous colony organisation (e.g. Figure 2.2). Initial nest sizes are selected from a uniform distribution between 0 and 100,000.

Add connections

At the start of each season, each nest forms connections. These can connect the nest to food sources and - if the nest has a polydomous colony organisation - to other nests sharing the same colony identity. We assume that nests make more connections linearly depending on their population size. The number of connections T_i that each nest is assigned in each season depends on the size of the nest N_i (Table AA3) and the number of ants needed to fully exploit a single food source in the constant food source condition ($\frac{F}{Q}$) (Equation 1). Finally, T_i is rounded up to the nearest integer to give the number of connections that each nest is assigned. The number of connections that each nest adds is calculated by deducting the count of any connections that the nest has from previous seasons from T_i.

$$T_i = \lceil N_i \frac{Q}{F} \rceil \tag{1}$$

The connections are then allocated. Nests are unable to detect how much food is available at a food source or nest until they are connected to it. However, nests are more likely to connect to food sources or other nests that are nearby. Thus, connections that would have a shorter length (L_{ij} for a connection between nests i and j) are more likely to form than more distant connections. Formally, whenever nest i makes a new connection, the probability that the connection is to nest or resource j depends on the relative inverse squared distance of j from i, as quantified by Equation 2.

$$P(connection\ from\ i\ to\ j) = \frac{{L_{ij}}^{-2}}{\sum_{n=1}^{\infty} {L_{in}}^{-2}} \tag{2}$$

Nest growth calculations

Once the network is established, changes in nest size are calculated for that season using a set of differential equations (Equations 3-7) modelling the continuous time dynamics of nests connected to a fixed set of nests and food sources throughout that season. These equations assume that nests take food from all food sources to which they are connected and - if they are polydomous - from nests to which they have an outgoing connection. The rate R_{ij} that each nest i receives food from a connection to j depends on the number of ants A_{ij} it commits to the connection (Equation 3), the amount of competition for that resource (Equation 4), the length of the connection (L_{ij}), and the quantity of food that is available (see Equation 5).

We assume, for simplicity, that each nest commits an equal number of workers to each of its foraging trails. Therefore, number of ants (A_{ij}) that a nest i commits to each of its connections j is calculated by dividing the total nest population size (N_i) by the number of outgoing connections the nest has (T_i) (Equation 3).

$$A_{ij} = \frac{N_i}{T_i} \tag{3}$$

Nests from different colonies compete for food from food sources, but do not compete for territory. We assume that the colony that has the largest number of ants foraging on a food source is the only colony able to take any food. However, for all colonies, the number of ants that take any food from the food source *j* is reduced proportionately to the sum of the effort by the other colonies that do not receive any food (Equation 4). We provide a sensitivity analysis of the inclusion of competition in Appendix 3.

$$C_{ij} = \begin{cases} A_{ij} - \sum_{n=1}^{\infty} A_{nj}, & \text{if } C_{ij}>0 \\ 0, & \text{otherwise} \end{cases} \tag{4}$$

The rate at which food is taken by a foraging nest *i* from each food source or nest *j* to which it is connected is calculated by multiplying the number of individuals A_{ij} from the nest committed to the connection, after costs of competition (C_{ij}), by the quantity of food each individual can carry (Q). However, the rate at which the foraging nest receives food (R_{ij}) is less than that taken from the food source or nest that it is foraging on, because of foraging costs. The foraging costs of a trail between nest *i* and nest or food source *j* are dependent on the number of foraging individuals (A_{ij}) and the distance to the food source (L_{ij}), which are multiplied by a constant connection cost (E_{ij}), which is representative of energetic and trail maintenance costs, may differ for foraging trails and internest trails (Equation 5). The quantity of food it is possible to take from a food source is also limited by productivity (quantified by a rate F_i for food source *i*), whereas, for other nests, the quantity is limited to a certain portion (H) of the nest population size (N_i).

$$R_{ij} =$$

$$\begin{cases} QC_{ij} - A_{ij}L_{ij}E_{ij}, & \text{if j is food source and } R_{ij} < F_j\text{, or if j is nest and } R_{ij} < N_jH \\ F_j - A_{ij}L_{ij}E_{ij}, & \text{otherwise, if j is a food source} \\ N_jH - A_{ij}L_{ij}E_{ij}, & \text{otherwise, if j is a nest} \end{cases} \tag{5}$$

Once R_{ij} and C_{ij} have been derived for all *i* and *j*, the rates of nest growth are calculated (Equation 6 and 7). The growth rate G_i (Equation 6) of each nest *i* is determined by summing together all the food received from foraging, taking away the food lost to other nests and taking away a constant loss rate (*V*) (Table AA2). Once this has been done, nest size is constrained by adjusting G_i using a simple assumption of local logistic growth with *K* as the nest-level carrying capacity for each nest (Equation 7). This is incorporated to account for nest size constraints such as structural limitations (Robinson 2014).

$$G_i = \sum_{n=1}^{\infty} R_{in} - \sum_{n=1}^{\infty} R_{ni} + \sum_{n=1}^{\infty} A_{ni}L_{ni}E_{ni} - VN_i \tag{6}$$

$$\frac{dN_i}{dt} = G_i - G_i\frac{N_i}{K} \tag{7}$$

The differential equations of nest growth are run for all the nests simultaneously over a fixed time interval for a given season (see Table AA1; the units are arbitrary but may be considered as representing “weeks” during each seasonal “year”). The differential equations are solved using an ODE solver, built under the deSolve package (Karline Soetaert 2010).

Remove dead nests

At the end of the season, each nest which has a size below a threshold (*D*) is removed from future seasons. Additionally, each nest may ‘die’ stochastically, independent of size, with the probability of this occurring in each season determined by *P* (Table AA2). Nests that die through this process are also removed from future seasons.

Add new nests

At the end of each season each nest can "parent" (produce through budding) a new nest, independently of all other nests, with a probability determined by dividing the nest's current size by a constant (*U*), meaning that larger nests are more likely to create new nests (Equation 8). If a new nest is created, the location of the new nest depends on the colony organisation of the parent nest. If the parent nest is polydomous, the distance to the new nest from the parent nest is taken from a gamma distribution (see Parameterisation). The angle between the parent nest and the new nest is taken at random from a uniform distribution between 0^o and 360^o. The new nest location may be outside of the 50x50m environment. However, if the parent nest is monodomous, the new nest is given a random location in the arena. This method of nest foundation imitates independent foundation, whereby queens fly from the parent nest to found a new nest, which is commonly found in monodomous species (Pamilo and Rosengren 1984). Once created, each new nest is given an initial size which is a fixed proportion *S* of the size of the parent nest. The size of the parent nest is reduced by the same amount. If the parent nest is polydomous then the new nest inherits the same colony identity and is also polydomous; otherwise it is given a new colony identity. However, every time a new nest is created there is a small probability (*M*) that the new nest will differ in colony organisation from the parent. If this occurs, then the new nest will have a new colony identity and its colony organisation will be different to that of its parent nest: if the parent was monodomous the new nest will form a new polydomous colony and if the parent was polydomous the new nest will form a new monodomous colony. Mutation of strategy in this way permits opportunities for either strategy to be re-established if it ever reaches extinction.

$$P(i\ parents\ a\ nest) = \frac{N_i}{U} \quad \textbf{(8)}$$

Remove connections

To prepare for a new season, each connection can be either maintained or removed. Whether a connection is removed depends on the estimated profitability of the trail. The profitability of each connection from nest i to nest j (R_{ij}) is calculated by Equation 5. We then take the inverse of R_{ij} and multiply it by the nest size of i (N_i), and a constant (Z) (Equation 9). Z is set to a value whereby more profitable connections are less likely to be removed than those with low profitability. Each connection that is entirely unprofitable ($R_{ij} < 0$) is removed. If a connection is removed then it is not possible for the nest to remake the same connection in the next season. However, the connection may be made in any subsequent seasons.

$$P(ij = 1) = min\ (Z\frac{R_{ij}}{N_i}, 1) \quad \textbf{(9)}$$

Update seasons

At the end of each season a season counter is updated. If the counter has not reached the final season (Table AA1) the model will continue to Process 2, otherwise the replicate will be finished and will output the results.

Table AA2. Parameters, values and their units

Letter	Description	Value	Units
F_i	Rate of productivity of food source *i*	Constant - 4000; Fluctuating - selected from a uniform distribution between 0 and 8000 every season	resources t^{-1}

K	Carrying capacity	Low - 30,000; High - 300,000	ants
Q	Rate at which an ant can transport food to its nest along an outgoing connection	0.1	resources ant^{-1} t^{-1}
H	Rate of population size shared by polydomous nests	0.01	portion t^{-1}
V	Rate of colony loss	0.05	portion ^{1} t^{-1}
U	Constant to adjust probability of creating new nest	K x 20	ants
S	Portion of a parent nest that is donated to a new nest	0.1	
D	Minimum population threshold for nest survival to next season	1000	ants
Z	Constant to adjust probability of removing connection based on profitability	0.001	
E_{ij}	Connection cost for a connection from nest *i* to nest or food source *j*	Internest - 0.00001 Foraging (low) - 0.00001; Foraging (high) - 0.00005	resources t^{-1}
M	Probability of strategy mutation	0.1	
P	Probability of stochastic nest death	0; 0.01	

Table AA3. Output variables

Letter	Description	Units
N_i	Nest size of nest *i*	ants
T_i	Number of outgoing connections from nest *i*	
L_{ij}	Length of connection from nest *i* to nest or food source *j*	cm
A_{ij}	Number of ants committed to a connection from nest *i* to nest or food source *j*	ants
C_{ij}	Number of ants committed to a connection after costs of competition from nest *i* to nest or food source *j*	ants
R_{ij}	Resources received from a connection from nest *i* to nest or food source *j*	resources

Parameterisation

The distance from a parent to a new nest for polydomous nests has been parameterised using six years of data on 10 colonies of polydomous wood ants (Ellis and Robinson

(2015), DB *unpublished data*), which indicated a gamma distribution (fit described in Appendix D).

Appendix B - Food source placement

Food sources are placed according to two different conditions: random and clustered. In the random condition food source locations are taken from a uniform distribution covering the whole arena (e.g. Figure AB1A). In the clustered condition food sources are grouped into four clusters. Each cluster is given a centre, which is taken from a uniform distribution covering the whole arena, but with a minimum distance of 10m between them. Each food source is then given a location taken from a normal distribution with a mean that is equal to the cluster centre and a standard deviation of 2.5m (e.g. Figure AB1B). If either of the x or y coordinates of any of the food sources is outside of the arena they are changed so that they are on the border of the arena.

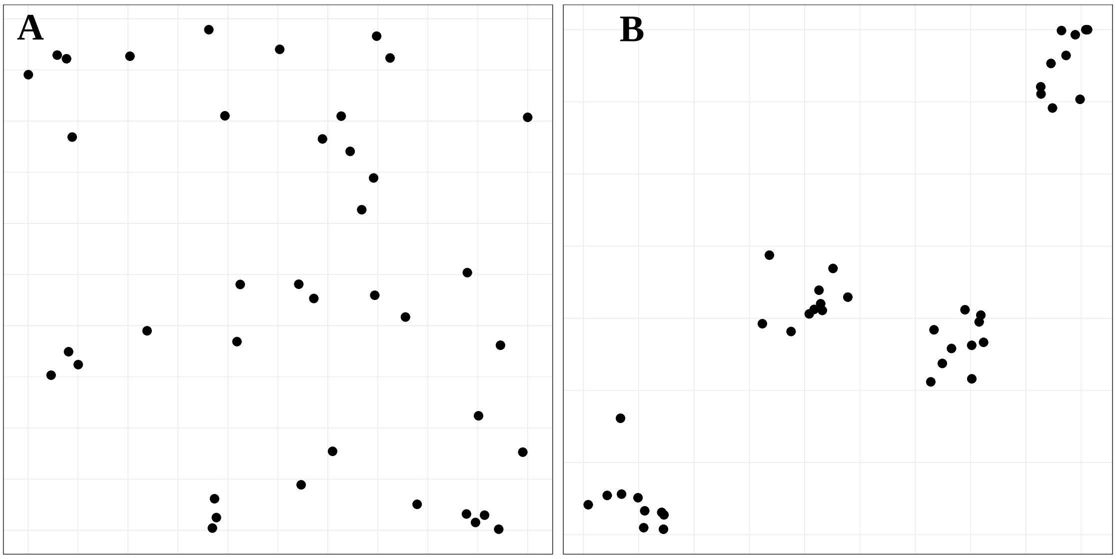


Figure AB1. Examples of the distribution of food sources for the random (A) and clustered (B) conditions. Each point is an individual food source.

Appendix C - Sensitivity analysis of competition

To identify whether there is any effect of competition in the model on the relative success of polydomy and monodomy we repeat the method used in the main body of the paper, but remove direct competition (Equation 4) from the model. This was the only change we made to the model.

When competition is removed, we find that in many of the conditions the population of nests grows very large. Consequently, competition is likely to be an important interaction to include in the model to prevent the population growing in an unrestricted way, as is likely to also be the case in many natural systems. Removing competition resulted in both a higher number of nests (Figure AC1) and a higher total population (Figure AC2). In addition, removal of competition appears to be favourable to monodomy (Figure AC3/AC4). This effect is unsurprising as competition is likely to be important to many of the advantages that polydomy appears to convey (e.g. resource monopolization). Consequently, the way that ant colonies compete is likely to play an important role in determining whether polydomy or monodomy is a better strategy.

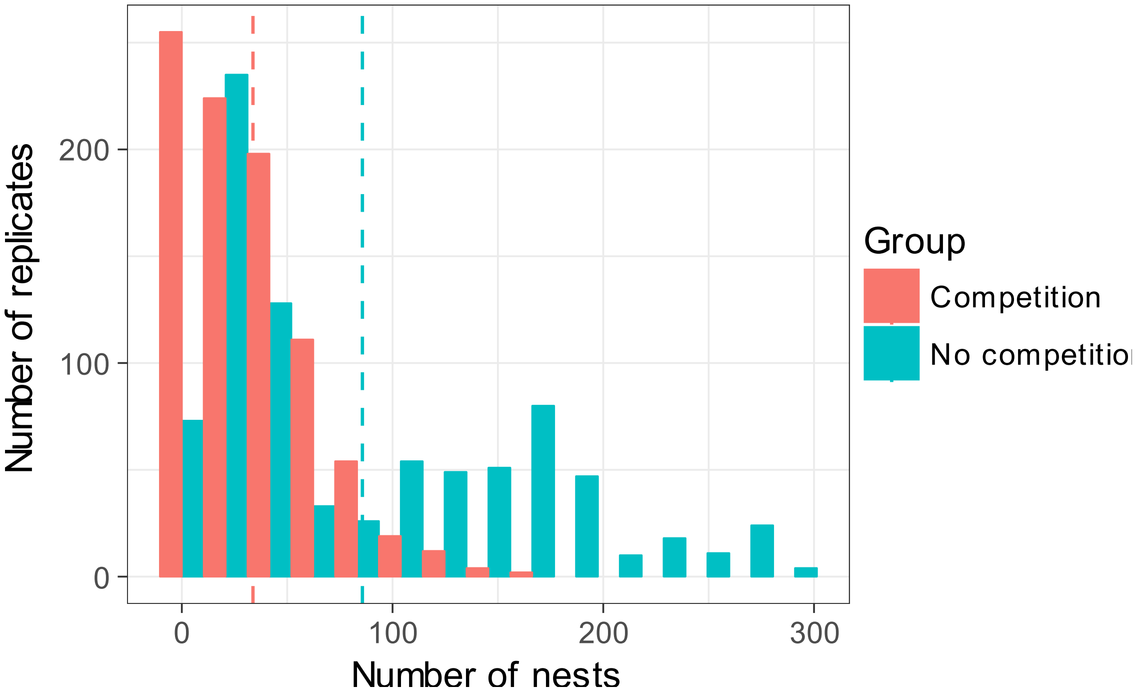


Figure AC1 Distribution of the number of nests in the final season of all replicates when competition is included (red) and when it is removed (blue). Vertical dashed lines represent median values.

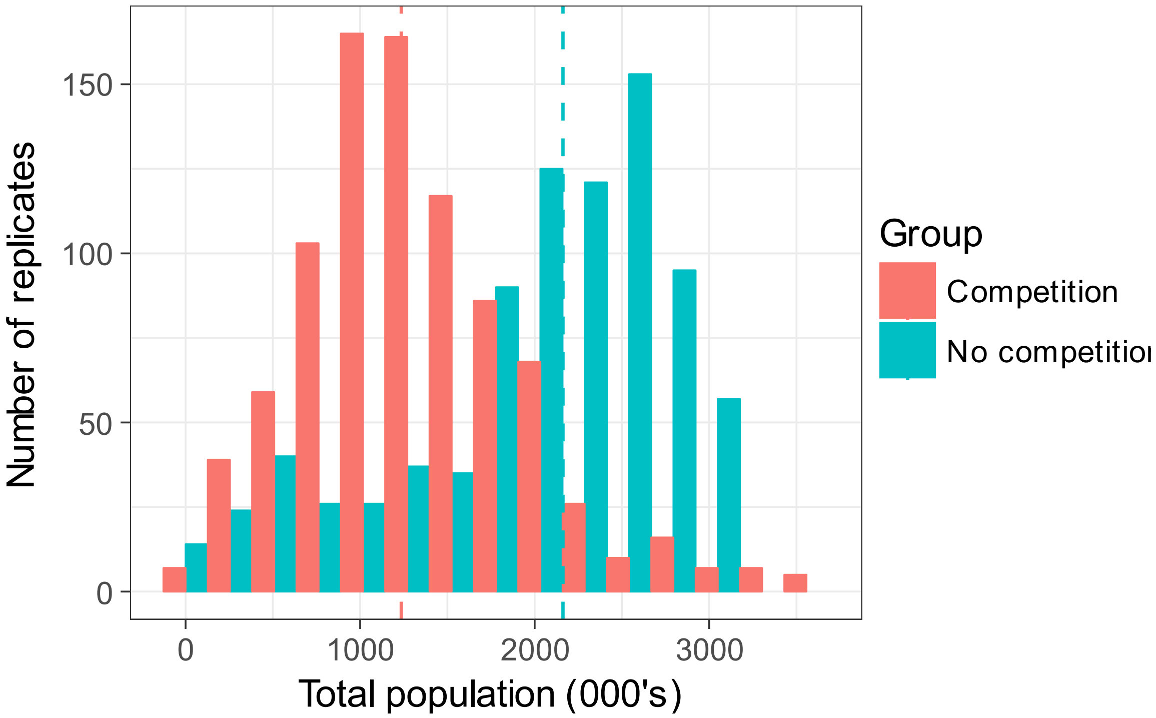


Figure AC2 Distribution of the total population size in the final season of all replicates when competition is included (red) and when it is removed (blue). Vertical dashed lines represent median values.

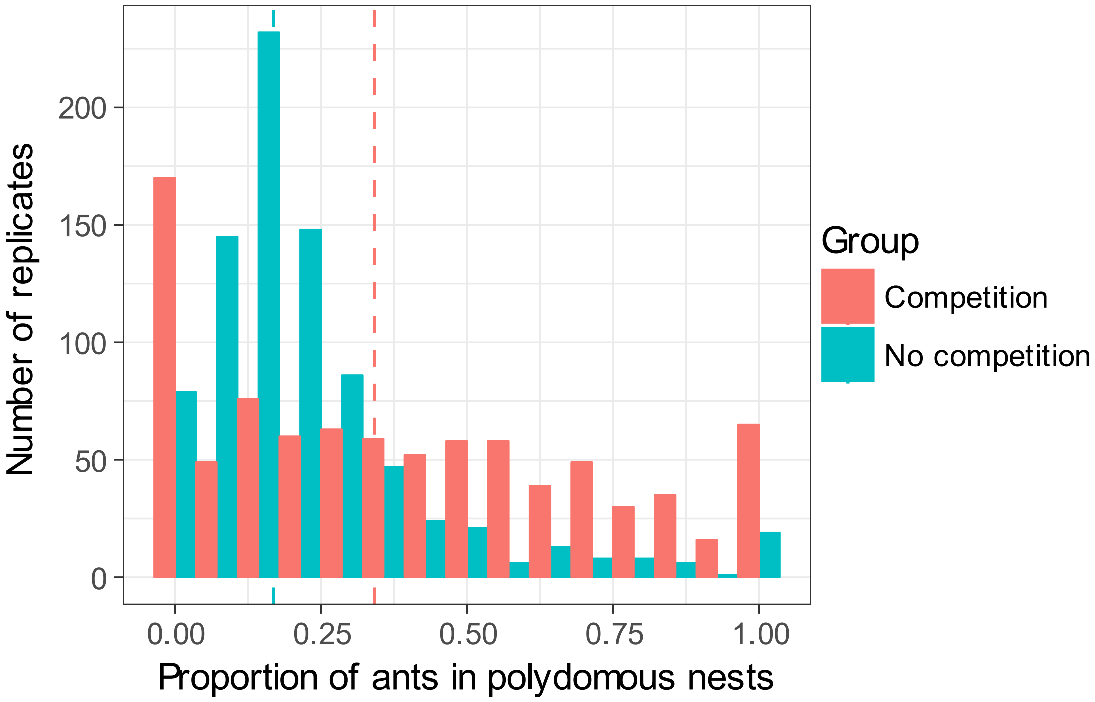


Figure AC3 Distribution of the proportion of the population in polydomous nests in the final season of all replicates when competition is included (red) and when it is removed (blue). Vertical dashed lines represent median values.

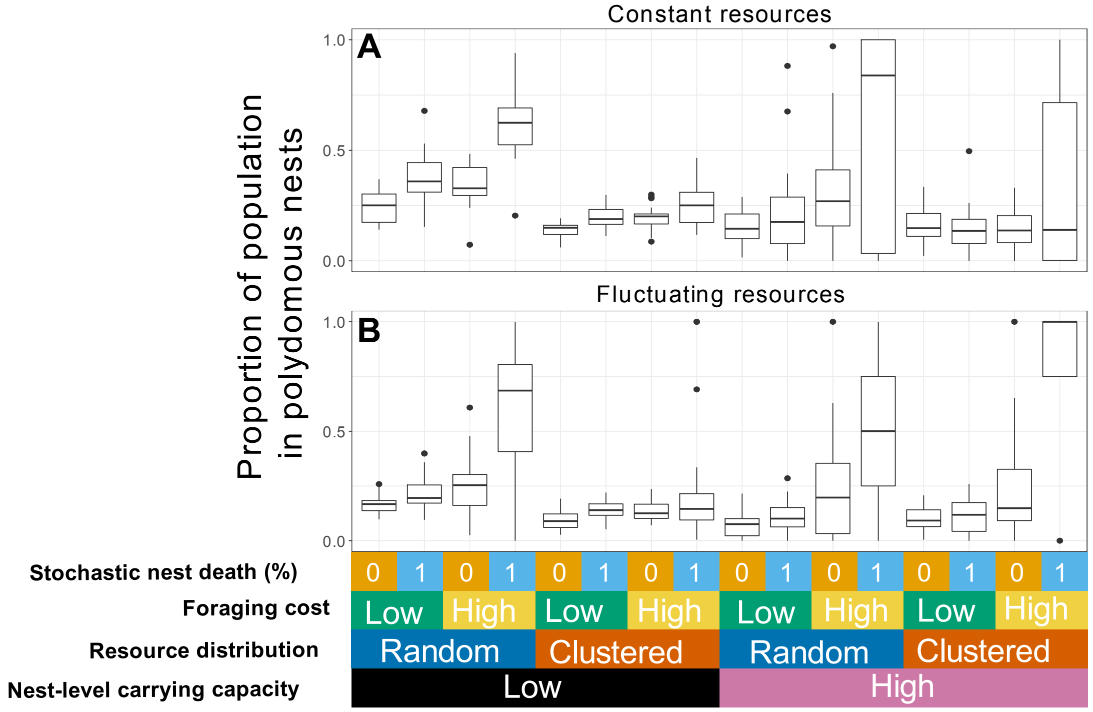


Figure AC4 The proportion of the population in polydomous nests at the end of 500 seasons in each condition when competition is removed. A shows conditions where food sources have a constant availability and B shows conditions where food sources fluctuate in availability. Middle lines represent median values, lower and upper hinges represent 25th and 75th percentiles respectively and whiskers reach to the lowest (lower whisker) or highest (higher whisker) value, with a maximum reach of 1.5 x IQR from the hinge. Values outside of this range are plotted as outliers.

Appendix D - Fit of gamma distribution to founding data

Over the last seven years data on 10 polydomous colonies of northern hairy wood ants (*Formica lugubris*) have been collected (Ellis et al. 2015; DB *unpublished data*). We identified cases where new nests had been founded and the identity of the parent nest was clear. We measured the distance from the parent nest to the new nest and fit a gamma distribution to these distances. Diagnostic plots of the model fit indicated a good fit (Figure AD1).

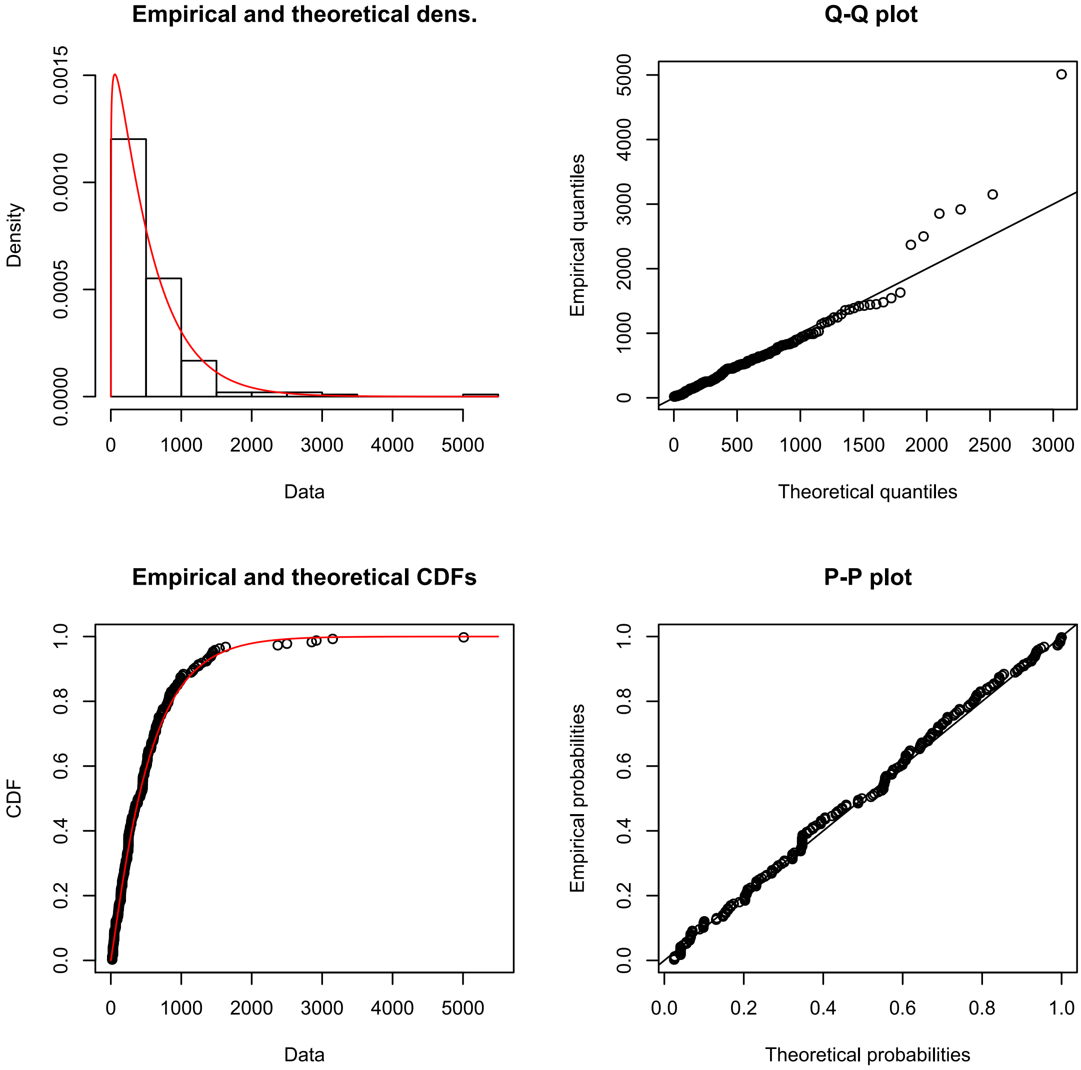


Figure AD1. Fit of the gamma model to the founding distance data.

Appendix E - Model selection

We fit a binomial general linear model with a logit link to the data and selected a final model by first fitting all single factors and two-way interactions between factors in the data and then removing factors in step-wise fashion, only keeping any changes that resulted in a model with a lower AIC. Each of the models tested and their AICs are as follows:

Table AE1. AIC for different models used in the model selection. Predictors are coded as follows: S = food stochasticity, K = nest-level carrying capacity, F = foraging trail cost, D = stochastic nest death and R = food distribution. Letters separated by a colon indicate an interaction between the two factors. The final model is indicated in bold text.

Model	Predictors	AIC
1	Single: S, K, F, D, R Interactions: S:K, S:F, S:D, S:R, K:F, K:D, K:R, F:D, F:R, D:R	945.95
2	Single: S, K, F, D, R Interactions: S:K, S:F, S:D, S:R, K:F, K:D, K:R, F:D, D:R	944.44
3	Single: S, K, F, D, R Interactions: S:K, S:F, S:R, K:F, K:D, K:R, F:D, D:R	942.42
4	Single: S, K, F, D, R Interactions S:K, S:F, S:R, K:F, K:D, K:R, F:D	939.54
5	Single: S, K, F, D, R Interactions: S:K, S:F, S:R, K:F, K:D, K:R	938.2
6	Single: S, K, F, D, R Interactions: S:K, S:F, S:R, K:D, K:R	935.32
7	**Single: S, K, F, D, R** **Interactions: S:K, S:R, K:D, K:R**	**934.67**
8	Single: S, K, F, D, R Interactions: S:K, S:F, K:D, K:R	952.46

Appendix F - Population extinction in different conditions of the model

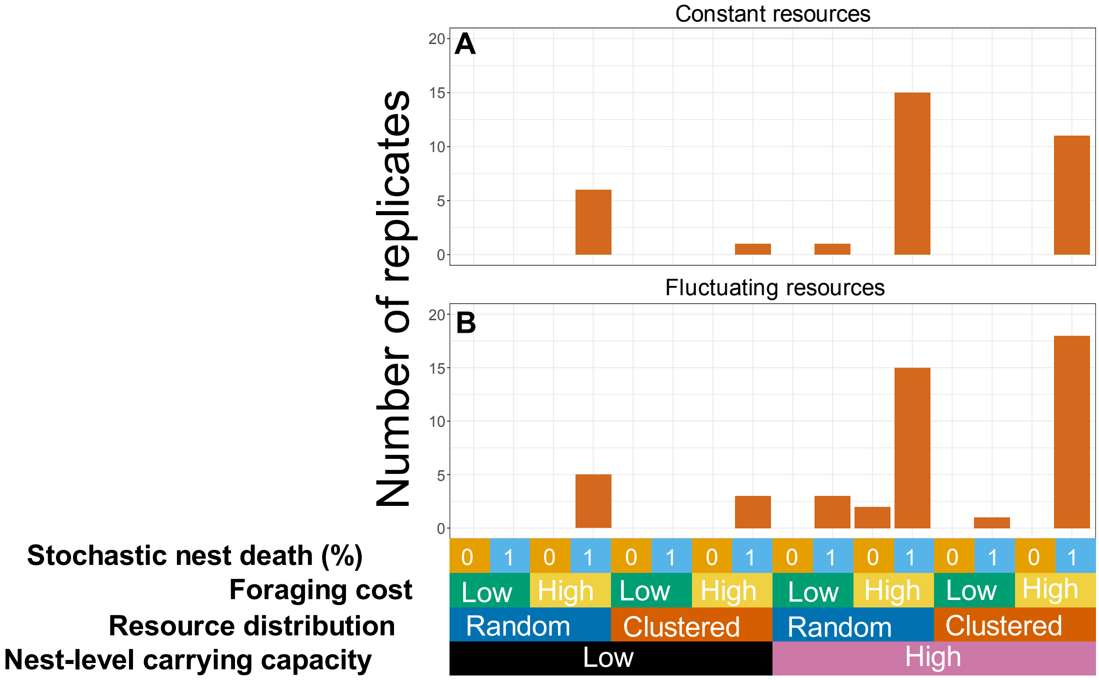


Figure AF1. The number of replicates, out of 30, in which the population did not survive until the final season in each condition when food is either constant (A) or fluctuating (B) in availability.

Appendix G – Growth of different sized nests in response to different quantities of food

Here we present a simple mathematical model demonstrating the growth of individual nests when presented with food sources of different size. The model uses the same equations as in the main model. However, in each simulation there is only a single nest alive that is placed 5m from two food sources that each have the same size, which is determined by the condition (Table AG1). Consequently, there is no competition or internest sharing. For simplicity, we also remove the ability of nests to parent new nests.

Table AG1 Parameters, their values and units used in model for Appendix G.

Letter	Description	Value	Units
F_i	Rate of productivity of food source *i*	0; 8,000	Resources t^{-1}
K	Carrying capacity	300,000	Ants
Q	Rate at which an ant can transport food to its nest along an outgoing connection	0.1	Resources ant^{-1} t^{-1}
V	Rate of colony loss	0.05	Portion ^{1} t^{-1}
E	Connection cost	0.00005	Resources t^{-1}

We test the growth of two different sized nests (small = 5,000, large = 100,000) under two different conditions of F_i, which represent situations where food sources fluctuate to be either unproductive or very productive in the 'fluctuating' condition in the main model. We run the model for a length of 200 arbitrary units of time.

The results from this basic model are presented in Figure AG1 and demonstrate that smaller nests grow more slowly when presented with very productive food sources, but also become extinct faster when presented with unproductive food sources.

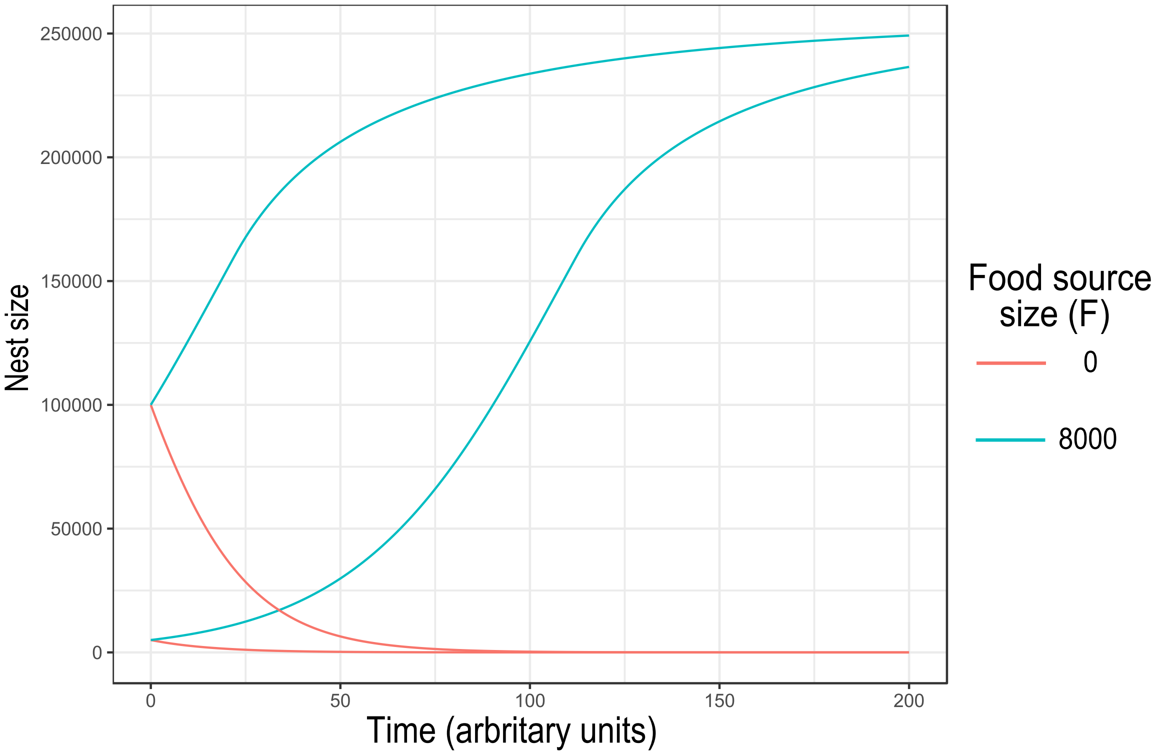


Figure AG1. Growth of nests of different population size when presented with different food sources.

Appendix H - Response of cumulative mean of frequency of polydomy to number of replicates

Here we present a plot demonstrating how cumulative mean of the frequency of polydomy changes as more replicates are added to each condition. In most of the conditions, there is very little change in the mean as more replicates are added once there are 30 replicates. Consequently, 30 replicates is sufficient to give reliable results.

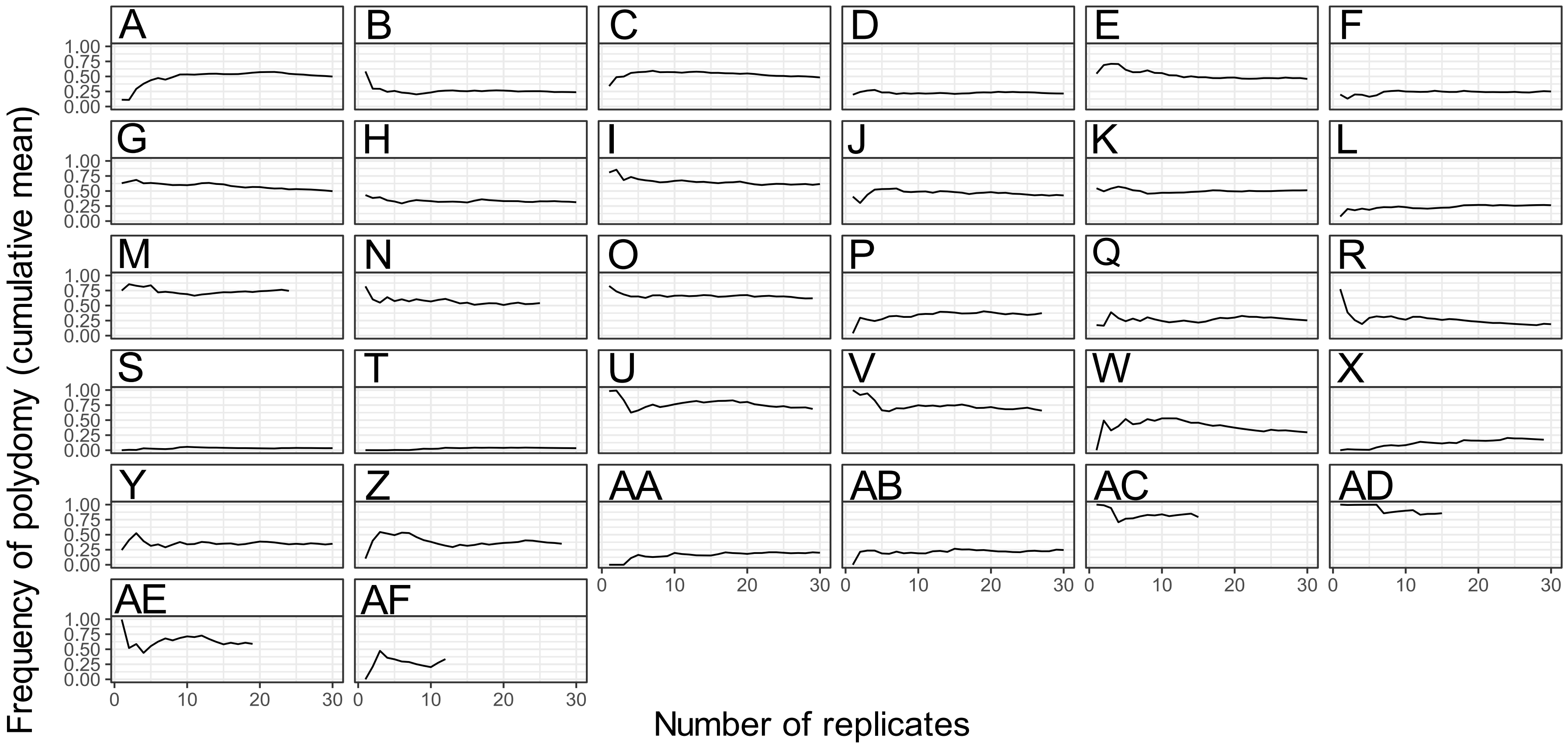


Figure AH1 Change in the cumulative mean of frequency of polydomy as more replicates are added in each condition. Conditions are given in Table AH1. Some conditions have fewer than 30 records as populations did not always survive to the final timestep (see Appendix F).

Table AH1. Conditions for Figure AH1

Letter	Nest-level carrying capacity	Foraging cost	Stochastic nest death	Distribution of resources	Food source productivity
A	Low	Low	None	Clustered	Constant
B	Low	Low	None	Clustered	Fluctuating
C	Low	Low	None	Random	Constant
D	Low	Low	None	Random	Fluctuating
E	Low	Low	1%	Clustered	Constant
F	Low	Low	1%	Clustered	Fluctuating
G	Low	Low	1%	Random	Constant
H	Low	Low	1%	Random	Fluctuating
I	Low	High	None	Clustered	Constant
J	Low	High	None	Clustered	Fluctuating
K	Low	High	None	Random	Constant
L	Low	High	None	Random	Fluctuating
M	Low	High	1%	Clustered	Constant
N	Low	High	1%	Clustered	Fluctuating
O	Low	High	1%	Random	Constant
P	Low	High	1%	Random	Fluctuating
Q	High	Low	None	Clustered	Constant
R	High	Low	None	Clustered	Fluctuating
S	High	Low	None	Random	Constant
T	High	Low	None	Random	Fluctuating

U	High	Low	1%	Clustered	Constant
V	High	Low	1%	Clustered	Fluctuating
W	High	Low	1%	Random	Constant
X	High	Low	1%	Random	Fluctuating
Y	High	High	None	Clustered	Constant
Z	High	High	None	Clustered	Fluctuating
AA	High	High	None	Random	Constant
AB	High	High	None	Random	Fluctuating
AC	High	High	1%	Clustered	Constant
AD	High	High	1%	Clustered	Fluctuating
AE	High	High	1%	Random	Constant
AF	High	High	1%	Random	Fluctuating

Appendix I - Sensitivity analyses for parameters

Here we present sensitivity analyses for parameters in the model. For each parameter we ran 30 replicates where we decreased the parameter by 10% and 30 replicates where we increased the parameter by 10%. All parameters other than the focal parameter were not changed. We performed a linear model on the results with each of the results to check whether any of the parameters heavily influenced the proportion of population in polydomous nests. The p-values from the linear model were then corrected for multiple comparisons using the *Bonferonni-Holm* method. The sensitivity analyses demonstrate that none of the parameters heavily influence the frequency of polydomy in the model (Figure AI1/Table AI1).

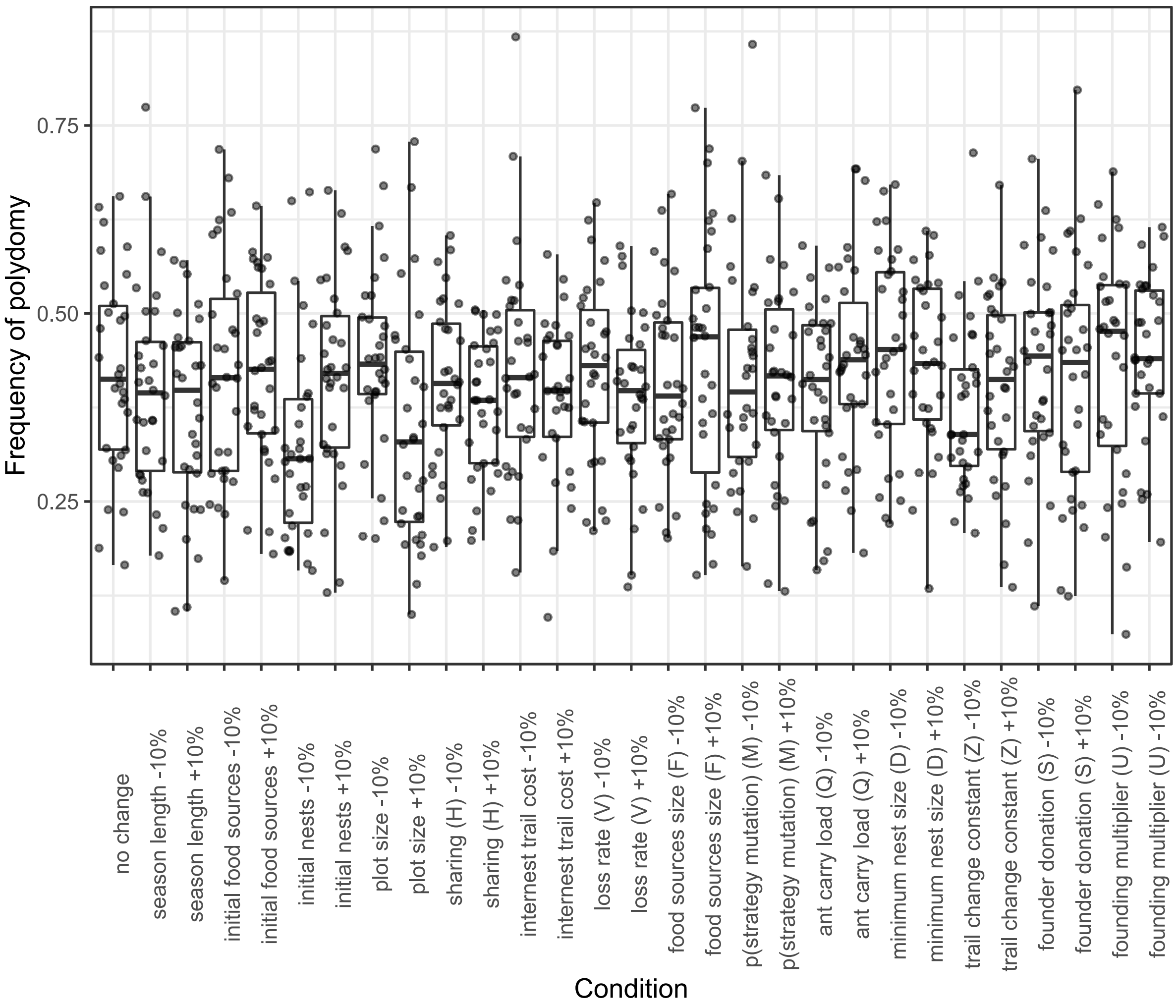


Figure AI1. Results of sensitivity analyses the model. Letters for parameters are given where relevant. Middle lines represent median values, lower and upper hinges represent 25th and 75th percentiles respectively and whiskers reach to the lowest (lower whisker) or highest (higher whisker) value, with a maximum reach of 1.5 x IQR from the hinge. All values are plotted as jittered points.

Table AI1. Results from the linear model of sensitivity analyses. The frequency of polydomy in each condition is compared against the frequency of polydomy in the control condition.

Condition	**Estimate**	**Standard error**	**T**	**Adjusted p-value**
season length -10%	-0.02	0.03	-0.70	0.98
season length +10%	-0.05	0.03	-1.55	0.87
initial food sources -10%	0.00	0.03	0.00	1.00
initial food sources +10%	0.00	0.03	-0.03	1.00
initial nests -10%	-0.09	0.03	-2.80	0.08
initial nests +10%	-0.01	0.03	-0.23	0.98
plot size -10%	0.01	0.03	0.37	0.98
plot size +10%	-0.08	0.03	-2.29	0.22
sharing (H) -10%	-0.01	0.03	-0.40	0.98
sharing (H) +10%	-0.05	0.03	-1.33	0.88
internest trail cost -10%	0.00	0.03	0.05	1.00
internest trail cost +10%	-0.03	0.03	-1.01	0.98
loss rate (V) -10%	0.00	0.03	0.01	1.00
loss rate (V) +10%	-0.04	0.03	-1.04	0.98
food sources size (F) -10%	-0.01	0.03	-0.41	0.98
food sources size (F) +10%	0.01	0.03	0.42	0.98
p(strategy mutation) (M) -10%	-0.01	0.03	-0.20	0.98
p(strategy mutation) (M) +10%	-0.01	0.03	-0.41	0.98
ant carry load (Q) -10%	-0.03	0.03	-0.77	0.98
ant carry load (Q) +10%	0.02	0.03	0.60	0.98
minimum nest size (D) -10%	0.03	0.03	0.83	0.98
minimum nest size (D) +10%	0.01	0.03	0.32	0.98
trail change constant (Z) -10%	-0.05	0.03	-1.44	0.87
trail change constant (Z) +10%	-0.02	0.03	-0.59	0.98
founder donation (S) -10%	0.01	0.03	0.22	0.98
founder donation (S) +10%	-0.01	0.03	-0.31	0.98
founding multiplier (U) -10%	0.01	0.03	0.30	0.98
founding multiplier (U) -10%	0.02	0.03	0.65	0.98

Appendix J - mapping dates for each colony

Table AJ1. Mapping dates for each colony. Green shading indicate that maps were collected for that date and grey shading indicates that no maps were collected for that date.

2012-2016, 2019								
Colony	26/07/12	31/05/13	28/08/13	26/05/14	25/08/14	25/08/15	22/08/16	27/06/19
2a	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2b	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
3	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
4	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
6	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
7	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
9	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
12	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2017								
Colony	23/05	01/06	14/06	25/06	12/07	28/07	10/08	22/08
2a	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2b	▨	🟩	▨	▨	▨	▨	🟩	▨
3	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
4	▨	🟩	▨	▨	▨	▨	🟩	▨
6	▨	🟩	▨	▨	▨	▨	🟩	▨
7	▨	🟩	▨	▨	▨	▨	🟩	▨

8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
9	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
10	⬜	🟩	⬜	⬜	⬜	⬜	🟩	⬜
12	⬜	🟩	⬜	⬜	⬜	⬜	🟩	⬜
2018								
Colony	08/05	23/05	12/06	28/06	20/07	02/08	17/08	30/08
2a	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2b	⬜	⬜	🟩	⬜	⬜	⬜	⬜	⬜
3	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
4	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
6	⬜	⬜	🟩	⬜	⬜	⬜	⬜	⬜
7	⬜	⬜	🟩	⬜	⬜	⬜	⬜	⬜
8	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
9	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
10	⬜	⬜	🟩	⬜	⬜	⬜	⬜	⬜
12	⬜	⬜	🟩	⬜	⬜	⬜	⬜	⬜

Appendix K - model fitting details

Table AK1 Description of models fitted

Model	Data used	Model type	Response variable	Fixed effects	Random effects (groups)	Samples	Levene's test for equal variances
1	17/18 4 colony subset	LMM	Log(Proportion of sub-colony population on foraging trails)	Stage of season	Colony (4), year (2)	84	F = 2.33 p = 0.13
2	17/18 4 colony subset	LMM	Log(Proportion of sub-colony population on internest trails)	Stage of season	Colony (4), year (2)	84	F = 1.09 p = 0.30
3	17/18 4 colony subset	LMM	Log(Proportion of sub-colony population on any trails)	Stage of season	Colony (4), year (2)	84	F = 0.25 p = 0.62
4	17/18 4 colony subset	Poisson GLMM	Number of nests founded by colonies	Stage of season	Colony (4), year (2)	16	F = 3.46 p = 0.084
5	17/18 4 colony subset	Poisson GLMM	Number of nests abandoned by colonies	Stage of season	Colony (4), year (2)	14	F = 0.69 p = 0.42
6	17/18 4 colony subset	Poisson GLMM	Number of trails added in colonies	Stage of season	Colony (4), year (2)	16	F = 0.0018 p = 0.97
7	17/18 4 colony subset	Poisson GLMM	Number of trails removed in colonies	Stage of season	Colony (4), year (2)	16	F = 1.06 p = 0.32
8	Full dataset	Binomial GLMM	Binomial indicating if a sub-colony was a founder	Log (Population size of sub-colony)	NestId (293), timepoint (10)	1024	N/A (numeric fixed effect)
9	Full dataset	Binomial GLMM	Binomial indicating if a sub-colony had abandoned their nest	Log (Population size of sub-colony)	NestId (293), timepoint (10)	993	N/A (numeric fixed effect)
10	17/18 4 colony subset	Binomial GLMM	Binomial indicating if new sub-colonies had abandoned their nest over the winter	Stage of season	Colony (4), year (2)	37	F = 1.13 p = 0.29

Table AK2 Description of model results

Model	Effect	Estimate	Standard Error	Test statistic	p-value
1	Intercept	-6.46	0.62	T = 10.48	< 0.01
	Stage of season	-0.79	0.86	T = -0.92	0.36
2	Intercept	-3.67	0.42	T = -8.78	< 0.001
	Stage of season	0.59	0.58	T = 1.02	0.31
3	Intercept	-2.70	0.36	T = -7.47	< 0.01
	Stage of season	0.070	0.41	T = 0.17	0.86
4	Intercept	1.00	0.33	Z = 3.05	0.0023
	Stage of season	-0.61	0.34	Z = -1.80	0.072
5	Intercept	0.25	0.35	Z = 0.70	0.49
	Stage of season	0.80	0.40	Z = 1.99	0.046
6	Intercept	2.39	0.46	Z = 5.16	< 0.001
	Stage of season	-0.62	0.16	Z = -3.94	< 0.001
7	Intercept	2.35	0.42	Z = 5.60	< 0.001
	Stage of season	-0.33	0.15	Z = -2.1	0.027
8	Intercept	-4.93	0.66	Z = -7.42	< 0.001
	Log(Population size of sub-colony)	0.32	0.064	Z = 4.94	< 0.001
9	Intercept	-4.65	0.70	Z = -6.63	< 0.001
	Log(Population size of sub-colony)	0.61	0.075	Z = 8.11	< 0.001
10	Intercept	-0.93	1.21	Z = -0.77	0.44
	Stage of season	3.02	1.18	Z = 2.55	0.011

Appendix L - distribution fitting of number of nests founded by each sub-colony

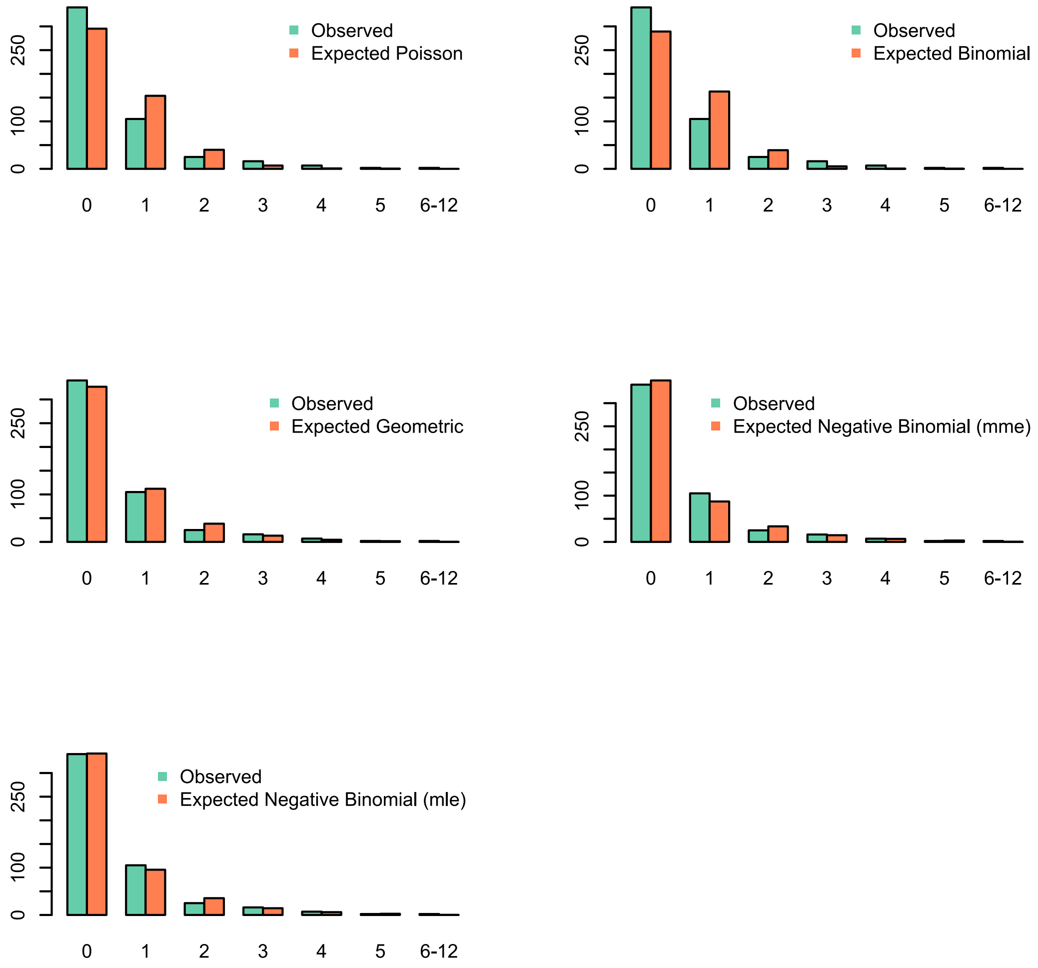


Figure AL1 Results of fitting number of nests founded by each sub-colony in a season to established distributions. Negative binomial fit using MLE provides a very close fit to the real distribution.

Table AL1 Results of fitting number of nests founded by each sub-colony in a season to established distributions. Negative binomial fit using MLE provides the best fit

	Distribution				
	Poisson	**Binomial**	**Geometric**	**Negative Binomial (MME)**	**Negative Binomial (MLE)**
Sum of residuals	0.01	0	0.78	2.64	1.85
Chi-squared statistic	27.85	34.95	6.12	4.86	4.25
p-value	0	0	0.41	0.56	0.64
MSE	678.28	899.5	60.13	67.76	29.67

Appendix M

PCRs were carried out in three panels for each individual. The primers and their respective labels were as follows:

1: Fl12 6-FAM, Fe13 NED, Fe11 PET ja Fl20 VIC

2: Fe7 6-FAM, Fl21 6-FAM, Fe37 NED ja Fe19 PET

3: Fe16 6-FAM, Fe38 NED ja Fe21 PET

The PCR cycle was:

Step1: Initial denaturation 95℃ for 15minutes

Step2: Denaturation 95℃ for 30 seconds

Step3: Annealing 55℃ for 90 seconds

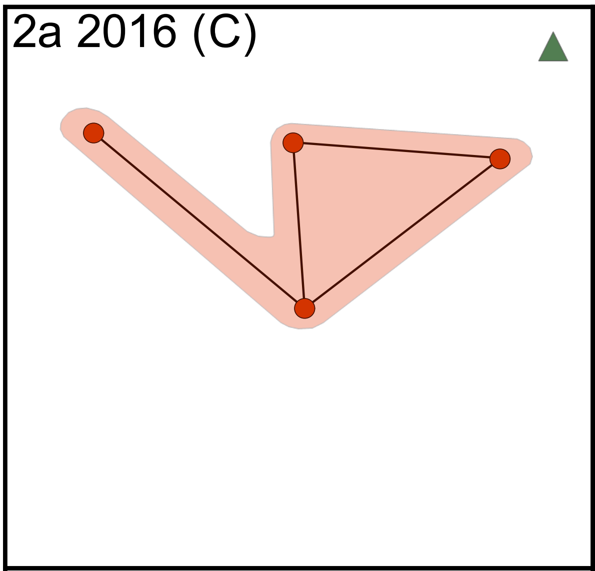
Step4: Extension 72℃ for 30 seconds

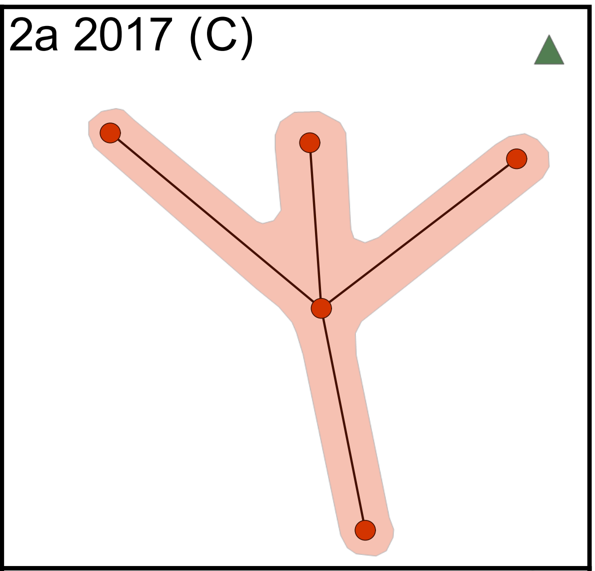
Step5: Repeat steps 2-4 28 times

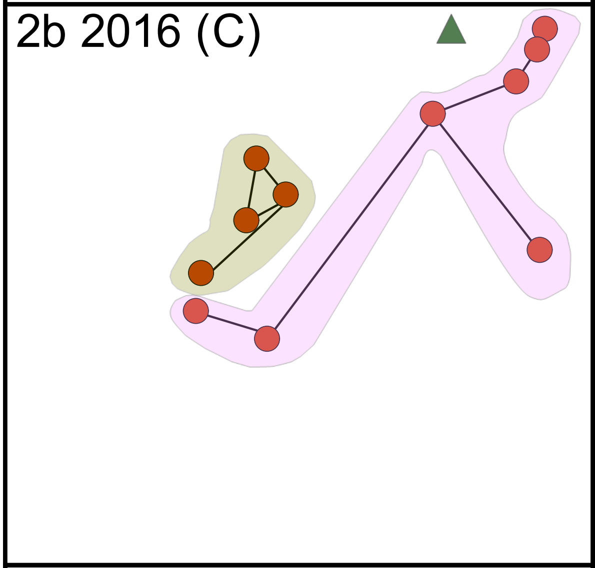
Step6: Final elongation 60℃ for 30 minutes

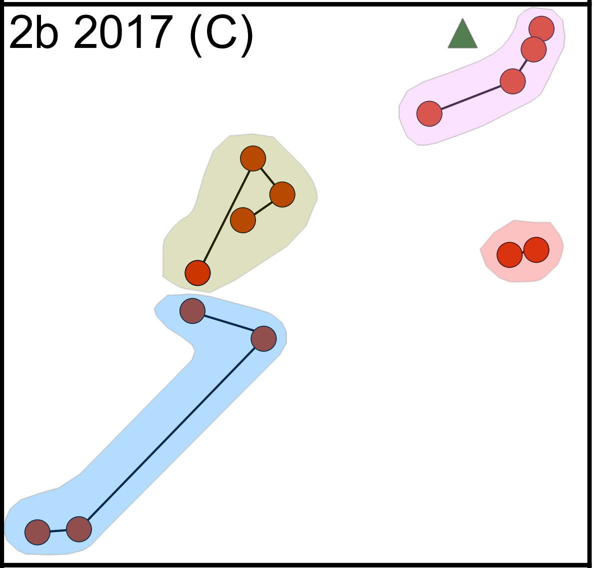
Step7: Cooling 15℃ for 5 minutes

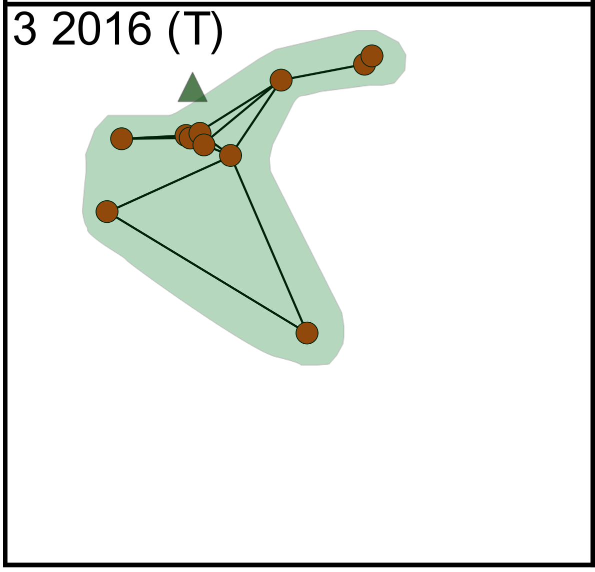
Appendix N - colony inter-nest maps

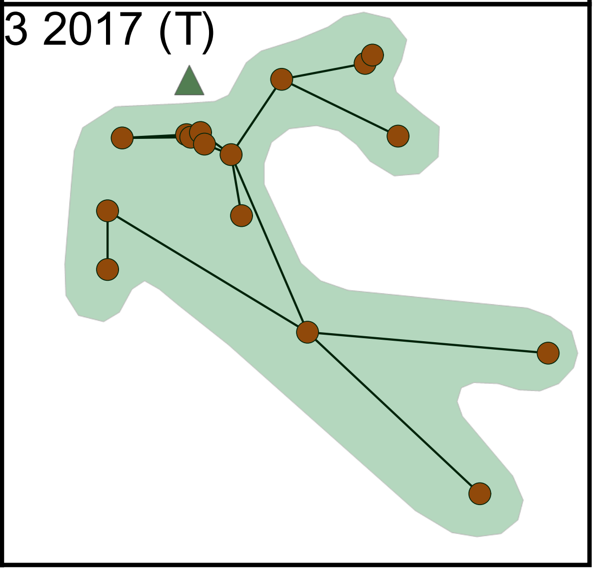

2a 2016 (C)

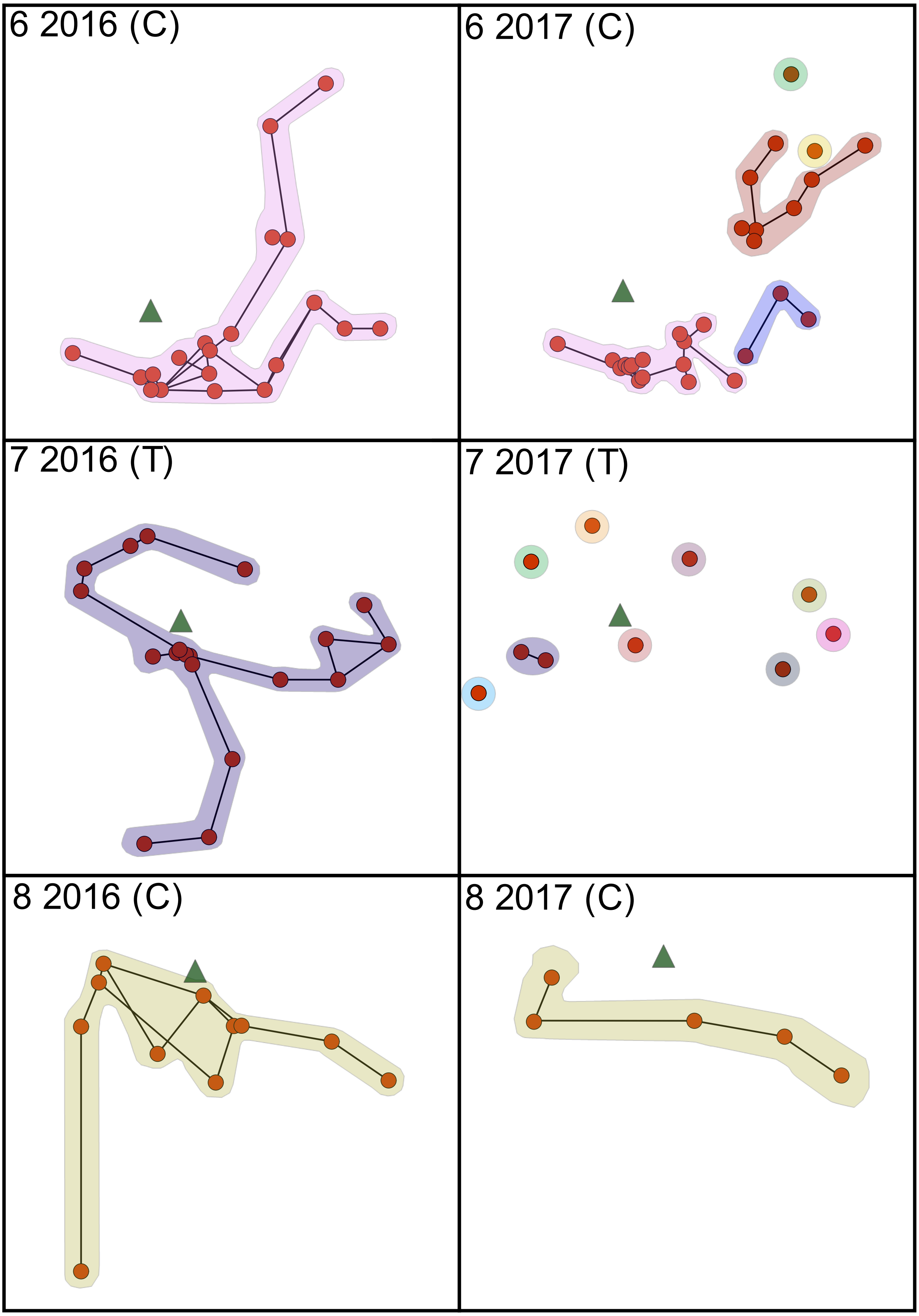

2a 2017 (C)


2b 2016 (C)


2b 2017 (C)


3 2016 (T)


3 2017 (T)


6 2016 (C)
6 2017 (C)
7 2016 (T)
7 2017 (T)
8 2016 (C)
8 2017 (C)

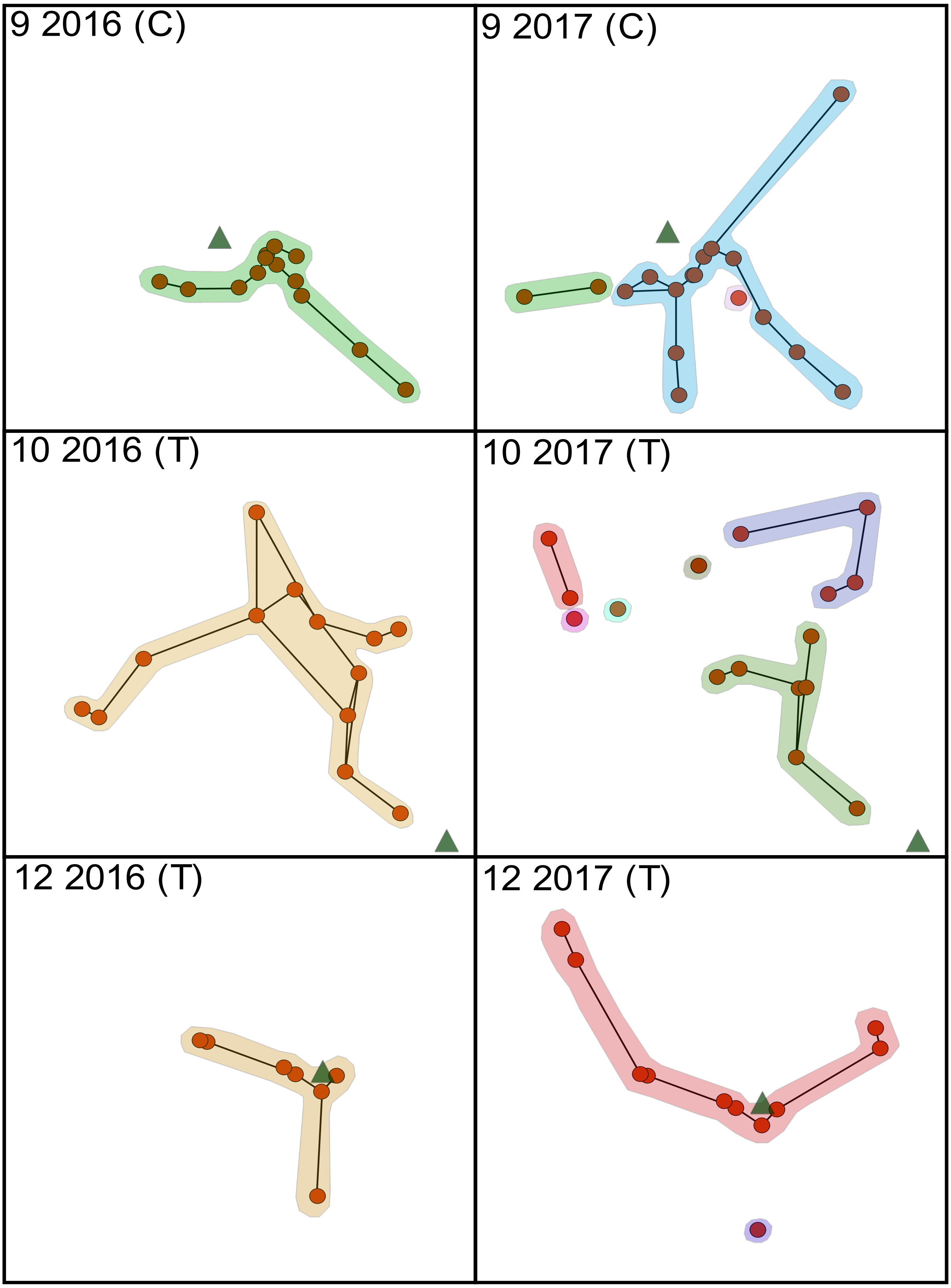
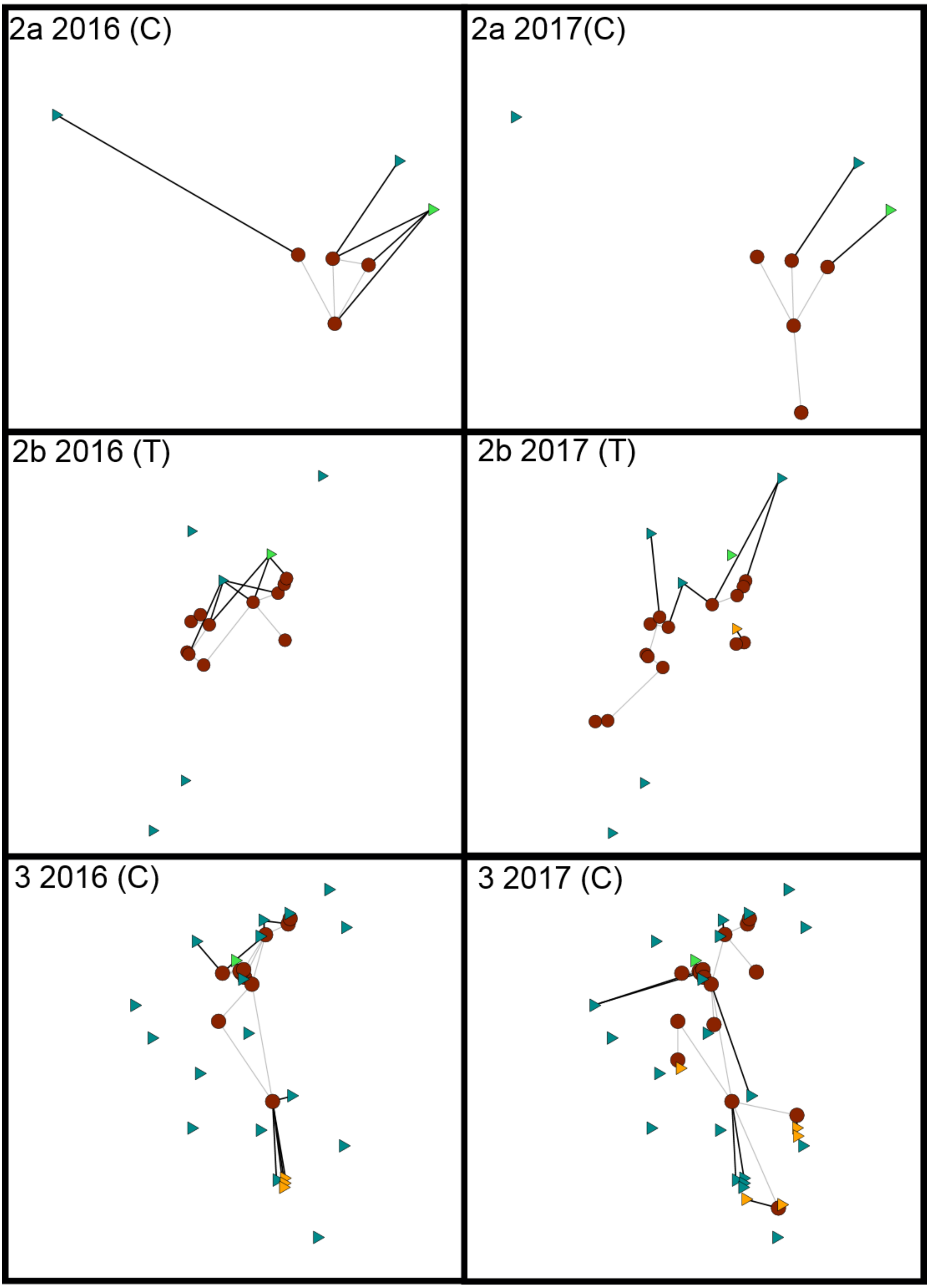
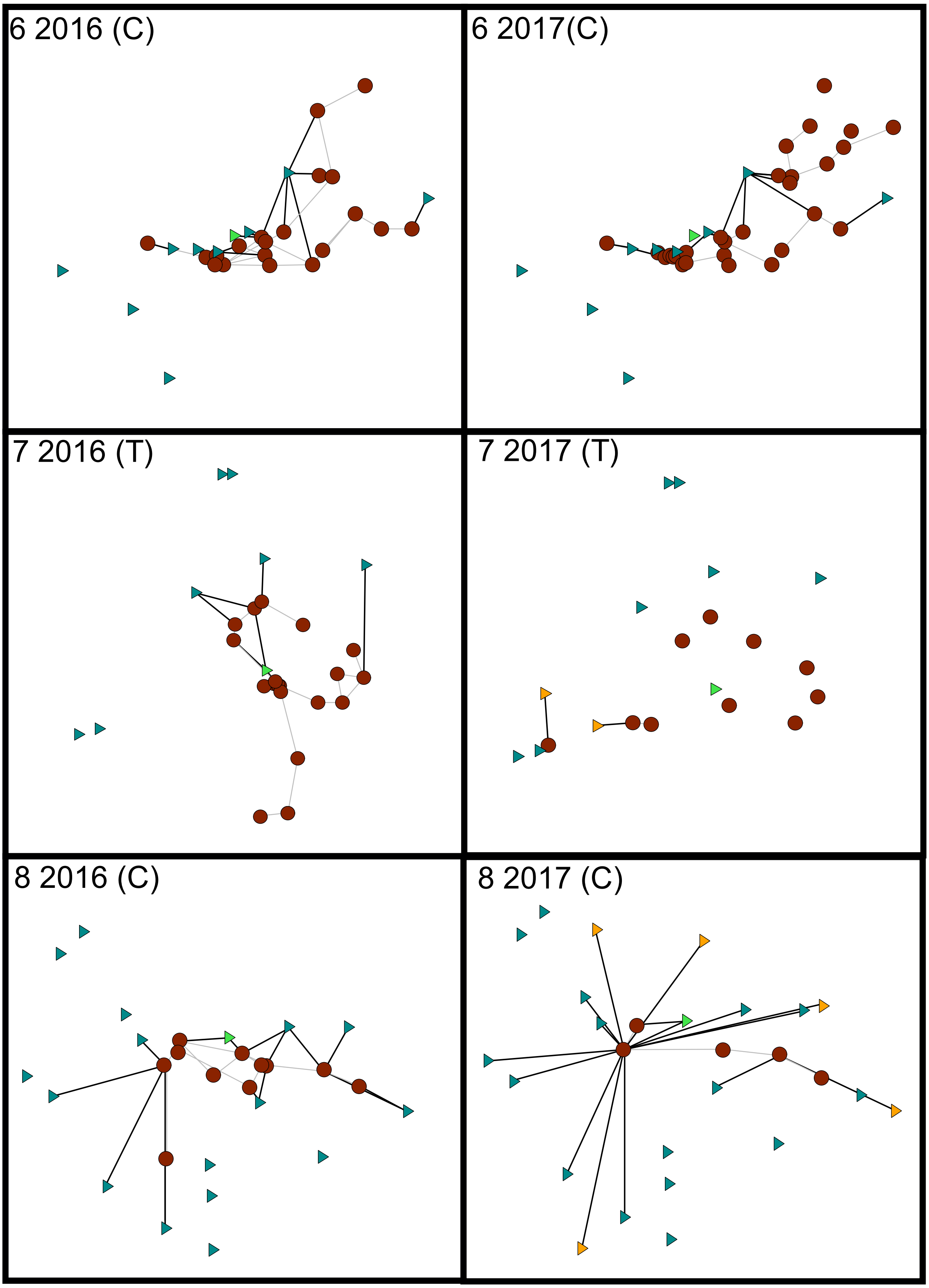


Figure AM1. Colony maps before (2016) and after (2017) focal trees were either excluded in treatment condition (indicated with T) or unchanged in control conditions (indicated with C). Red nodes indicate nests, black edges indicate trails, green triangles indicate focal trees and coloured polygons indicate independent network components.

Appendix O - colony foraging maps




6 2016 (C)
6 2017(C)
7 2016 (T)
7 2017 (T)
8 2016 (C)
8 2017 (C)

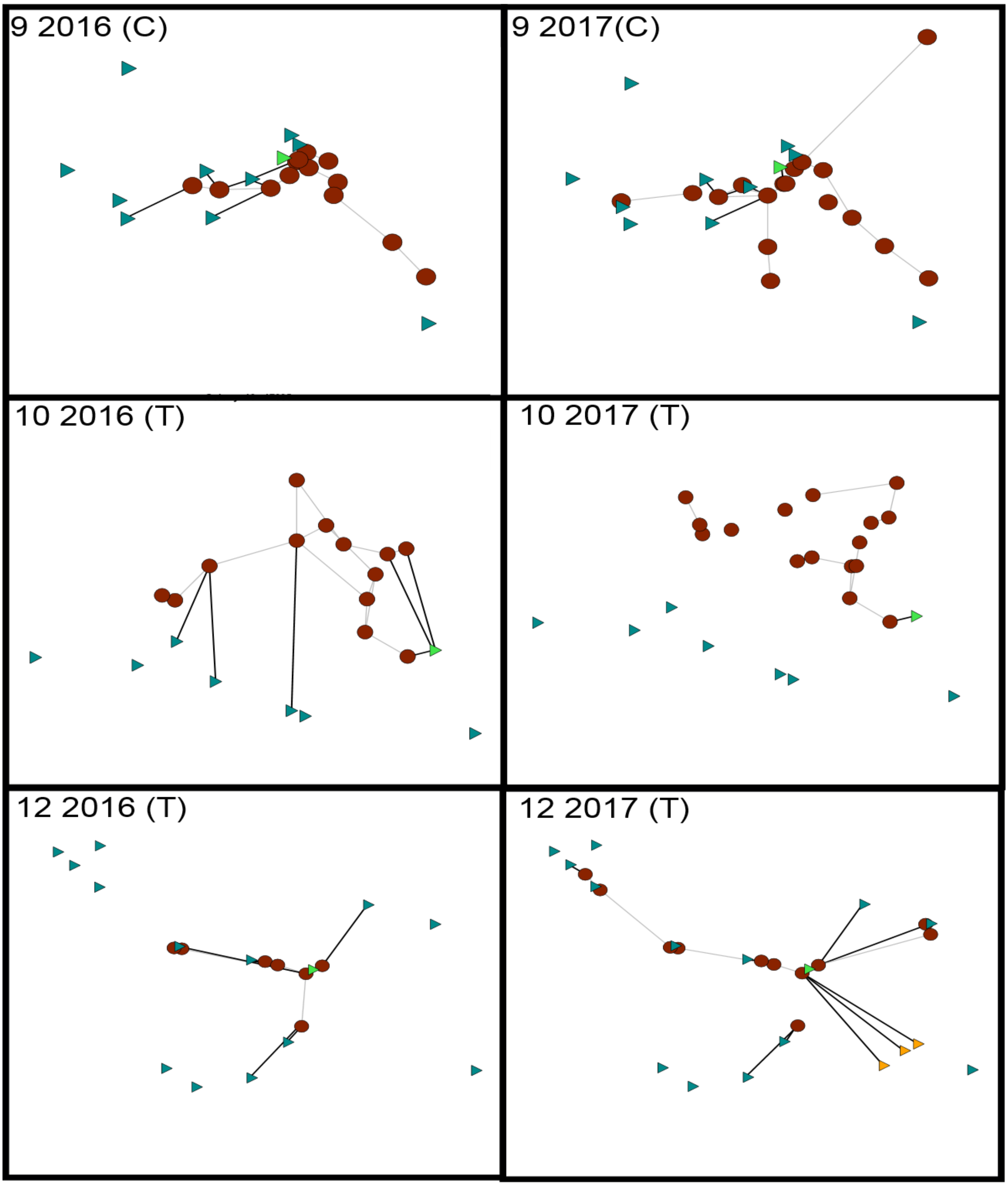


Figure A2.1. Colony foraging maps before (2016) and after (2017) focal trees were either excluded in treatment condition (indicated with T) or unchanged in control conditions (indicated with C). Red nodes indicate nests, black edges indicate foraging trails, grey edges indicate inter-nest trails, bright green triangles indicate focal trees, yellow triangles indicate newly foraged trees and blue tree indicate trees that were neither new or focal.

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