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An experimental investigation of task allocation in the ant Leptothorax albipennis

Backen, S. J.

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An Experimental Investigation of Task Allocation in the ant *Leptothorax albipennis*

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To my family...Mum, Dad, Claire, Wills, Jez, (and not forgetting Bella), for whom I feel no need to express any poetic sentiments, because there is true feeling and understanding

And to Dr. Robin Wootton (always 'G' to me)...a source of inspiration
From Dorabella

And Dr. Danny Kelly – who understands what it takes
- with much love

"[The man of system] seems to imagine that he can arrange the different members of a great society with as much ease as the hand arranges the different pieces upon a chessboard; he does not consider that the pieces upon the chessboard have no other principle of motion besides that which the hand impresses upon them; but that, in the great chessboard of human society, every single piece has a principle of motion of its own, altogether different from that which the legislator might choose to impress upon it."

Adam Smith, Theory of Moral Sentiments (1759)

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Abstract

Division of labour is of fundamental importance in biology. In this thesis, I review the success of the eusocial insects and discuss the proximate mechanisms that underpin the ultimate explanations of this success. I conclude that the presence of a reproductive division of labour, one of the three 'defining traits' of eusociality (Wilson, 1971 p.4), and the consequent facilitation of divisions among other sorts of work, is likely to be the primary cause of the success of the eusocial insects. Division of labour allows a colony to perform tasks more efficiently than would be the case for a colony of generalists. Spatial efficiency is fundamental to the selection pressures that have driven the evolution of division of labour (Bourke and Franks, 1995). Individuals in colonies of *Leptothorax* ants exhibit spatial fidelity to limited zones in the nest (Sendova-Franks and Franks, 1995a). Furthermore, workers re-establish these positions after emigration to a new nest site, and resume their tasks. This phenomenon, *Social Resilience*, may be fundamental to maintaining the efficiency of division of labour. I describe experiments in which I manipulated the social structure of *Leptothorax albipennis* colonies. These experiments utilise *sociotomy* techniques to provide new insights into the spatial organisation of tasks within these colonies, and to test the limits of social resilience (Backen *et al.*, 2000). The results from these experiments suggest strongly that relative specialisation is dependent on experience, rather than being directly dependent upon age, and moreover, that spatial organisation is established prior to task allocation. I conclude that spatial organisation is dependent on experience, rather than age, and task specialisation occurs as a consequence of task demand. Furthermore, I show, for the first time, that the mechanisms underlying worker spatial organisation and task specialisation do not require templates that are external to the workers.

Chapter 1

An Introduction to Social Insects

1.1 Introduction to Social Insects – A Unique Success Story

Social insects are undisputedly successful organisms. Wilson (1990, 1992), found that social insects were exemplary in every one of the four categories by which he defined success. These categories are stated below:

❶ **Dominance** - defined as "relative abundance, especially as it affects the appropriation of biomass and energy and impacts the life and evolution of the remainder of the biota" (Wilson, 1990, 1992 p.1).
❷ **Diversity** - defined as number of species.
❸ **Wide geographical distribution.**
❹ **Persistence** throughout geological time.

Social insects can be examined with reference to each of the categories above. Firstly, with regards to ❶ above, there is no doubt that the social insects are ecologically '**dominant**' organisms. The pie charts in Figure 1, demonstrate the huge proportion of total animal biomass attributed to insects, and of this, to ants, termites, stingless bees, and social wasps (adapted from Gadagkar, 1995).

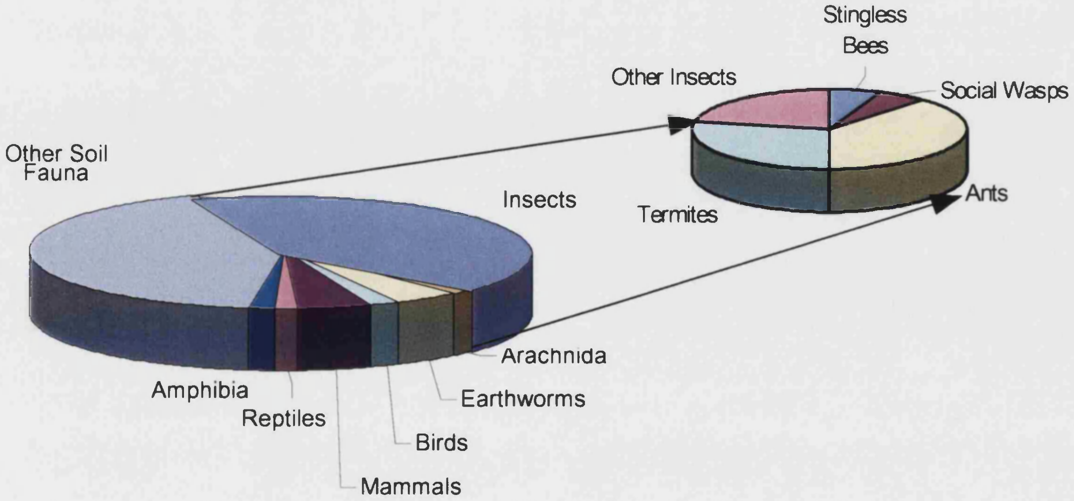


Figure 1: Pie charts showing the proportion of biomass attributed to insects and social insects

Social insects have attained an extraordinary abundance. Densities of 130,000 termites, and 16.6 million ants, per hectare, have been recorded in the litter of tropical forests on Barro Colorado Island, Panama. In East Africa densities of 47 million ants per hectare (consisting of just three arboreal species) and in Trinidad, from 1.8 million to 180 million termites, have been measured (Baroni Urbani *et al.*, 1978).

The impact of social insects on their environment is even more impressive. They are among the leading predators of other invertebrates in most terrestrial environments (Hölldobler and Wilson, 1990). They may also act as scavengers, feasting on arthropod or small vertebrate remains; or compete with granivorous mammals for seeds in the deserts of the south western United States (Wilson, 1990, 1992). Other social insect species move a comparable amount of soil to earthworms in woodlands in New England, and surpass them in tropical forests (Wilson, 1985). In fact, the biomass and energy consumption of social insects exceeds those of vertebrates in most terrestrial habitats (Wilson, 1971, p.1).

❷ Social insects, particularly the ants, are incredibly **diverse**. Wilson (1987) noted that the described world ant fauna consists of approximately 300 genera and 8800 species. He estimates that fewer than 100 ant genera remain unrecognised, but that the number of undescribed species is very large, and that there could be 20,000 extant species. The Formicidae contain more species and genera than the rest of the social insects combined (Wilson, 1971 p.27).

❸ **The geographical range** inhabited by social insects extends from the Arctic Circle through to Tasmania, Tierra del Fuego, and southern Africa (Wilson, 1971 p.27). In fact, four of the Formicidae genera alone, namely *Camponotus*, *Crematogaster*, *Hypoponera*, and *Pheidole*, are to be found across this entire range (Wilson, 1987).

❹ Are the social insects a **persistent** clade throughout **geological time**? Persistence is the most important measure of success in strictly Darwinian terms.

The first termites arose in the Jurassic or early Cretaceous, approximately 200 million years ago, and the ants, the social wasps and the social bees, in the Cretaceous, about 100 million years later (Wilson, 1990, 1992). All the ants arose from the single genus *Sphecomyrma* (Wilson, 1987). By the mid-Eocene at least three of the modern formicid subfamilies had evolved, the Myrmicinae, Dolichoderinae and the Formicinae (Wilson, 1987). The Formicidae, therefore, are older than all mammalian orders except the Marsupalia, and only one of the orders that emerged at the same time as the ants, the Didelphidae or opossums, are extant, and thus as old as the ants.

1.2 Why are the Eusocial Insects so Successful?

i. Definition of Eusociality

The success of the social insects has been established, but what are the reasons for their success? The social insects are more than just 'social' – they are **'Eusocial'**. Wilson's (1971) hypothesis that eusociality has arisen twelve times in the Hymenoptera, and once in the Protoblattoid line from which the Termites (order Isoptera) arose, is now viewed to be an underestimation (Bourke and Franks, 1995 p.93). However, eusociality is not a phenomenon limited to the eusocial insects. There is evidence that eusociality has also arisen in aphids and naked mole-rats (Benton and Foster, 1992; Jarvis et al., 1994). Eusociality may, therefore, be a more common phenomenon than has been previously thought. But what exactly is eusociality? Initially I will concentrate on the traditional view (most espoused by Wilson), of the definition of eusociality. However, there is a substantial and continuing debate over the extent of eusociality and how the scale between co-operative breeding, sociality and eusociality has been delimited. This will be examined in detail in section 1.2 (iv), below.

Wilson (1971 p.4) defined eusociality by the presence of three traits in individuals of the same species:

- **Co-operative care of the young;**
- **Overlap of at least two generations** which are capable of assisting in colony labour, so that offspring assist their parents at some stage of their life;
- **Presence of a reproductive division of labour**, with more or less sterile individuals working on behalf of fecund individuals.

ii. Altruism Cannot be Explained by Individual-Level Selection – Can it be Explained by Gene-Level Selection?

The eusocial insects have been shown to be *successful* (above), in all possible senses of the word. The crucial question is - how does the presence of these three traits, the classical defining traits of eusociality, lead to such undeniable success? In order to begin to answer this question, I will pose another. How do these societies exist at all? It is paradoxical that individuals within the society will co-operate with other individuals, to the extent that they help raise the young of the others, whilst apparently forgoing the chance to reproduce. In other words, why do these co-operating individuals behave altruistically and suffer a cost associated with this behaviour, which appears to enhance the fitness of others? More specifically, how did the eusocial Hymenoptera evolve?

There is no simple answer to this question. In fact, Darwin was so perplexed by it that he viewed the problem as being potentially *"fatal to my whole theory"* (Darwin, 1859 p. 236). How had natural selection resulted in these 'generous' individuals? How could such societies evolve by the process of natural selection?

The reason Darwin was unable to explain the behaviours that he observed, was due to the way he thought selection acted. He envisaged natural selection acting on the *individual.* If the individual is the *'unit of self-interest'*, the occurrence of altruism contradicted his predictions that each individual should behave so as to maximise its number of offspring (Bourke and Franks, 1995 p. 11). Darwin tried to resolve the problem by hypothesising that workers could evolve if they were *"profitable to the community"* (Darwin, 1859 p.236).

Gene Selectionism is, arguably, the biggest revolution in biological thought since Darwin's theory of evolution by natural selection. Introduced by Hamilton (1964 a, b) and Williams (1966), and championed by Dawkins (1976, 1982a, 1986), the theory advocates a gene-centred view of the mechanism of natural selection. Bourke and Franks (1995 p.4) note that gene selectionism or the **'selfish gene'** perspective is still controversial, *"nevertheless, a look at today's behavioural ecology textbooks suggests that it is already the dominant mode of evolutionary explanation."*

The individual is not the fundamental unit of selection, according to gene selectionism. Instead, the unit of selection is a **replicator** – an entity which can make copies of itself (Dawkins, 1982a, p.293, b). Simply (as reviewed by Bourke and Franks 1995), Dawkins' "replicator version" of natural selection theory consists of replicators (structures which show high-fidelity copying), combined with a degree of error (or mutation) in the process of copying, which results in undirected variation in each generation of replicators. The structure and properties of replicators influence their survival and rate of replication. Consequently, there is an accumulation of favourable mutations within replicators that will lead to the appearance of adaptive complexity benefiting the replicators. The process of adaptation is not simple however and cannot arise in one stage. This is too improbable according to Dawkins (1986), and adaptation can only arise from successive bouts of selection, or **cumulative selection**.

Replicators are usually lengths of DNA or genes (hence the term 'selfish gene'). Individuals, as single bodies, are regarded, not as the units of selection themselves, but as '*vehicles*' for replicators (Dawkins, 1982a, b). Alterations to bodies are not transmitted in reproduction, unlike alterations in replicators. Groups, also, are not replicators (Bourke and Franks, 1995 p.7), they too are vehicles for replicators, as alterations to group structure are not conserved. It may be beneficial to genes to instruct their bodily vehicles to form groups or societies. In fact, it is probable that replicators can arise spontaneously by '**single-step selection**' (Dawkins, 1986), unlike bodies, that are too complicated. This has led people to conclude that life originated as **free replicators** or '**naked genes**', (Dawkins, 1982b; Szathmary and Maynard Smith, 1997; refined by Lifson and Lifson, 1999) which then evolved to acquire their cellular and bodily vehicles.

Can gene selectionism resolve Darwin's *"one special difficulty"* (1859 p.236)? That is, can it explain altruism?

iii. The Basis of Altruism

Altruism is defined as occurring when:

> ***" an individual behaves in such a way that the result is an increase in the survival or offspring production of another individual and a decrease in its own survival or offspring production"***
>
> Bourke and Franks, 1995 p.11

There are three possible ways in which altruism may evolve.

❶ **Kin Selection**

❷ **Manipulation or Social (or brood) Parasitism**

❸ **Via Delayed Benefits - mutualism**
- reciprocal altruism

❶ Kin Selection Theory

Kin selection theory was originally formulated by Hamilton (1963, 1964a, b) as '**Inclusive Fitness Theory**', and was designated '**Kin Selection**' by Maynard Smith (1964). In fact the idea that kinship motivated social interaction came from Aristotle (Platt, 1912) in *De Generatione Animalium*, where he stated that "*The bees attend upon their kings because they are their offspring*". Darwin himself appreciated that altruistic behaviour could evolve via benefits to relatives (1859), but Hamilton was the first to formalise the theory.

Kin selection is defined as:

> ***"the natural selection of genes for social actions via the sharing of genes between the performer of the action and its relatives (kin)"***
>
> Bourke and Franks, 1995

There are four, mutually exclusive, types of social actions, which are defined by whether the actor increases or decreases his personal fitness and that of the recipient, due to the action. Altruism (as defined above) is a social action in which the actor loses and the recipient gains.

Hamilton's rule describes the conditions required for the spread of a gene for altruistic action. It states that a gene for altruistic action will spread through a population if the following inequality is satisfied:

$$rb - c > 0$$

- **r** = the regression relatedness between the altruist and the beneficiary (a measure of the genetic similarity between the two individuals, formally defined in kin selection theory as a regression coefficient).
- **b** = the benefit of altruism in terms of the extra offspring gained by the beneficiary.
- **c** = the cost of altruism in terms of the offspring production lost by the altruist.

Hamilton's rule explains how a gene for altruism can spread under kin selection because the gene causes its bearer to care for individuals which have a greater than average chance of sharing the same gene as they are related (the relatedness coefficient does not have to be high, but it does have to be greater than zero). Consequently, what the gene 'loses' in terms of offspring of the actor caused by its altruistic behaviour, it redeems many times over because of the enhanced survival and reprodution of the beneficiaries (Bourke and Franks, 1995 p.22). In effect the gene for social action spreads because it promotes care for copies of itself (Dawkins, 1979).

Hamilton therefore solved Darwin's '*one special difficulty*' of how characteristics that lowered an individual's personal fitness could evolve by replacing the individual-centered perspective with the perspective of the gene which coded for the characteristic. It is not personal fitness that matters in this case, but **inclusive fitness**, a term which represents an individual's personal genetic representation in its own offspring, and also, an individual's genetic representation in its relatives (Hamilton, 1964a, b). Consequently, Williams (1966) and Dawkins (1976) have reformulated natural selection theory itself in terms of genetics.

❷ Manipulation or Social (or brood) Parasitism

Social Parasitism typically involves the exploitation of a pre-existing altruism. An example is the feeding by an adult bird of the offspring of a brood parasite, such as a cuckoo (Krebs and Davies, 1981 p.279). Manipulation may also be intra-specific, for example, female starlings lay eggs in the nests of conspecifics in order to avoid the costs of incubation and parental care (Yom-Tov, 1980). These interactions thus involve an individual being 'tricked' into behaving for another individual's benefit.

Parental Manipulation has been suggested as an alternative hypothesis for the basis of eusociality (Alexander, 1974). The theory suggests that workers help raise the queen's offspring because she manipulated them into doing so, by, for example, under-feeding them, or otherwise dominating them, and consequently preventing their reproduction directly. Bourke and Franks (1995 p.36) accept that parental manipulation theory may have been important in eusocial insect evolution, but only when it is viewed as part of kin selection theory (see above). After all, by definition, parental manipulation can only occur amongst kin. Also the offspring may be selected to resist manipulation and not accept it passively. Thus Bourke and Franks conclude that parental manipulation theory is best included as part of *kin conflict theory* (Trivers, 1974; Trivers and Hare, 1976), the branch of kin selection theory dealing with evolutionary conflicts of interest between relatives (discussed later).

❸ Altrusim via Delayed Benefits

An individual may join a social group as a helper and may later inherit the position of breeder. Consequently this is also known as the '**hopeful reproductive**' strategy or '**mutualism**' (West-Eberhard, 1978).

Reciprocal altruism occurs when an individual makes a sacrifice in return for a future favour from the recipient. Of course, there is always the possibility that one individual may cheat. A beneficiary may never return the favour. However, the Prisoner's Dilemma model shows that, under certain conditions, reciprocity can be evolutionarily stable (Axelrod and Hamilton, 1981).

Reciprocity, when altruism is rewarded in kind, is difficult to apply to many examples, and it has been argued that rewards may be acquired indirectly, or may not involve the return of altruism at all (Roberts, 1998). Introducing differences in individual generosity and partner choice into reciprocity models can lead to escalation in altruistic behaviour. Individuals may then compete for the most altruistic partners and non-altruists may become ostracised. Thus, there are indirect rewards for altruistic behaviour, as altruists tend to be chosen as mates, by **Competitive Altruism.**

Of course, mutualism or reciprocal altruism may occur between relatives, so these two strategies are not mutually exclusive to kin selection. Alexander (1974) notes that as the 'altruist' receives delayed benefits over the course of its lifetime, this is only altruism in the short-term sense, and therefore is more accurately classified as **'co-operation'**.

Caution is advised when applying the principles of reciprocal co-operation, as has been seen recently in the sentinel behaviour of meerkats (*Suricatta suricatta*, Clutton-Brock, 1999). Explanations of such 'look-out' behaviour, which frequently occurs amongst group members of some animal societies, have relied on reciprocal behaviour (and kin selection in larger groups, Krebs and Davies, 1981 p.279). Recent models suggest that guarding may be an individual's optimal activity when its stomach is full, if no other animal is on guard. Meerkat guards position themselves in safe sites, and even solitary individuals spend part of their time on guard. Hence individuals previously though to be showing altruistic behaviour may actually be behaving selfishly.

iv. The Distiction Between Sociality and Eusociality

The eusocial members of the Hymenoptera and the termites are not the only animals to fulfil Wilson's (1971) three defining traits. Other animals that are considered to be eusocial include aphids (Aoki, 1987; Itô, 1989; Benton and Foster, 1992), ambrosia beetles (Kent and Simpson, 1992), thrips (Crespi, 1992), and bathyergid mole-rats (Jarvis *et al.*, 1994). There has been considerable controversy as to the extent and definition of eusociality, and whether it in fact applies to a much larger number of species than previously thought.

There has been a recent flurry of papers arguing for the alteration of the definition of eusociality, with particular reference to aspects of reproduction, considered to be key in determining eusociality (but see Costa and Fitzgerald, 1996). Crespi and Yanega (1995) argue that alloparental care and the evolution of caste differentiation are the key aspects by which to define eusociality, and that all social species lacking one of these properties should be classified as **communal**. Gadagkar (1994) focuses on the trait of co-operative breeding as the defining characteristic of eusociality, leading to expansion of the definition to include the co-operatively breeding birds and mammals. He uses the trait of caste differentiation to distinguish between '*advanced*' and '*primitive*' eusocial societies.

Taking into consideration the diversity of social forms and behaviours, the expansion of eusociality concept can be regarded as recognising a continuum – '***the eusocial continuum***' (Sherman *et al.*, 1995). Sherman *et al.*, (1995) argue that reproductive co-operation is the primary phenomena, and that it varies continously among social species. The measure of the degree of reproductive co-operation is the '***index of reproductive skew***', which measures lifetime reproductive success among colony members. Reproductive skew varies from zero, where all colony members reproduce and co-operate in brood rearing, to one, where one dominant reproductive is assisted in brood rearing by non-reproductive helpers. Thus joint-nesting breeders, such as black-tailed prairie dogs (*Cynomys ludovicianus*) and acorn woodpeckers (*Melanerpes formicivorous*), are at the lower end of the continuum, and colonies with a single reproductive female, such as the honeybee (*Apis mellifera*), are at the upper end.

There are two conclusions that can be drawn from the eusociality continuum debate. Firstly, there is general agreement (but see Costa and Fitzgerald, 1996) that it is the reproductive component of the definition of eusociality that is of the most importance, and of this reproductive skew is an important measure of the degree of reproductive co-operation. Consequently it can be concluded that the presence of a reproductive division of labour, and subsequent caste differentiation, is the crucial trait. Division of labour is the single trait essential to the advanced modes of life of colonial existence, which are so clearly demonstrated in the ants (Oster and Wilson, 1978). Secondly, the widening of the scope of the eusociality concept results in studies of the 'traditionally' eusocial organisms (*sensu* Wilson, 1971) having implications for other organisms.

v. Does Kin Selection Explain The Evolution of Eusociality?

Of the three defining traits of eusociality, it has been seen that the presence of a reproductive division of labour is the most paradoxical and arguably, the most important (see above). Natural selection favours maximum genetic contribution to the next generation, so how can totally sterile individuals evolve? Moreover, these non-reproductives often act as helpers, facilitating the upbringing of the offspring of the reproductive(s) – how are these genes for 'helping' passed on to the next generation as these individuals do not reproduce? In attempting to resolve this apparent paradox, we are in fact asking how eusociality could have evolved. The exact mechanism of the evolution of eusociality, in terms of the actual pathways of evolution, have been the subject of continous debate (Michener, 1969; Wilson, 1971 p. 99; Lin and Michener, 1972; Starr, 1979; Seger, 1991). However a distiction must be drawn between '*Pathways to Eusociality*' and '*Theories for Eusociality*' (Bourke and Franks, 1995 p.71), and what concerns us here is the biological theory of how eusociality could have evolved, not the actual pathway(s) selected.

Traditionally there have been three explanations for the evolution of eusociality:

- Kin Selection
- Parental Manipulation
- Mutualism

There has been some confusion between explanations for altruistic behaviour, and explanations for eusociality. In referring to the definition of eusociality in the strict sense we are not referring to group-living, which may evolve via factors other than reproductive altruism. We can re-define the options above, such that, parental manipulation is seen as a sub-section of kin selection (as discussed above), and mutualism is seen as a possible precurser to the evolution of eusociality by kin selection, rather than an alternative to it (Bourke and Franks, 1995 p70). Strictly, mutualistic helpers are not truly altruistic, as they cooperate only in the short term, and do not suffer a decrease in personal fitness relative to the fitness of solitary breeders. They join the group originally beacause their personal fitness from joining exceeds that of attempting to breed alone. The important point is that these non-breeding group members could go on to be selected to be altruistic in the correct sense, if they were related to the breeders, and Hamilton's rule was satisfied.

Bourke and Franks (1995 p.71) conclude that:

> ***"The only explanation for true reproductive altruism in eusocial colonies is kin selection".***

Furthermore, in the Hymenoptera there is a further genetic predispostion towards reproductive altruism: **haplodiploidy**. Hamilton (1964b, 1972) suggested this theory to provide an explanation as to why there have been several, independent origins of eusociality, and, why workers are always female, as opposed to termite workers which can be of either sex.

Production of females is by fertilization by haploid male gametes of (meiotically produced) haploid female gametes. However, haplodiploidy, which occurs in all the Hymenoptera, involves the production of males from unfertilized haploid eggs, consequently, males are haploid. This has the effect of creating unusual coefficients of relatedness between family members. A female with a haploid father shares all of his genes, as oppose to the fifty percent she would share with him if he were diploid. The other half of the female's genetic complement comes from her mother, who is diploid, and consequently she has a fifty percent chance of sharing any one of her mother's genes. All of this female's sisters derive half their genes from their diploid mother, and the sisters therefore have a fifty percent chance of sharing any one of their mother's genes with one of their sisters. Hence, half of the sister's genetic complement is always identical (from their father), whilst the other half has a fifty percent chance of being shared. Haplodiploidy results in sisters being more closely related to each other ($r = 0.75$) than they are to their own daughters ($r = 0.5$). Theoretically, therefore, a female worker can make a greater genetic profit by rearing a reproductive sister, than she could if she produced a daughter of her own. In other words, a gene for caring for females would be more strongly selected if sisters rather than daughters received care. This, of course, assumes that that the colony has a single queen who mates with only a single male. This reasoning led Hamilton (1964b) to conclude that:

> ***"The haplodiploidy hypothesis therefore proposes that high sister-sister relatedness facilitates the evolution by kin selection of reproductive altruism among Hymenopteran females".***

The haplodiploidy hypothesis can explain why only females are workers. Males are not more closely related to their sibs than to their own offspring, and consequently there is no genetic predisposition to helper behaviour by males (Bourke and Franks, 1995 p.82).

I must sound a note of caution at this point. As noted by Bourke and Franks (1995 p.77), haplodiploidy forms a subset of kin selection theory, and is not its equivalent. Kin selection can apply in many circumstances of relatedness, and in fact, can result in altruistic behaviour when relatedness is low, if the ecological conditions are correct and Hamilton's rule is satisfied. For example, a very efficient organisation, for example by division of labour (see Chapter 2) could result in altruistic behaviour, even if relatedness is not that high. It is therefore more accurate to say that haplodiploidy predisposes the Hymenoptera to eusociality, it does not *cause* eusociality to evolve (Krebs and Davies, 1981 p.331). The haplodiploidy hypothesis may therefore explain why sterile castes evolved multiply, and independently, in the Hymenoptera. In fact it is obvious that haplodiploidy does not cause eusociality to evolve, as not all haplodiploid insects have sterile castes (examples include species of bumble bees, stingless bees, honey bees, vespine wasps, and ants, see Bourke, 1988), and in the termites, sterile castes have evolved in diploid species.

The haplodiploidy hypothesis is not without its problems, however. For example, the calculated relatedness coefficients on which it is based, are, as has already been stated, based on monogynous colonies with a singly-mated queen (monandrous). In the eusocial Hymenoptera it is not uncommon for colonies to have multiple queens (polygyny) and/or for multiple matings to occur (polyandry). Both of these occurences have the effect of reducing intra-colony relatedness. There could be biological conditions such that Hamilton's rule is still satisfied at these low levels of relatedness, for example, workers may still help because of their, and the queen's, morphological specialisation for particular roles (Bourke and Franks, 1995 p.85). This, however, depends on polygyny and polyandry being derived states, as the morphological specialisations evolved first under monogynous, monandrous conditions. Polygyny, at least in ants, could be a derived condition (Hölldobler and Wilson, 1977, Section 8.4).

If this is not the case, polygyny and polyandry may not represent too much of a problem for the haplodiploidy hypothesis, whether these biological specialisations are a contributary factor or not. This is because under any number of queen matings, relatedness between sisters is still higher than in diploids, and consequently haplodiploidy is more conducive to helping than diploidy (Sudd and Franks, 1987 p.5). Bourke and Franks (1995 p.87) conclude that it remains possible that haplodiploidy, despite low relatedness, promoted eusocial evolution.

Until such time as there is an experimental approach to measuring the benefits and costs of helping in solitary and facultatively social bees and wasps, and we have more data on the genetic structure, social structure and sex allocation in these species, the status of the haplodiploidy hypothesis as a contributary factor to the evolution of eusociality in the Hymenoptera is uncertain. In a review of alternative factors that could promote eusociality, or account for female-only workers, in the Hymenoptera, Bourke and Franks (1995 p.97) conclude that the key features of eusociality in the Hymenoptera could stem from traits aside from haplodiploidy, or from features of haplodipoidy other than those that affect relatedness. However, if haplodiploidy does promote the evolution of eusociality through its effects on relatedness, these other features would reinforce this effect. As Wilson (1976 p.208) stated, many factors could combine to push a species over the ***"eusociality threshold"***.

vi. Advantages of Eusocial Life

Having reviewed the unique success of the eusocial insects, and how eusociality might have evolved, I return to the question posed in section 1.2 (ii) - How does the presence of the three defining traits of eusociality, namely:

❑ **Co-operative care of the young;**

❑ **Overlap of at least two generations;**

❑ **Presence of a reproductive division of labour;**

...lead to such undeniable success? The main advantages conferred by eusociality are the following:

❶ The death of an individual is less consequential to her inclusive fitness than the death of a solitary animal, as a direct result of kin selection.

❷ Colonial life leads to superior resource inheritance compared to a solitary existence. Once the colony is established it has extremely high defensive capabilities. This allows the inheritance of prime nest sites and feeding areas from one generation to the next.

❸ There is a high degree of social homeostasis within colonies leading to a closer approximation to optimal conditions for growth and reproduction. This is because eusocial colonies are often large and long-lasting structures, and this provides opportunities for the microclimate of the colony to be regulated.

❹ Operations within the colony can be carried out concurrently, rather than sequentially (Oster and Wilson, 1978 p.10), and there is the capacity for greater colony efficiency through specialisation and the division of labour.

The existence of a division of labour within the colony is one of the defining factors of eusociality in terms of reproduction. Crucially, this functional division in the organisation of work in eusocial insect colonies allows the evolution of divisions of labour amongst other sorts of work. The division of labour is key to this discussion and is considered a probable cause of the ecological success of eusocial insects (Hölldobler and Wilson, 1990, Ch.8; Sendova-Franks and Franks, 1999). This is examined in more detail in Chapter 2.

1.3 Conflicts of Interest – Causes and Effects

The eusocial insects have evolved highly co-ordinated and functionally organised societies. However, these societies, although ultimately unified, are not always completely harmonius. Whatever the influence of haplodiploidy on the evolution of eusociality in the Hymnoptera, it is clear that it has other effects, which stem from the asymmetrical coefficients of relatedness it causes. These effects can cause apparent **conflicts**. Conflicts over sex allocation (or sex ratios) are an example of **kin conflict**, a branch of kin selection theory dealing with evolutionary disagreements between individual kin or classes of kin.

Kin conflict arises not only from differences in relatedness with sexual offspring, but also from productivity (Bourke and Franks, 1995, p.220). Most kin conflict in eusocial insect societies arises from sex ratio conflict, and it is important to note that relatedness is not the only factor involved in deciding the outcome of these conflicts. However, Hamilton's rule is a good predictor of the outcome (Godfray and Parker, 1992). Some of the more prominent potential conflicts are outlined below. The field is still fraught with controversy about the balance of power between the queen and the workers, and the mechanisms by which any control could function. However, it is crucial to realise that intra-colony conflict can and does occur in eusocial insect societies, as this can shed light on other important behaviours, such as the division of labour (see section 2.4ii).

i. Conflicts over Sex Allocation

Females are more closely related to their sisters (r = 0.75) than their brothers (r = 0.25), which suggests females should try to create a situation in the colony in which the sex ratio is biased towards females. Trivers and Hare (1976) expanded Hamilton's original idea, explaining that in monogynous monandrous colonies with random mating and sterile workers, the stable population sex ratio for the female workers is 3 : 1, females : males, whereas the stable population sex ratio for the queen is 1 : 1, females : males. Consequently there is the potential for worker-queen conflict of interest over sex allocation.

Experimentally conflict is not easy to determine, partly because there is a distiction between *potential* conflict predicted by the kin structure of a group, and *actual* conflict (Ratnieks and Reeve, 1992). Additional factors may stop actual conflict from occuring. However, the outcome of studies of sex allocation in ant colonies generally indicate that workers 'win' this conflict, perhaps by manipulating sex allocation according to relatedness asymmetry (Keller et al., 1996), and have at least partial control of sex allocation. This conclusion has been reached by studies across species, and more recently, in intraspecific studies (thus eliminating variation due to life history and breeding system effects, Chapuisat and Keller, 1999). Sex ratios observed in ants broadly endorse the predictions of Trivers and Hare (1976).

It must be noted that the predictions of Trivers and Hare (1976) were made under conditions of monogynous, monandrous colonies with sterile workers and random mating. Confirming that a species precisely fits these conditions is difficult, and may account for a slightly less female biased sex ratio than Trivers and Hare (1976) predicted if the workers dominated the conflict (for example Boomsma, 1989; Pamillo, 1990; Vargo, 1996). It has also been considered possible that the queen shares the control of sex allocation, to some degree, with the workers (Matessi and Eshel, 1992).

ii. Conflicts over Reproduction

Females are more closely related to their own sons (r = 0.5) than to their brothers (r = 0.25). As the unfertilized female workers are rarely completely sterile (Bourke, 1988) and can, theoretically, produce haploid male offspring, they could be predicted to gain a genetic advantage by retaining this ability, and trying to replace their brothers (the queen's sons) with their own sons. The ability for workers to reproduce has been maintained, perhaps in case the colony loses its queen, and is unable to adopt another one. This is more likely to happen in monogynous colonies, and reproductive workers are consequently more common here (Bourke, 1988).

Under monogynous, monandrous conditions, workers are also, on average, more closely related to other worker's sons (r = c. 0.375) than to the queen's sons (r = 0.25). Despite this workers should lay their own haploid male eggs if possible, an not tolerate laying by other workers. This is known as **'worker policing'** (Ratnieks, 1988; Ratnieks and Reeve, 1992) and there is strong evidence for it in honey bees (for example Ratnieks, 1993; Visscher, 1998). There is less evidence for it in ants, and Bourke and Franks (1995 p.236) note that data on genetic and social structure is required from more species if the worker policing hypothesis is to be tested sufficiently in ants.

There is evidence for actual conflict over male production in colonies of three, monogynous, leptothoracine species, and in some ponerine species (for a review see Bourke and Franks, 1995 pp.243-244). Reproductive workers form dominance hierarchies in which rank is correlated with the degree of ovary development.

Again the distinction between potential and actual conflict is emphasised. Both worker reproduction and worker policing are associated with a cost, and if the overall productivity of the colony is reduced, great difficulty exists in the evolution of these traits (Ratnieks, 1988; Pamilo, 1991). Costs to colony productivity may be why actual conflict over male production declines under multiple mating, and why monogynous ants show little evidence of worker reproduction in queenright colonies (Bourke and Franks, 1995 p.235). Queen (or gamergate, Tsuji et al., 1999) inhibition of worker reproduction may also be a factor (Bourke and Franks, 1995 p.256), particularly in smaller colonies (Arevalo et al., 1998).

Smaller and simpler colonies are characterised by:

lower **morphological** and **reproductive skew** (morphological differences between reproductives and workers, and the degree of sharing of reproduction between individuals, respectively);

no physical caste polymorphism;

relatively simple nests and communication (Bourke, 1999).

Such colonies, for example members of the leptothoracines, have been hypothesised to experience a high degree of conflict over reproduction and caste determination, as the chances of an individual becoming a replacement reproductive is higher, and there is decreased selection for worker policing, compared to more complex, larger, colonies (Bourke, 1999). The maintenance of reproductive potential (or **totipotency**, Crespi and Yanega, 1995) may be why there is a characteristically low morphological skew in small societies. This may lead to additional effects on the organization of worker behaviour in these colonies. Small societies are less well insulated against variation in the environment and thus workers should remain relatively plastic in their ability to perform different tasks (see section 2.2vii. on flexibility). Larger, more complex, colonies can take advantage of their greater buffering abilites to evolve more specialised workers, for example, via physical polymorphism (Bourke, 1999).

In conclusion, potential kin conflict is a universal feature of the Hymenopteran societies (Bourke and Franks, 1995 p.154), and actual conflict may occur. Kin conflict has far-reaching effects, and influences the organisation of work in the colony. In simpler colonies (*sensu* Bourke, 1999) individuals maintain a high degree of totipotency, and are consequently less able to irreversibly specialise in particular types of work. The evolutionary origin of the correlation between age and task, see section 2.2ii., may be explained by fitness interests. Younger workers tend to perform tasks inside the nest, whilst older ones work outside, which is potentially more hazardous. Workers with a chance of replacing the queen or producing males, may be selected to perform less risky tasks.

1.4. Gene Selection, Kin Selection, Group Selection and the Superorganism

The field of evolutionary biology, particularly in relation to the evolution of eusocial insect societies, is still invigorated by controversy. One such controversy arises from questions concerning the level at which selection acts. It has been argued that selection acts at the level of the group (Wynne-Edwards, 1962, 1963; D.S. Wilson, 1975), best understood as the concept that animals act for the good of their group or local population (species-advantage theories are effectively redundant, but see Gould and Lloyd, 1999). It has been also suggested that selection could act at the level of the colony, viewed as a special case of group selection (Seeley, 1997; Moritz and Fuchs, 1998); individual (Darwin, 1859); or gene (or replicator, see above, Williams, 1966; Dawkins, 1976, 1982a, 1986).

Most authors now accept that selection acts at least at the level of the gene, and that this explains the evolution of altruism and the evolution of eusocial insect societies (see above). However, this does not automatically make the other suggestions 'incorrect'. In a review of group (and hence colony) selection arguments, Bourke and Franks (1995 p.39) concluded that with certain qualifications, there is no fundamental clash between gene and colony-level selection. They considered the '*components of selection*' approach (Wade, 1980), which states that as a gene for altruism will always (by definition) decrease in frequency in groups, the only way the gene can spread is if the groups with altruists are more productive than the groups without. In other words, the positive 'between-group' component of selection exceeds the negative 'within-group' component. Bourke and Franks (1995) concluded that as the 'components of selection' method shows that altruism is only favoured if the conditions of Hamilton's rule are met, it is the mathematical equivalent of kin selection theory, and both are methods of modelling gene selection. Thus, in populations structured by relatedness, group selection can provide an alternative way of looking at the evolution of altruism, rather than a competing theory.

The existence of this variety of conceptual frameworks for analysing evolution may be seen as an advantage, even a "blessing" (D.S. Wilson and Sober, 1989), as a single framework may be limited. This pluralistic approach may eliminate overly intense competition between theories, which are not, in fact, mutually exclusive. This may lead to more helpful insights in studying the evolution of eusociality.

There is more general controversy concerning the '*unit of selection*'. Dawkins regards the gene (a replicator) as the unit of selection (1978, 1982a), whereas proponents of '*levels of selection*' theory consider all levels within the biological hierarchy of organisation as potential units of selection. Dugatkin and Reeve (1994) recognise that the concept of the body or group of bodies being 'vehicles' for genes recognises the biological hierarchy. Gene selectionists advocate this 'level of vehicle' view, which recognises the gene level as a unique and special case (Bourke and Franks, 1995 p.55). They, therefore, conclude that the 'levels of selection' viewpoint can be reconciled with gene selectionist arguments, and that differences arise from semantics. This is by no means universally accepted. Gould and Lloyd (1999), believe that '*interactors*' (Hull, 1980), not replicators, constitute the unit of selection, and that these interactors may exist at several levels of the biological hierarchy (genes, organisms, demes and species). Whatever one's view (and pluralism, as it leads to different methods of examining the evolution of eusociality, rather than competition between theories, seems preferable in its contribution to progress), useful concepts have emerged. Whether it is accepted as a genuine unit of selection, or a useful analogy, the **superorganism** concept is enjoying a revival (e.g Seeley, 1989; D.S. Wilson and Sober, 1989).

Eusocial insect colonies can be viewed as being analogous to autonomous organisms. The term '*Superorganism*' refers to this (Wheeler, 1911). Organisation is such that natural selection confers on groups the functional properties normally associated with individual organisms (D.S. Wilson and Sober, 1989). Consequently just as individual organisms can be viewed as vehicles for the propagation of their own genes, the eusocial insect colony can be viewed as a vehicle, made up of many individual parts, which together form a co-operative unit to propagate their genes (Seeley, 1989). Conflicts between members of the colony, which, previously, might have seemed to undermine the superorganism concept, can be understood in terms of levels of selection theory.

The superorganism concept can be used to explore key aspects of eusocial insect evolution. In fact, one of the defining characteristics of eusociality, the division of labour, is not limited only to eusocial insects. The selective advantage conferred by gains in efficiency due to a division of labour may have driven the evolution of the eukaryotic cell and metazoan life (Bonner, 1988)

The selection pressures that have led to the evolution of morphogenesis are likely to be the same as those fundamental to the evolution of '**sociogenesis**' in eusocial insects. Studying the sociogenesis of a colony of eusocial insects could be beneficial in improving our understanding of developmental and differentiative processes in metazoans (Wilson, 1985; Bourke and Franks, 1995 p402; Sendova-Franks and Franks, 1999). The term 'sociogenesis' refers to the process by which the colony members undergo changes in caste, behaviour, and physical location incident to colonial development (Wilson, 1985). The absence of physical connections among the components of a eusocial insect colony means colonies can be experimentally manipulated relatively easily. They can be taken apart to see how they function – **sociotomy** experiments (Lachaud and Fresneau, 1987); moreover they can also be put back together in new ways – **pseudomutant** experiments (Wilson, 1980a,b).

Chapter 2

An Introduction to Division of Labour

2.1 Proximate and Ultimate Explanations - Division of Labour

It is arguably ‘unfashionable’ to study social physiology and mechanisms, as many evolutionary and molecular biologists consider **‘ultimate’** questions to be more important (Franks, 1999). The success of the eusocial insects can be explained by both **‘ultimate’** (‘why?’) and **‘proximate’** (‘how?’) factors, but the two are inextricably linked. In Chapter 1, I posed the question ‘why are the eusocial insects so successful?’ I have shown that this question may be answered by ‘ultimate’ explanations, with reference to kin selection: the presence of a reproductive division of labour is a key factor in the success of the eusocial insects. However, the question cannot be fully answered without reference to ‘proximate’ explanations. Studies of social physiology: information flow and collective decision making, explain how the eusocial insects work together, and how this may lead to enhanced efficiency, and ultimately, to the evolution of the eusocial way of life (Franks, 1999). This thesis is concerned, therefore, with the proximate mechanisms that underpin the ultimate explanations of the success of eusocial insects.

The division of reproduction is not completely discrete: workers may sometimes reproduce, and conflicts may arise within the colony. However, the existence of a relatively numerous, and essentially non-reproductive sub-set of the colony results in the ability of these individuals to perform tasks other than reproduction, and specialise in these tasks. Consequently, these jobs are performed more efficiently than they would be by a colony of generalists, each of whom could perform every task in the colony. This leads to the evolution of highly organised and structured societies where division of labour also exists amongst non-reproductive tasks. Groups of task specialists within the eusocial insect colony are known as a **‘caste’**.

This chapter is concerned with proximate explanations of the success of the eusocial insects. I examine the concept of caste, its definition, ways in which individuals can be specialised, and how this enhances the efficiency of the colony. I argue that the division of labour among individuals that are essentially non-reproductive is also a key factor in the ultimate success of the eusocial insects (Oster and Wilson, 1978; Wilson, 1987, 1990). Lastly, Chapter 2 examines the possible mechanisms of the division of labour and processes that may enhance the division of labour and, consequently, colony efficiency.

i. Introduction of the Concept of Caste and its Definition

The term 'caste' originates from the Hindu social system, where it refers to:

> ***"A system of social classification in humans, in which membership is determined culturally by birth and remains fixed; the group is ranked in a hierarchy of groups in the system"***
>
> The Concise Oxford Dictionary of Zoology

Wilson adapted the definition of caste to the biological sense as:

> ***"a set of individuals, smaller than the society itself, which is limited, more or less strictly to one or more roles. Where role is defined as a pattern of behaviours, which particular individuals may or may not display, the caste is defined obversely as a set of individuals characterised by their limitation to certain roles."***
>
> Wilson, 1975

This definition is highly tautological and of little functional value. Franks (1998) defines caste among non-reproductives, more simply, as being used:

> ***"to discriminate between different worker forms or workers that exhibit different behaviour.....the clearest differences are seen between different worker morphs."***
>
> Franks, 1998

Thus the concept refers to a morphological division in some species, where the workers are physically specialised to undertake particular jobs. The existence of a behavioural division of labour is known as **temporal polyethism**; the case where individuals are morphologically specialised as **physical polyethism**. Physical polyethism can, by definition, only arise in species in which groups of individuals are physically distinct from each other. Such species are said to exhibit **physical polymorphism**. Physical polymorphism is restricted to twenty percent of extant ant genera (Bourke and Franks, 1995).

Physical classes amongst workers are seldom discreet, and usually vary along a continuum (Calabi, 1988). There are usually two or three physical castes per species (Oster and Wilson, 1978 p.181; Tofts and Franks, 1992), although some species have been shown to have four physical castes (*Atta sexdens*, Wilson, 1980a; *Eciton burchelli*, Franks, 1985). By contrast, most ant species have an essentially monomorphic worker population, within which all non-reproductive individuals are similar. The division of labour in such colonies is temporal; an individual changes the tasks it performs with time (Bourke and Franks, 1995 p.404). Temporal polyethism also exists within physical castes of polymorphic ant colonies. If the tasks performed are a direct function of the individual's age, this is termed **age polyethism** (see section 2.2ii).

The degree of morphological skew between queens and workers is a crucial factor when examining the division of labour. Wheeler (1986) notes that in '*primitively eusocial*' species there are no apparent differences in external structure between queens and workers, whilst in '*highly eusocial*' species, workers and queens differ markedly in morphology. Bourke (1999) uses the degree of morphological skew to define these societies alternatively as '*simple*' (those with few or no morphological differences between reproductive individuals and workers) and '*complex*' (those with wide morphological differences and worker polymorphism), respectively. He states that '*complex societies almost certainly evolved from simple ones*', and that this is associated with a loss of '**totipotency**' (the ability of an individual to adopt both reproductive and helper roles, Crespi and Yanega, 1995). The concept of totipotency is not new. Oster and Wilson (1978 p.7) note that division of labour is accompanied by a reduction in the behavioural repertory of individuals, and that the whole colony is equivalent to a '**totipotent solitary individual**' but with a '*much higher ergonomic efficiency*'.

That a low degree of morphological skew, and consequent retention of totipotency, can lead to conflicts over reproduction has already been noted (section 1.3). This situation can lead to the establishment of a dominance hierarchy of worker status regarding reproduction (Heinze and Oberstadt, 1999). It has also been stressed that workers in these 'simple' societies should retain plasticity in their abilities to perform tasks. In 'complex' societies, the greater buffering abilites of the colony to cope with variation in the environment, generated by superior numbers, has led to the evolution of more specialised workers, for example, via physical polymorphism (Bourke, 1999). Specialised workers are restricted in the tasks they can perform because of their morphological differences (even though physical caste varies along a continuum leading to variation within a physical caste in the ability to perform a task). The definition of caste is more difficult in the simpler societies, as variations in morphology cannot be used as an index. How is work organised in these colonies and how are efficiencies gained (and they must be, or there would be no division of labour), despite reproductive conflicts? There is a conspicuous lack of experimental data in this field.

ii. How Does Division of Labour Lead to Increased Efficiency?

➊ Efficiency Gains Compared to Solitary Individuals

How can work be organised more efficiently in eusocial insect colonies, allowing the evolution of the highly specialised arrangements of divided labour? A solitary individual can only meet one contingency at any one time, and must switch from one task to another in order to carry out all the functions necessary for survival. A colony, alternatively, can meet a number of contingencies at the same time, and consequently the responses of the colony are more massive, prompt and thorough (Oster and Wilson, 1978 p.11). The chance of failure at a particular task is substantially reduced by this '**parallel**' arrangement. If one individual fails at a task, another is likely to succeed. Conversely, failure of the solitary individual to complete one of a series of operations, leads to the failure of the whole enterprise.

Figure 2 (a), below, represents a '**series**' operation in which one individual must complete every task in a series sequentially. Also shown is a '**parallel-series**' operation, Figure 2 (b), in which two individuals are attempting to solve the same number of tasks. However a more realistic model of a eusocial insect colony is a '**series-parallel**' operation, Figure 2 (c), in which each individual may complete a single act, or not, and it only matters that the sequence be performed by any combination of the individuals (Oster and Wilson, 1978 p.12).

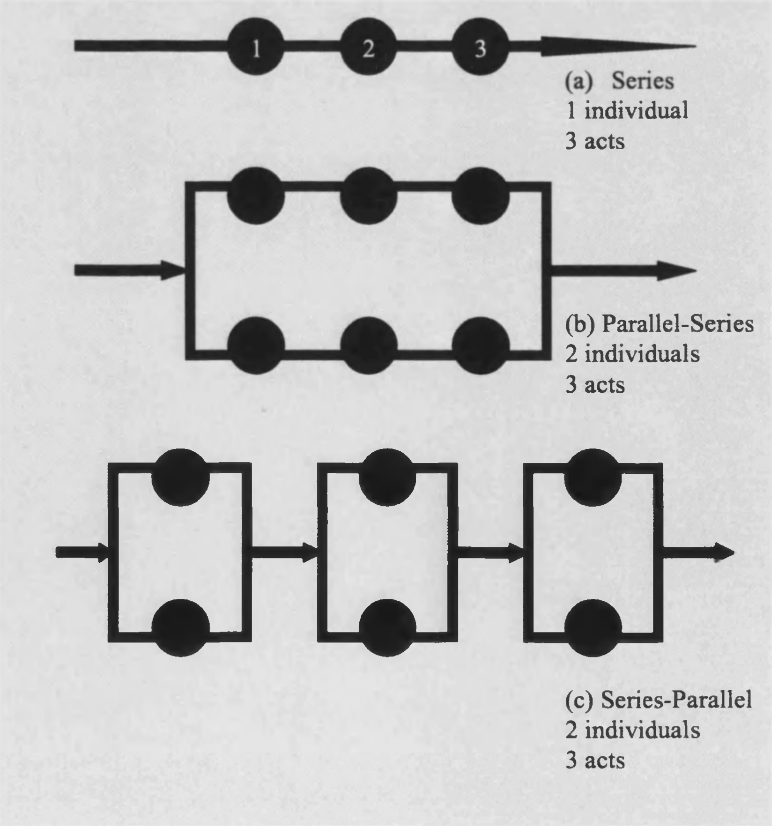


Figure 2: A comparison of behavioural sequences (from Oster and Wilson, 1978).

Oster and Wilson (1978) formalised these models by calculating the probability of successful performance of the final task in the sequence for a given probability, p_{ij}, of ant *j* performing task *i* successfully, see Figure 3.

(a)

$$P\,(\text{success}) = \prod_{i=1}^{n} p_i$$

$$P\,(\text{series}) = (0.2)^3 = 0.008$$

(b)

$$P\,(\text{success}) = 1 - \prod_{j=1}^{m}\left(1 - \prod_{i=1}^{n} p_{ij}\right)$$

$$P\,(\text{parallel-series}) = 1 - [\,1 - (0.2)^3\,]^2 = 0.016$$

(c)

$$P\,(\text{success}) = \prod_{i=1}^{n}\left(1 - \prod_{j=1}^{m} (1 - p_{ij})\right)$$

$$P\,(\text{Series-parallel}) = [\,1 - (\,1 - 0.2)^2]^3 = 0.047$$

Key

Series

n distinct acts are required for success

p_i = probability of performing the *i*th task correctly

P = probability of overall success

Parallel

m individuals, acting independently

p_{ij} = probability that the *j*th individual will perform the *i*th act successfully

Figure 3: A comparison of the reliability of behavioural sequences (from Oster and Wilson, 1978).

In general, the reliability of (c) is greater than (b), which is, in turn, greater than (a). Failure at one stage of the completion of a task does not render the whole task a failure. Different permutations of individuals at these different stages can be utilised to ensure tasks are completed more reliably, and the colony can meet a number of possible contingencies at the same time if a number of individuals are all acting independently. These are the efficiency gains made by a colony compared to a solitary individual. But the eusocial insects could exhibit much greater efficiencies than those that arise simply because of their superior numbers. These efficiencies arise directly from division of labour. But how exactly does a division of labour lead to increased efficiency? Specialisation and spatial efficiency may be key to this.

❷ Efficiency Gains from Specialisation

Specialisation by individuals within groups is considered crucial in many of the major transitions in the evolution of life (Szathmáry and Maynard Smith, 1995). Examples include the transition from single-celled to multicellular organisms, or from solitary to social organisms. Efficiency gains due to specialisation are key to the selective advantages of both multicellular and social systems and lead to an effective and efficient division of labour. Efficiencies can be gained, for example, in terms of the probability of success in completion of the task, or the speed at which it is accomplished.

There are many mechanisms by which specialisation could arise. It has been seen that individuals are sometimes physically specialised to the task, their morphology enabling them to complete the task more effectively. If not morphologically specialised, however, individuals can still be behaviourally specialised, and thus be more effective at performing the task. What is the basis of this specialisation? Physiological factors, for example, genetics, age, or hormones, could be crucial. Individuals could simply become better at a task the more they perform it (skill acquisition, Bourke and Franks, 1995 p.401; Elizabeth Langridge, *pers. comm.*). Recent papers have suggested that specialisation for particular tasks, resulting from differing propensities of individuals to do these jobs could explain task allocation where learning could play a vital role (Bonabeau *et al.*, 1996; Spencer *et al.*, 1998; Theraulaz *et al.*, 1998). This is discussed in section 2.2.

❸ Efficiency Gains from Spatial Organisation

Ants live in a highly structured world. Complex foraging patterns have been the focus of much interest (for example Hölldobler and Wilson, 1990 pp.378-388). However, it has long been known that the internal environment of the nest is also highly structured and complex (Wilson, 1971 pp.310-315), and this in fact allows social homeostasis and is therefore one of the key advantages of eusocial life (see section 1.2.vi.❸ above).

The biotic, as well as the abiotic, components of the nest demonstrate structure. The brood pile and younger workers are often found at the centre of the nest. Progressively older workers can be found further and further towards the nest's extremities, the oldest workers tending to be the foragers, spending a high proportion of their time external to the nest (Wilson, 1985). This is known as **centrifugal polyethism,** and the influence of this on the organisation of work within the colony is examined in greater detail below.

It is reasonable to assume from these findings that ants may use structure, and their ability to explore space, in some way, in the organisation of the colony. There are considerable efficiencies to be gained from this. Time and energy will be saved if the colony has a structure that negates the need for individuals to travel round the nest finding and performing tasks. If individuals remain faithful to a particular area, where they perform particular tasks, they will be more efficient workers.

Until recently empirical data in this field has been rare. However, Franks and Sendova-Franks (1992) studied the spatial arrangement of individuals and brood in *Leptothorax unifasciatus* colonies and have revealed that there was a consistent arrangement. The brood was sorted into concentric circles, with the smallest items (eggs and microlarvae) in the middle and the largest larvae on the outside. It has been shown that this organisation allows the brood items with the greatest requirements to be fed and groomed first. Sendova-Franks and Franks (1993, 1995a) demonstrated that the movement of individual workers was restricted spatially to particular areas of the nest. These were termed spatial fidelity zones (SFZ's). Sendova-Franks and Franks (1995a) established that there is a strong relationship between the frequency at which individuals performed particular types of task, and their SFZ. Sendova-Franks and Franks (1994) suggested that the re-establishment of the relative positions of workers SFZ's has implications for the role of learning in the maintenance of an efficient division of labour. It has also been shown that the relative spatial positions of the workers (Sendova-Franks and Franks, 1994), and the brood types (Franks and Sendova-Franks, 1992), are re-adopted after an emigration, a phenomenon known as **Social Resilience.**

Colonies of the harvester ant, *Pogonomyrmex badius*, have also been shown to have considerable regularity in patterns of distribution of chambers, workers, callow workers, brood and seeds (Tschinkel, 1999). Chamber area, number, and the proportion of older workers decreased with depth within the nest, whereas the proportion of callow workers increased towards the bottom of the nest, resulting in workers age being inversely related to the depth at which the workers were found. The pattern of behaviour of these workers was correlated with their age, and may be explained by the structure of the nest.

The implications of this are that structure within nests may be crucial in explaining some of the efficiencies gained from the division of labour within ant colonies. Until recently this field has been largely ignored. How this high degree of structure might arise and be maintained is examined in section 2.4 (below). It is clear structure needs to be considered as an important factor and may lead to a division of labour more efficient than previously thought.

2.2 The Mechanism of Division of Labour

i. Division of Labour Based on Reproductive Benefits

The presence of a reproductive division of labour in the eusocial hymenoptera allows the further division of labour amongst '*quotidian*' tasks by non-reproductive individuals. However, in many species, some workers maintain functionality of their ovaries, although worker reproduction is normally suppressed by the egg-laying queen (Powell and Tschinkel, 1999). This maintenance of reproductive plasticity is particularly prominent in species which exhibit a low degree of morphological skew (Bourke, 1999).

West-Eberhard proposed a hypothesis to explain the ultimate evolutionary origins of both reproductive and non-reproductive divisions of labour involving differences in reproductive ability (West-Eberhard, 1981). She argued that individuals in which direct reproduction has been suppressed, opt to assist in non-reproductive tasks to salvage indirect reproductive benefits. This hypothesis also claims to explain centrifugal patterns of temporal polyethism (West-Eberhard, 1979, 1981). The behavioural role of these individuals is determined to maximise their reproductive payoff, in view of their decreasing reproductive potential. Thus, individuals with the highest degree of functionality of their ovaries should remain near to the centre of the nest, to maximise their potential reproductive benefits, and consequently perform brood-related tasks. Conversely, as reproductive potential declines, inclusive fitness is maximised by helping raise related brood by carrying out external tasks such as defence and foraging.

This hypothesis does not explain division of labour in which workers are completely non-reproductive in the presence of a queen, or those in which workers are sterile (Bourke and Franks, 1995 p.406). However, reproductive status may prove to have an influence in the proximate cause of the organisation seen in eusocial insect colonies. The role of dominance hierarchies in mechanisms of task allocation, in colonies with a low morphological skew, is discussed later (see section 2.4ii).

ii. Age Polyethism

It has often been hypothesised, that divisions of labour among workers are based on their age, specifically that there is a causal relationship between an individual's age and the task she performs. This stems from the occurrence of centrifugal polyethism in many eusocial insect colonies. Newly emerged callows remain within the nest, near to the queen and the brood pile, and perform tasks specific to this area, such as nurse work. Slightly older workers remain in the nest, further from the brood pile and nearer to the nest entrance, and perform tasks such as nest maintenance. The oldest workers act as foragers, external to the nest.

The phenomenon whereby workers change their tasks specifically as a function of their age is termed **age polyethism** and is not the same as **temporal polyethism** (Bourke and Franks, 1995; Franks *et al.*, 1997). It must be emphasised that age polyethism refers strictly to a causal relationship between age and task.

Temporal polyethism involving a correlation between age and task has been demonstrated in honey bees (Free, 1965; Wilson, 1971 p.174; Seeley, 1982; Seeley and Kolmes, 1991; Robinson, 1992); wasps (Naug and Gadagkar, 1998a); and a large number of ant species (Wilson, 1976; Lenoir, 1979a, b; McDonald and Topoff, 1985; Calabi and Traniello, 1988, 1989a; Hölldobler and Wilson, 1990 pp.312-317; Dejean and Lachaud, 1991; Pratt *et al.*, 1994; Gordon, 1995; Masuko, 1996). It is this frequently observed correlation between age and task that has established the view of age polyethism. However, the correlation observed is often weak (Calabi *et al.*, 1983; Sendova-Franks and Franks, 1993, 1994), and does not in itself imply causation. That there is a correlation in many cases between the age of an individual and the task it performs is not disputed. What is disputed (for example, Bourke and Franks, 1995 pp.406-408) is that the age itself causes the individual to perform a particular task.

There is, however, much evidence of physiological correlates of behavioural development in some eusocial insect species (Traniello and Rosengaus, 1997). Most of this research has concentrated on honey bees (*Apis mellifera*) and it is well-established that genetic variation among worker honey bees (reviewed by Page and Robinson, 1991; Robinson, 1992; Moritz and Southwick, 1992) and hormones such as juvenile hormones (reviewed by Fahrbach and Robinson, 1996; Robinson and Vargo, 1997), influence the division of labour in this species. More recently it has been suggested that nutrient status, in terms of levels of lipid stored, may influence the role of workers in colonies of the ant *Leptothorax albipennis* (Blanchard *et al.*, 2000).

iii. The Influence of Genetic Variation on the Division of Labour in Honey Bees

The genetics of honey bee colonies is complicated because queens exhibit polyandry. They mate with between seven and seventeen drones (Page, 1986), and consequently colonies exist as subfamilies, or patrilines. There is evidence for differences in worker behaviour between these subfamilies, which has been attributed to genetic variation between them. Experiments where ten different colonies, each with three constituent subfamilies, were examined with respect to five different tasks, demonstrated differences in subfamily representation for some behaviours (Robinson and Page, 1988, 1989). Specifically, there was variability between undertaking or guarding behaviour, and between foraging for pollen or nectar, activities that are performed at similar ages. There is some evidence that there is variation between different subfamilies for some other tasks (queen larval care, Page *et al.*, 1989; allogrooming, Frumhoff and Baker, 1988; and defensive behaviour, Breed *et al.*, 1991). Calderone *et al.* (1989) showed that there were also genotypic differentiation for guarding, nectar foraging and pollen foraging, in colonies derived from naturally mated queens.

The evidence that honey bee workers of different patrilines tend to perform different tasks in the colony, reviewed above, has led to the suggestion that polyandry has evolved to provide the colony with a precisely optimal mix of worker genotypes (Frumhoff and Baker, 1988; Robinson and Page, 1988). This is disputed by Bourke and Franks (1995 p.414) for the reason that it is highly unlikely the queen could chose to mate with precisely the right combination of males to achieve this.

There is also evidence for genotypic differences in the age of performance for some tasks, including the age at which the onset of foraging occurs (Calderone and Page, 1988, 1992). And similar results from cross-fostered Africanized and European honey bees (Winston and Katz, 1982) led Robinson (1992) to conclude that there is also genotypic variability for the rate of behavioural development.

iv. Hormonal Control of Age Polyethism in Honey Bees

Juvenile hormones (JH), synthesized and released by the corpora allata, play a fundamental role in the division of labour in honey bees (Rutz *et al.*, 1976; Fluri *et al.*, 1982; Robinson, 1985, 1992; Seeley, 1985 p.31; Robinson and Page, 1989;

Robinson *et al.*, 1989, 1994; Robinson and Vargo, 1997). Titers of juvenile hormone III have been shown to increase as the adult worker bee ages: low titers are associated with behaviour in the nest, for example, brood care; higher titers (reached at about three weeks of age) with the onset of foraging. JH is thought to affect the timing of behavioural development and behavioural transitions, particularly the transition to foraging behaviour (Robinson and Huang, 1998). Treatment with JH (Jaycox, 1976), a JH mimic (Jaycox *et al.*, 1974), and a JH analog (Robinson, 1987a, b; Sasagawa *et al.*, 1989) induces precocious foraging in honey bees.

JH is thought to co-ordinate exocrine and behavioural development, allowing changes in the activity of some exocrine glands, that are associated with age polyethism, to proceed in step with behavioural transitions (Robinson and Huang, 1998). The hypopharyngeal gland, which produces the major component of larval food, and is well developed in nurse bees, degenerates in foragers, and becomes involved with the conversion of nectar into honey (Winston, 1987). JH (Rutz *et al.*, 1974, 1976), JH mimic (Jaycox *et al.*, 1974), or JH analog (Sasagawa *et al.*, 1986) treatment induces the hypopharyngeal gland to degenerate prematurely. Moreover, removal of the corpora allata blocks the degeneration of the hypopharyngeal gland, and subsequent application of JH causes it to subsequently degenerate (Imboden and Lüscher, 1975). The former delays (but does not prevent) the onset of foraging, the delay being eliminated with the subsequent application of JH (Sullivan *et al.*, 1996).

v. The influence of Genetics and Hormones on the Division of Labour in Other Eusocial Insect Species

There is evidence that genetic variability affects worker behavioural traits, and consequently the division of labour, in honey bees. Task performance at a given age, and the rate of behavioural development, may both be influenced by genotype (Robinson, 1992). This influence may not be crucial as levels of genetic variability in natural populations may be less than in the commercially manipulated colonies of North America. However there is some evidence for genetic variability for behavioural traits in natural populations (see above).

Genetic variability in behavioural traits has been shown to a limited extent in some species of ants. Stuart and Page (1991) showed that in experimental colonies derived from pairs of field-collected parental colonies, there were differences in the tendency to forage or remain in the nest between similarly aged workers.

It is possible that JH may play a role in the division of labour of other eusocial insects. O'Donnell and Jeanne (1993) have found evidence that a JH analog accelerates the rate of polyethism in the wasp *Polybia occidentalis*. The age at which workers first performed acts in seven behavioural categories was negatively correlated with the dose of the analog. However, there is evidence that JH does not have any affect on division of labour in the so-called '*primitively eusocial*' Hymenoptera (Cameron and Robinson, 1990), and Robinson (1992) concludes that JH is involved only in species with strong '*age polyethism*'. O'Donnell and Jeanne (1993) suggest that JH control of polyethism evolved independently in the '*advanced*' species of Apidae and Vespidae.

vi. The Necessity of Colony Plasticity and Individual Flexibility

Colonies of eusocial insects must exhibit inherent plasticity in order to survive and achieve their indisputable success. If the division of labour is discretised and rigid, relatively large gains in efficiency will be lost as the system may eventually break down due to its lack of plasticity. This becomes even more likely due to the unpredictable, and often catastrophic, nature of the environments in which eusocial insects live. Colonies must respond to changing internal and external conditions and consequently the behaviour of individual workers must be flexible. There must be mechanisms controlling the adjustment of worker activity levels, or, in the case of colonies exhibiting temporal polyethism, mechanisms controlling the allocation of individuals to different tasks (Seeley, 1995 p.241).

There is no evidence to indicate that there are colony leaders to co-ordinate such responses (Seeley, 1989; Wilson and Hölldobler, 1988), and it is unlikely single individuals in large colonies can perceive the requirements of the entire colony and co-ordinate their responses. How flexible are eusocial insect colonies and how can task allocation accommodate this flexibility? I use the same categorisation of tasks as Robinson (1992).

vii. How Flexible are Eusocial Insect Colonies?

❶ Performance of Tasks Outside Normal Caste Repertoire

The flexibility of the division of labour has been shown in experiments where colony demography has been manipulated resulting in accelerated, retarded, or even reversed behavioural development.

Precocious foraging by younger individuals can be induced by the removal of the foraging caste in honey bees (reviewed in Free, 1979). This has also been shown in some species of ants. Calabi and Traniello (1989b) showed that young workers of *Pheidole dentata* competently perform typical older-worker tasks when the older workers are removed. There is also evidence for this in the red imported fire ant, *Solenopsis invicta* (Mirenda and Vinson, 1981; Sorenson *et al.*, 1984). Work on the ant *Novomessor albisetosus* showed the removal of all the older workers resulted in callows developing in a third of the normal time (McDonald and Topoff, 1985). Colonies might be predominantly composed of young individuals when they are newly founded; when a surge in birth rate occurs (Oster and Wilson, 1978); or if a large number of foragers have been lost, for example, to predators (Robinson, 1992). Precocious foraging has been induced in starved *Apis mellifera* colonies (Schulz *et al.*, 1998), demonstrating that colony nutritional status affects behavioural development, rather than only modulating the activity of bees already competent to forage.

Retarded behavioural development may also occur. In honey bee colonies with an ageing population, nurses may continue to care for brood past the age at which they would normally change to foraging tasks. This could occur naturally after a new colony has been founded from a swarm and the younger workers have not yet eclosed (Robinson, 1992). The over-aged nurses are comprised of the youngest members of the colony that are present (Robinson *et al.*, 1989; Naumann and Winston, 1990). Retarded behavioural development has been experimentally induced in older workers of *Pheidole dentata* (Calabi and Traniello 1989b) and *Novomessor albisetosus* (McDonald and Topoff, 1985).

Reversion of behaviour from foraging tasks to nurse-work in colonies from which the young individuals have been experimentally removed has been demonstrated in honey bees (Robinson *et al.*, 1992), and the ants *Pheidole dentata* (Calabi and Traniello, 1989b) and *Solenopsis invicta* (Sorenson *et al.*, 1984). Robinson (1992) speculated on possible scenarios for natural behavioural reversion in honey bees. It may occur during changes in social organisation associated with over-wintering of perennial colonies, or changes from comb construction to brood care in newly swarm-founded colonies (as the comb must be built before there can be any brood to rear).

In colonies of species exhibiting physical polymorphism among the workers, physical castes may be induced, to a certain extent, to perform the tasks of other physical castes. It is evident that in polymorphic colonies some size classes cannot physically perform the jobs of other size classes. However, when colonies of a species of the seed-harvester ant, *Pheidole pubiventris*, were deprived of the minor caste the majors performed nearly the whole repertory of minor tasks (Wilson, 1984). Wilson (1983) demonstrated that workers in adjacent size classes could perform foraging tasks when the foragers of the leaf-cutter ant, *Atta cephalotes*, were removed. The fact that workers can be adopted from other physical castes is striking as it demonstrates that individuals can perform tasks that would never normally be expressed. However, there is a cost associated with the performance of tasks by other physically specialised individuals. Experiments on the fire ant, *Solenopsis invicta* (Porter and Tschinkel, 1985), have shown that colonies in which the caste structure has been manipulated (specifically where a normally polymorphic colony has been artificially manipulated forming colonies of monomorphic workers from each size class) are less efficient (as measured by brood production).

Workers that change their typical behaviour in response to changing colony requirements appear to retain caste specific differences despite their apparent shift (Robinson, 1992). Individually marked workers of *Neoponera apicalis*, which showed accelerated or reversed behavioural development when colonies were divided into fractions composed of either young or old individuals, resumed 'normal' behaviour when the colonies were re-united (Lachaud and Fresneau, 1987). The majors of *Pheidole* colonies that had performed minors tasks reverted to their original tasks once the minors were replaced (Wilson, 1984).

❷ Task Switching Within Normal Caste Repertoire

Gordon (1986, 1987, 1989) demonstrated that when the environment of colonies of harvester ants, *Pogonomyrmex barbatus,* was manipulated so that demands on a particular caste were altered, the number of ants engaged in other activities also altered. By marking workers performing exterior tasks of foraging, patrolling, nest maintenance or midden upkeep, she showed that an experimentally induced increase in the need for a particular task led to task switching by other workers and a decrease in the numbers of workers performing the other exterior tasks. Natural variation in task demand has also been shown to induce task switching (Gordon, 1991). It should be noted that task switching does not occur in all directions. Gordon (1989) showed that ants that exhibited switching from nest maintenance to another task do not switch back to nest maintenance. This is discussed further below with reference to the 'roles' of workers, section viii, below.

❸ Increased Activity Levels Within Normal Caste Repertoire

The plasticity required by changing environmental conditions can sometimes be achieved by individuals in the colony increasing their activity. Pendrel and Plowright (1981) found larval feeding by workers increased after half the worker population of *Bombus terrestris* colonies were removed. Experimentally induced damage of nests of *Polybia occidentalis* colonies led to an increase in the rates of nest-material collection in individuals already performing this task (O'Donnell and Jeanne, 1990).

Wilson performed key pseudomutant experiments on *Atta cephalotes,* a species of leaf-cutter ant with a degree of physical polymorphism, where he removed the foraging caste. They showed that colonies did not respond by adding workers from adjacent size classes to the foraging force, but that excess workers from adjacent size classes were already present on the foraging area, and also that the remaining foragers increased their activity (Wilson, 1983). Wilson (1984) showed that major workers could serve as an '*emergency stand-by caste*' in three species of the seed harvester ant, *Pheidole.* When the ratio of minor to major workers was lowered to below 1: 1 (from the usual 3 : 1 to 20 : 1, depending on species), the majors shifted their behaviour to performing nearly all the tasks the minors had performed.

Plasticity has also been shown to occur via increases in activity levels in honey bee colonies. Increases in the activity of wax-producers (Naumann and Winston, 1990) in response to manipulations has been demonstrated, although this was coupled by shifts in temporal castes. Kolmes and Winston (1988) showed that honey bees increase their activity in response to relatively moderate manipulations. They concluded that changing colony requirements induced by moderate manipulations led to the increase of activity at particular tasks, whereas more drastic manipulations (for example, removal of all or most of the young /old workers) led to dramatic changes in the age of task performance.

❹ Changes in the Proportion of Individuals Working

Studies have shown that a large proportion of the workforce in eusocial insect colonies is inactive at any one time (Herbers and Cunningham, 1983, *Leptothorax longispinosus*; Kolmes, 1985, *Apis mellifera*). It has been suggested (Lindauer, 1952; Michener, 1964) that inactive workers could form a reserve force, which could be employed if colony requirements change. There is support for this hypothesis (for example, Jaycox, 1970; Wilson, 1983; Lenoir, 1987; Gordon, 1989;

Breed *et al.*, 1990), and it has been shown that changing colony conditions causes inactive workers to become active (Gordon, 1989; von Frisch, 1967).

❺ Caste and Worker Behavioural Flexibility

Colonies could respond to changing conditions by the alteration of ratios of physical castes produced by the colony. This response to changing colony requirements would be much slower to take effect because of the time taken to rear new workers. There is evidence that physical caste ratios change in response to changing environmental conditions in some species of ant (Wilson, 1983; Wheeler and Nijhout, 1984; Porter and Tschinkel, 1985; Walker and Stamps, 1986; Passera *et al.*, 1996). There is not always consequent bias in the production of brood towards an experimentally reduced size class (Wilson, 1984; Johnston and Wilson, 1985).

The results of these experiments emphasise the impressive flexibility of eusocial insect species, and demonstrate how the work done by members of the colony is greatly influenced by the needs of the colony. Kolmes and Winston (1988) concluded that honey bee colonies are '*resilient, rather than maximally efficient*', and colonies have been shown to exhibit a '*far-reaching fluidity*' (Lindauer, 1953) in their division of labour. Work done at any time is determined rather by the current needs of the colony (Lindauer, 1953; Winston and Fergusson, 1985) than by the physiological or anatomical state of the bees.

viii. Task Allocation and the Division of Labour – Roles and Tasks

The title of this thesis *is 'an experimental investigation of task allocation'*. Strictly, '**task allocation**' refers to '*the process that adjusts the numbers of workers engaged in each task*' (Gordon, 1999). Task allocation is inextricably linked to studies of division of labour. Gordon (1999) argues that task allocation consists of two components: firstly, what determines an individual's task, characterised by the division of labour; and secondly, what determines whether, at a particular instant, that individual is active. I argue that it is incorrect for the latter component of task allocation to be considered without reference to division of labour. The consideration of task allocation without reference to division of labour occurs because studies of task allocation often concentrate on groups that comprise individuals which perform the same role, for example the work of Gordon, cited above.

The distinction between **'task'** and **'role'** is an important one. Blanchard *et al.* (2000) emphasise the distinction, noting that whereas '*individuals can change between tasks within a role rapidly and in response to local demand, role change is accompanied by physiological changes and occurs more slowly'*. Seeley (1982) defined four stages in the development of a worker in honey bee colonies: brood and queen nursing; grooming and feeding other workers and nest maintenance; dealing with incoming forage; and finally, foraging. During these stages a number of different tasks may be performed, which, together, constitute the role (Blanchard *et al.*, 2000).

It is important to note that physiological changes may well be involved in role determination or progression. The involvement of genetic influences and Juvenile hormones has been discussed in some detail, above. I have shown that JH titres may influence role, but as Blanchard *et al.* (2000) note, it is less clear what factors may influence JH titre. Another physiological factor that may prove to be important is nutrient status. Blanchard *et al.* (2000) have determined that worker lipid store is negatively correlated with worker foraging propensity. This recent work on lipid levels has indicated that this may indeed be a fundamental factor, as differences in lipid storage have also been shown to be correlated with spatial position in the nest (the relevance of which is discussed later), and the amount of activity in the nest. However, further work is required.

Many of the experiments reviewed above, have concentrated on task allocation within particular roles, and this explains the apparent sliding scale of reaction by the colony to an increasing severity of manipulations. Relatively moderate manipulations on honey bee colonies (Kolmes and Winston, 1988) cause the remainder of the manipulated caste to increase their activity. This has also been shown in some species of ant (see above), and this may be considered to be the second component of task allocation. In the case of more serious manipulations, the first component of task allocation is affected, and the age at which workers perform tasks alters, or workers may be adopted from reserves in other size classes in the case of polymorphic species. In fact, role progression itself is considered more labile than previously thought (Blanchard *et al.*, 2000).

Few experiments in which colonies have been sufficiently severely manipulated to affect task allocation as regards role, appear to have been performed on small monomorphic colonies of ants. The effect of the loss of a behavioural class, or 'role' group, is relatively greater in such colonies. Moreover, individuals are not physically specialised, and may maintain totipotency between reproductive and helper roles. How flexible are individuals in roles in such 'simple' (*sensu* Bourke, 1999) colonies? How do external influences affect role, and how do role distributions respond to the changing needs of the colony (Blanchard *et al.*, 2000)? By what mechanism could the division of labour function so as to allow these colonies sufficient plasticity to respond to changing environmental condition, against which they have a relatively smaller buffer?

ix. A Re-examination of Age Polyethism

Robinson (1987) and Robinson *et al.* (1989) concluded that JH mediates apparent age polyethism in honey bees. Can a system of age polyethism, mediated by changes in JH titre, produce the plasticity seen in honey bee colonies? There is a growing body of evidence that challenges the causal link between age and task. It has been shown that when role deviates from the expected age-determined pattern, JH tracks role, rather than age (Withers *et al.*, 1993). Calderone (1998) recently concluded that the role of age in division of labour remains unresolved, and that possible 'age-neutral' mechanisms demand further study.

The demonstration of plasticity and individual flexibility in eusocial insects goes against the idea of a relatively rigid, age-determined division of labour.

Bourke and Franks (1995 p.407) point out that correlations demonstrated between the variation of physiological factors such as JH titre, patriline membership, and age and task, have yet to show causality. Bourke and Franks (1995 p.415) also argue that if temporal polyethism is a developmental process, physiological changes should occur in individuals prior to a change in their task, when task demand is held constant. In their view, individuals should change tasks (and undergo any corresponding physiological changes), only if task demand changes.

It is also noted that correlations between task performance and age are often weak and that there is considerable variation in the age at which each task is performed (Calabi, 1983; Sendova-Franks and Franks, 1993, 1994). There have been attempts to account for the latter by considering relative age (age ranks), rather than fixed age (Naug and Gadagkar 1998a,b, 1999). Experiments on the 'primitively eusocial' wasp, *Ropalidia marginata*, showed that both the probability of a certain task being performed, and the rate of task performance, showed age-dependent patterns, but more of the variance was explained by relative age rather than absolute age (Naug and Gadagkar, 1998a). The flexibility of a system based on this mechanism was demonstrated by artificially creating young-cohort colonies of the wasp, which showed premature foraging (Naug and Gadagkar, 1998b). There is an effectively constant difference in relative age for individuals in these artificially created colonies. Frequency of task performance increased with absolute age, but the probability of task performance did not. Naug and Gadagkar (1998b) concluded, therefore, that probability of task performance must increase with relative age – although this is only implied by their experimental results. They argue that small perturbations in colony conditions could be accommodated by changes in the frequency of task performance, but larger changes could not be, as they would require substantial changes in absolute age. Changes in the probability of task performance, governed by relative age, would instead be important in coping with large fluctuation in colony conditions.

These results are important primarily because they demonstrate a strong division of labour in a primitively eusocial insect in which individual workers have not lost the ability to reproduce. That the probability of task performance is controlled by relative age is only implied by these results, as there is no difference in relative age between the workers in the artificially created young-cohort colonies. It is interesting that there is little or no apparent spatial structure in the organisation of tasks in this species, as (a) the queen does not lay eggs in a single place, (b) the workers do not sort the brood, and (c) the queen and workers move over the entire surface of the nest every day (Naug and Gadagkar, 1998a). The possible role of spatial organisation in the division of labour has already been discussed in terms of possible efficiencies gained, and is discussed further later.

The 'traditional' view of age polyethism has been modified due to the results of the multitude of experimental studies of the division of labour. There is currently a tendency towards an acceptance of a correlation between 'roles', division of labour in its broader sense, and age, rather than a rigid and discretised progression through a finite series of tasks ranging from nurse work to foraging. Even advocates of age polyethism based on physiological factors, for example Calderone and Page (1996), discuss age-based task allocation with reference to *'(at least) one age-related transition in the composition of a worker's behavioural repertoire'*, i.e the transition for nest activities to foraging. More recently, authors have conceded that, in fact, the timing of this transition may be determined more by the environment and physiological processes than by age (Calderone, 1998).

x. Regulation of Colony Plasticity by Centralised Control

It has been demonstrated that interactions between workers play a central role in colony activity. There is no colony leader directing activities. However, there is evidence that the integration of activity in eusocial insect colonies may be more centralised in the case of small, primitively social species (Robinson, 1992). In sweat bees (Breed and Gamboa, 1977), and polistine wasps (Reeve and Gamboa, 1983, 1987; Gamboa *et al.*, 1990) queens may act as central pacemakers modulating worker activity. It has been shown that the experimental removal of foragers from a colony of polistine wasps, results in increased activity and aggressive interactions by the queen (Gamboa *et al.*, 1990) leading to increased foraging behaviour by workers. It is important to note that the queen may play a more important role in regulating behavioural plasticity in small 'simple' colonies than in larger, highly eusocial species.

This finding is in contrast to the results of a study on the 'primitively' eusocial wasp *Ropalidia marginata* (Premnath *et al.*, 1995). In this species the absence of the queen does not affect colony maintenance activities such as foraging and brood care, although it does lead to an increase in aggressive interactions by one individual, who will later become the queen, if the original is not replaced. Premnath *et al* conclude that the queen does not play a significant role in the regulation of colony activities. Instead, the workers regulate task performance themselves by various mechanisms, including dominance interactions. Recent work has shown that reproduction-based dominance interactions in the ant *Odontomachus brunneus*, control worker movement and location (Powell and Tschinkel, 1999), and the authors claim that it is this that mechanistically governs task allocation. The influence of worker movement and location on task allocation is discussed in detail later.

2.3 Self-Organisation

i. An Introduction

A functional definition of self-organisation is:

> ***"a mechanism for building pattern at the global (collective) level by means of multiple interactions among components at the individual level. The components interact through local, often simple, rules that do not directly, explicitly code for the pattern. By pattern, we mean a definite arrangement of parts in space, or in time, or both."***
>
> Camazine and Deneubourg, 1994

Although, originally conceived in the context of physics and chemistry to describe how microscopic processes give rise to macroscopic structures, the theory provides a concise description of various collective phenomena in eusocial insects (Bonabeau *et al.*, 1997). Self-organisation has been used to explain the formation of foraging patterns and forage selection in eusocial insects (Deneubourg and Goss, 1989; Camazine and Sneyd, 1991; Franks *et al.*, 1991); rythmical patterns of activity in *Leptothorax* ants (Franks *et al.*, 1990; Cole, 1991; Hatcher *et al.*, 1992; Boi *et al.*, 1999; modelled in Goss and Deneubourg, 1988); and building behaviour (Deneubourg and Franks, 1995; Theraulaz and Bonabeau, 1995a; specifically, in honey bees, Skarka *et al.*, 1990; wasps, Theraulaz and Bonabeau, 1995b; and *Leptothorax* ants, Franks *et al.*, 1992). Self-organisation theory has also been applied to modelling particular aspects of colony organisation, for example, hierarchical differentiation (Bonabeau *et al.*, 1995, 1996a; Theraulaz *et al.*, 1991, 1995); and division of labour (for example, Tofts, 1993; Franks and Tofts, 1994; Deneubourg *et al.*, 1987; Bonabeau *et al.*, 1998; Page and Mitchell, 1998; Spencer *et al.*, 1998).

The realisation that complex colony behaviour can be understood in terms of relatively simple individual behaviour is a great step forward in the field, and has stimulated a flurry of studies, see above. The main appeal of the self-organisation approach is its independence from the accumulation and processing of huge amounts of complex information by individuals or any central influence by the queen (Bonabeau *et al.*, 1998). There are, however, two important points to note before undertaking to explain phenomena observed in the eusocial insect field by the principle of self-organisation. Firstly, self-organisation does not preclude external influences on the colony, and in fact, a relevant explanation of collective phenomena in eusocial insects involving self-organisation invokes a combination of internal and external factors. Secondly, self-organisation may function in concert with other processes to produce collective behaviour. Structure in the environment may prove to function as a template, and this provides a link between internal and external factors. Organisation using templates is discussed in some detail in section 2.4i., below. There may be considerable differences in processes of decision-making between large (maximum population > 1000, *sensu* Franks, 1999) and small (maximum population ca. 100, *sensu* Franks, 1999) colonies. Franks (1999) deduces that decision-making by individuals in larger colonies tends to be based more on disseminated and carefully sampled information, whereas individuals in smaller colonies tend to be independent decision-makers. Again, there is a requirement for more studies on small, 'simple' (*sensu* Bourke, 1999), colonies.

ii. Mechanisms for Division of Labour involving Self-organisation

The models outlined below are examples of the application of recent advances in theories that interactions between large numbers of relatively simple entities on a local level can lead to collective behaviour that is both robust and flexible (Bonabeau *et al.*, 1997). Such self-organisation is a decentralised process, and may provide us with a deeper understanding of the basis of the division of labour, based on algorithms that could drive a self-organising, self-tuning, self-correcting division of labour (Deneubourg *et al.*, 1987; Tofts and Franks, 1992; Tofts, 1993; Bourke and Franks, 1995). This contrasts with the view that either, organisation is centralised and there is a colony leader directing activities (but see 2.2x., above), or, that individual workers acquire information as to the needs of the entire colony (for example, by patrolling, Lindauer, 1952), and act accordingly. The latter is considered unlikely (see, for example, Huang and Robinson, 1999), although the travel patterns of honey bees within the hive may reflect some gleaning of information from different locations (Seeley, 1995 p.246). Individuals may instead acquire information on colony needs indirectly, via interactions with other workers in their immediate environment, or through some component of the shared environment (Bonabeau, 1998; Seeley, 1995 p.250; Gordon, 1999). Seeley (1995 p.251) concludes that more information within honey bee colonies, and colonies of other eusocial insects, is exchanged indirectly, rather than directly.

iii. Interaction Patterns and Task Allocation

In section 2.2vii. ❷, I discussed the flexibility exhibited by colonies of harvester ants evident from task switching in response to a changing environment. Task allocation in this sense can be explained by the principles of self-organisation, as a consequence of simple decisions by individuals. This work stems from the observation that individual task decisions are based on more than an independent assessment of the environment, but also on signals and cues from other workers which may be packaged as chemical, mechanical, or visual messages (Gordon, 1999). Mathematical models (Pacala *et al.*, 1996) have determined that the pattern of interactions experienced by a worker has important implications for processes of task allocation. Importantly, the interaction in which the worker is involved, may itself, alter the environment, and thus influence the pattern of interactions and future task decisions by individuals.

Recent empirical work has shown that interaction patterns do indeed affect task decisions amongst harvester ants (Gordon and Mendiabadi, 1999). However, this work only examines tasks involved with midden work (the sorting and piling of the refuse pile). Further work on the role of interaction patterns in task allocation in the broader sense, discussed in section 2.2viii, is required in species with large, and species with small, colonies.

iv. Models Based on Response Thresholds

Response thresholds could form the basis of a mechanism that accounts for both the specialisation exhibited and the flexibility seen in the division of labour in eusocial insect societies. The idea is that workers have a threshold for performing a task, which can be exceeded by a stimulus, causing the individual to perform that task. Thus, the determinants of a worker's performance are first, the rules governing a worker's behaviour (its 'behavioural program') and, second, the needs of the colony. The tasks performed at any one time are thus not a fixed behavioural characteristic, but are strongly influenced by the worker's environment (Beshers *et al.*, 1999). The behavioural program of the worker includes a response threshold for every task in the repertoire, and it performs a task if the threshold for it is exceeded; the default state of the workers being not to attempt to perform any task. The stimuli relate to the labour needs of the colony, and are thus influenced by fluctuating conditions. This idea has generated a number of models of task allocation, some in concert with other physiological factors such as hormonal regulation.

➊ The Activator-Inhibitor Model – Hormonally Regulated Plasticity

Robinson *et al.* (1994) argue that temporal polyethism in honey bees is, essentially, a developmental process, but that they can still explain the plasticity exhibited by the colonies (Robinson, 1992; Robinson and Huang, 1998). They argue that genotypic differences in the rate of behavioural development predispose individuals to respond to changing environmental conditions in predictable ways. For example, in single-cohort colonies of honey bees, consisting only of newly eclosed individuals, where precocious foraging is thus induced, workers of certain sub-families are more likely to become precocious foragers than workers of other sub-families (Robinson *et al.*, 1989; Page *et al.*, 1992). Workers of other sub-families are more likely to continue as over-aged nurses as the colony ages (Robinson *et al.*, 1989).

Environmental conditions induce changes in JH titers, which result, directly or indirectly, in altered age polyethism in honey bees (Robinson and Huang, 1998). Response thresholds for task-related stimuli are modulated, and this influences what task a bee will perform (Huang and Robinson, 1999). These changes may be more likely to occur in certain social subfamilies, owing to variation in the rate of behavioural development resulting from genetic differences (Robinson and Page, 1989).

The '**activator-inhibitor**' model (Huang and Robinson, 1992) proposes a mechanism by which this scenario could work. JH functions as an '*activator*', originally conceived to promote behavioural development, now thought, in fact, to influence the rate of behavioural development through roles (Robinson and Huang, 1998). Newly eclosed workers have low levels of the hormone, which are programmed to increase with age, and once they reach a critical level, the worker becomes a forager. The levels of an '*inhibitor*', an as-yet unidentified factor, which unlike the activator, can be transferred from bee to bee, modulate this increase. This inhibitor has been hypothesised to be a chemical or a behaviour. In a situation of regular development, levels of the inhibitor are high in foragers, and consequently other individuals are prevented from becoming foragers. Huang and Robinson (1999) show that this model can explain how the colony achieves plasticity. The increase of activator levels with age can be delayed by social interactions. Manipulation of colonies causing precocious forager development can be explained as an attempt to reduce levels of the inhibitor, whereas individuals become foragers at older ages when levels of the inhibitor are increased. This model also incorporates the genetic aspects of the division of labour, as it is hypothesised that genetic variation for the production of, or sensitivity to, the activator and inhibitor can explain genetic variation in rate of behavioural development (Huang and Robinson, 1999).

Naug and Gadagkar (1999) applied Huang and Robinson's (1992) activator-inhibitor model, in the form of a numerical computer simulation, to the system of division of labour they had studied in the 'primitively eusocial' wasp, *Ropalidia* *marginata* (Naug and Gadagkar, 1998a, b). The simulation model demonstrated that the precocious foraging seen in *Ropalidia marginata* (Naug and Gadagkar, 1998b) could be understood in terms of the activator-inhibitor model, where levels of activator and inhibitor increased in an age-dependent way, and the inhibitor was exchanged among individuals through random individual interactions. Behavioural reversion has not yet been demonstrated in this species.

The presence of an activator or an inhibitor has not been experimentally demonstrated in *Ropalidia marginata*. Moreover, although JH is the obvious candidate for activator in honey bees, the inhibitor is currently unidentified. It has been shown that physical contact is required for such social inhibition to occur, and that removing the mandibular gland of older bees renders them less inhibitory (Huang *et al.*, 1998). It has also recently become clear that there must be other factors involved in activation (Huang and Robinson, 1999). The control of age-related division of labour is more complex than suggested by the activator-inhibitor model and its regulation has been concluded to be a multifactorial process (Huang and Robinson, 1999).

❷ The Fixed and Variable Response Threshold Models

There have been other attempts to use a response-threshold-type model to explain task allocation. In a recent paper, Bonabeau *et al.* (1996b) have suggested that task allocation could be accounted for if individuals had different response thresholds for task-related stimuli. Consequently, if the intensity of a particular stimulus exceeds the individual's response threshold then the individual has a high probability of doing that particular task. The model might account for some of the observations on the ant species *Pheidole* (Wilson, 1984), assuming ants belonging to different castes have different response thresholds, and that these thresholds are fixed. Alternatively, a variable threshold model has been introduced whereby performing a task reduces the appropriate threshold, and not performing it, increases it. Under these conditions of the model, specialised workers, that is, workers that are more sensitive to stimuli associated with particular behaviour, are produced, in a group where all are initially identical

(Theraulaz *et al.*, 1998).

It is argued that variation in response thresholds can account for all the key aspects of behaviour of workers, in the context of a division of labour (Beshers *et al.*, 1999). Flexibility to short-term variation in colony requirements can be explained by higher stimulus levels that stimulate workers to do other tasks, resulting from increased need for that task to be performed. If tasks are not performed stimuli may increase even further (Calabi, 1988; Robinson and Page, 1989). Longer-term changes could be accommodated by changes in workers thresholds. Beshers *et al.* (1999) argue that other peculiarities of worker behaviour such as specialisation, elitism, and idiosyncrasy, can also be explained. Specialisation results from workers with low thresholds for a small set of tasks. Elitism, the unusually high frequency of performance of a number of tasks by a worker (Oster and Wilson, 1978), is explained by low thresholds for the tasks performed. Idiosyncrasy (Jaisson *et al.*, 1988) variation in task performance not explained by age or morphological polyethism, could be due to individual deviation from the response threshold norms for the particular age or physical caste.

v. The Foraging for Work Algorithm

❶ The Model

Tofts and Franks (1992) developed a model of the mechanism of task allocation, based on self-organisation (see also Tofts, 1993; Franks and Tofts, 1994). They argue that although a correlation has often been demonstrated between age and caste, the existence of a correlation does not necessarily imply causation. This argument also applies to the physiological and genetic correlates of task performance demonstrated in honey bees, discussed above. Tofts and Franks' model suggests instead that task allocation, and a correlation between age and task, could result from individuals responding in the short term to local events and workloads in light of their previous experience (Bourke and Franks, 1995). The model does not dispute that individuals undergo changes in terms of physiology and behaviour. Franks and Tofts (1994) argue that these changes are not necessarily a result of some internal program, but depend largely on the individual's experience within its society. The model is known as the '**Foraging-for-work algorithm**' (Tofts and Franks, 1992; Tofts, 1993; Franks and Tofts, 1994).

In greater detail, the algorithm satisfies the essential conditions that (1) if the work force is of sufficient size, all the required work will be done; (2) no individual is overloaded; and (3) individuals do not waste time needlessly looking for work to do (Franks and Tofts, 1994). The model regards the work as involving abstract 'tokens', making it easier to evaluate in mathematical terms. The model makes two working assumptions. Firstly, that all individuals are identical, and secondly, that each individual is unaware of its own age.

The diagram below (from Bourke and Franks, 1995, after Tofts, 1993) illustrates how the model works. It envisages the organisation as a production line housed in a series of linked 'rooms' containing the ants. Tokens pass along the line, and each ant receiving the token performs work on it before passing it further down the line.

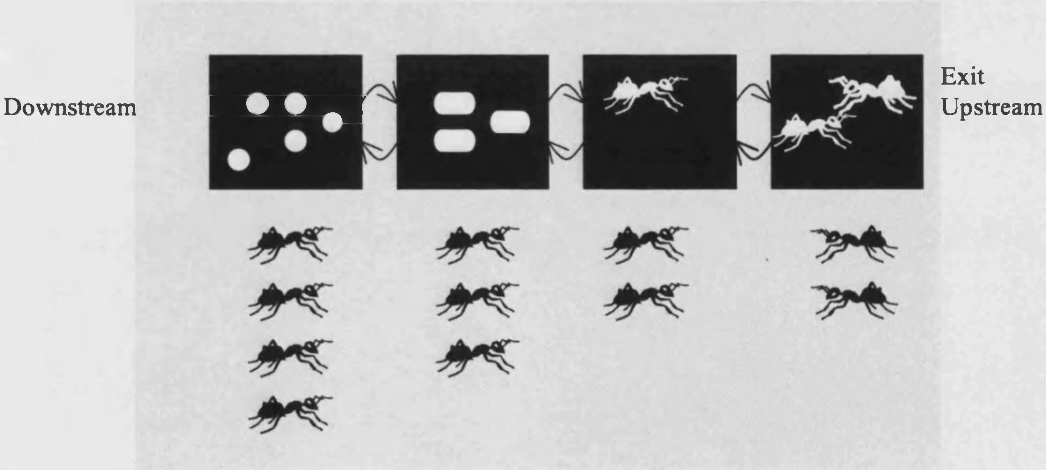


Figure 4: A representative diagram of the Foraging-for-work algorithm. From left to right the tasks shown: care of eggs; care of larvae; care of callow workers; and trophallaxis from foragers

exchanging food with ants within the nest.

The algorithm can respond to changing demands and acts to maximise the throughput of tokens through the system. For example, if an ant in a particular work station receives a reduced amount of tokens coming downstream, or tokens are taken at a reduced amount from the work station, the ant moves, with some probability, after a certain number of these occurrences is reached, either up or down a work station, depending on the stimulus. The basic algorithm is therefore:

1. Attempt to take a token from upstream (work on it) and pass the product downstream.

2. If this succeeds, then stay where you are and remember that you found work.

3. Keep a cumulative score of each time a direction fails to give or take work.

4. If the number of failures for either direction exceeds a critical amount, move in that direction with some probability (Bourke and Franks, 1995 p.410).

Consequently, instead of workers allocating tasks, tasks allocate workers (Franks and Tofts, 1994). One of the most important aspects of this model is the inclusion of spatial considerations. The organisation of tasks is envisaged as a linear sequence of work-stations. Such a linear spatial organisation has been shown to exist in nests. For example, there is a linear sequence of different tasks, extending from the brood pile to the outside of the nest.

The mechanism is robust enough to allocate tasks even if some individuals do not act altruistically. For example, some workers may reproduce, or may become fixated on a particular task through learning or, as in morphological castes, some individuals may become physically specialised to perform particular tasks

(Bourke and Franks, 1995). The model provides a comprehensive explanation of how colonies can be flexible. The colony could still function even if a minority of the ants were specialised only to perform one task. However, as the environment is variable, the colony would be most efficient if the ants could perform a variety of tasks (Bourke and Franks, 1995). As larger colonies have greater resources they will be more greatly buffered against variation in the environment. Consequently, individuals in smaller colonies should show greater behavioural flexibility than ants in larger colonies which could afford to become more specialised.

The model can also explain the correlation between age and task observed in so many ant colonies. If the production line is a linear sequence, for example from the center of the brood pile to the outside of the nest, younger workers will be positioned closest to the brood pile, whereas increasingly older workers are further and further away, because callows eclose on the brood pile. As more callows eclose, older ants may be displaced further towards the outside of the nest (Bourke and Franks, 1995). This demonstrates how the observation of a correlation between age and task may not necessarily be causal but could merely be a by-product of a self-organising process.

❷ Beyond the Foraging-for-work Algorithm

The Foraging-for-work algorithm has proved extremely controversial. Objections to the model have been based on the assumptions of the Foraging-for-work Algorithm, and general confusion over caste theory (Robinson *et al.*, 1994; Calderone, 1995; Calderone and Page, 1996; Traniello and Rosengaus, 1997). I argue that critical authors have misunderstood the original purpose of the model. The model established that a basic algorithm generating an efficient, flexible and robust system for task allocation, is at least theoretically possible (Franks and Tofts, 1994). Moreover, this simple algorithm could generate the kind of temporal patterns of behaviour observed in real colonies (Tofts, 1993). The Foraging-for-work Algorithm, therefore, indicates that correlations between age and task may be the outcome of more generic and simple principles of organisation (Franks and Tofts, 1994).

The model was designed to examine a possible mechanism of task allocation that applied to eusocial animals in general, and particular developmental constraints were not therefore invoked. The general principles can be applied, for example, to the eusocial Hymenoptera and the Naked Mole Rats (Tofts and Franks, 1992). I argue that the discussion of these principles in relation to widely differing groups emphasises the fundamental nature of such mechanisms. The discussion should not be seen as an attempt to 'champion' this exact algorithm and apply it to all eusocial systems in an attempt to explain the task allocation processes without appreciating the subtlety and variety of different species, as some authors have implied (for example Traniello and Rosengaus, 1997). Franks and Tofts (1994) do not preclude the existence in real colonies of developmental and physiological constraints. Even some critics (Calderone, 1998) have conceded that they consider the basic principle that an age-neutral mechanism can generate age polyethism, '*is an important contribution that demands further study*'.

The real value of the Foraging-for-work algorithm in studies of task allocation can be summarised as the following conclusions:

> Observed correlations between age, or physiological or developmental indicators of age, and task, do not necessarily imply causation, and such correlations may be a result of the organisation process.
>
> Flexibile and robust mechanisms of task allocation can be explained by more generic and simple principles of organisation.
>
> Learning may be an important additional mechanism in task allocation.

Theraulaz *et al.* (1998) suggest that the reinforcement mechanism combined with the Foraging-for-work algorithm (Tofts and Franks, 1992) could reinforce and stabilise patterns of temporal polyethism. Indeed, genetically determined response thresholds may explain the link between particular genotypes and specialised jobs. Franks and Tofts (1994) argue that physiological factors may also be involved, but that these are additional mechanisms, and not the main organisational principle of division of labour. Learning, or physiological task fixation (the physiological counter-part of learning), may also be important additional mechanisms (Franks and Tofts, 1994). Individuals may be physiologically adapted to the current task. The Foraging-for-work model can also explain the division of labour amongst closely similar age cohorts, if amplification of differences via positive feedback mechanisms such as learning, are considered (Deneubourg *et al.*, 1987; Deneubourg and Goss, 1989; Tofts and Franks, 1992).

2.4 Mechanisms involving Self-organisation with Other Processes – Beyond Self-organisation?

i. Self-organisation and the Environment – The Role of Templates

Self-organisation may act in concert with other mechanisms, to shape collective activities (Bonabeau *et al.*, 1997; Bonabeau, 1998), see section 2.3i. I have discussed the role of genetically determined response thresholds in relation to self-organising mechanisms of task allocation such as the Foraging-for-work algorithm, above. The importance of environmental influences on self-organising mechanisms has also been emphasised.

The environment may play a more concrete role in the production of collective behaviour by self-organising mechanisms. Environmental factors can act as a **template**. A consistent theme in this chapter has been the emphasis of structure in the eusocial insect world. The importance of templates in colony organisation has long been appreciated. Some ant species utilise temperature and humidity gradients in building their nests, and spatially distributing brood (*Ancantholepsis custodiens*, Brian, 1983; and *Myrmica rubra*, Ceusters, 1986). An individual (the queen) may act as a template, for example in the construction of the royal chamber in the termite, *Macrotermes subhyalinus*. A pheromone gradient is established around the physogastric queen, which influences worker building activity (Bonabeau *et al.*, 1998). The use of gradients of carbon dioxide gas or pheromones, as templates, has been hypothesised to explain the building behaviour exhibited by colonies of *Leptothoracine* ants (Deneubourg and Franks, 1995; Franks and Deneubourg, 1997). Templates may also be involved in generating the patterns of synchronised activity found in some ant nests (Cole, 1991). The possible role of carbon dioxide gradients in relation to activity cycles is discussed further in Cox and Blanchard (2000).

It is important to note that templates that result from the activities of a colony, not only influence the future activities of the colony, but may also be changed by the action of individuals. This is a phenomenon known as **stigmergy** (Grassé, 1959). This applies to any template which arises from the activity of the colony, and is not simply a result of heterogeneity in the environment (Bonabeau, 1998). Such templates provide a mechanism whereby individuals could influence one another, indirectly, via the environment, using cues.

Could templates be involved in a possible mechanism for task allocation? I have mentioned that templates play a role in some aspects of building behaviour in the Isoptera and the eusocial Hymenoptera, and may be involved in activity cycles in ants. The role of templates in task allocation at the more fundamental and universal level, has not been thoroughly examined. Recently, however, Cox and Blanchard, (2000), have attempted to investigate the possible role of carbon dioxide gradients in nests of the ant genus *Leptothorax.* They explored the spatial and temporal patterns of carbon dioxide diffusion in nests of these ants. Cox and Blanchard, (2000), concluded that templates based on carbon dioxide gradients may play a role, not only in building behaviour and activity cycles, but may also explain the phenomenon known as **social resilience**, and consequently have implications for task allocation (see sections 2.4i. and 2.4iv., below).

ii. Self-organisation and Dominance Interactions

There is evidence that the ultimate evolutionary cause of division of labour in the eusocial hymenoptera may result from variation in reproductive potential of individuals and reproductive benefits to workers (West-Eberhard, 1981), discussed in section 2.2i., above. I discussed potential and actual conflict in eusocial insect colonies in section 1.3, above. There is evidence for actual reproductive conflict over reproduction in three species of leptothoracine ants, and some ponerine species (see Bourke and Franks, 1995 pp.243-244 for a review).

Until recently, however, this hypothesis did not provide, at a proximate level, any algorithm or organisational procedure for generating the complex multi-stage division of labour observed in eusocial insect colonies (Bourke and Franks, 1995 p.406). It has recently been suggested that reproduction-based dominance interactions control worker movement and location, and mechanistically govern task allocation and establish the division of labour amongst non-reproductive tasks (Powell and Tschinkel, 1999). In the ant *Odontomachus brunneus*, movement toward the brood was mostly preceded by victory in a pairwise dominance interaction, and, conversly, movement away from the brood, by losing such an interaction. This provides a mechanism whereby organisation exibiting a correlation between rank and task can be established: the more subordinate an individual, the more peripheral her location. This is another example of a self-organising process combined with variation in reproductive dominance resulting in complex organisation at the colony level. The authors conclude that reproductive conflict may function as a mechanism to explain both reproductive and non-reproductive division of labour. They claim that the study also shows that the role adopted by each individual is driven by self-organising mechanisms resulting from the actions of nestmates, not by considerations of inclusive fitness benefits. Powell and Tschinkel (1999), therefore, conclude that '*alternative behavioural mechanisms may underlie the ultimate cause proposed by West-Eberhard (1981)*'. Interaction-based task allocation also claims to explain the correlation often observed between age and task.

This mechanism may prove to be important in generating the complex organisation and division of labour observed in eusocial insect colonies. It may also provide an explanation for the flexibility of colonies to environmental fluctuations: rates of social interaction may reallocate workers in accordance with changing colony requirements, and for social resilience (see below). However, as noted by the authors, it requires rigorous theoretical and empirical testing. The mechanism may be of greater import when considering 'simple' (*sensu* Bourke, 1999) species, in which there is relatively less morpholgical skew, and there is evidence that colonies establish an organisation in which role is correlated with rank. Powell and Tschinkel (1999) argue that dominance interactions may occur in more 'complex' species by more covert interactions: vigour of antennal contact; pheromonal communication; or trophollaxis. These interactions too, require further study. Powell and Tschinkel (1999) accept, however, that there are limits to the role of interaction-based task allocation, and that task allocation is likely to be a multi-layered process in more 'complex' groups, built on this base mechanism. As previously noted, 'simple' societies have a relatively small 'buffer' against changing environmental conditions, and colony requirements. The maintenance of behavioural 'totipotency' and low morphological skew exhibited by these colonies, allows mechanisms such as interaction based task allocation to function to provide the increased colony plasticity required.

iii. Self-organisation and Nutrient Status

Blanchard *et al.* (2000) proposed that variation in lipid stores may provide a mechanism for role determination in eusocial insect colonies. I have already mentioned the correlation between nutrient status and foraging propensity, and spatial factors (see section 2.2ii.). Blanchard *et al.* (2000), propose that lipid stores influence behavioural predisposition, and that changes in nutrient status should result in changes in role (and in JH titre in some eusocial insects). The mechanism allows colonies to respond to changes in resource flow and consumption, as the lipid levels of individuals change. Briefly, the ratio of nurses to foragers depends on the balance between the rate of colony food acquisition (a function of forager number and success) and the rate of energy consumption by colony sinks (brood, sexual adults, and the queen). In situations of plentiful resources, demand by the sinks is reduced, the lipid levels of foragers increases as they cannot pass on collected food, and they consequently become nurses. Conversely, when resources are limited (or if forager mortality is high), demand increases, and nurses become foragers as their lipid levels drop to satiate this need.

Blanchard *et al.* (2000), also claim that this mechanism can explain the fates of callows in single-age cohort colonies. Those callows that demonstrate precocious foraging in such circumstances, are hypothesised to be those with initially lower lipid levels (Blanchard *et al.*, 2000). The authors also accept that colony role structure responds to factors other than changes in nutrient demand. That changes in reproductive status (resulting, for example, from the loss of the queen) may also have an effect, is conceded.

iv. Social Resilience – a Mechanism for Maintaining an Efficient Division of Labour?

❶ An Introduction to Social Resilience

Spatial organisation in eusocial insect colonies has been discussed in the context of the consequent efficiencies gained (section 2.1ii.❸, above). Here I introduced the concept of social resilience, the ability of *Leptothorax* ant colonies to re-assemble after dissociation, for example, after emigration to a new nest site. The implications arising from the phenomenon of social resilience are now starting to be appreciated.

The fidelity of individual workers to particular positions in nests of *Leptothorax unifasciatus* colonies (manifested as Spatial Fidelity Zones, reviewed above) is evidence for the fundamental importance of spatial structure in eusocial insect colonies. Moreover, the resilience of this spatial structure to massive disruptions, such as an emigration, has prompted the suggestion that social resilience has implications for the division of labour (Sendova-Franks and Franks, 1994). Social resilience may be a key mechanism to maintain the efficiency of the division of labour, which has been seen to be the primary cause of the ecological success of the eusocial insects. After an emigration, social resilience prevents time and resources being wasted in worker respecialisation. This ability is particularly important in ants such as those of the genus *Leptothorax*, for which emigrations may be a part of their fundamental biology (Sendova-Franks and Franks, 1994, 1995a). Moreover, social resilience may apply to other sorts of disruptions, and could explain how an efficient division of labour is maintained throughout colony ontogeny, and through environmental fluctuations.

The concept of spatial efficiency is fundamental to self-organising models of task allocation, such as the Foraging-for-work algorithm. The principles of the Foraging-for-work algorithm provide an explanation of how the appropriate number of ants could be allocated to each task after colony re-assembly. Moreover, neither the mechanism of task allocation, nor the mechanism for the reassembly of colony structure, can be based on age (Sendova-Franks and Franks, 1994). The reassembly of the structure exhibited in the social resilience of some ant colonies may work on similar principles to task allocation in response to the changing requirements of the colony.

❷ Reassembly and Social Resilience

It has been suggested (Sendova-Franks and Franks, 1999; Backen *et al.*, 2000) that the ability of *Leptothorax* ant colonies to re-adopt their previous spatial positions relative to one another after dissociation, the phenomenon known as *social resilience* (Sendova-Franks and Franks, 1994), results from some form of assembly process. This process is analogous to the reassembly exhibited by sponge cells of the genus *Sycon*, when they reassemble after dissociation (Huxley, 1912), and is fundamental to biological organisation at many levels (see Sendova-Franks and Franks, 1999, for a full discussion). A distinction is drawn between **self-assembly**, where only the constituents in the final structure take part in the re-assembly process, and **template-directed assembly**, in which case the information is imposed externally by a template not present in the final structure. It is noted that "*self-assembly and self-organisation are not mutually exchangeable, but often self-assembly processes are self-organising*" (Sendova-Franks and Franks, 1999). Template-directed assembly can also be combined with self-organisation (reviewed in section 2.4i., above), for example: the basis of nest building in termites (Franks and Deneubourg, 1997).

The role of templates in social resilience needs further investigation. The recent work by Cox and Blanchard (2000) has indicated that carbon dioxide gradients may act as 'collapsible skeletons' in ant nests. They established that the ants would have to be faithful to concentrations within 0.000001*μmol* of their preferred concentration for social resilience to occur through this mechanism!

❸ Self-Assembly – A Possible Sorting Mechanism?

There is growing evidence that mechanisms underlying social resilience constitute a self-assembly process (Sendova-Franks and Franks, 1999). Such mechanisms would allow workers to re-establish their relative spatial positions, and resume their task specialisations, in the absence of information external to the workers themselves. Conversely, template-directed assembly indicates that the re-assembly of the colony's worker population does require external information. There have always been parallels between colonies of eusocial insects and autonomous organisms, and the importance of self-assembly in metazoans is now also beginning to be appreciated (see Sendova-Franks and Franks, 1999, for a review).

The work in this thesis (see also Backen *et al.*, 2000, Appendix A) tests the 'limits of social resilience' in 'simple' (*sensu* Bourke, 1999) ant colonies and demonstrates the importance of self-assembly as a mechanism of social resilience. It has not previously been established that social resilience can occur in the absence of possible templates. The presence of strong spatial patterns of, for example, brood, observed in some eusocial insect nests, has led to speculation that templates may be involved in the organisation of division of labour. The presence of the queen, or chemicals emitted by her, are also possible sources of templates. Recent work by Cox and Blanchard (2000) investigates the role of gas gradients as a template in ants, and the authors claim that such templates may provide a mechanism for social resilience. The experiments of Backen *et al.* (2000), and the work in this thesis, are the first experiments to utilise sociotomy techniques to determine the extent to which social resilience occurs despite the absence of a number of key colony components, namely, the queen and the brood (that could act as templates), as well as a proportion of the workforce. The results indicate the fundamental robustness of social resilience. They demonstrate for the first time that social resilience can occur in the absence of physical templates. The flexibility of workers in response to changes in task demand is not precluded by the robustness of social resilience. This flexibility is thoroughly investigated.

Chapter 3

The Experiments

3.1 Introduction to the Experiments

i. A Unique Approach

It has been seen that division of labour is key to the phenomenal success of eusocial insects. Chapter 2 examined how division of labour leads to the increased efficiency of the colony, and explained possible mechanisms by which this may be achieved and maintained, whether the division of labour is physical, or temporal. The phenomenon of *social resilience* was introduced, and this was discussed in terms of its relation to the division of labour, and the possible processes by which this may function. There are several areas where empirical work is lacking.

It has already been noted that whilst a large amount of work has concentrated on polymorphic species of ants, with relatively large colonies (*sensu* Franks, 1999: maximum population > 1000), little work has been done with smaller, monomorphic species (*sensu* Franks, 1999: maximum population ca. 100). Recently there has been a lot of theoretical modelling of proximate mechanisms, and a 'bottom up' approach has been favoured (for example, Tofts and Franks, 1992; Tofts, 1993; Bonabeau *et al.*, 1998; Spencer *et al.*, 1998; Theraulaz *et al.*, 1998; Blanchard *et al.*, 2000; Cox and Blanchard, 2000). Various models and hypotheses have been suggested concerning the mechanisms of task allocation, but few have been thoroughly examined experimentally. Attempts to test hypotheses concerning the division of labour and its mechanisms have been hindered by the selection of species that have very large colonies and complex nest structures so that the colony cannot be easily manipulated, for example *Atta cephalotes* (Wilson, 1980a, b, 1983), species of *Pheidole* (Wilson, 1984, 1985) and harvester ants, *Pogonomyrmex barbatus* (Gordon, 1989, 1991). Furthermore, only a small number of individuals from these colonies can be examined at any one time. Experiments have therefore been restricted either to examining in detail the behaviour of a small number of individuals, or examining the behaviour of whole groups of individuals, eliminating individual detail.

Recent studies have had success in providing sound empirical data on task allocation and the division of labour, using ants of the genus *Leptothorax* (Franks *et al*, 1990, *L. acervorum*; Franks and Sendova-Franks, 1992; Sendova-Franks and Franks, 1993, 1994, 1995a, b, c, *L. unifasciatus*; Backen *et al.*, 2000; Blanchard *et al.*, 2000; Cox and Blanchard, 2000, *L. albipennis*). *Leptothorax* ants have proved to be ideal experimental animals. All the individuals in the colony can be uniquely marked with minute paint spots and consequently the behaviour of all the individuals in the colony can be followed continuously over long periods of time if required. Colonies are kept in the laboratory in very thin nests, which mimic the geometry and scale of the nests of the ants in the field, see Figures 5 and 6, below. Uniquely, this facilitates the collection of photographic or video footage of all the individuals in the colony, inside, as well as outside the nest.

ii. The Aim of the Experiments

The following experiments are designed to examine division of labour in *Leptothorax albipennis*, a species which occurs as small, 'simple' (*sensu* Bourke, 1999) colonies. Using sociotomy techniques, the colonies are drastically manipulated by dividing them into fractions. Colonies are either divided by task (experiments 1 and 2), or by age (experiment 3). The experiments aim to test the following hypotheses:

❶ Behavioural Flexibility and Social Resilience

In small colonies of ants, the effect of the loss of a group of individuals is relatively great, and buffering effects against environmental change are relatively small. I hypothesise that such colonies must exhibit plasticity in their division of labour, and flexibility in terms of the tasks individuals perform. Such species should exhibit low levels of physical specialisation, and are often characterised by a low level of morphological skew (or high levels of totipotency).

What is the extent of colony plasticity and individual flexibility – Dividing the colony into fractions based on behavioural task:

Does the removal of individuals associated with external activity induce the remaining individuals in such a colony to exhibit precocious external activity?

Do removed individuals experience behavioural reversion and become associated with nurse-work?

What happens when the fractions of the colony are re-united? If workers re-specialise following sociotomy, do they revert to their original role, or do they maintain their new role after re-unification?

What is the extent of colony plasticity and individual flexibility – Dividing the colony into fractions based on age:

Can an artificially constructed colony of 'all-young' individuals establish and maintain a viable division of labour? Do some of these individuals carry out precocious external activity?

If the 'all-young' cohort is removed from a colony, do the remaining individuals experience behavioural reversion?

When these fractions are re-united do workers re-specialise or maintain their current tasks?

What is the extent of spatial organisation within such colonies and what role does spatial organisation play in the division of labour, efficiency and flexibility?

Are tasks spatially organised?

> Do colonies exhibit social resilience? What is the spatial structure of fractions of colonies, divided on the basis of behavioural task or age?
>
> When the fractions are re-united, do workers re-organise their relative spatial distribution to that observed prior to sociotomy, or do they maintain the spatial organisation observed when they existed as separate fractions?

❷ The Influence of the Queen

There is evidence that the integration of activity in eusocial insect colonies may be more centralised in the case of small, primitively social species (Robinson, 1992). As reviewed in Chapter 2, section 2.2x., there is evidence that queens may act as central pacemakers modulating worker activity, in sweat bees (Breed and Gamboa, 1977), and polistine wasps (Reeve and Gamboa, 1983, 1987; Gamboa *et al.*, 1990). It was shown that the experimental removal of foragers from a colony of polistine wasps, resulted in increased activity and aggressive interactions initiated by the queen (Gamboa *et al.*, 1990) leading to increased foraging behaviour by workers. It was also noted that the queen may play a more important role in regulating behavioural plasticity in small 'simple' colonies than in larger, highly eusocial species. Conversely, in another 'primitively eusocial' species, the wasp, *Ropalidia marginata,* the absence of the queen does not affect colony maintenance activities such as foraging and brood care. However, her absence does lead to an increase in aggressive interactions by one individual, who will later become the queen, if the original is not replaced.

I discussed above (section 2.4i. and iv.) the possible role of templates in explaining the phenomenon known as **social resilience**, and the consequent implications for task allocation. The role of templates in task allocation at the more fundamental and universal level, has not been thoroughly examined. The queen, or gradients of chemicals emitted by the queen, are obvious sources of possible templates in ant colonies. I, therefore, also investigate the role and behaviour of the queen.

> Does the queen play a significant role in the regulation of colony activities?
>
> How is the behaviour of the queen, in terms of her spatial distribution and interactions, affected by sociotomy?
>
> Does the absence of the queen have any effect on the specialisation and social resilience of workers, and therefore indicate that she may play a role in the mechanisms of these processes?

❸ The Influence of the Brood

Franks and Sendova-Franks (1992) showed that the spatial arrangement of brood, as well as individuals, is very consistent in *Leptothorax unifasciatus* colonies. The brood is sorted into concentric circles, with the smallest items (eggs and microlarvae) in the middle and the largest larvae on the outside. Such a highly structured arrangement could also be utilised as a template for re-organisation.

> Does the presence/absence of the brood have any effect on the specialisation and social resilience of workers, and therefore indicate that it may play a role in the mechanisms of these processes?

❶ The Influence of Interactions Experienced by Workers

Individuals task decisions are influenced by signals and cues from other workers which may be packaged as chemical, mechanical, or visual messages (Gordon, 1999). The pattern of interactions experienced by a worker has important implications on processes of task allocation. This has only been studied in relation to specific tasks, and task switching within a normal caste repertoire

(Pacala *et al.*, 1996; Gordon and Mendiabadi, 1999), and the how the pattern of interactions experienced by workers in the colony is affected by sociotomy, has not been investigated.

It has been suggested that reproduction-based dominance interactions control worker movement and location, and mehanistically govern task allocation and establish the division of labour amongst non-reproductive tasks (Powell and Tschinkel, 1999). This may also provide an explanation for the flexibility of colonies to environmental fluctuations, and for social resilience: rates of social interaction may reallocate workers in accordance with changing colony requirements. It is therefore of interest to investigate any patterns of interaction experienced by the workers in colonies in which the queen is not present, when dominance interactions may influence task allocation.

> How does the pattern of interaction between the workers change as a consequence of sociotomy?
>
> How are any patterns of interaction between the workers affected by the absence of the queen (and the brood) in fractions of the colony?
>
> What are the implications of any patterns of interaction for processes of task allocation , in the presence, and in the absence of the queen?

iii. *Leptothorax albipennis* – The Experimental Animal

The leptothoracine ants belong to the tribe *Formicoxenini* (Bolton, 1995), part of the subfamily Myrmicinae (Hölldobler and Wilson, 1990). There are two principal subgenera, *Leptothorax (Myrafant)* and *Leptothorax (Leptothorax).* They have become very important in experimental studies because of their ease of collection and maintenance in the laboratory, and the wide variety of social structure and life-history traits displayed by the tribe (Bourke and Franks, 1995). In a review of the life histories of leptothoracine ants, Bourke and Franks (1995) note several common features of these ants which make them suitable for experimental studies. The colonies are generally small, only about 500 workers in the largest colonies, and consist of relatively small individuals. The nests also contain one or more queens, and a varying amount of brood. Their preferred nest sites are hollow cavities for example in decaying plant material or natural crevices in rocks. Nest emigrations are common and nest sites may be abandoned more than once within the lifespan of the colony, a period of approximately twelve years. The availability of suitable nest sites may be limited and due to this, and that many species do not repair damaged nests, competition is intense. To compensate for this the life history features of colony foundation, polygyny and polydomy have evolved (Bourke and Franks, 1995).

Leptothoracine ants exhibit a low degree of morphological skew - queens and workers are very similar in size. Queens live much longer than workers, but there is an orphanage period after the queen dies when the last batch of workers is still alive. Worker reproduction may be very common in this period (Bourke, 1988).

In the following experiments ant colonies were collected from Portland Bill in Dorset, and kept in the laboratory. The species of ant used, *Leptothorax albipennis* (formally *tuberointerruptus*, Orledge, 1998), naturally lives in rock crevices. This species of ant has previously been used in studies of building behaviour (Franks *et al.*, 1992). The main advantage of this species is that it can easily be kept in the laboratory by the construction of an artificial nest (described in detail below) and the nesting conditions in the laboratory mimic substantially the natural field conditions. The artificial nest is constructed from two glass slides between which is sandwiched a thin piece of cardboard with a hole cut in it which forms the single nest chamber. Consequently the ants can be easily observed within the nest and this can provide a unique insight into the internal workings of the ant colony. This experimental set-up can be used very effectively to study the division of labour both within and outside the nest. Such colonies can be used to test hypotheses about the division of labour and task allocation by facilitating the collection of data on the spatial positions of individuals in the nest, and their behaviour, which can be recorded photographically. Individuals can be marked with minute, unique, paint spots, consequently making identification possible, see

Figure 5, below.

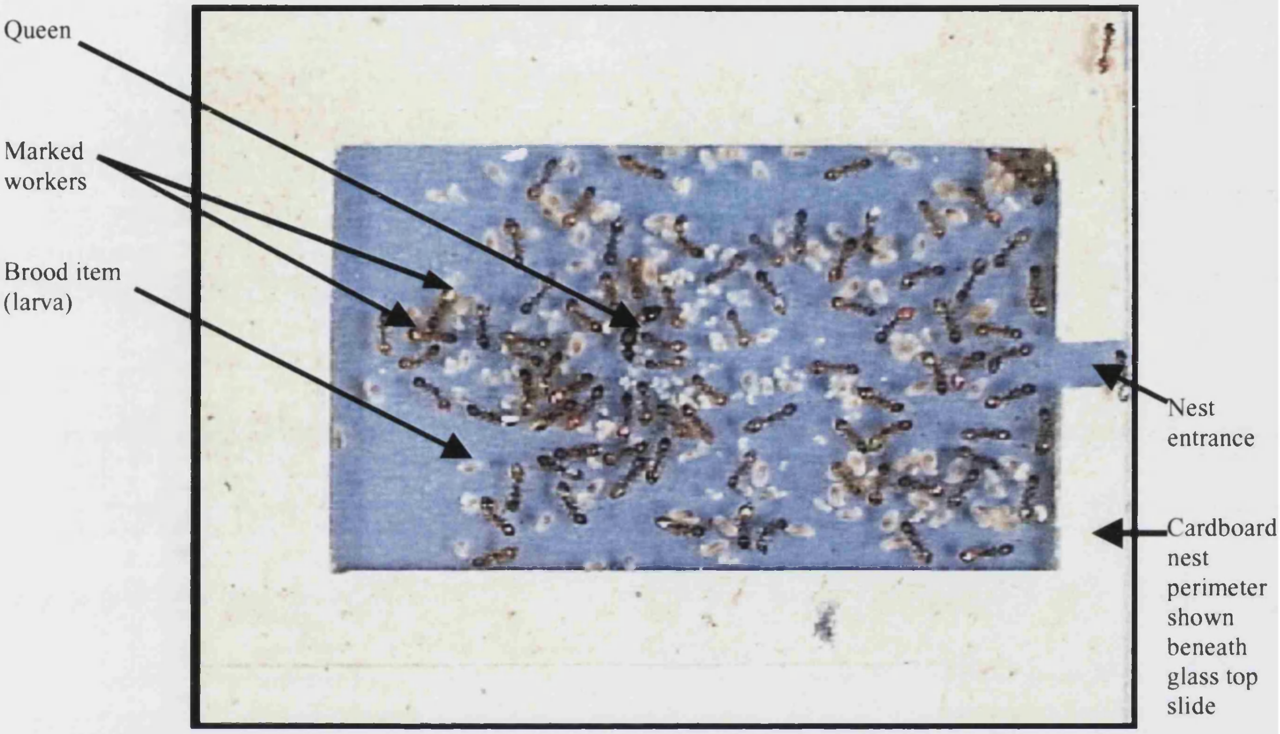


Figure 5: Digital image of Leptothorax albipennis colony within an artificial nest

3.2 Experimental Techniques

i. Collection and Maintenance of Colonies

Colonies of *Leptothorax albipennis* were collected from Portland Bill in Dorset, during April 1997 (used in experiment 1) and April 1998 (used in experiments 2 and 3). Preferred nest sites are natural crevices in rock from which complete colonies may be aspirated after prising the crevices open with a knife. Several more colonies were collected than were needed for the experiments, allowing for selection of suitable colonies in the laboratory. One selection criterion was the presence of a single queen (some *Leptothorax albipennis* colonies have more than one queen present, although there is nearly always only a single functional queen, the remainder being virgins, Ana Sendova-Franks, *pers. comm*). Colonies consisted of approximately 100 workers (see Appendix B, Table 1 for population counts), and also contained an amount of brood, at various stages of development. In each of the three experiments, four different colonies were used as experimental colonies, and there was one Control colony for each experiment.

The artificial nest was constructed from two glass slides, each 50mm by 75mm, which were used to sandwich a piece of cardboard 0.8mm thick, from which had been cut a chamber 36mm by 23 mm and an entrance tunnel 2mm by 4mm, in the positions shown in Figure 6, below.

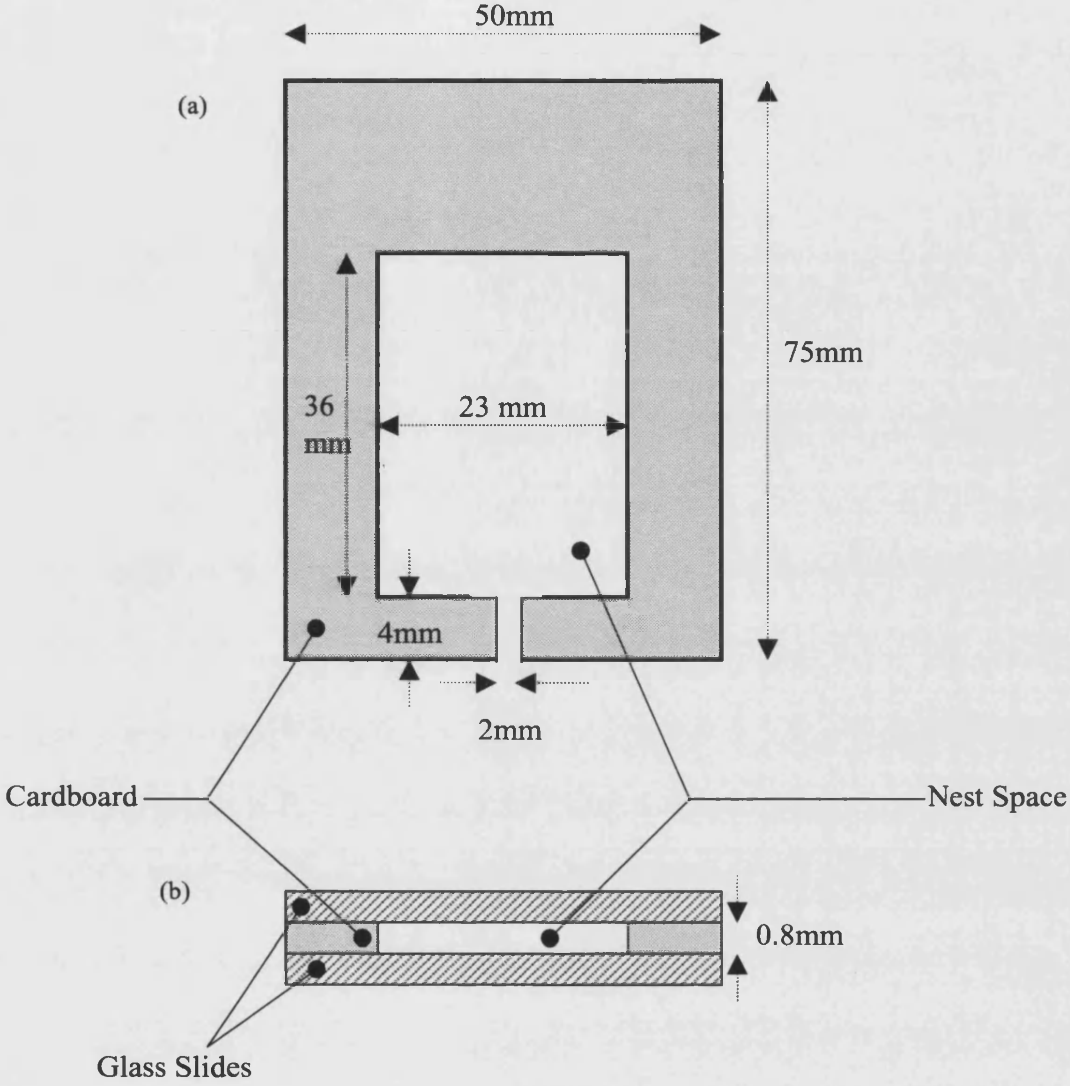


Figure 6: (a) A diagrammatic representation of an artificial nest (b) A diagrammatic representation of a cross-section of a nest

Before construction of the nest the glass slides were wiped with ethanol, carefully washed with water and left to dry. The cardboard was cut as shown above using a sharp scalpel and a metal ruler. A piece of sellotape, approximately 65mm long, was placed down each of the long sides of the nest, and folded over the other side to form a seal. It was ensured that no air spaces had been created in the sellotape where ants might get trapped.

Each nest was placed in a square petri-dish (10cm x10cm x 2cm), the sides of which were coated with Fluon®, so that the ants could not climb out. The ants readily inhabited this nest after the colony was placed in the arena. The ants were fed on a supply of dilute honey water (1 part honey, 10 parts water) and three *Drosophila* larvae per colony. The food supply was replenished weekly. Fresh water was readily available.

ii. Marking

❶ Experiment 1

All the individuals in the five queenright colonies (four experimental, one Control) were uniquely marked with minute spots of paint (PACTRA R/C polycarbonate, ketone-soluble, model paint), according to the methods described in detail in Sendova-Franks and Franks (1993), during the week starting 28th April 1997. Trials of different colour combinations of the available paints, on spare colonies, limited the colour choice to nine possible paints, which effectively contrasted with each other and showed up well on both the ants' cuticle, and the photographic slides. Different combinations of two, and three colours were used to mark the gasters of the ants, allowing unique identification of all the individuals in the colony.

The process of marking the workforce of each colony proceeded as follows:

Workers outside the old nest were picked up, using a fine paintbrush, marked, and placed on top of a new nest in a separate foraging arena (petri-dish). After all the external workers had been marked, the old nest was opened and the top slide placed facing upwards in then old arena next to the bottom slide (this was done as some brood items remained stuck to the top slide). The remaining workers were removed one by one, and marked, then placed on the top slide of the new nest, in the new arena. This continued until the stage where it was possible to remove the queen and brood from the old arena without disturbing any remaining workers. The queen and brood were gently removed, and placed on the top slide of the new nest. Any workers remaining in the old arena were removed, marked, and placed on the top slide in the new nest. By this time the workers in the new arena had begun to emigrate into the new nest, together with the brood and the queen. This procedure was also followed for experiments 2 and 3.

❷ Experiment 2

Five queenright colonies (four experimental, one Control) were marked using *Pactra* paints, employing a combination of marks on thorax and and the right and left side of the gaster to enable each worker to be identified individually. This is an elaboration on the marking technique in experiment 1, and allowed individuals to be identified with a greater fidelity. Individuals could still be identified even if one of the marks became dislodged, due to the variations of positions and colour of the marks (after Blanchard, 1996, see Figure 7, below). It can be seen from Figure 7 that the total number of permutations of colour and position possible, allowing the identification of individuals even if one of the marks has been removed, is eighty-one. If the total number of workers to be marked exceeded eighty-one, the remaining individuals were marked according to the scheme in Figure 7, but with each thorax mark moved one place to the right in the this table. This allowed identification down to two individuals if one of the marks was dislodged. This marking scheme theoretically allowed individuals to be identified with a higher degree of fidelity than in experiment 1, as they could still be identified even if they lost one mark. Furthermore, individual variation in the exact shape of marks (which was recorded) meant that some individuals could still be identified if they lost two marks.

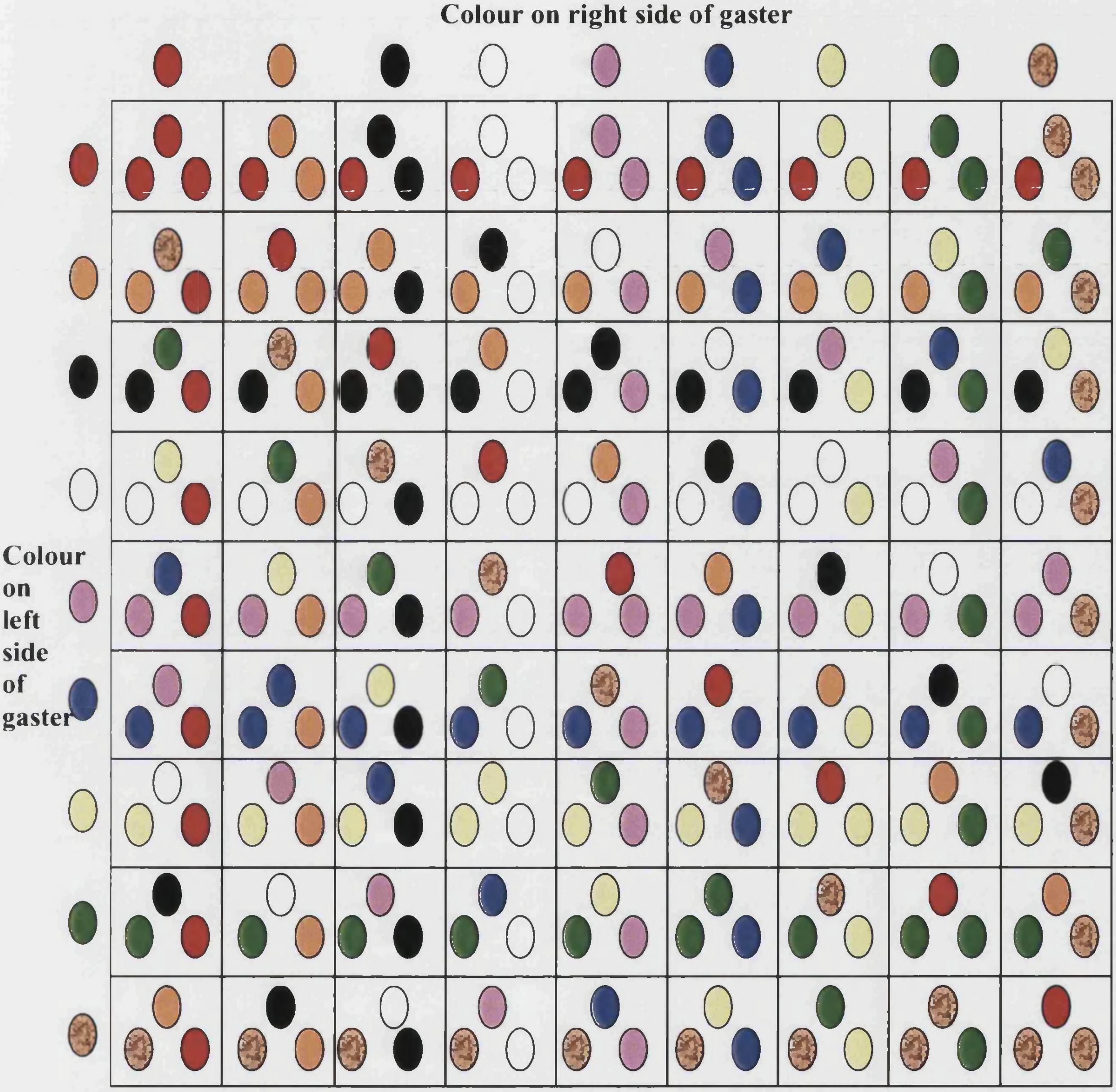


Figure 7: Table showing combinations of colour and position used to mark workers in experiments 1 and 2. The mark on the thorax (the top mark of the three shown for each individual) was determined by taking the colours in the same order as they are on the index for the right side, and applying this at the diagonal (after Blanchard, 1996)

A key was constructed enabling each individual to be identified from its marks. Three further colonies (known as donors) were fed with honey water and water containing Fat Red (Sigma chemicals) a vital dye known to cause coloration of the brood (Andrew Bourke, *pers. comm.*).

❸ Experiment 3

Five colonies containing a high proportion of mature pupae were used in the experiment and each colony was divided into two fractions – 'callows' and 'older workers', according to procedures outlined in 3.3iii., below. At the appropriate stage of the procedure, both fractions of each colony were marked using colour permutations, as in experiment 2. Marking was carried out such that individuals could be uniquely distinguished not only from the other individuals in their own fraction, but also from all the individuals in the other corresponding fraction of their colony, as the old and young fractions were later to be united.

iii. Obtaining and Analysing the Results

❶ Producing a Photographic Record

Photographs were taken as required by the experimental procedures outlined below, using an Olympus OM-2 camera focused through a Zeiss binocular microscope (Magnification was x 0.8). Two Olympus T32 electronic flashes and Kodachrome colour slide film (ISO 64: 36 exposures) were employed.

The camera was focused so that its field of view was fractionally larger than the interior of the nest. Immediately after each photograph was taken the identities of the individuals in the entrance tunnel (not in the camera's field of view) and outside the nest were determined and recorded. The positions of the brood were determined from the photographic record for each colony, from every fifth slide, throughout an experimental session.

All three experiments were divided into sessions, each session lasting 15 days. Photographs were taken at randomly selected times, four times per day, yielding sixty photographs per colony during each session of the experiment.

❷ Producing a Record of Individual Identities and Spatial Positions - Digitising the Slides

After the experimental sessions were completed, each slide was digitised using a computer with a frame-grabber and digitising program, that recorded the x and y co-ordinates of any selected point within a previously identified area. This area corresponded to the cavity of the nest, and had dimensions of 618 x 387 pixels, where 1mm = 17 pixels. Note, in all tables and graphs in Chapters 4-8, distance units are given in pixels. Each slide was individually positioned on a Perspex sheet above a light box. A video camera, positioned above the Perspex sheet, was focused on the slide image. The video camera was connected to the computer, and the frame-grabber allowed the computer to 'capture' the image.

After the image on a slide was 'captured' on the computer, the cursor was positioned over each individual's petiole, and it was selected. If the ant could be identified from its marking, the identification number of the ant was determined by examining the markings using a slide projector. This code was typed into the computer. Unidentifiable workers were all given the identification code '0'. The queen in each colony, determined by her slightly greater size and darker colour, was also digitised and given the code '150'. Unidentified callows (distinguishable by their pale colour) were given the code '149', and any males the code '151'. In this way a data file of worker's identities and co-ordinates was produced for all the individuals within the nest for every slide.

The brood was digitised from every fifth slide in a session, using the centre of each item, and given a code specific to one of five types: eggs or microlarvae (hard to distinguish from each other), medium larvae, large larvae, pre-pupae, and pupae (after Sendova-Franks and Franks, 1993). The mean position of the eggs and microlarvae was distinguished as the centre of the brood pile. In the results worker's positions are given relative to this centre. In experiment 1, the subsidiary fractions of the colonies did not contain any brood, and worker's positions are therefore given relative to the centre of the brood pile before the colony was separated (session 1).

❽ Producing a Record of Individual Identities and Behaviour

A behavioural record was created for each identifiable worker in each colony for each of the different experimental sessions. Behaviour was determined directly from each slide. Acts of behaviour were defined as observed contact between the mouthparts of the worker and a nestmate, or item of brood. As in Sendova-Franks and Franks (1995a) there were ten possible behavioural acts: contact with each of the five types of brood; contact with an old worker; contact with a young worker; contact with the queen; trophallaxis with a larva; trophallaxis with a worker. During data analysis the categories were pooled (as in Sendova-Franks and Franks, 1995a). Behavioural acts were therefore classified as one of the following categories:

Brood-related tasks (classified as contact with any of the five types of brood item, or trophallaxis with a larva);

worker-worker interaction (classified as contact or trophallaxis with old or young workers);

worker-queen interaction (classified as contact or trophallaxis with the queen).

A further category,

external activity,

was created from the record produced when individuals were recorded as being in the nest entrance or outside the nest, which was determined immediately after each photograph was taken.

❹ Analysing the Results

The raw data was manipulated using programs written in the UNIX language '***awk***', and in '***C***'. The description of an ant's zone of movement was simplified to one dimension (after Sendova-Franks and Franks, 1995a). This was done because the colonies organisation is radial, in a similar way to that of *Leptothorax unifasciatus* (Sendova-Franks and Franks, 1995a). The brood of these colonies is arranged in a radial pattern (as has been discussed in the introduction, see section 2.1ii. ❸ – Efficiency Gains from Spatial Organisation). Colonies of *Leptothorax unifasciatus* and *Leptothorax albipennis* tend to build annular nest walls if they are given the opportunity to build their own nests, whether in laboratory or field conditions (Franks and Sendova-Franks, 1992; Franks *et al.*, 1992). Sendova-Franks and Franks (1995a) noted that in artificial nests, the location of the brood pile can vary, as can its arrangement, which can be in the form of concentric rings, semi-rings, or straight bands (see Figure 8, below). Thus, Sendova-Franks and Franks (1995a) concluded that the single nest entrance is a major departure from radial structure, even in cases where the brood is arranged in concentric rings. Therefore they state that measuring the spatial position of ants in terms of distance from the brood pile is a highly simplified, but perfectly adequate measure of their position.

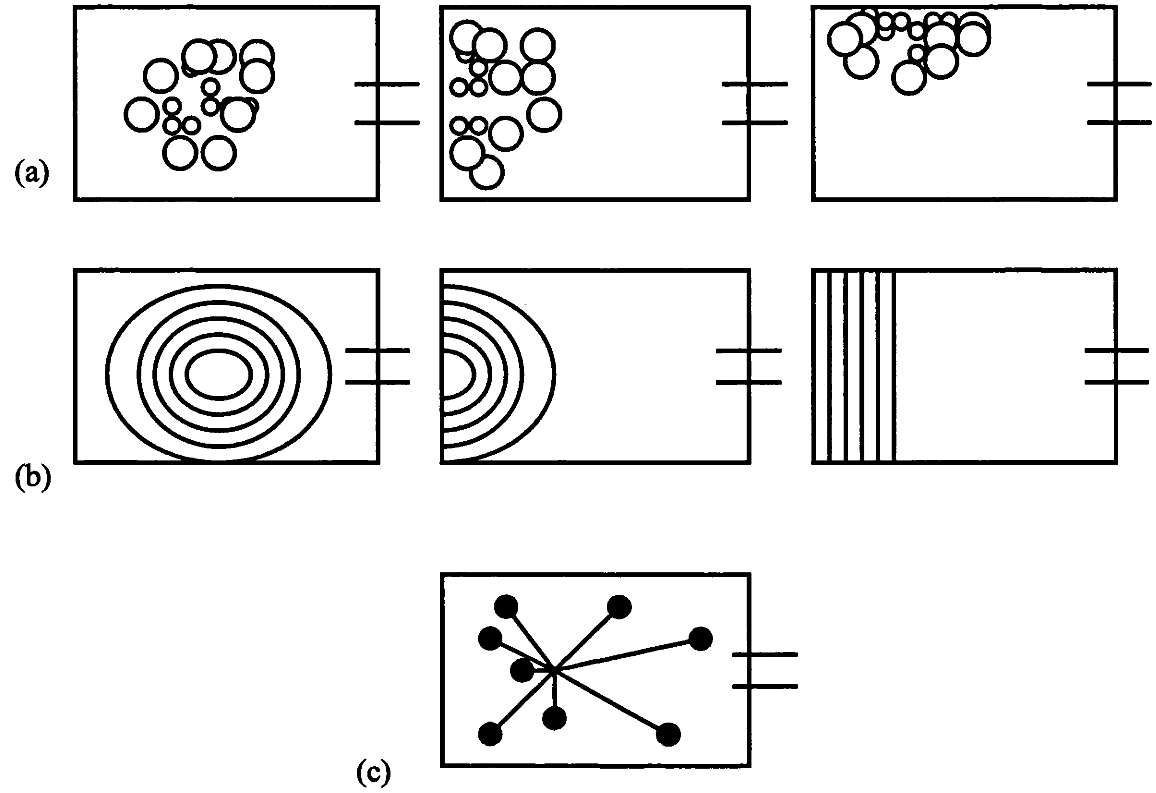


Figure 8: Position of the brood pile can vary: (a) in its location within the nest; and (b) in the shape of its distribution. (c) To minimise the effect of nest geometry on the shape of an ant's zone of movement, the spatial distribution of an ant's position in the nest was described in terms of her distances from the centre of the colony: the centre of the distribution of eggs and microlarvae. From Sendova-Franks and Franks (1995a).

Taking the brood pile as the reference point from which to measure an ant's position is used for a second reason. The brood pile is taken to be the "*biological centre of the colony*" (Sendova-Franks and Franks, 1995a). A large amount of the work undertaken by the colony occurs here, and it is the place where the queen is most often situated. As in Sendova-Franks and Franks (1995a) the mean co-ordinates of the eggs and microlarvae brood category were taken to be the mean centre of the brood pile, in view of the different shapes the brood pile could take. As noted by Sendova-Franks and Franks (1995a) the eggs and microlarvae form a small, homogenous, group in the middle of the brood pile, and hence their centroid is more representative of the biological centre of the colony, than the mean position of all the items of brood.

Consequently, the distance of each ant from the centre of the brood pile was measured over each session of observation. For each session, the median position of each individual was calculated.

3.3 Detailed Description of Each Experiment

i. Experiment 1 - 1997

The experiment was divided into four *sessions*, each of fifteen days duration.

The first session consisted of observations of un-manipulated colonies.
During session two, external workers were removed sequentially from the colony into a new nest in a separate arena, according to the sampling procedure outlined below.
During session three the '*subsidiary*' and '*original*' fractions of the colonies were maintained separately.
In the fourth session, the fractions of the colonies were re-united and maintained for the duration of the session.

Data were obtained by the procedures described in section 3.2, and were used to investigate the following:

What is the extent of colony plasticity and individual flexibility when the colony is divided into fractions based on behavioural task?

Does the removal of workers associated with external activity induce the remaining individuals in the colony to exhibit precocious external activity?

What happens when the fractions of the colony are re-united? If workers re-specialise following sociotomy, do they revert to their original role, or do they maintain their new role after re-unification?

What is the extent of spatial organisation within such colonies and what role does spatial organisation play in the division of labour, efficiency and flexibility?

Are tasks spatially organised?

Do colonies exhibit social resilience? What is the spatial structure of fractions of colonies, divided on the basis of behavioural task?

When the fractions are re-united, do workers re-organise their relative spatial distribution to that observed prior to sociotomy, or do they maintain the spatial organisation observed when they existed as separate fractions?

Does the queen play a significant role in the regulation of colony activities?

How is the behaviour of the queen, in terms of her spatial distribution and interactions, affected by sociotomy?

Does the absence of the queen in the subsidiary fractions during session 3, have any effect on the specialisation and social resilience of workers, and therefore indicate that she may play a role in the mechanisms of these processes?

How does the pattern of interaction among the workers change as a consequence of the sociotomy of the colonies?

How are any patterns of interaction among workers affected by the absence of the queen, and the brood, in the subsidiary fractions during session 3?

What are the implications of any patterns in interactions for processes of task allocation, in the presence, and in the absence of the queen?

Procedure

Colonies 1, 2, 3, and 4 were manipulated experimentally, Colony C_1 was the Control.

Session 1:

Procedure: No experimental treatment was carried out on the colonies.

Photography: The day was divided into 8 periods from 9am to 5pm. Using a random number generator 4 of these 8 periods were selected for each of fifteen days that made up the session. At the beginning of each selected period a photograph was taken of the inside of the nest of each experimental colony for the duration of session 1, thus yielding four slides per colony per day, and a total of 60 slides per colony during each session.

Session 2:

Procedure: Experimental manipulations were carried out on Colonies 1, 2, 3 and 4. The colonies were starved of food for 48 hours prior to the start of session 2. On day 1 of session 2, fresh honey solution was placed in the foraging arena of each colony, simultaneously. This was time = zero. The colonies were observed. Any workers that ventured outside the nest entrance were removed, using a fine paintbrush, just before they re-entered the nest, in order to standardise the procedure of removing them. These workers were classed as 'externals'. The time at which each individual was removed was recorded in hours and minutes. This procedure was carried out simultaneously for each of the four experimental colonies (a volunteer was sequestered for the procedure as it is only possible for the experimenter to carry out this procedure on a maximum of two colonies at one time). The externals that were removed from each of the four colonies were placed in a new arena with a new nest identical to their original home. The externals readily emigrated into their new nest. Thus four 'subsidiary' colonies were produced (1d, 2d, 3d, and 4d).

After three hours the rate at which ants ventured outside the nest had dramatically reduced. A sampling technique was adopted for the remainder of session 2. Before sampling began, the identity of all the individuals in the subsidiary colonies was determined from their marks.

Sampling technique: Externals were removed as before and placed in the relevant subsidiary colony. As individuals were removed they were identified from their marks, and the time at which they were removed was also recorded. This procedure was carried out for Colonies 1 and 2 simultaneously for twenty minutes (between the hour and twenty minutes past the hour), and Colonies 3 and 4 for twenty minutes (between thirty minutes and fifty minutes past the hour), in every hour, for eight hours a day. Sampling was continued until the end of session 2. Food and water were replaced in the foraging arena of each nest every night, and honey solution was present throughout sampling (this also provided a source of water).

Photography: In session 2, no photographs were taken during the first three hours (before sampling started). During the remainder of session 2, photographs were taken of the original colonies during four, randomly allocated, sampling periods. This occurred between 20 and 30 minutes past the relevant hour for Colonies C_1, 2, and 3, and between 50 minutes past the hour and the start of the next hour for Colonies 4 and 5. Consequently four slides per day, per colony, were produced.

Session 3:

Procedure: No experimental treatment was carried out on either of the fractions of the colonies.

Photography: During session 3 photographs of the subsidiary and original fractions of the colonies were taken at the same rate as in session 1.

Session 4:

Procedure: At the start of session 4, workers from the subsidiary colonies were placed into the foraging arena of the appropriate original colonies. The colonies were left to 'settle' for 24 hours.

Photography: During session 4, photographs were taken of the Control and the four re-united colonies at a rate of four per day for each colony, in randomly chosen periods, as before.

The Control:

The Control colony, Colony C_1, controlled for the effect of physical removal of a portion of the colony workforce, and for seasonal changes in task demand, or organisation. Colony C_1 was treated identically to the experimental colonies during marking and session 1 of the experiment. At the start of session 2, the colony was allowed to emigrate to a new nest. During this emigration approximately one third of the workers (the first third to exit the original nest during the emigration) were removed (using a fine paintbrush) to a separate nest, in order to control for the effect of the physical act of removal of workers from the colony. After one week in the new nest (during which photographs of the original colony were taken at the same rate as for the other nests), the removed workers were returned to the foraging arena of the original nest. The colony was left to settle for 24 hours after which photographs continued to be taken at the same rate as for the experimental colonies for the remainder of session 2, and for sessions 3 and 4.

ii. Experiment 2 – 1998

The following experiment is an extension of experiment 1, and examines further the division of labour in *Leptothorax albipennis.* The procedure was similar to that of experiment 1, but with the addition that foreign, stained, conspecific brood was placed in the nests containing the subsidiary fractions of the colonies. This experiment examined further the flexibility of worker behaviour and the degree to which the tasks performed changed, depending on the requirements of the colony. It is hypothesised that some individuals associated with external activity in the subsidiary colony were sufficiently flexible to change their behaviour from that of performing external tasks, behaviour classically associated with older workers, to nurse work, classically associated with the youngest workers. This experiment also examined how behavioural reversion, if it occurred, affected the organisation of tasks when the fractions of the colony were re-united. The experiment also examined spatial organisation within the colony.

Preliminary tests on the effect of foreign, stained brood showed that workers did not discriminate between stained, foreign brood and their own un-stained brood, and there was a Control in experiment 2 to test for this. Foreign, conspecific brood was used instead of brood taken from the original colonies in order to avoid disruption to the original fractions of the colony.

The experiment was divided into four *sessions*, each of fifteen days duration.

The first session consisted of observations of un-manipulated colonies.
During session two, external workers were removed sequentially from the colony into a new nest in a separate arena according to the sampling procedure outlined below. Stained, foreign brood had been placed on top of the nest to which externals were removed.
During session three these '*subsidiary*' and '*original*' fractions of the colonies were maintained separately.

In the fourth session, the colonies were re-united (without the dyed, foreign brood in order to keep task demand in the original colony constant) and maintained for the duration of the session.

Data obtained by the procedures above, were used to investigate the following:

What is the extent of colony plasticity and individual flexibility when colonies are divided on the basis of behavioural task?

Does the removal of individuals associated with external activity induce the remaining individuals in the colony to exhibit precocious external activity?

Do removed individuals experience behavioural reversion and become associated with nurse-work?

What happens when the fractions of the colony are re-united? If workers re-specialise following sociotomy, do they revert to their original role, or do they maintain their new role after re-unification?

What is the extent of spatial organisation within such colonies and what role does spatial organisation play in the division of labour, efficiency and flexibility?

Are tasks spatially organised?

Do colonies exhibit social resilience? What is the spatial structure of fractions of colonies, divided on the basis of behavioural task?

When the fractions are re-united, do workers re-organise their relative spatial distribution to that observed prior to sociotomy, or do they maintain the spatial organisation observed when they existed as separate fractions?

Does the queen play a significant role in the regulation of colony activities?

How is the behaviour of the queen, in terms of her spatial distribution and interactions, affected by sociotomy?

Does the absence of the queen in the subsidiary fraction during session 3, have any effect on the specialisation and social resilience of workers, despite the presence of brood in these fractions, and therefore indicate that she may play a role in the mechanisms of these processes?

How does the pattern of interaction among the workers change as a consequence of sociotomy?

How are any patterns of interaction among the workers affected by the absence of the queen in the subsidiary fractions during session 3, of the colony, despite the presence of brood?

What are the implications of any patterns in interactions for processes of task allocation, in the presence, and in the absence of the queen?

Procedure

Colonies 5, 6, 7, and 8 were manipulated experimentally, Colony C_2 was the Control.

Session 1:

Procedure: No experimental treatment was carried out on the colonies.

Photography: The day was divided into 8 periods from 9am to 5pm. Using a random number generator 4 of these 8 periods were selected for each of fifteen days that made up the session. For each selected period a photograph was taken of the inside of the nest of each experimental colony (and the Control).

Session 2:

Procedure: On day 1 of session 2, at 9am, Experimental Colonies 5 and 6 were simultaneously observed for 50 minutes. Any workers that ventured outside the nest were removed to new nests using a fine paintbrush just before they re-entered the nest. These workers were classed as 'externals'. In the last 10 minutes of this hour the identities of the removed workers were recorded, and the time at which they were removed was recorded as period 1, session 2. From 10am till 10.50am, Colonies 7 and 8 were observed and manipulated in the same way, and the identities recorded in the last 10 minutes, the time also recorded as period 1, session 2. The experimental colonies were observed and manipulated in this way, for a total of 3 hours a day each (i.e a total of 6 hours of observation). The time and identities were recorded in a similar way (as period 1-3 on each day). This was continued for 3 weeks, the colonies were alternated each day, so the 2 colonies that were observed in the first hour on day 1, were observed in the second hour on day 2.

On the last day of session 2 approximately half the brood (stained) from a donor colony was removed and placed on top of the upper slide in the subsidiary nest of an experimental colony. This was done for each of the subsidiary nests of the 4 experimental colonies. It was ensured that there was no bias towards particular types of brood.

Photography: No photographs were taken during session 2.

Session 3:

Procedure: The original and subsidiary fractions of each colony were maintained in different nests.

Photography: Photographs were taken of both fractions in the same way as in session 1.

Session 4:

Procedure: On day 1 of session 4, each subsidiary nest was opened and the stained foreign brood removed and discarded. The bottom slide of the subsidiary nest was placed on top of the nest in the original colony allowing externals to re-emigrate back into the original colony. Any workers that were in the foraging arena in the subsidiary colonies were removed and placed in the foraging arena of the original nest, into which they then readily emigrated. The colonies were given 24 hours to settle.

Photography: After the initial 24 hour period, photographs were taken in the same way as in sessions 1 and 3, for the duration of session 4.

Control:

Four days prior to the start of the experiment, the Control colony was allowed to emigrate to a new nest. During the emigration, approximately half the brood was also removed at this stage, in order to maintain the original ratio of workers to brood when the stained, foreign brood replaced it.

At the beginning of session 1 approximately half the stained brood was removed from one of the donor colonies and placed on top of the nest of the control colony. When removing brood it was ensured that there had been no bias towards brood of particular types. During session 1 and sessions 3 and 4, the Control colony was photographed as for the experimental colonies. This controlled for the effect of stained, foreign brood on the colony.

On day 1 of session 2, the Control colony was allowed to emigrate to a new nest. During this emigration approximately the first third of the workers to leave the original nest were removed. The fractions of the nest were maintained for one week. The workers in the subsidiary nest were then replaced on top of the original nest and the colony was maintained for the remainder of the duration of session 2. The colony was photographed in the same way as the experimental colonies for the duration of sessions 3 and 4. Colony C_2 therefore also controlled for the physical effect of the temporary removal of a portion of the workforce.

During the experiment, the queen in Colony C_2 died. However, this did not occur until after the end of session 1, and the colony therefore still served as a control for the effects of stained foreign brood in terms of the subsequent organisation of the workers. This is discussed further in Chapter 4. The remaining results from this control should be disregarded (they are given for the sake of completeness), as they may have been affected by the loss of the queen, or any subsequent senescence of this colony. Colony C_1, (from experiment 1), serves as an adequate control for the remainder of the experiment. The results from the experimental colonies in experiment 2 are therefore compared to this control, Colony C_1.

The experiment yielded 60 slides per experimental colony in sessions 1 and 4, and 60 slides of each of the original and subsidiary colonies in session 3. The Control colony yielded 60 slides during sessions 1, 3 and 4 of the experiment. The experiment yielded a total of 1140 slides.

iii. Experiment 3 – 1998

The following experiment is a further examination of the division of labour in *Leptothorax albipennis*. The colonies were again divided into fractions, but this time, on the basis of the age of the workers, rather than directly by their behavioural task. The aim was to examine whether a colony of callows could establish a stable division of labour, and if they could, to examine its properties in terms of individual behaviour and spatial organisation. The overall plan of the experiment was basically similar to experiments 1 and 2, except that there was no initial session where the colonies had not been divided. The colony was divided before the callows actually eclosed so that they had no experience of being in a colony in its original state (it was necessary to put fifteen older workers in the callow fractions initially to aid the eclosion of the callows, but these were removed one week after the majority of the pupae had eclosed). The two fractions of the colonies ('callow' and 'older') were later united, and further data were collected at this stage.

The experiment was divided into three *sessions*, each of fifteen days duration.

Session 1 began after the colonies had been separated into 'callow' and 'older' fractions, maintained for 2 weeks, then opened and marked. Session 1 consisted of fifteen days of observations on the separate fractions of the colonies.
Session 2 began a month after session 1 finished. It consisted of fifteen days of observations on the separate fractions of the colonies. This session was included in order to give the fractions of the colonies sufficient time to maintain a stable division of labour.
At the beginning of session 3 the fractions of the colonies were re-united, and maintained for the duration of the session.

Data were obtained by the procedures described in detail below, and used to investigate the following:

What is the extent of colony plasticity and individual flexibility when colonies are divided on the basis of age?

> Can an artificially constructed colony of callow individuals establish and maintain a viable division of labour, despite the absence of the queen? Do some of these individuals carry out precocious external activity?
>
> If the callow cohort is removed from a colony, do the remaining individuals experience behavioural reversion and become associated with nurse-work?
>
> When the fractions are united, do workers re-organise their relative spatial distribution to that observed in the Control colony that has not undergone sociotomy, or do they maintain the spatial organisation observed when they existed as separate fractions?

What is the extent of spatial organisation within such colonies and what role does spatial organisation play in the division of labour, efficiency and flexibility?

> Are tasks spatially organised?
>
> Do colonies exhibit social resilience? What is the spatial structure of fractions of colonies, divided on the basis of age?
>
> When the fractions are united, do workers re-organise their relative spatial distribution to that observed in the Control colony, that has not undergone sociotomy, or do they maintain the spatial organisation observed when they existed as separate fractions?

Is the pattern of the interactions of callow workers with the queen in the united fractions the same as the patterns observed for the callows in the

Control colony, which has not undergone sociotomy?

How does the pattern of interaction among the workers change as a consequence of sociotomy?

How are any patterns of interaction among the workers affected by the absence of the queen in the subsidiary fractions during session 3, of the colony, despite the presence of brood?

What are the implications of any patterns in interactions for processes of task allocation, in the presence, and in the absence of the queen?

Procedure

Colonies 9, 10, 11, and 12 were manipulated experimentally, Colony C_3 was the Control.

The experiment began when there were a large number of mature pupae present in each of the nests, but no callows had eclosed (any callows that did eclose were removed from the experiment).

On day 1, each of the four experimental colonies was emigrated. During the emigration the first 15 older workers to emerge from a nest were removed to a petri dish containing a new nest. The mature pupae were also all removed to the new nest. The remainder of the experimental colony was then allowed to emigrate to a second new nest. The subsidiary colonies (henceforth 'callow' colonies) were maintained.

After almost all the pupae in the callow colonies had eclosed, a fraction of the brood in the original colonies, approximately proportional to the size of the callow fractions compared to the original fractions, was placed on the top slide of each of the callow nests. The colonies were left for a further week, allowing the workers to bring the brood into the callow nests. After this week, the 15 older workers were removed from each colony and discarded. The callow colony was maintained to determine if the callows established a division of labour (indicated by some of them starting to perform external activities, for example, foraging). This took two weeks.

The nests containing the callow colonies were then opened and each individual was uniquely marked according to the scheme outlined in section 3.2ii.❸. The colonies were allowed to emigrate to new nests and left for 48 hours to 'settle'. The nests with the original ('older') fractions of the colonies were also opened and marked in the same way, after which they were allowed to emigrate to new nests, and left to settle for 48 hours.

Session 1:

Procedure: The 'older' and 'callow' fractions of each colony were maintained separately for the duration of session 1.

Photography: Photography now began at the rate of 4 slides (both original and subsidiary fractions) per day. The photographs were taken at randomly chosen intervals during the day, as in experiments 1 and 2, for the duration of session 1.

Session 2:

Procedure: One month was allowed to elapse after the end of session 1, during which the separate fractions of the colonies were maintained in the usual way. After this period session 2 began. The separate fractions of the colonies were maintained for the duration of session 2.

Photography: Photographs were taken at randomly chosen intervals during the day as before, for the duration of session 2.

Session 3:

Procedure: The nests containing the 'callow' fractions of the colonies were opened and the bottom slide placed on top of the corresponding nest containing the 'older' fraction. The colonies were left for 48 hours, during which the callows readily emigrated into the original nest, together with the brood. The united colonies were then maintained for the remaining duration of session 3.

Photography: Photographs were taken at the same rate, as in sessions 1 and 2, for the remaining duration of the session.

Control:

Colony C_3, the Control was maintained until the stage in the experiment where 'callow' fractions of the colonies were marked. At this stage the Control colony was also opened and marked, then allowed to emigrate to a new nest. It was possible to discriminate between callows and adults at this stage due to the callows lighter pigmentation. At the beginning of session 1, photography began at the same rate as for the experimental colonies. Photography continued for the duration of session 1, and for the duration of sessions 2 and 3.

Figure 9, below, is a diagrammatic representation of the methods of all three experiments, which can be used as a quick reference if required.

iv. Summary of Methodology for Quick Reference

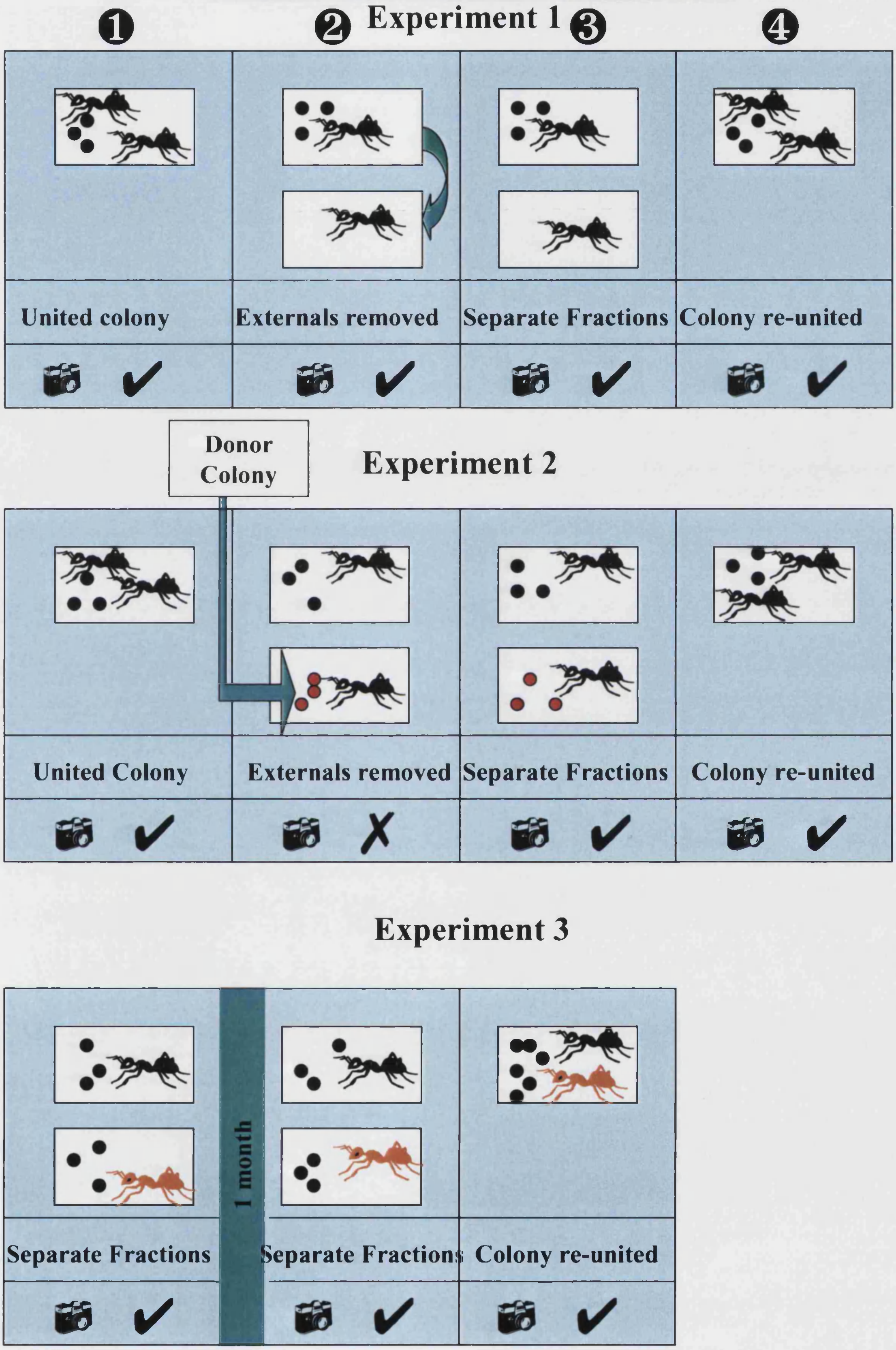


Figure 9: Methods Summary, indicating main events in each session of the three experiments

Chapter 4

An Investigation of the Spatial Organisation of Brood-Related Tasks and External Activity

The aim of the following chapter is to determine the extent of spatial organisation of brood-related, and external tasks, in colonies of *Leptothorax albipennis*. I investigate spatial organisation of these tasks during all sessions of experiments 1, 2 and 3 to determine the effect of sociotomy. The results are compared to the Control colonies in each case.

Analysis is carried out as follows (see section 3.2iii for further details):

The frequency at which individual workers perform brood-related tasks, or external activity, is determined during each session

> The performance of a brood-related task is established by interaction between an item of brood and a worker, defined as contact between the mouthparts of a worker and the item of brood determined from a photograph during that session.
>
> The performance of external activity is defined as the occurrence of a worker outside the nest, or in the entrance tunnel of the nest, when a photograph was taken.

The median position of each individual is calculated as the median of its distribution of distances from the centre of the brood pile.

> The centre of the brood pile is calculated as the mean of the co-ordinates of the eggs and microlarvae.
>
> This position is referred to as the 'colony centre'.

The relationship between the frequency at which individuals perform these tasks, and their median distance from the centre of the brood pile is examined by plotting graphs of these variables for the Control colonies, and for example experimental colonies. The centre of the brood pile is represented by the origin of each graph.

I analyse the relationship between the log of the frequency at which individuals carry out brood-related tasks, and their median distance from the colony centre, using linear regression analysis, for some example colonies. However, the data does not meet all the assumptions for type I linear regression analyses, and I therefore choose to examine correlations between the variables. This is the main tool of analysis, both in this chapter, and in subsequent chapters. Other methods of statistical analyses are introduced in further chapters as required. Correlation analyses are carried out as follows:

Individuals are ranked with respect to the frequency at which they carry out brood-related, or external tasks, and with respect to their median distance from the colony centre.

I calculate the Spearman's rank correlation coefficient for the relationship between these two rankings. In all cases where $n > 10$, the relationship is tested as an ordinary product-moment correlation coefficient (Sokal and Rohlf, 1981 p. 607). Where $n \leq 10$, the Spearman's rank correlation coefficient is calculated according to the following formula:

$$\boxed{r_s = 1 - \frac{6 \sum d^2}{n(n^2 - 1)}}$$

In the graphs of the relationship between frequency at which individuals perform the behaviours and their distance from the colony centre during sessions 3 and 4, of experiments 1 and 2, individuals that were removed as 'externals' during session 2 are represented in red. Other individuals are represented in black.

In the graphs of these relationships during sessions 1, 2 and 3, of experiment 3, including the Control colony, callows are represented in red, whereas older individuals are represented in black.

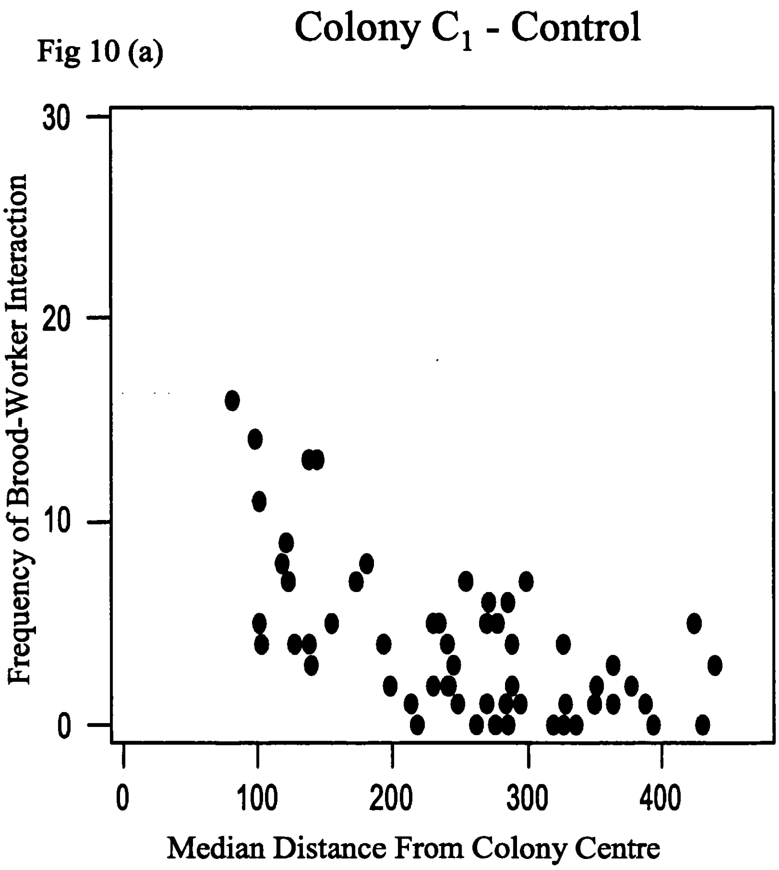
4.1. An Investigation of the Spatial Organisation of Tasks in Colonies with a Manipulated Task Structure

i. The spatial organisation of brood-related tasks

Experiment 1

The graphs below show the relationship between the frequency at which individual workers perform brood-related tasks and the median position of the workers in the colony during session 1, when colonies are un-manipulated, in experiment 1. The Control colony (Colony C_1), and an example of one of the experimental colonies (Colony 3), are shown.

Fig 10 (a)



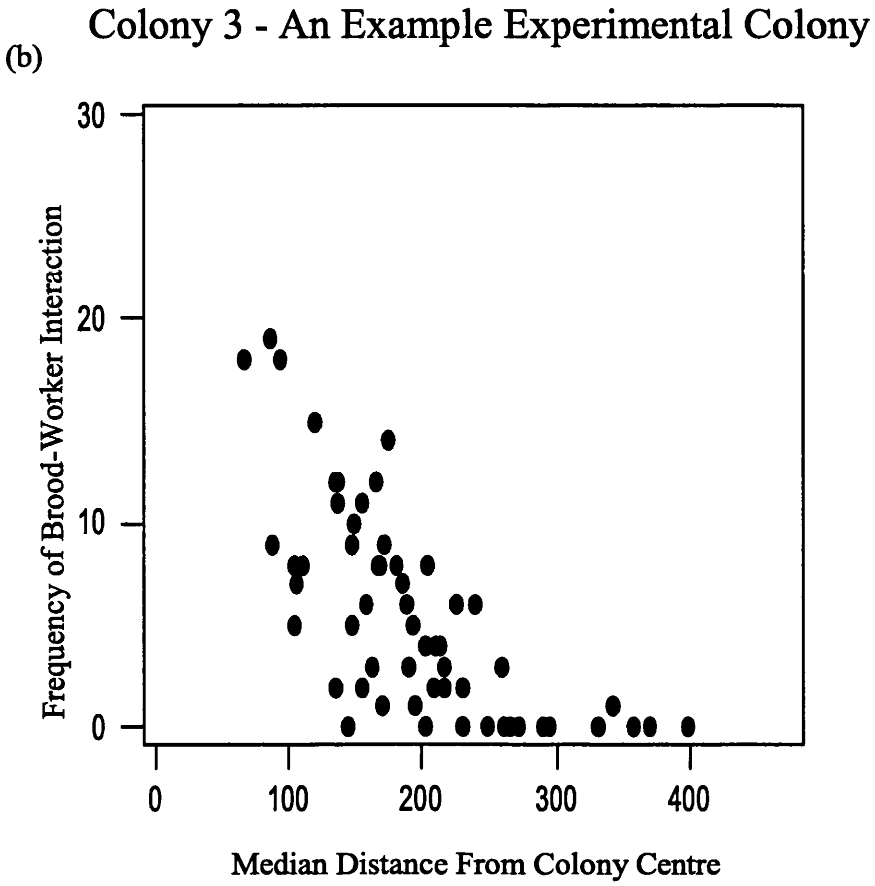


Figure 10 : (a) The relationship between the frequency of brood-worker interaction and the median distance from the colony centre for Colony C_1 (the Control), session 1; and (b), Colony 3, an example experimental colony.

The frequency of performance of brood-related tasks decreases as median distance from the brood pile increases for the Control colony, and for all the experimental colonies. A typical experimental plot is shown in Figure 10 (b), above. Workers with a median position above a certain threshold distance from the brood pile perform brood-related tasks at a frequency of zero, or very close to zero.

When the frequency at which individuals perform brood-related tasks is logged and plotted against their median distance from the centre of the colony, see Figures 11 (a) and (b), below, a linear regression model fits the data well.

Control (Colony C_1) :

log *f* brood-related tasks = 1.07 - 0.00222 Median distance from colony centre

$R^2 = 34.2\%$; df = 47; $P < 0.01$

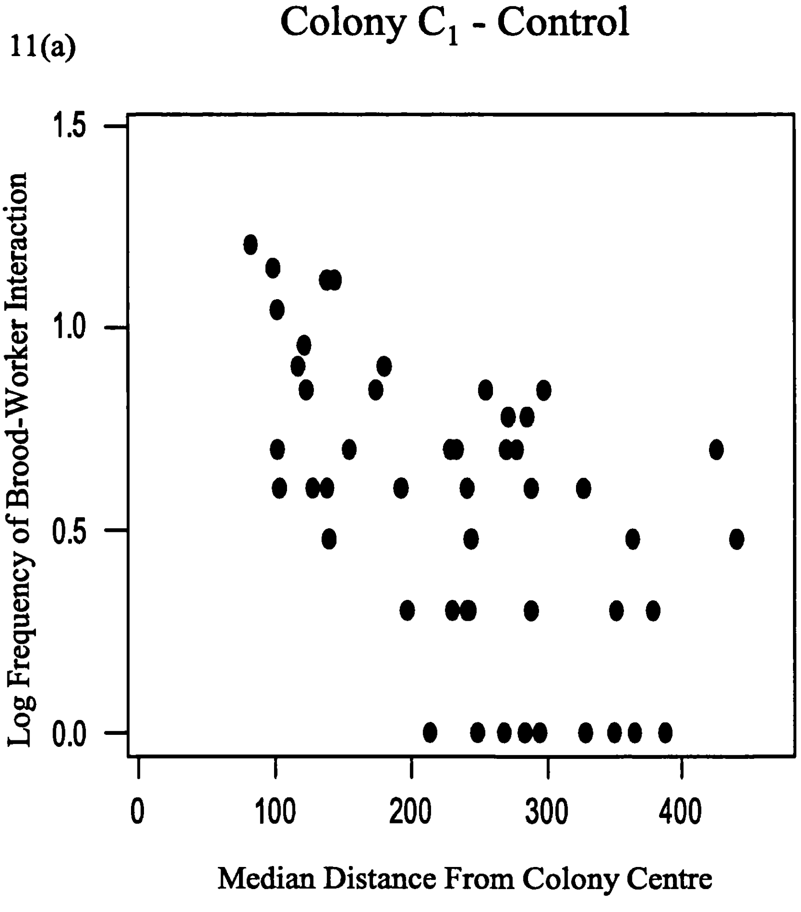
Colony 3:

log *f* brood-related tasks = 1.44 - 0.00413 Median distance from colony centre

$R^2 = 39.9\%$; df = 43; $P < 0.01$

Therefore the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre closely approximates an exponential decay.

Fig 11(a)



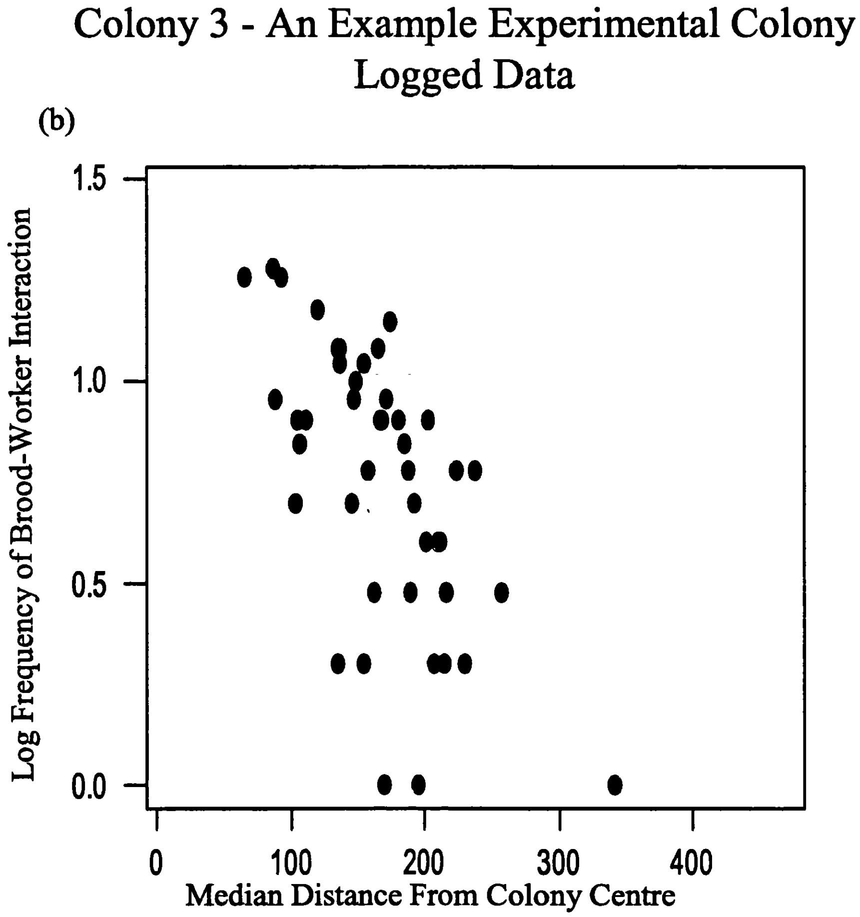


Figure 11 : The relationship between log frequency of brood-worker interaction and the median distance from the colony centre for (a) Colony C_1 (the Control); and (b) Colony 3, an example experimental colony.

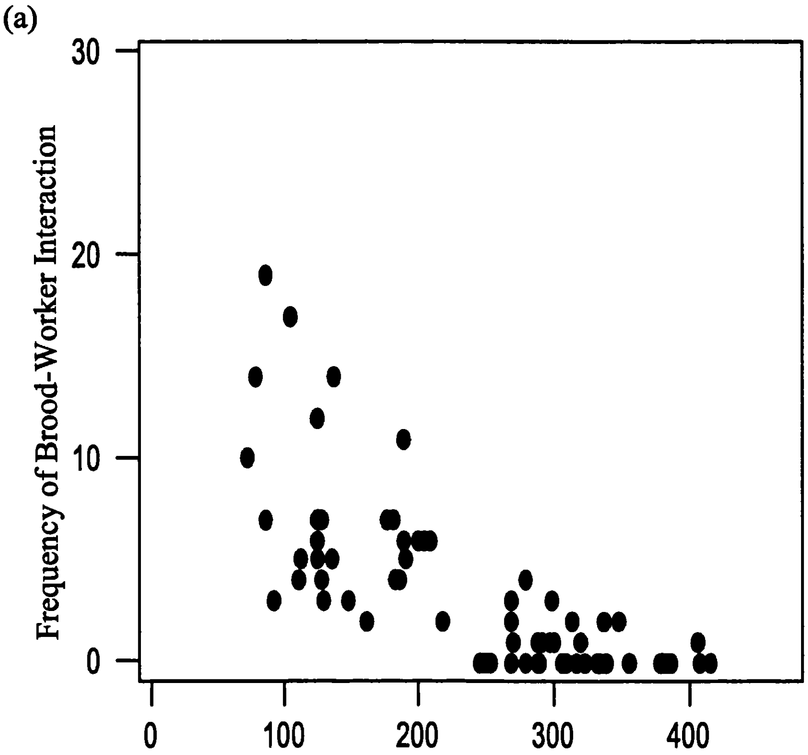
Experiment 2

A similar relationship between the frequency at which brood-related tasks are performed and the position of the individual in the colony is established for the Control colony, and the experimental colonies in session 1, experiment 2. A typical experimental plot is shown in Figure 12 (a), below. When the frequency of brood-related tasks is logged, Figure 12 (b), a linear regression model fits the data well.

log f brood-related tasks = 1.22 - 0.00318 Median distance from colony centre

$R^2 = 58.8$; df = 44; $P < 0.01$

Therefore the relationship for this colony also closely approximates an exponential decay.


Colony 8 - An Example Experimental Colony

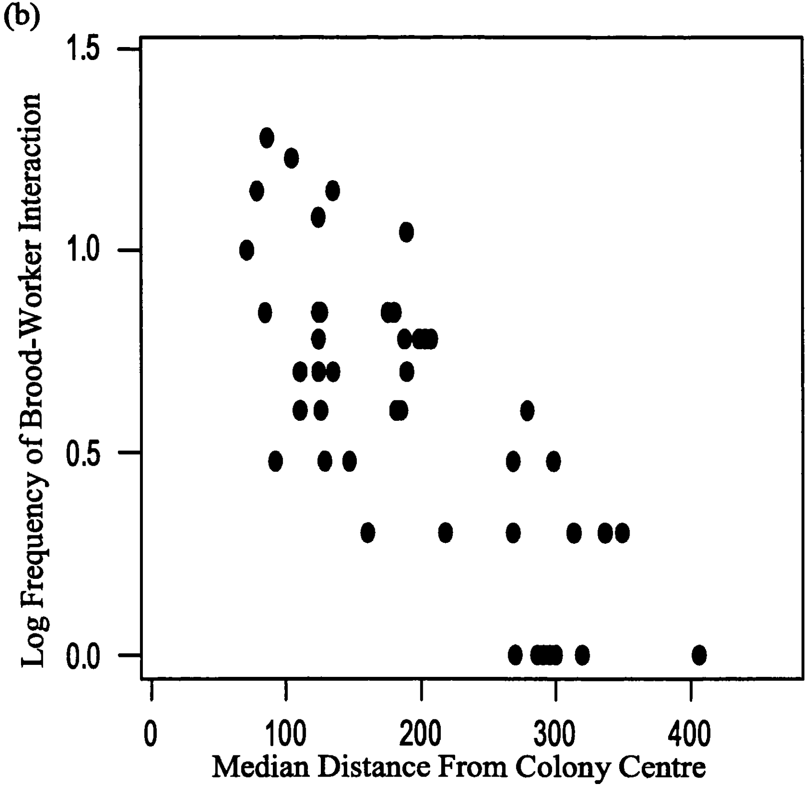

Colony 8 - An Example Experimental Colony
Logged Data

Figure 12 : (a) The relationship between frequency of brood-worker interaction and the median distance from the colony centre; and (b) log frequency of brood-worker interaction and the median distance from the colony centre, for Colony 8, an example experimental colony.

The relationship between the frequency at which brood-related tasks are performed and spatial position is further examined by carrying out correlation analyses according to the procedure described at the beginning of this chapter. The results of these analyses are given in Table 1, below, for experiments 1 and 2, during session 1. There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative spatial position, for all the colonies.

Colony	**Session**	r_s	**n**	**Significance Level** $p<$
Experiment 1				
C_1	1	-0.596	58	0.01
1	1	-0.765	64	0.01
2	1	-0.873	68	0.01
3	1	-0.746	58	0.01
4	1	-0.821	52	0.01
Experiment 2				
C_2	1	-0.673	17	0.01
5	1	-0.587	34	0.01
6	1	-0.730	25	0.01
7	1	-0.781	57	0.01
8	1	-0.803	72	0.01

Table 1: The relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiments 1 and 2. Tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607). r_s *= Spearman's rank correlation coefficient*

What is the effect of sociotomy on the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre?

I examine the spatial organisation of brood-related tasks in sessions 2 and 4 of experiment 1, and session 4 of experiment 2, to determine whether the relationship is preserved throughout the experiments, despite removal of workers associated with external activity from the experimental colonies, in session 2, and their re-unification in session 4. Furthermore, I examine the spatial organisation of brood-related tasks in session 3, where colonies are maintained as two separate fractions - the original fraction, and the subsidiary fraction (consisting of the removed workers). The spatial organisation of workers with respect to brood-related tasks in the subsidiary fractions, during session 3, can only be examined in experiment 2, as foreign brood is provided. No brood is provided for the subsidiary fractions during session 3, in experiment 1.

Experiment 1

The graphs below show the relationship between the frequency at which individual workers perform brood-related tasks, and the median position of the workers in the colony during:

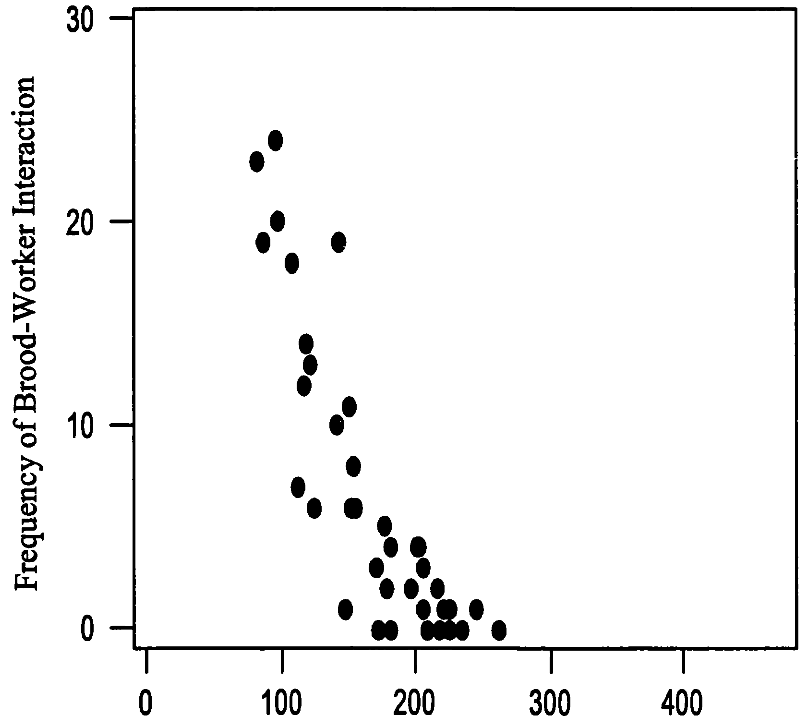
session 2, when workers associated with external activity are being removed sequentially from the experimental colonies;
session 3, when the original fractions of the colony are maintained separately from the removed individuals in the subsidiary fractions;
session 4, when the two fractions of the colonies are re-united.

The Control colony (Colony C_1), and an example of one of the experimental colonies (Colony 3), are shown in Figures 13, 14, and 15.

Session 2

Colony C_1 - Control

(a)



Colony 3 - An Example Experimental Colony

(b)

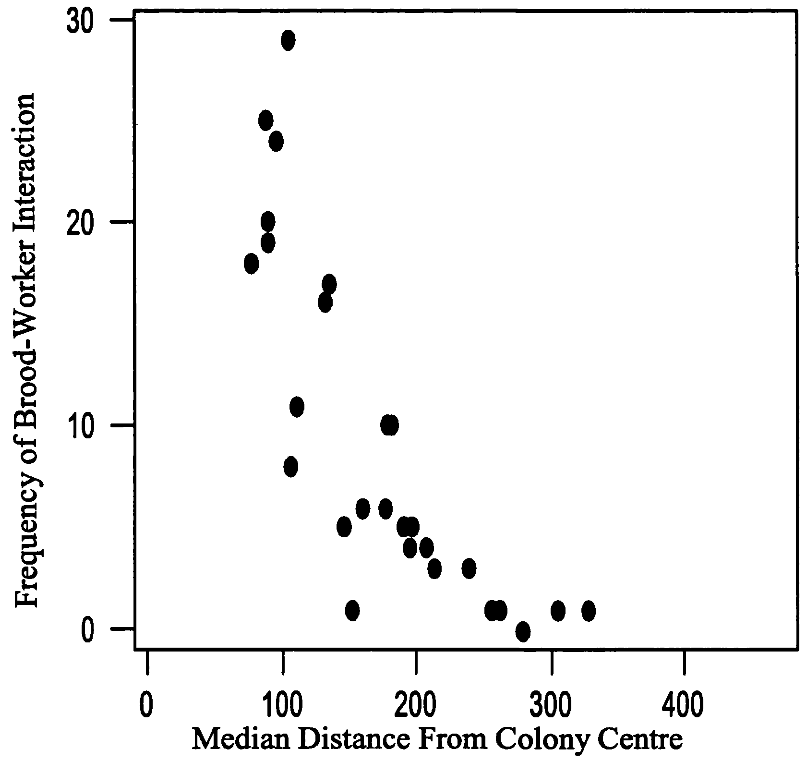
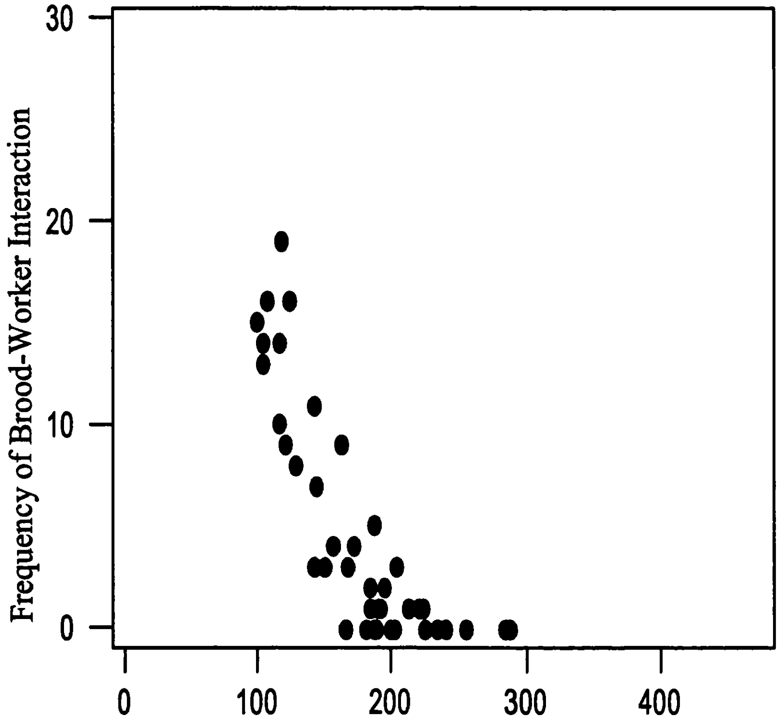


Figure 13: The relationship between the frequency of brood-worker interactions and the median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, for session 2..

Session 3

(a) Colony C_1 - Control



Colony 3 - An Example Experimental Colony

(b) Original Fraction Only

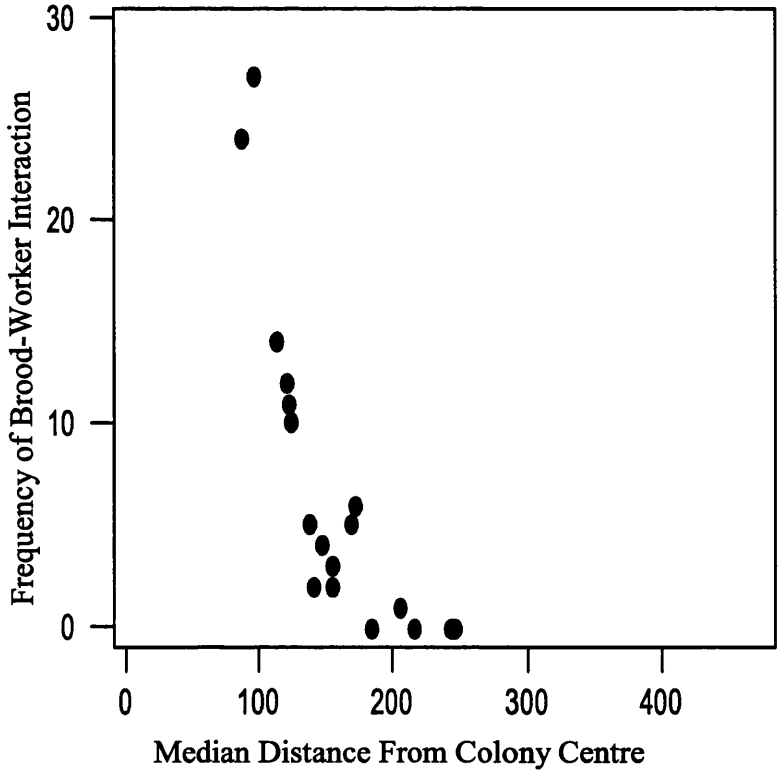
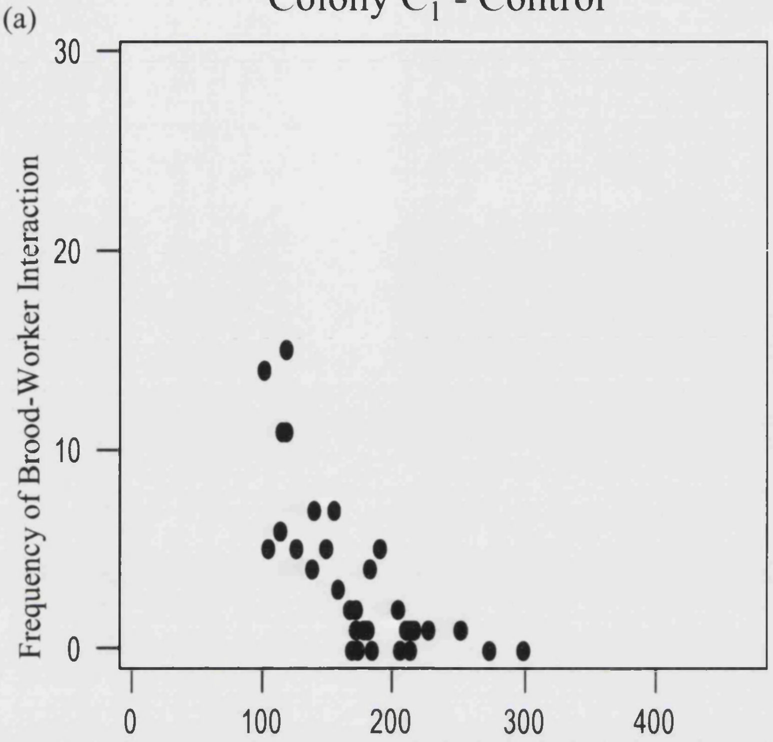


Figure 14: The relationship between the frequency of brood-worker interaction and the median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, for session 3.

Session 4

Colony C_1 - Control



Colony 3 - An Example Experimental Colony
Re-united Fractions

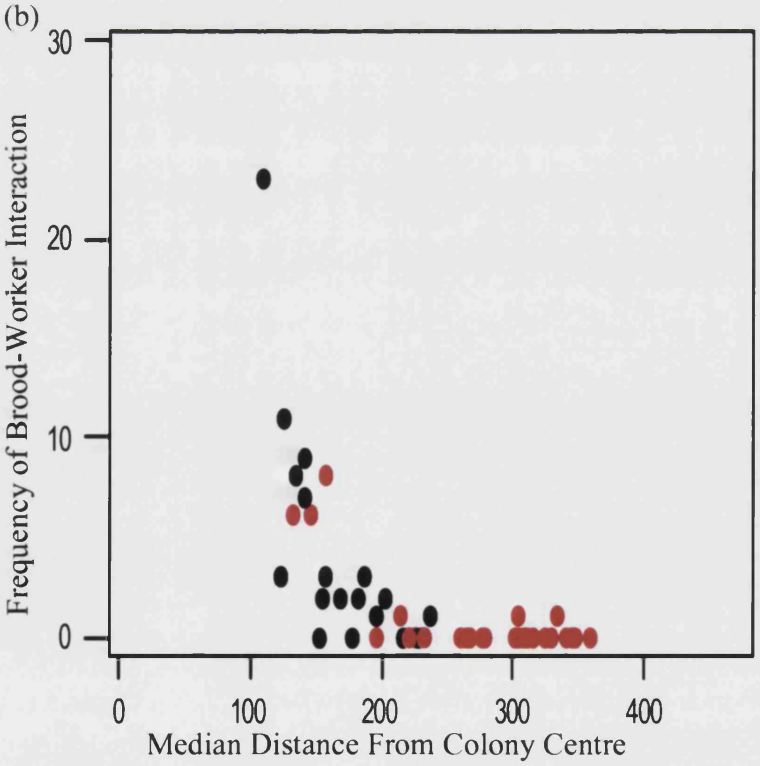


Figure 15: The relationship between the frequency of brood-worker interaction and the median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, for session 4. Individuals that were in the subsidiary fraction of the colony during session 3 are shown in red, individuals that were in the original fraction, in black.

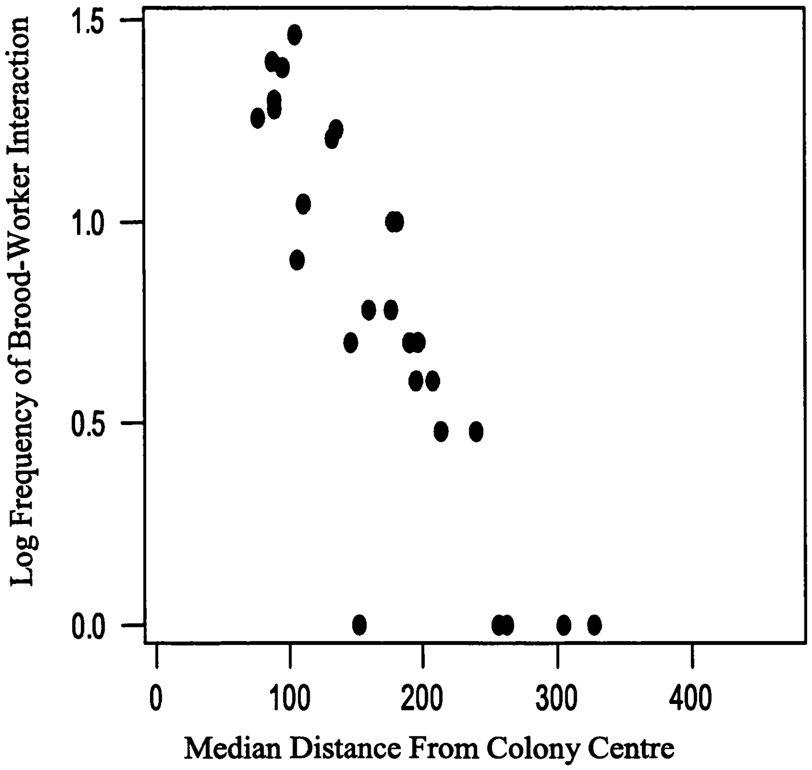
Logged Data

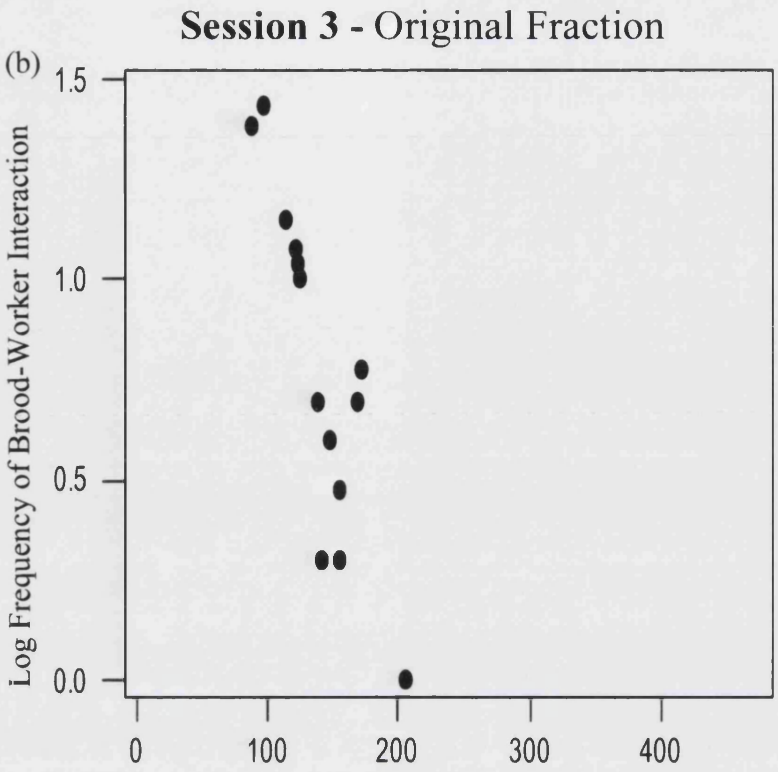
When the frequency at which individuals perform brood-related tasks is logged and plotted against their median distance from the centre of the colony, sessions 2, 3 and 4 also typically show a close approximation to exponential decay, as is shown for an example experimental colony in Figure 16, below.

Colony 3 - An Example Experimental Colony

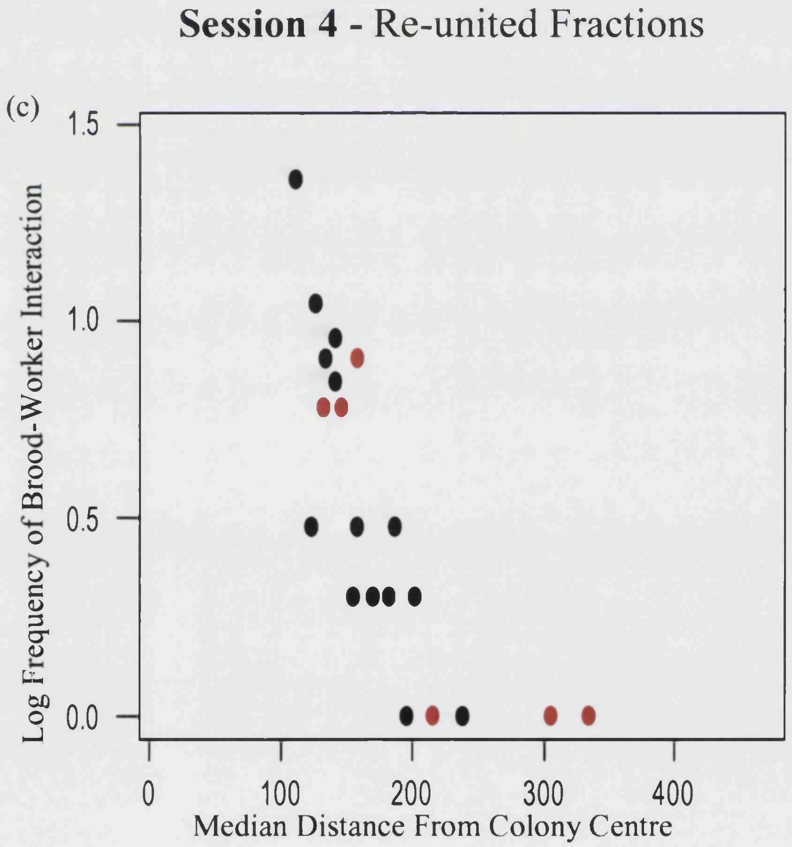
Fig 16(a)

Session 2





Session 3 - Original Fraction



Session 4 - Re-united Fractions

Figure 16: The relationship between logged frequency of brood-worker interaction and the median distance from the colony centre for Colony 3, an example experimental colony, for sessions (a) 2; (b) 3 (original fraction only); and (c) 4. Individuals that were in the subsidiary fraction of the colony during session 3, are shown in red, individuals that were in the original fraction, in black in (c).

The results of correlation analyses on these data are shown in Table 2, below, for experiments 1 and 2. There is a significant negative correlation between the relative frequency at which brood-related tasks are performed and relative median distance from the colony centre, for all the colonies in experiment 1, for session 2, session 3 (original fraction) except for colony 4, and session 4.

Colony/session	r_s	n	Significance level p<
Colony C_1			
Session 2	-0.876	40	0.01
Session 3	-0.846	40	0.01
Session 4	-0.774	33	0.01
Colony 1			
Session 2	-0.913	23	0.01
Session 3 – original	-0.792	8	0.05
Session 3 - subsidiary	-	-	-
Session 4	-0.865	30	0.01
Colony 2			
Session 2	-0.917	29	0.01
Session 3 – original	-0.869	19	0.01
Session 3 – subsidiary	-	-	-
Session 4	-0.913	31	0.01
Colony 3			
Session 2	-0.894	27	0.01
Session 3 – original	-0.910	18	0.01
Session 3 – subsidiary	-	-	-
Session 4	-0.737	48	0.01
Colony 4			
Session 2	-0.787	18	0.01
Session 3 – original	-0.700	5	NS
Session 3 – subsidiary	-	-	-
Session 4	-0.844	22	0.01

Table 2: The relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiment 1, sessions 2, 3 and 4, calculated as Spearman's rank correlation coefficient (r_s), and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where $n \leq 10$
NS = non-significant.

Experiment 2

The graphs below show the relationship between the frequency at which individual workers perform brood-related tasks, and the median position of the workers in the colony during:

session 3, original fractions, from which individuals associated with external activity have been removed;

session 3, subsidiary fractions, in which removed 'externals' are maintained in a separate nest containing stained, foreign, conspecific brood.

session 4, when the two fractions of the colonies are re-united.

Figure 17 shows example plots of the relationship between the frequency at which brood-related tasks are performed by individuals, and their median distance from the colony centre, for example experimental colonies: Colony 7, during session 3 (a) original and (b) subsidiary fractions and (c) session 4; and Colony 8, during (d) session 4.

Colony 7 - An Example Experimental Colony

Fig 17(a)

Session 3 - Original Fraction

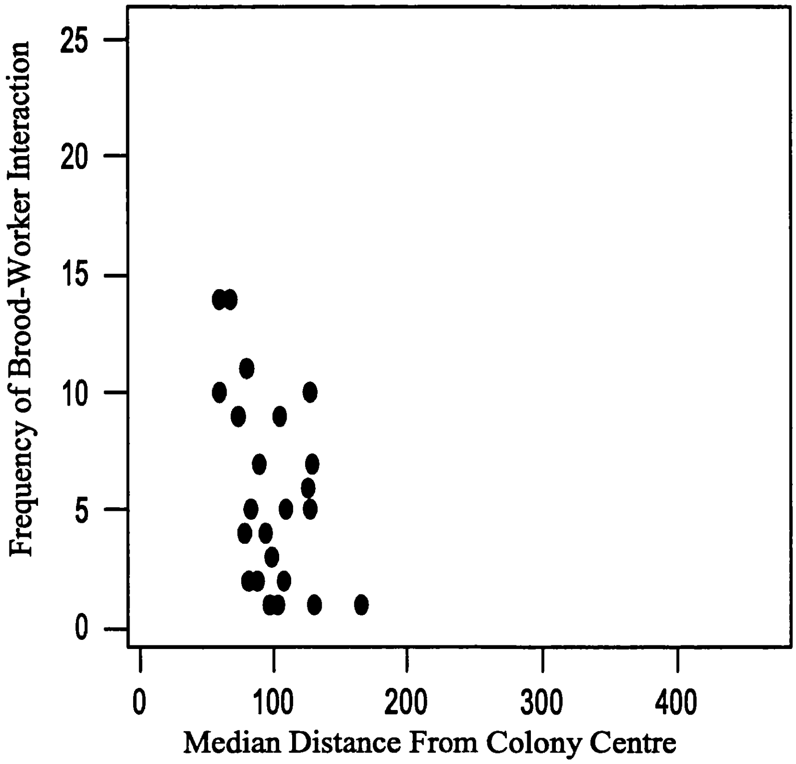
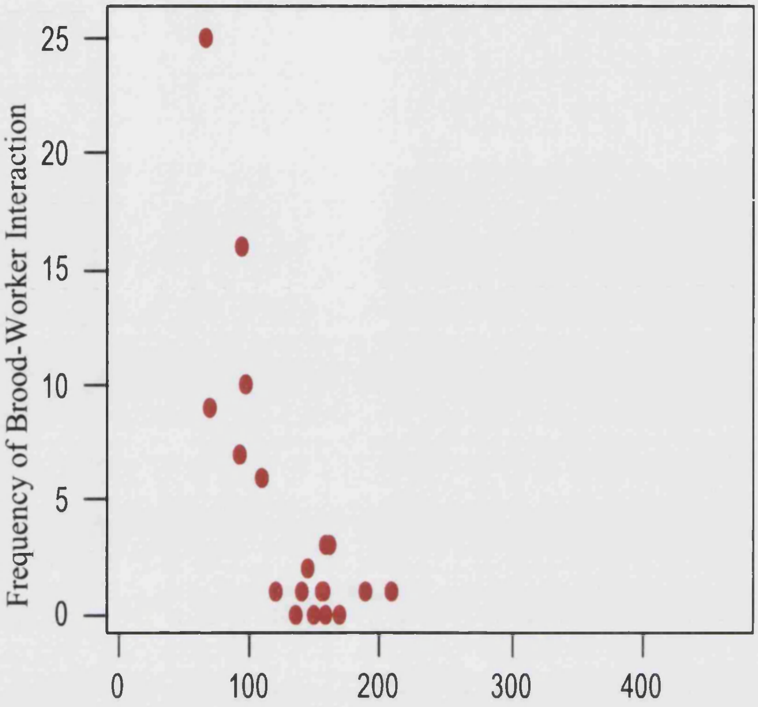
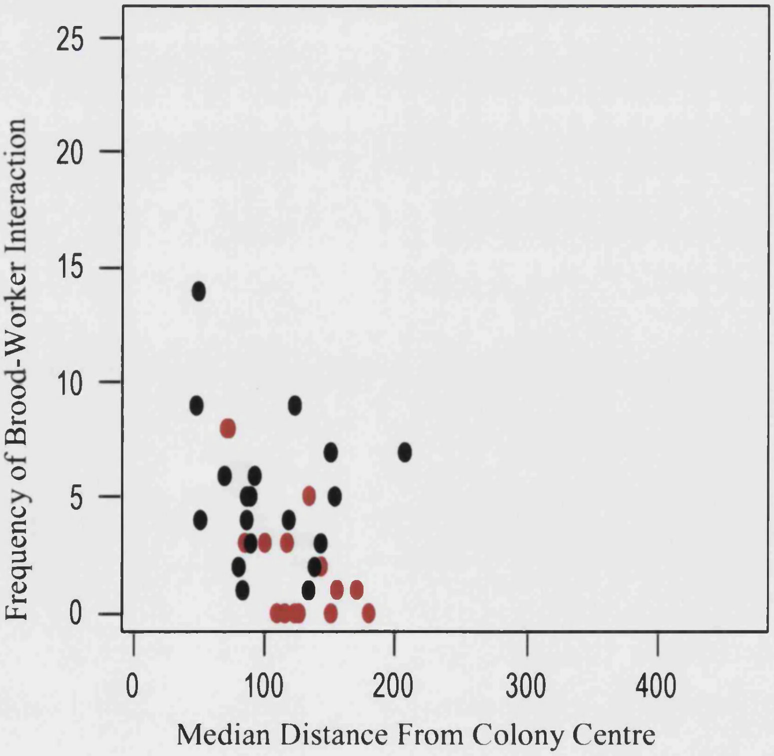


Fig 17 (b) **Session 3** - Subsidiary Fraction



Session 4 - Re-united Fractions

Fig 17(c)



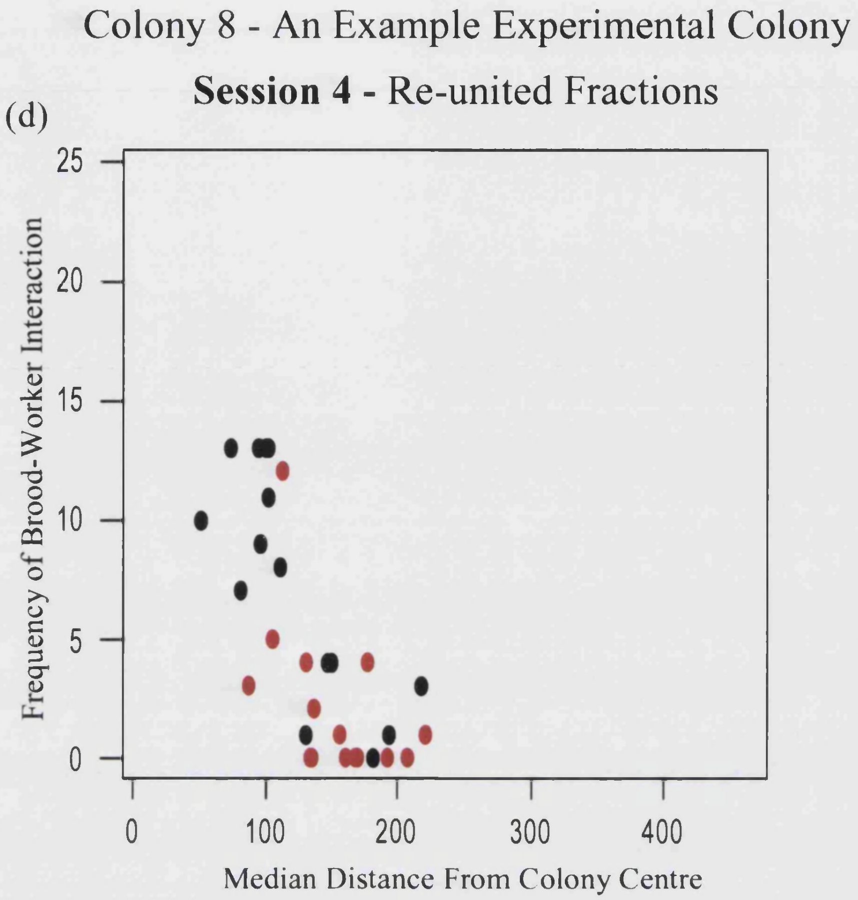


Figure 17: The relationship between the frequency of brood-worker interactions and the median distance from the colony centre for example experimental colonies: Colony 7, sessions (a) 3 (original fraction); (b) 3 (subsidiary fraction); and (c) 4; Colony 8, session (d) 4. Individuals that were in the subsidiary fraction of the colonies during session 3, are shown in red, individuals that were in the original fraction, in black.

The results of correlation analyses are shown in Table 3, below. There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks in the original fractions in session 3 and their relative median distance from the colony centre in the experimental colonies which contained sufficiently high numbers of individuals that have retained their marks. This is also the case in the subsidiary fractions, which contained stained, foreign brood, in session 3, (discussed further in section 4.3iii). There is also a significant negative correlation between the variables for these colonies during session 4, when the fractions of the colony are re-united.

Colony/session	r_s	n	Significance level p<
Colony C_2			
Session 3	-0.520	28	0.01
Session 4	-0.418	23	0.05
Colony 5			
Session 3 – original	-	-	-
Session 3 - subsidiary	-	1	-
Session 4	-	2	-
Colony 6			
Session 3 – original	-	1	-
Session 3 – subsidiary	-0.643	6	NS
Session 4	-0.866	7	0.05
Colony 7			
Session 3 – original	-0.443	23	0.05
Session 3 – subsidiary	-0.655	19	0.01
Session 4	-0.432	35	0.01
Colony 8			
Session 3 – original	-0.483	18	0.05
Session 3 – subsidiary	-0.663	19	0.01
Session 4	-0.732	31	0.01

Table 3: The relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiment 2, sessions 3 and 4, calculated as Spearman's rank correlation coefficient (r_s), and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where $n \leq 10$.

A Closer Examination of Session 4 - Re-united Colonies

Experiments 1 and 2

The spatial organisation of brood-related tasks in session 4, when the original and subsidiary fractions of the colonies are re-united is examined further. I calculate the Spearman's rank correlation coefficient separately for those individuals in session 4 that had remained in the original fraction of the colony during session 2, and for those that had been removed to the subsidiary fraction of the colony in session 2. The results are shown in Table 4, below.

Session 4 – Re-United Colonies				
Colony	**Fraction of Colony Individuals were in during Session 3**	**r_s**	**n**	**Significance Level p<**
Experiment 1				
1	Original	-0.714	6	NS
1	Subsidiary	-0.753	23	0.01
2	Original	-0.908	18	0.01
2	Subsidiary	-0.654	13	0.05
3	Original	-0.750	18	0.01
3	Subsidiary	-0.386	30	0.05
4	Original	-	4	-
4	Subsidiary	-0.872	18	0.01
Experiment 2				
5	Original	-	-	-
5	Subsidiary	-	-	-
6	Original	-	1	-
6	Subsidiary	-0.914	6	0.05
7	Original	-0.326	22	NS
7	Subsidiary	-0.239	13	NS
8	Original	-0.779	16	0.01
8	Subsidiary	-0.522	15	0.05

Table 4: The relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for re-united colonies in experiments 1 and 2, calculated separately for individuals in the original and subsidiary fractions of the colonies during session.3, calculated as Spearman's rank correlation coefficient (r_s), and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where $n \leq 10$. NS = non-significant. "-" indicates insufficient numbers to calculate r_s

There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative distance from the centre of the colony, for both subsets of individuals in session 4, in all the colonies except those in the original fraction in colony 1, and both subsets of Colony 7. These results are discussed in section 4.3v.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre between session 1 (un-manipulated colonies) and session 4 (after re-unification) I perform the following analysis.

1. I determine whether the correlation coefficients of the experimental colonies for the relationship in session 1, and in session 4, colonies can be considered samples from a population exhibiting a common correlation among the variables. The null hypothesis is that the *k* sampled correlation coefficients are homogenous. In effect I calculate the weighted sum of squares of the *z* values corresponding to the correlation coefficients. The sum of squares (χ^2) is calculated from the following equation:

$$\boxed{\chi^2 = \sum^{k} \left(\frac{z - z^{-}}{1 / \sqrt{n_{i-3}}} \right)^2 = \sum^{k} (n_i - 3)(z - z^{-})^2}$$

From Sokal and Rohlf (1995) p. 580

If I do not have sufficient evidence to reject the null hypothesis of homogeneity, I can consider the colonies to samples from a population exhibiting a common correlation.

2. I then compare the average *z* calculated for all colonies in session 1, with that of session 4 using a z transform. In this case, *n* is the mean number of individuals in the relevant colony and session that have retained their marks

The results of these comparisons are shown in Table 2a, below.

$\bar{z}$ session 1	$\bar{z}$ session 4	χ^2 session 1	Signif. level$_1$ p<	χ^2 session 4	Signif. level$_4$ p<	n_{mean1}	n_{mean4}	t_s	Signif. level p<
Exp 1									
-1.125	-1.216	5.48	NS	6.63	NS	57.5	29.8	0.38	NS
Exp 2									
-0.990	-0.723	4.32	NS	4.82	NS	44	21.3	-0.96	NS

Table 2a: Testing the relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiments 1 and 2, for combined colonies, compared between sessions 1 and 4. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for both sessions compared, and in both experiments. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled z for all the experimental colonies in session 1 and the pooled z for all the experimental colonies in session 4 (where colonies exhibit a significant correlational relationship between the frequency at which individuals perform brood-related tasks and their median distance from the centre of the brood pile), shows that there is no significant difference between the colonies before the manipulation and after it.

I also perform multiple comparisons between the pooled correlations for the relationship between the frequency at which individuals perform brood-related tasks in session 4 in the experimental colonies in experiment 1 compared to the pooled correlations for these relationships in the experimental colonies in experiment 2. The results are shown in Table 2b, below. The results indicate that there is no significant difference between the pooled correlational relationships. This is discussed further in Chapter 4.3v.

$\bar{z}_{exp1}$	$\bar{z}_{exp2}$	χ^{2exp1}	Signif. level$_1$ p<	χ^2_{exp2}	Signif. level$_2$ p<	n_{exp1}	n_{exp2}	t_s	Signif. level p<
Exp 1 and 2									
-1.216	-0.722	6.63	NS	4.82	NS	29.8	21.3	-1.629	0.05<0<0.1*

*Table 2b: Testing the relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiments 1 and 2, for combined colonies, compared between experiments for session 4. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables. * indicates one-tailed test.*

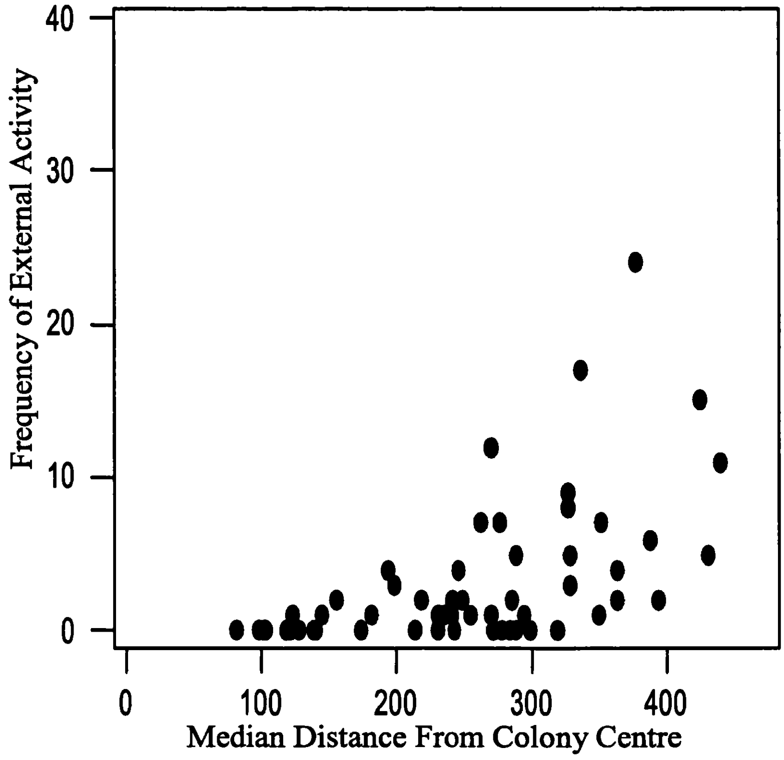
ii. The spatial organisation of external activity

Experiment 1

The graphs below show the relationship between the frequency at which individual workers perform tasks external to the nest and their median position in the colony during session 1, when colonies are un-manipulated, in experiment 1. The Control colony, Colony C_1, and an example of one of the experimental colonies, Colony 2, are shown.

Colony C_1 - Control

Fig 18(a)



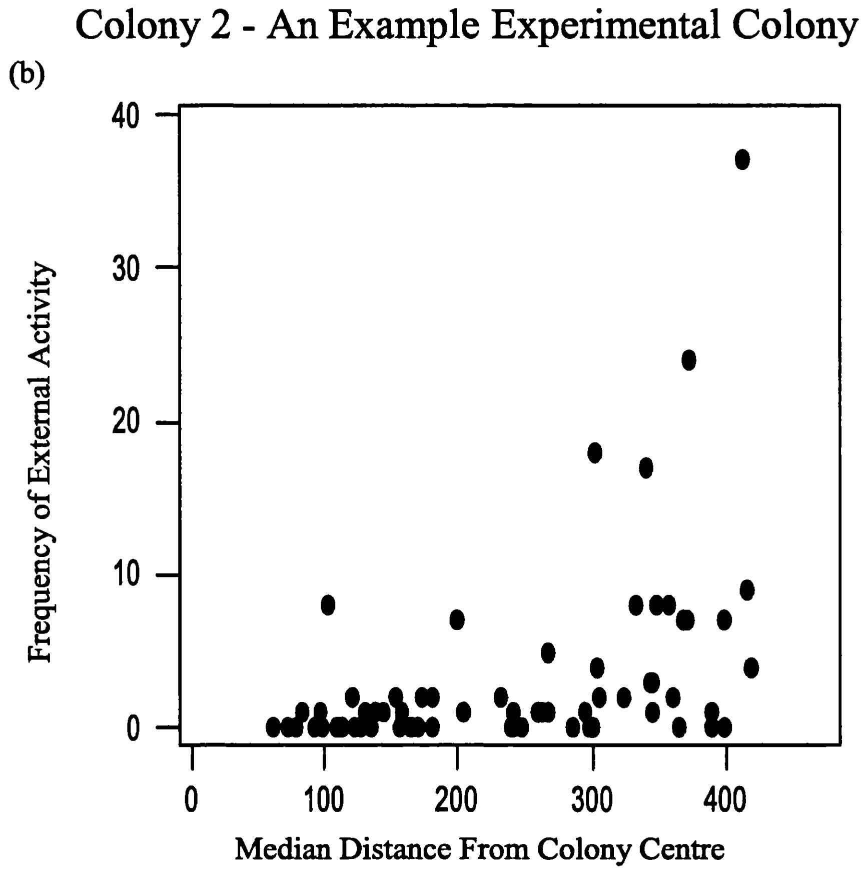


Figure 18 : The relationship between the frequency of external activity and the median distance from the colony centre in session 1 for (a) Colony C_1, the Control, and (b), Colony 2, an example experimental colony.

The frequency at which individuals perform external activity increases as median distance from the centre of the colony increases for the Control colony, and for all the experimental colonies. A typical experimental plot is shown in Figure 18 (b), above.

When the frequency at which individuals perform external activity is logged and plotted against their median distance from the centre of the colony, see Figures 19 (a) and (b), below, a linear regression model fits the data well.

Control (Colony C_1) :

log *f* external activity = - 0.337 + 0.00289 Median distance from colony centre

$R^2 = 32.0\%$; df = 35; $P < 0.01$

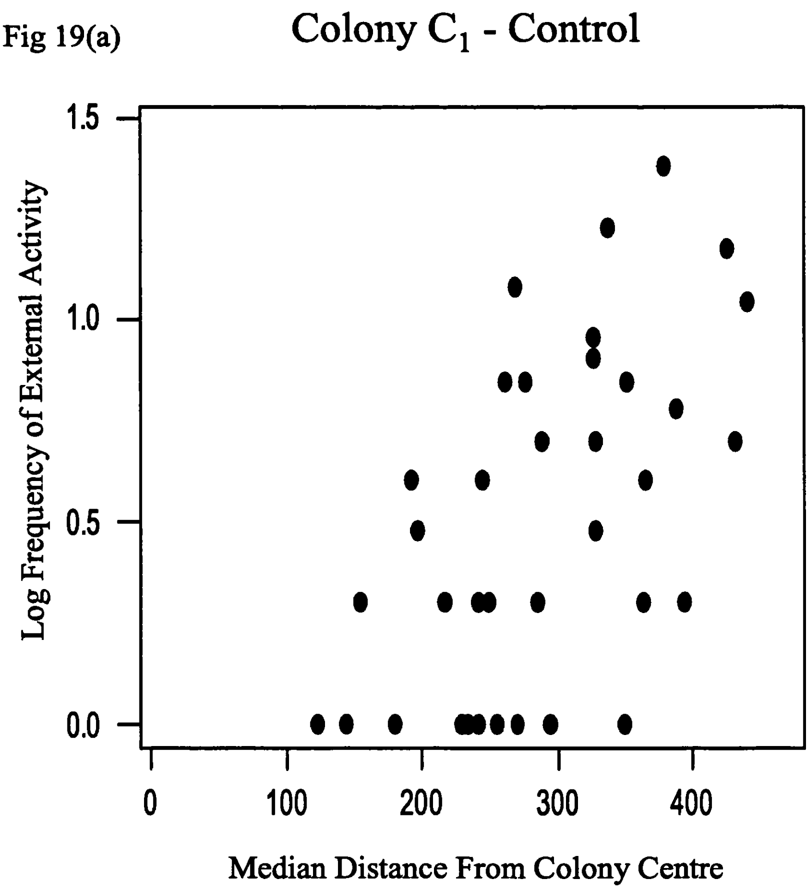
Colony 2:

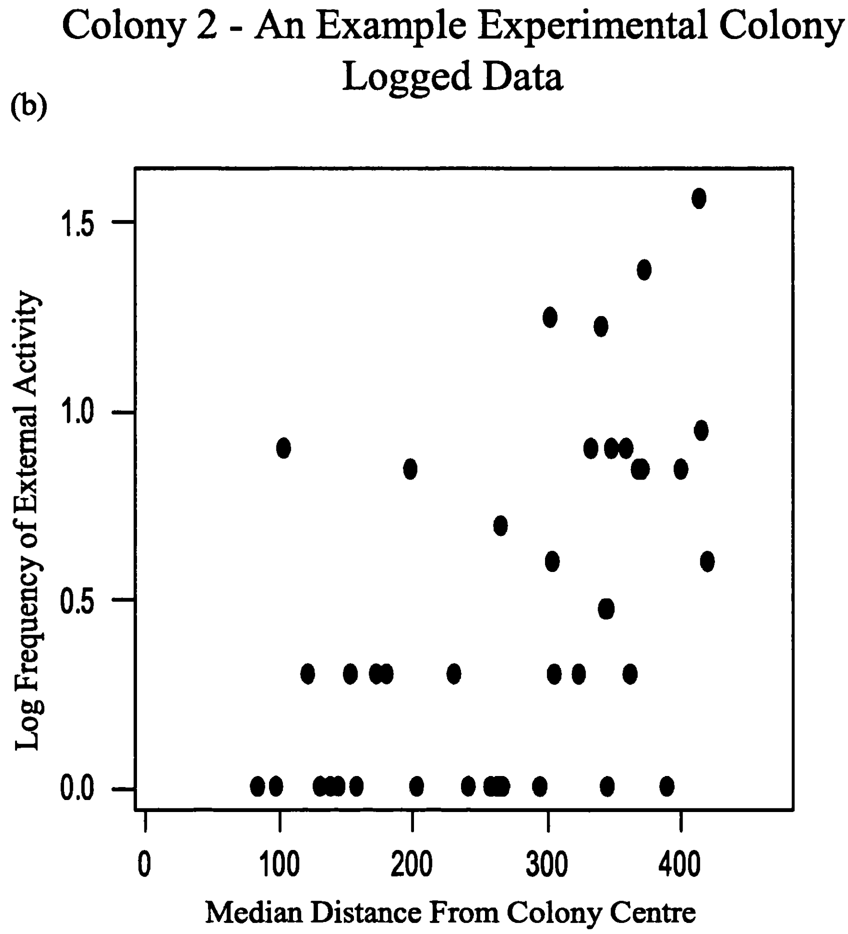
log *f* external activity = - 0.195 + 0.00240 Median distance from colony centre

$R^2 = 27.4\%$; df = 39; $P < 0.01$

Therefore the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre closely approximates an exponential growth.

Fig 19(a)




Colony 2 - An Example Experimental Colony
Logged Data

(b)

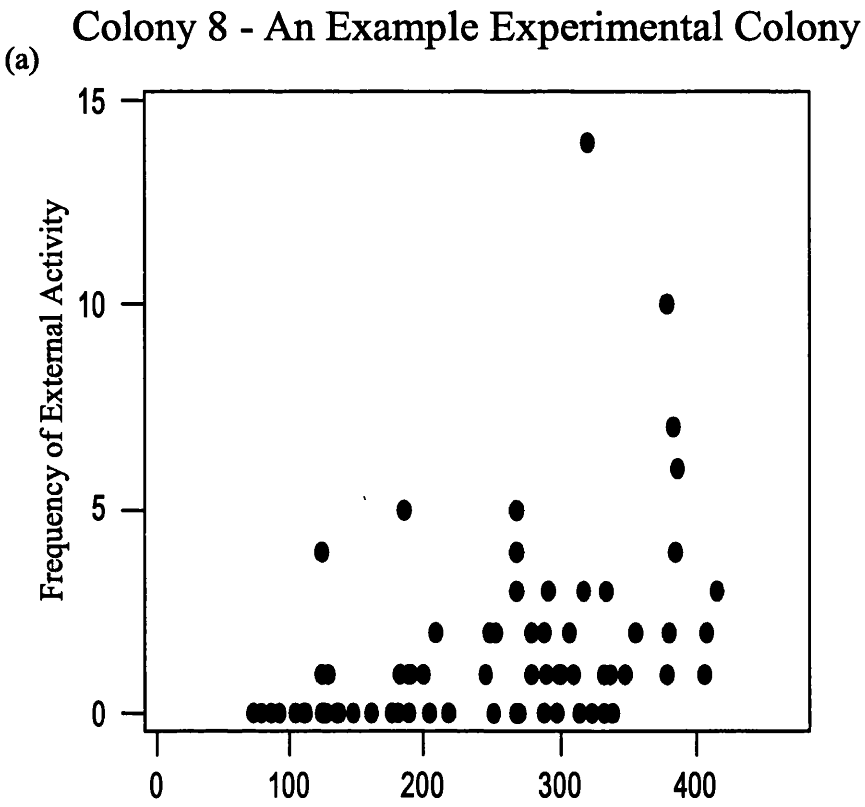
Figure 19: The relationship between log frequency of external activity and the median distance from the colony centre for (a) Colony C_1 (the Control); and (b) Colony 2, an example experimental colony.

Experiment 2

A similar relationship between the frequency of external activity and the position of an individual in the colony is established for the experimental colonies in session 1, experiment 2. A typical experimental plot is shown in Figure 20 (a), below. However, when the frequency of external activity is logged, Figure 20 (b), a linear regression model does not fit the data.

$R^2 = 6.3$; df = 38; $P > 0.05$

Therefore the relationship for this colony does not approximate an exponential growth.



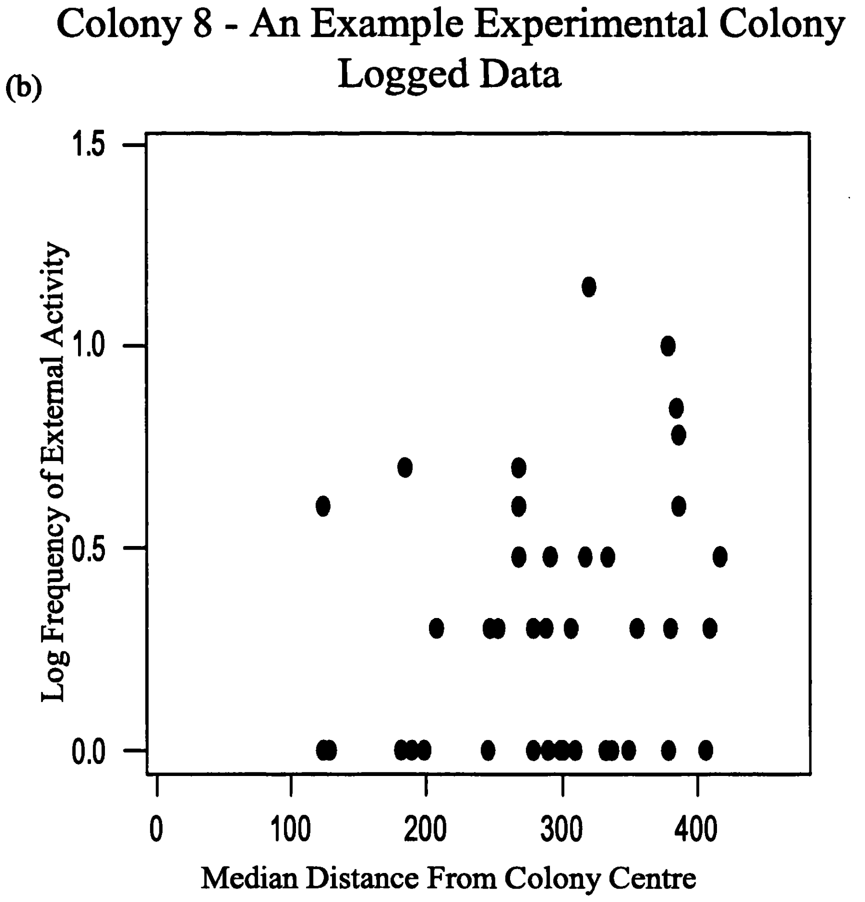


Figure 20 : (a) The relationship between frequency of external activity and the median distance from the colony centre; and (b) log frequency of external activity and the median distance from the colony centre, for Colony 8, an example experimental colony.

The relationship between the frequency of external activity and spatial position is further examined by carrying out correlation analyses according to the procedure described at the beginning of this chapter. The results of these analyses are given in Table 5, below, for experiments 1 and 2, during session 1. There is a significant, positive correlation between the relative frequency at which an individual performs external activity and her relative median distance from the colony centre, for all the colonies in experiment 1, and the experimental colonies in experiment 2 except Colony 5, see Table 5.

Colony	**Session**	r_s	**n**	**Significance Level p<**
Experiment 1				
C_1	1	0.636	58	0.01
1	1	0.571	64	0.01
2	1	0.504	68	0.01
3	1	0.365	58	0.01
4	1	0.600	52	0.01
Experiment 2				
C_2	1	0.322	17	NS
5	1	0.328	34	$0.05<p<0.1$
6	1	0.483	25	0.05
7	1	0.724	57	0.01
8	1	0.543	72	0.01

Table 5: The relationship between the frequency of external activity and the median distance of individuals from the colony centre for experiments 1 and 2, calculated as Spearman's rank correlation coefficient (r_s), and tested as an ordinary product-moment correlation coefficient as $n > 10$ (Sokal and Rohlf, 1981 p.607), NS = Non-significant.

Colony C_2 (the Control), experiment 2, does not show a significant correlation between the relative frequency of external activity and relative median distance from the colony centre. A closer examination of the results indicates very low levels of external activity in this colony, such that there is insufficient data for a correlation to be established. This is discussed further in section 4.3.ii.

What is the effect of sociotomy on the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre?

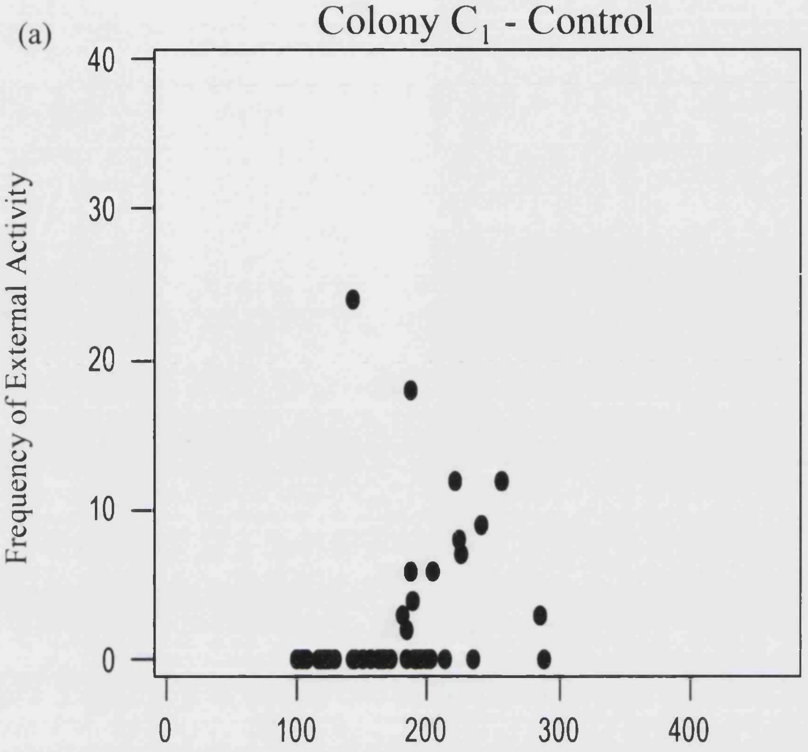
I examine the spatial organisation of external activity in sessions 2 and 4 of experiment 1, and session 4 of experiment 2, to determine whether the relationship is preserved throughout the experiments, despite removal of workers previously associated with external activity from the experimental colonies, in session 2, and the re-unification of the colonies in session 4. Furthermore, I examine the spatial organisation of external activity in session 3, where colonies are maintained as two separate fractions - the original fraction, and the subsidiary fraction.

Experiment 1

During session 2, when workers associated with external activity are being removed sequentially from the experimental colonies, no external activity is recorded. In session 3, when the original fractions of the colonies are maintained separately from the removed individuals in the subsidiary fractions, no external activity is recorded in the original fractions. Figure 21 (b), below, shows the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre in the subsidiary fraction during session 3. The colony centre in this session is taken as the colony centre for the colony during session 1 (as there is no brood present in the subsidiary fraction). Also shown is the graph of this relationship for the Control colony during session 3, Figure 21 (a), and the graphs for the Control and an example experimental colony, Colony 2, during session 4, Figures 22 (a) and (b), respectively.

Session 3

Colony C_1 - Control



Colony 2 - An Example Experimental Colony
Subsidiary Fraction

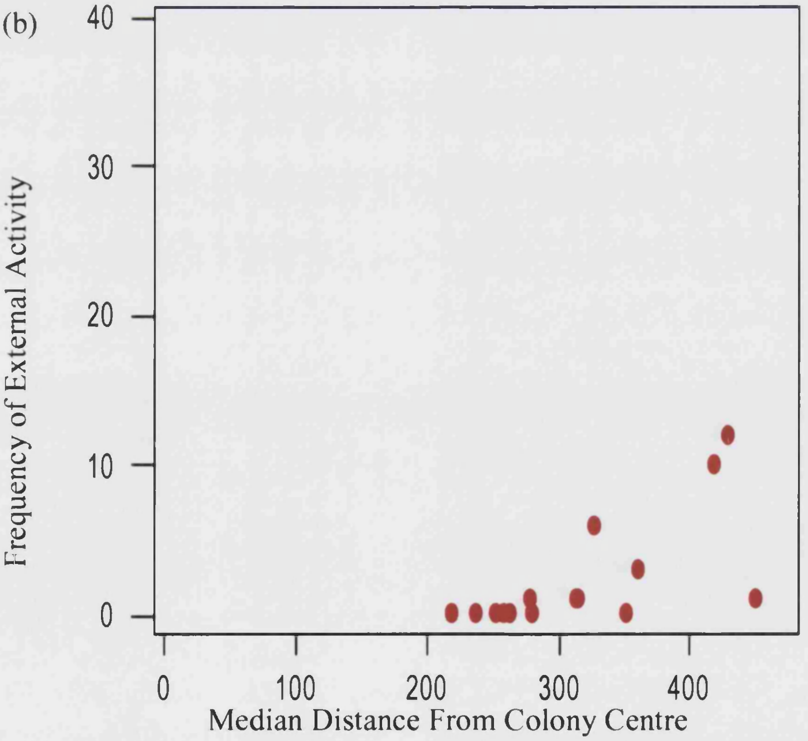
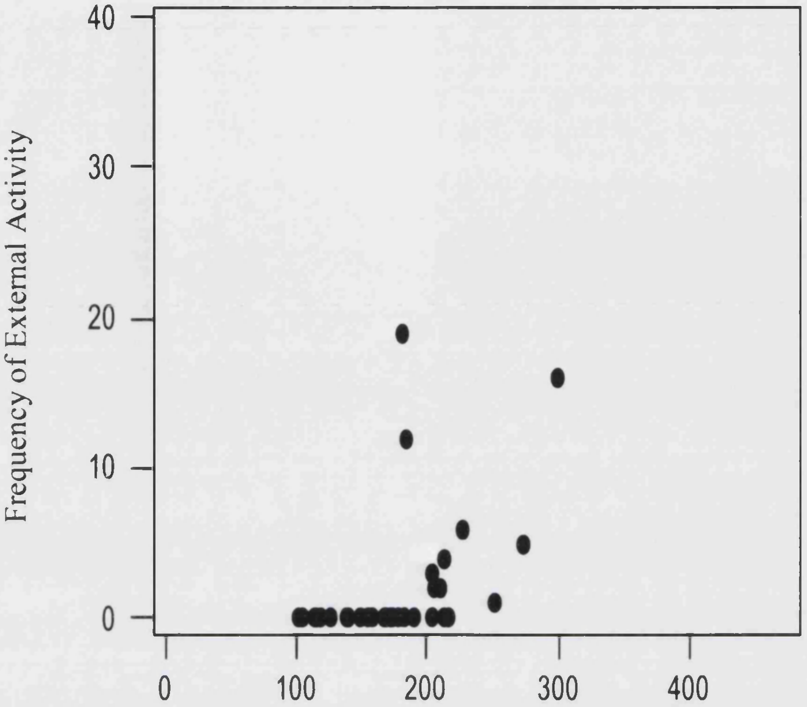


Figure 21: The relationship between the frequency of external activity and median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 2, an example experimental colony (subsidiary fraction only, as no external activity is recorded in the original fraction), for session 3. Individuals that are in the subsidiary fraction of Colony 2, are shown in red.

Session 4

(a) Colony C_1 - Control



Colony 2 - An Example Experimental Colony
(b) Re-united Fractions

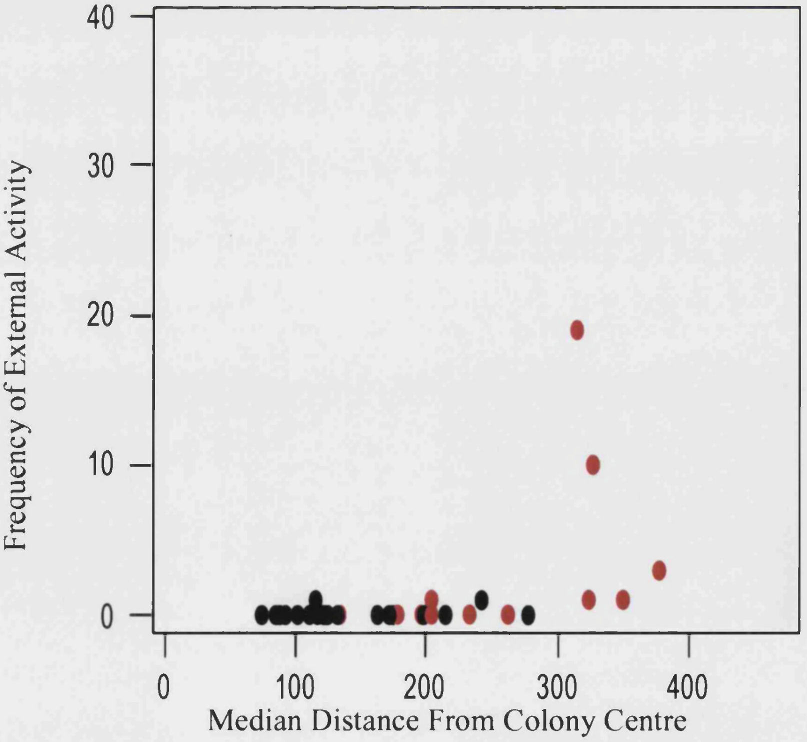


Figure 22: The relationship between the frequency of external activity and median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 2, an example experimental colony, for session 4. Individuals that are in the subsidiary fraction of Colony 2 during session 3 are shown in red.

The results of the correlation analyses are shown in Table 6, below, for each colony in experiment 1, for session 3 (subsidiary fractions), and session 4. There is insufficient external activity during the photographic sessions for this calculation during session 2 in the experimental colonies, or in the original fractions of the colonies during session 3.

There is a significant positive correlation between the relative frequency at which individuals perform external activity and their relative distance from the colony centre, for all the colonies in experiment 1, during session 3 (subsidiary fractions) and session 4, with the exception of Colony 4. A closer examination of the data for Colony 4 shows that in the subsidiary fraction during session 3, one individual has an extremely high frequency of external activity compared to the other individuals. If this individual is not considered in the analysis, the positive correlation between the relative frequency at which individuals perform external activity and their relative spatial position, is significant. The results obtained for this colony during session 4 are also skewed by the extremely high performance of external activity by three individuals (discussed further in sections 4.3vi).

I carry out the same analysis for the colonies in experiment 2, and the results are shown in Table 7. No external activity is recorded during the photographic sessions for the original fraction of experimental Colonies 5, 6 and 7, during session 3. External activity does occur during this session in Colony 8. The relative frequency at which individuals perform external activity and their relative spatial position are not significantly correlated because of the relatively low frequency of external activity exhibited during this session. A maximum of three incidents of external activity are recorded for any one individual during this session. This is discussed further in section 4.3iv.

Colony/session	r_s	n	Significance level p<
Colony C_1			
Session 2	0.514	40	0.01
Session 3	0.469	40	0.01
Session 4	0.660	33	0.01
Colony 1			
Session 3 - subsidiary	0.688	24	0.01
Session 4	0.534	30	0.01
Colony 2			
Session 3 – subsidiary	0.809	17	0.01
Session 4	0.581	31	0.01
Colony 3			
Session 3 – subsidiary	0.352	36	0.05
Session 4	0.669	48	0.01
Colony 4			
Session 3 – subsidiary	0.404	19	$0.05<p<0.1$
Session 4	0.340	22	NS

Table 6: The relationship between the frequency of external activity and the median distance of individuals from the colony centre for experiment 1, sessions 3 and 4, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607), NS = non-significant.

Colony/session	r_s	n	Significance level p<
Colony C_2			
Session 3	0.565	28	0.01
Session 4	0.438	23	0.05
Colony 5			
Session 3 - subsidiary	-	1	-
Session 4	-	2	-
Colony 6			
Session 3 – subsidiary	0.929	6	0.05
Session 4	0.714	7	0.05*
Colony 7			
Session 3 – subsidiary	0.612	19	0.01
Session 4	0.454	35	0.01
Colony 8			
Session 3 – original	0.182	18	NS
Session 3 – subsidiary	0.750	19	0.01
Session 4	0.439	31	0.05

Table 7: The relationship between the frequency of external activity and the median distance of individuals from the colony centre for experiment 2, sessions 3 and 4, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where n ≤ 10. NS = non-significant. '' indicates one-tailed test.*

A Closer Examination of Session 4 - Re-united Colonies

Experiments 1 and 2

The spatial organisation of external tasks in session 4, when the original and subsidiary fractions of the colonies have been re-united, is examined further. I calculate the Spearman's rank correlation coefficient separately for those individuals in session 4 that had remained in the original fraction of the colony during session 2, and for those that had been removed to the subsidiary fraction of the colony during session 2. The results are shown in Table 8, below.

Session 4 – Re-United Colonies				
Colony	**Fraction of Colony Individuals were in during Session 3**	**r_s**	**n**	**Significance Level p<**
Experiment 1				
1	Original	0.714	6	NS
1	Subsidiary	0.429	23	0.05
2	Original	0.170	18	NS
2	Subsidiary	0.734	13	0.01
3	Original	0.469	18	0.05
3	Subsidiary	0.467	30	0.01
4	Original	-	4	-
4	Subsidiary	0.282	18	NS
Experiment 2				
5	Original	-	-	-
5	Subsidiary	-	-	-
6	Original	-	1	-
6	Subsidiary	0.929	6	0.05
7	Original	0.017	22	NS
7	Subsidiary	0.800	13	0.01
8	Original	0.493	16	0.05<p<0.1
8	Subsidiary	0.068	15	NS

Table 8: The relationship between the frequency of external activity and the median distance of individuals from the colony centre for re-united colonies in experiments 1 and 2, calculated separately for individuals in the original and subsidiary fractions of the colonies during session.3, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where $n \leq 10$. NS = non-significant. '-' indicates insufficient numbers to calculate r_s

There is a significant positive correlation between the relative frequency at which individuals perform external activity and their relative distance from the colony centre in session 4, for individuals that are in the subsidiary fraction during session 3, except for Colonies 4 and 8. Of those that are in the original fraction during session 3, only Colony 3, experiment 1, shows a significant relationship between the relative frequency at which individuals perform external activity during session 4 and their relative median distance from the colony centre. These results are discussed in section 4.3vi.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre between session 1 (un-manipulated colonies) and session 4 (after re-unification) I perform the multiple comparison analysis detailed earlier in the chapter.

The results are shown in Table 8a, below.

z^{-} session 1	**z^{-} session 4**	**χ^2 session 1**	**Signif. level_1 p<**	**χ^2 session 4**	**Signif. level_4 p<**	**n_{mean1}**	**$n_{mean\,4}$**	**t_s**	**Signif. level p<**
Exp 1									
0.568	0.711	3.07	NS	0.85	NS	57.5	33.3	-0.63	NS
Exp 2									
0.711	0.507	3.74	NS	0.65	NS	48.3	21.3	0.74	NS

Table 8a: Testing the relationship between the frequency of performance of external activity and the median distance of individuals from the colony centre for experiments 1 and 2, for combined colonies, compared between sessions 1 and 4. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for both sessions compared, and in both experiments. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 and the pooled *z* for all the experimental colonies in session 4 (where colonies exhibit a significant correlational relationship between the frequency at which individuals perform external activity and their median distance from the centre of the brood pile), shows that there is no significant difference between the colonies before the manipulation and after it.

4.2 An Investigation of the Spatial Organisation of Tasks in Colonies with a Manipulated Age Structure

Experiment 3

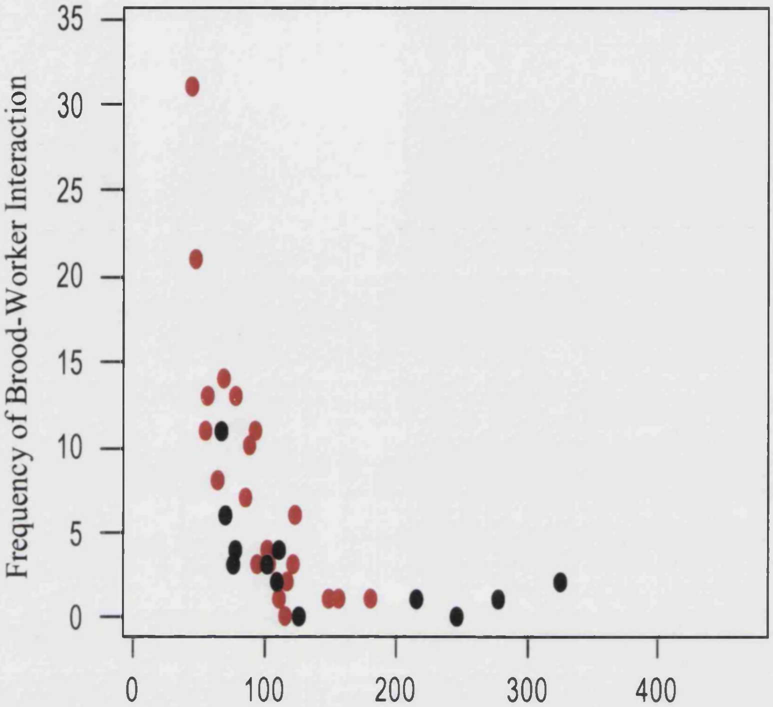
I analyse the results from experiment 3 in a similar way to those of experiments 1 and 2. The experimental colonies in experiment 3 are divided into two fractions on the basis of the age of the workers, rather than by their task. Recall that experiment 3 consists of three sessions. Session 1 starts after the original colonies have been divided into fractions consisting first of pupae and fifteen older workers, and second, of the remaining workers. After the eclosion of callows in the former (subsidiary) fraction, the fifteen older workers are removed and the colonies are marked and emigrated to a new nest. The workers that have not been removed (those in the 'original' fraction of the colony) are also marked and emigrated to a new nest, and session 1 subsequently commences. A month after the end of session 1, session 2 begins, during which no further manipulations are carried out on the two fractions of each colony. Prior to the beginning of session 3, the two fractions of each colony are united.

i. The Spatial Organisation of Brood-Related Tasks

The graphs below show the relationship between the frequency at which individual workers perform brood-related tasks and their median distance from the colony centre, during session 1, experiment 3. The Control colony (Colony C_3), and both fractions, original ('older') and subsidiary ('callow'), of a typical example of an experimental colony (Colony 11), are shown in Figures 23 (a), (b) and (c), respectively.

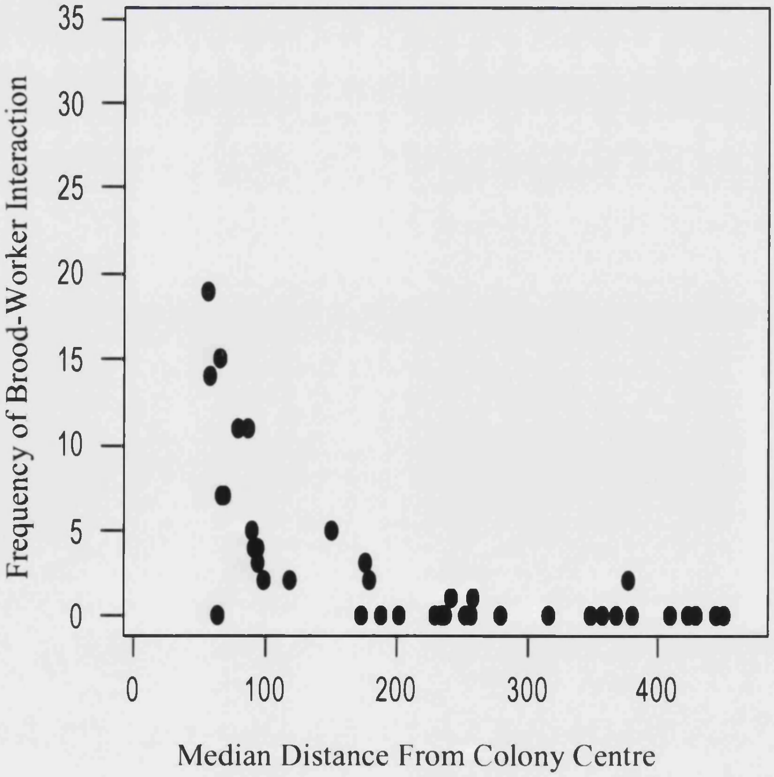
Colony C_3 - Control

Fig 23(a)



Colony 11 - An Example Experimental Colony
Original Fraction (Older Workers)

Fig 23(b)



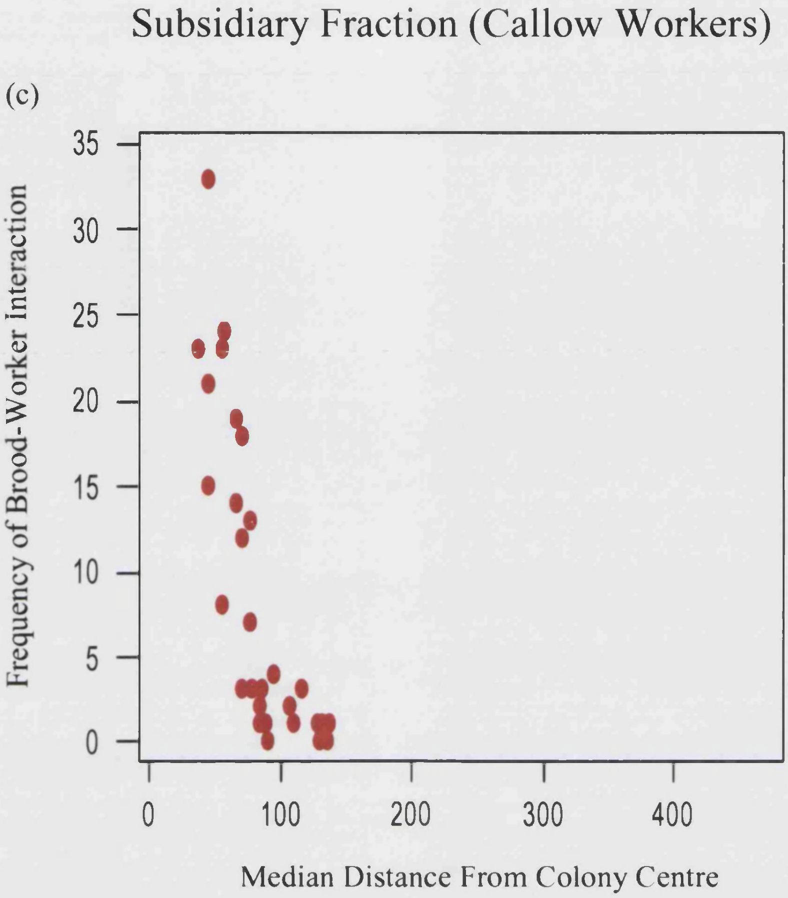


Figure 23: (a) The relationship between the frequency of brood-worker interaction and the median distance from the colony centre for Colony C_3, (the Control) session 1; (b), Colony 11, an example experimental colony, older workers; (c) Colony 11, callow workers. Callows are shown in red, older workers in black.

The frequency at which individuals perform brood-related tasks decreases as their median distance from the brood pile increases for the Control colony, and for all the experimental colonies. In the Control colony, callows (shown in red), tend to be located closer to the brood pile, and exhibit a higher frequency of performance of brood-related tasks than the older workers in the Control colony. In Figures 23 (b) and (c), workers with a median position above a certain threshold distance from the brood pile exhibit a frequency of brood-related tasks of zero, or very close to zero.

I carry out Mann Whitney U tests comparing the maximum frequency at which the callows perform brood-related tasks in session 1, compared to the maximum frequency at which the older workers perform these tasks in the same session.

The results are shown in Table 9a, below. The distribution of the maximum frequency at which brood-related tasks are performed is significantly higher among the callows, compared to the older workers.

Colony	Maximum f brood-related tasks – Older workers	Maximum f brood-related tasks – Callow workers	η_{older}	$\eta_{callows}$	W	Signif. p<
9	12	19	17	23	10.5	0.05
10	16	25				
11	19	33				
12	18	21				

Table 9a: A comparison of the distribution of the maximum frequency of performance of brood-related activity in session 1 (experiment 3), between the older workers and the callow workers using Mann-Whitney U Tests. η_{older} *= median frequency of brood-related tasks during session 1 – older workers.* η_{older} *= median frequency of brood-related tasks during session 1 – callow workers.. * = one-tailed test (null hypothesis* η_{older} *is not less than* η_{callow}, H_1 : η_{older} *is less than* η_{callow}).

When the frequency at which individuals perform brood-related tasks is logged, and plotted against their median distance from the colony centre, see Figures 24 (a), (b) and (c), below, a linear regression model fits the data well.

Control (Colony C_3):

log *f* brood-related tasks = 1.16 - 0.00490 Median distance from colony centre

$R^2 = 49.9\%$; df = 30; $P < 0.01$

Colony 11 - Older workers:

log *f* brood-related tasks = 1.06 - 0.00328 Median distance from colony centre

$R^2 = 51.1\%$; df = 17; $P < 0.01$

Colony 11 - Callow workers:

log *f* brood-related tasks = 2.02 - 0.0163 Median distance from colony centre

$R^2 = 73.5\%$; df = 25; $P < 0.01$

Therefore the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre closely approximates an exponential decay.

Fig 24(a)

Colony C_3 - Control
Logged Data

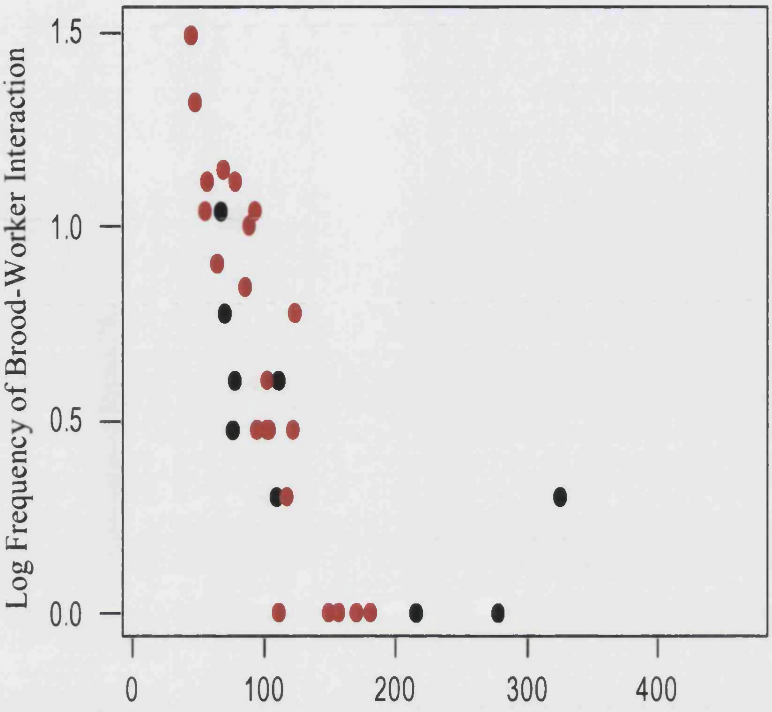
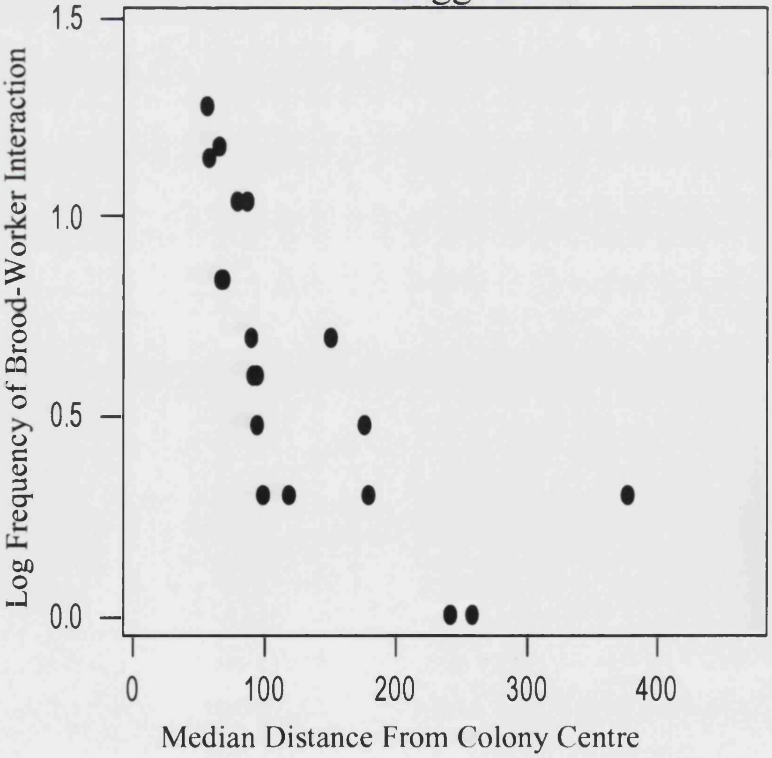


Fig 24(b)

Colony 11 - An Example Experimental Colony
Original Fraction (Older Workers)
Logged Data



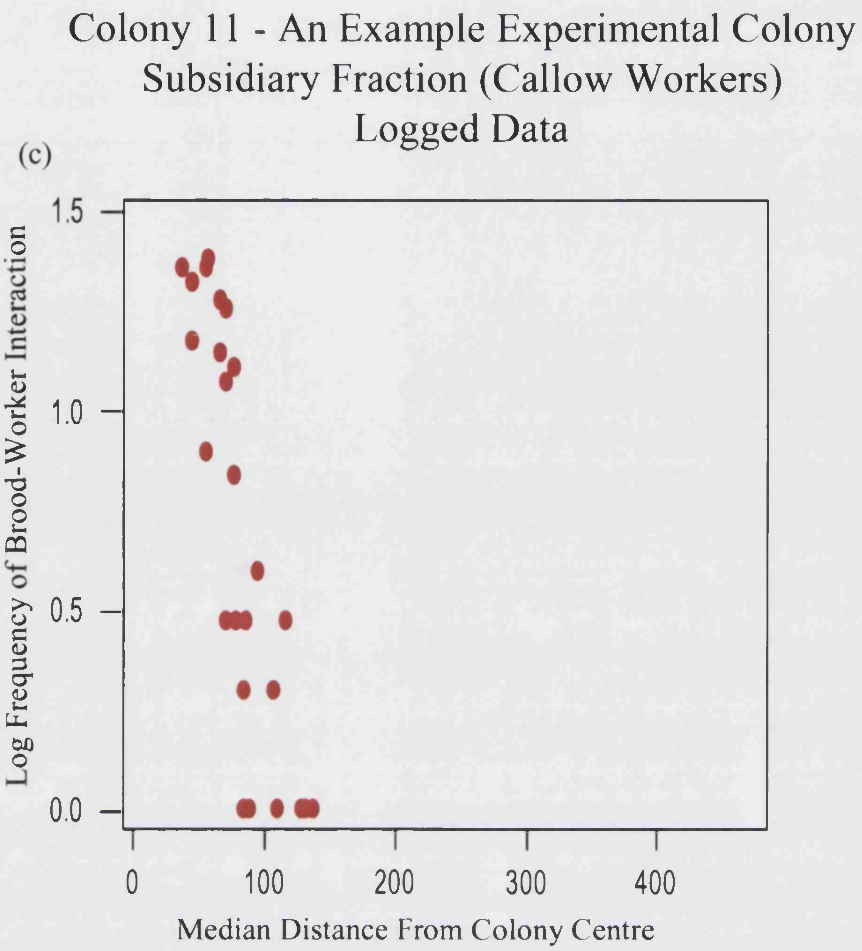


Figure 24 : (a) The relationship between the logged frequency of brood-worker interaction and the median distance from the colony centre for Colony C_3 (the Control); (b) Colony 11, an example experimental colony, older workers; (c) Colony 11, callow workers. Callows are shown in red.

The relationship between the frequency at which individuals perform brood-related tasks and their spatial position, is examined further by carrying out correlation analyses according to the procedure described at the beginning of the chapter. The results of these analyses are given in Table 9, for experiment 3 during session 1. There is a significant, negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative distance from the colony centre, for all the colonies in experiment 3 during session 1. A typical plot of the relationship is given below, Figure 25, for an example experimental colony, Colony 11 (a) 'older' fraction and (b) 'callow' fraction.

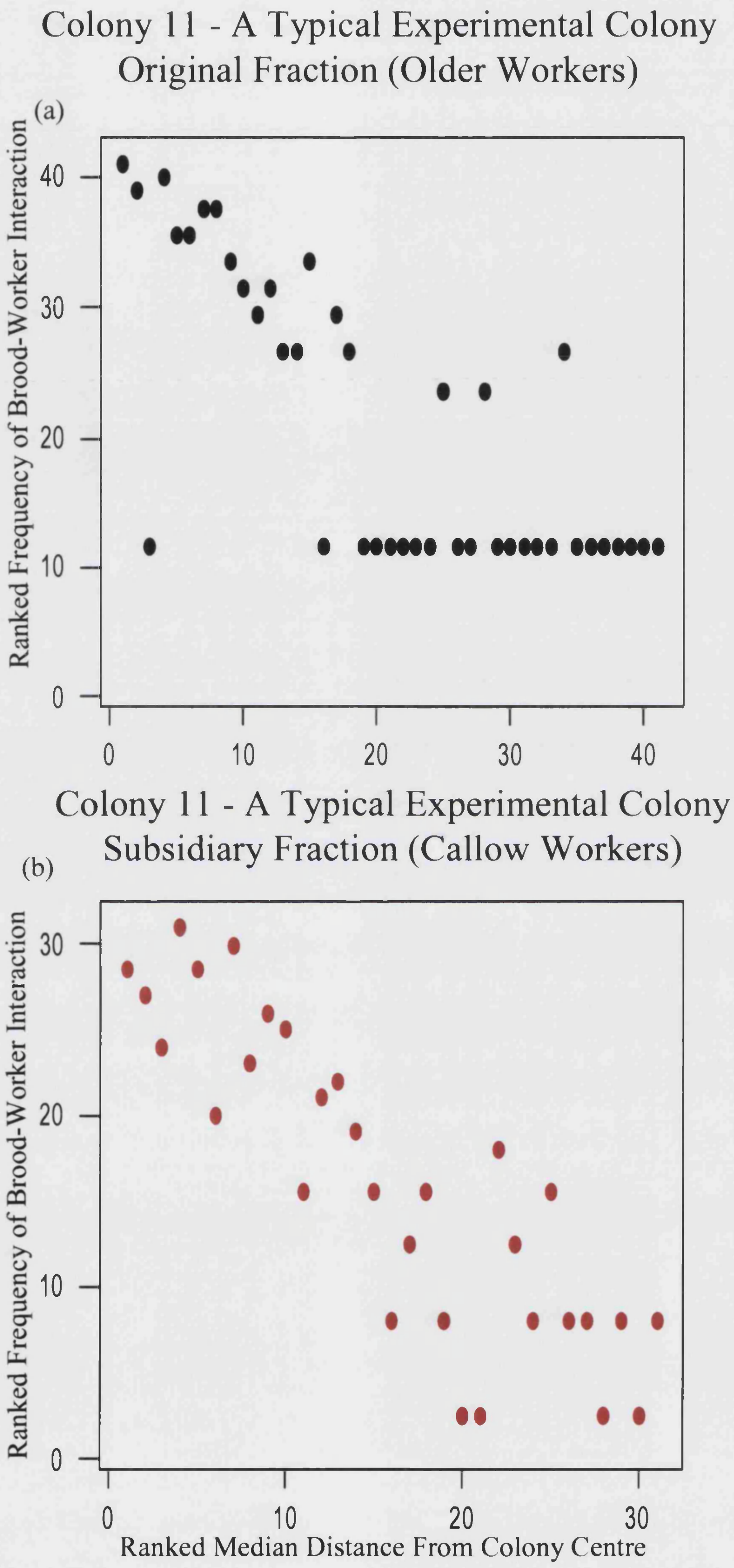


Figure 25 : (a) The relationship between ranked frequency of brood-worker interaction and the median distance from the colony centre for Colony 11, an example experimental colony, older workers; (b) Colony 11, callow workers (shown in red).

It is clear from Figure 25 (a) that a large number of individuals in the original fraction of the colony (containing the older workers), have the same rank. These individuals have a frequency of brood-worker interaction of zero. In the fraction containing the callows, such individuals are less common.

Colony	Session	r_s	n	Significance Level p<
Experiment 3				
C_3	1	-0.867	38	0.01
9 – Older Fraction	1	-0.692	37	0.01
9 – Callow Fraction	1	-0.808	59	0.01
10 – Older Fraction	1	-0.842	33	0.01
10 – Callow Fraction	1	-0.908	39	0.01
11 – Older Fraction	1	-0.765	41	0.01
11 – Callow Fraction	1	-0.863	31	0.01
12 – Older Fraction	1	-0.710	21	0.01
12 – Callow Fraction	1	-0.653	30	0.01

Table 9: The relationship between the frequency of performance of brood-related tasks and median distance of individuals from the colony centre for experiment 3, calculated as r_s*, Spearman's rank correlation coefficient, tested as an ordinary product-moment correlation coefficient as* $n > 10$ *(Sokal and Rohlf, 1981 p.607).*

What is the effect of uniting the 'Older' fractions with the 'Callow' fractions on the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre?

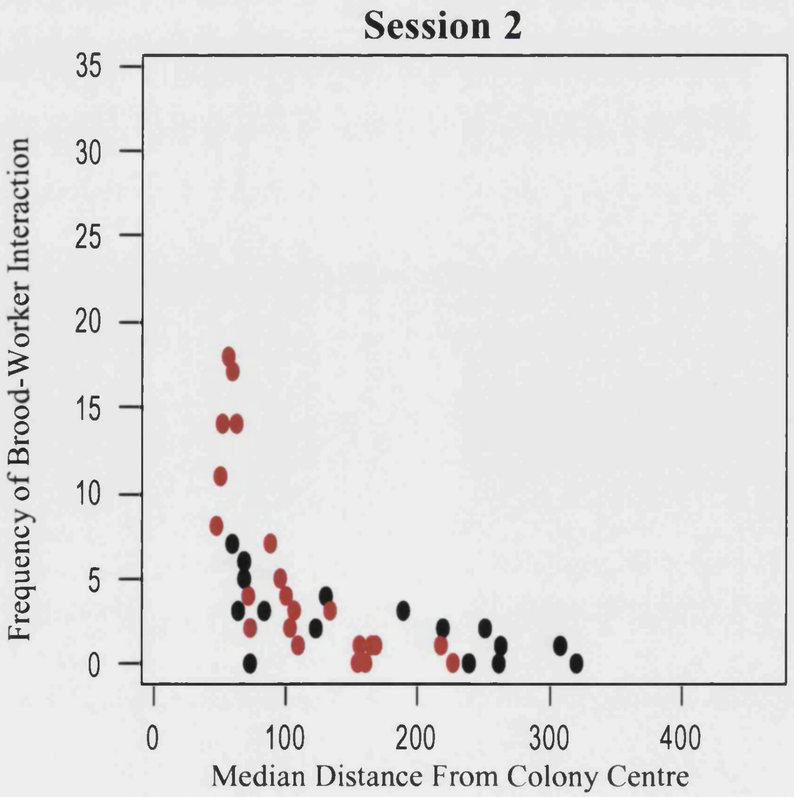
The graphs below show the relationship between the frequency at which individual workers perform brood-related tasks and their median position in the colony during:

session 2, which begins after 1 month has elapsed from the end of session 1, during which the two fractions of each colony are maintained separately.

session 3, when the two fractions of the colonies are united.

The relationship is shown in Figure 26 for (a) and (b) the Control colony (Colony C_3) during sessions 2 and 3, respectively, and (c), (d) and (e) a typical example of an experimental colony (Colony 11), during sessions 2 (original and subsidiary fractions) and 3, respectively.

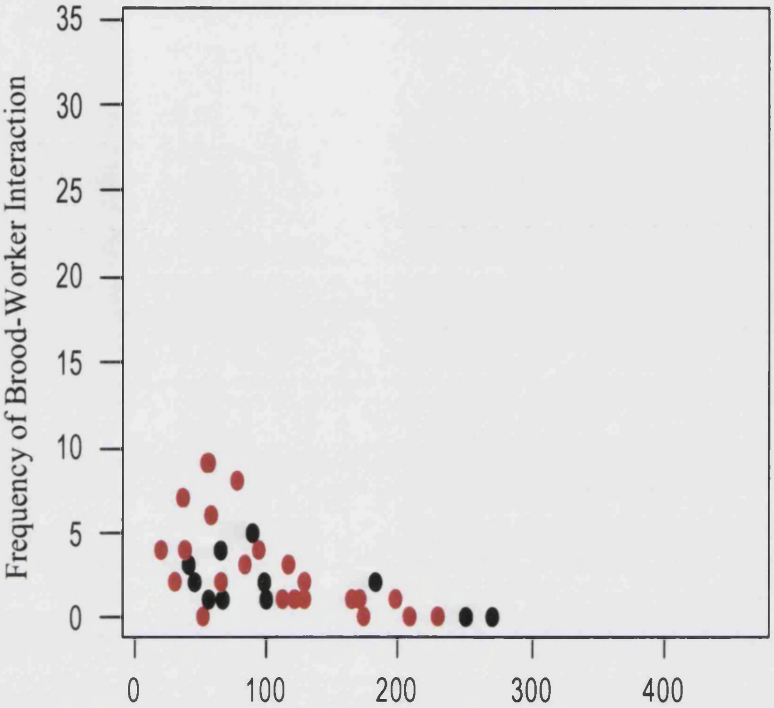
Fig 26(a) Colony C_3 - Control



Colony C_3 - Control

Fig 26(b)

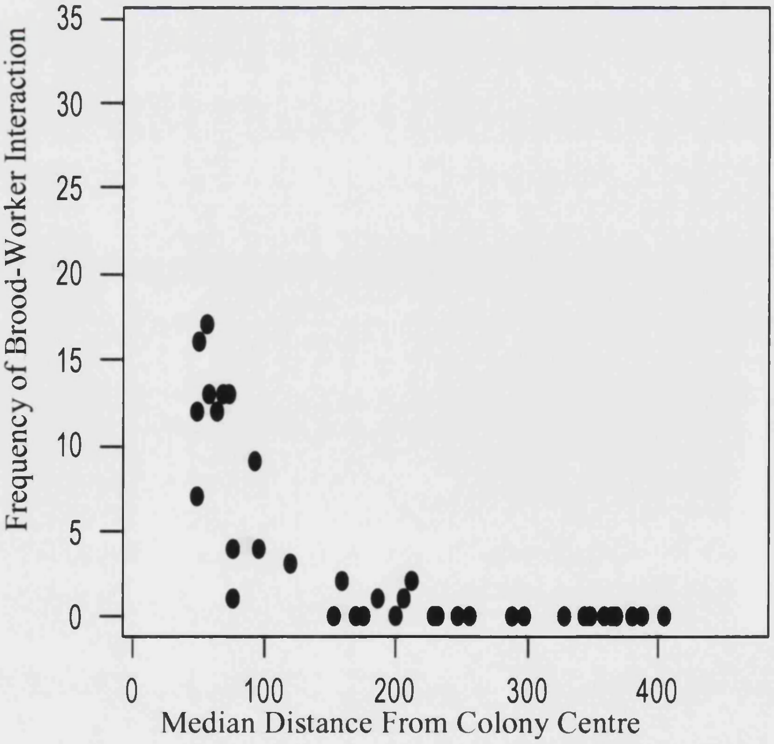
Session 3

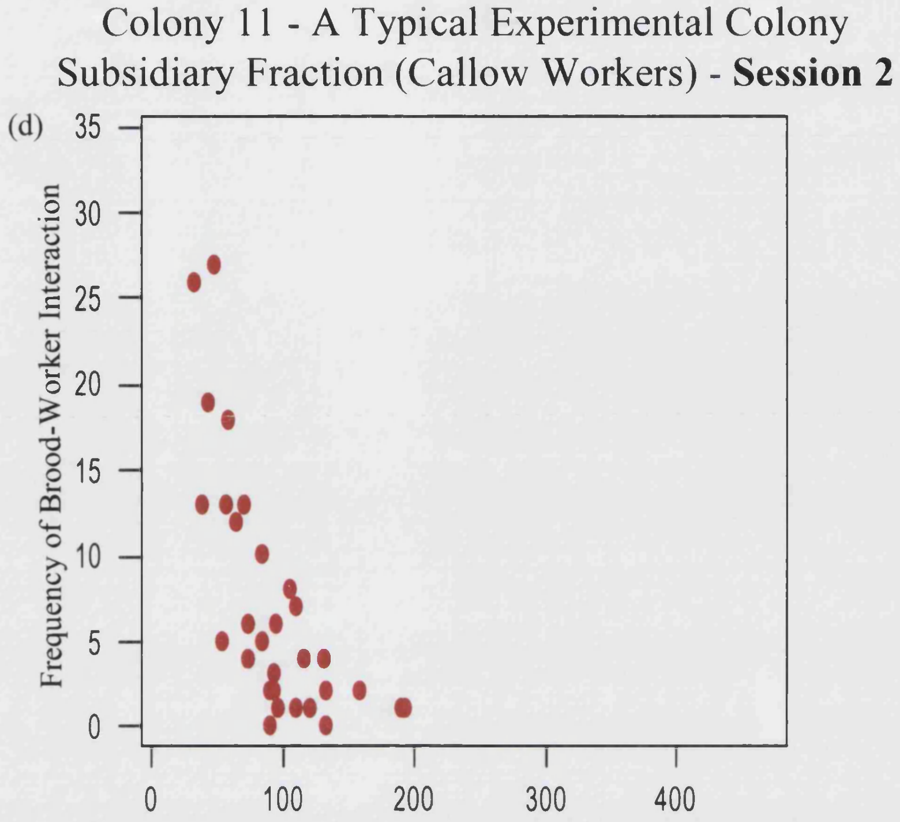


Colony 11 - A Typical Experimental Colony
Original Fraction (Older Workers)

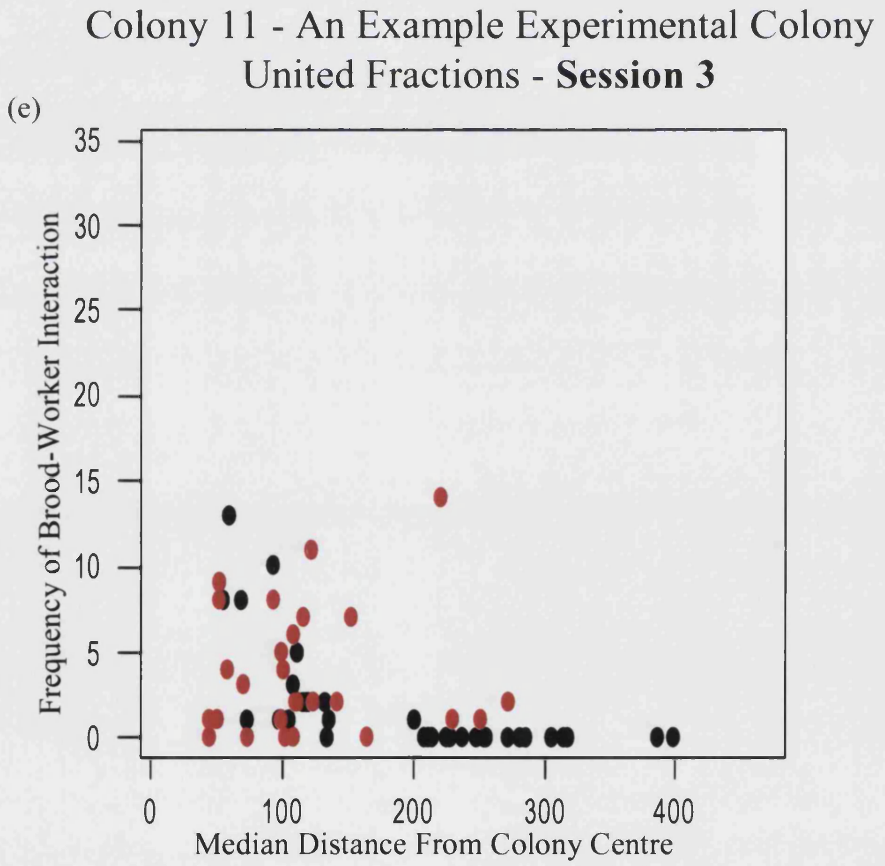
Fig 26(c)

Session 2





Colony 11 - A Typical Experimental Colony
Subsidiary Fraction (Callow Workers) - **Session 2**



Colony 11 - An Example Experimental Colony
United Fractions - **Session 3**

Figure 26 : (a) The relationship between the frequency of brood-worker interaction and the median distance from the colony centre for Colony C_3 (the Control), session 2; (b) session 3 (c) Colony 11, an example experimental colony, session 2, older fraction; (d) session 2, callow workers; (e) Session 3, re-united fractions. Callows are shown in red.

I compare the distribution of distances of the callows from the centre of the brood pile in session 1 with their distribution of distances from the centre of the brood pile in session 2, using a Mann Whitney U test. The results are shown in Table 10a, below. There is no significant difference between the distribution of distances of the callows from the centre of the brood pile in session 1 compared to session 2, for any of the experimental colonies in experiment 3.

Colony	$\eta_{\text{Distance session 1}}$	$\eta_{\text{Distance session 2}}$	W	Signif. p<
9	207.9	137.8	3277.5	NS
10	157.6	153.4	357.0	NS
11	83.3	89.8	801.0	NS
12	148.6	108.1	742.5	NS

Table 10a: A comparison of the distribution of distances from the centre of the brood pile for the callows in session 1 (experiment 3),compared with the distribution of distances from the centre of the brood pile during session 2, using Mann-Whitney U Tests. $\eta_{distance\ session\ 1}$ *= median distance from the centre of the brood pile during session 1 – callow workers.* $\eta_{distance\ session\ 2}$ *= median distance from the centre of the brood pile during session 1 – callow workers. * = one-tailed test (null hypothesis* $\eta_{distance\ session\ 1}$ *is not less than* $\eta_{distance\ session\ 2}$*,* H_1 *:* $\eta_{distance\ session\ 1}$ *is less than* $\eta_{distance\ session\ 2}$*).*

I perform a similar analysis for the workers in session 3. I compare the distribution of distances of the callows from the centre of the brood pile in session 3 with the distribution of distances of the older workers from the centre of the brood pile in session 3, using a Mann Whitney U test. The results are shown in Table 10b, below. There is no significant difference between the distribution of distances of the callows from the centre of the brood pile compared to the older workers in session 3 for two of the four experimental colonies in experiment 3. The distribution of distances of the older workers is significantly higher in session 3 compared to the callows for the remaining experimental colonies.

Colony	$\eta_{\text{callow sess3}}$	$\eta_{\text{older sess3}}$	W	Signif. p<
9	106.0	164.8	789.0	0.01
10	118.6	116.5	1035.0	NS
11	104.8	207.3	1302.0	0.01
12	136.7	132.9	284.5	NS

Table 10b: A comparison of the distribution of distances from the centre of the brood pile for the callows in session 3 (experiment 3),compared with the distribution of distances from the centre of the brood pile for the older workers, using Mann-Whitney U Tests. $\eta_{callow\ sess3}$ *= median distance from the centre of the brood pile during session 3 – callow workers.* $\eta_{older\ sess3}$ *= median distance from the centre of the brood pile during session 3 – older workers. * = one-tailed test (null hypothesis* $\eta_{callow\ sess3}$ *is not less than* $\eta_{older\ sess3}$*,* H_1 *:* $\eta_{callow\ sess3}$ *is less than* $\eta_{older\ sess3}$*).*

The results of correlation analyses on these data are shown in Table 10, below, for experiment 3, sessions 2 (original and subsidiary fractions), and session 3. There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative median distance from the colony centre for all the colonies during sessions 2 and 3.

Colony/session	**r_s**	**n**	**Significance level p<**
Colony C_3			
Session 2	-0.801	38	0.01
Session 3	-0.661	35	0.01
Colony 9			
Session 2 – Older Workers	-0.803	28	0.01
Session 2 – Callows	-0.894	57	0.01
Session 3	-0.829	65	0.01
Colony 10			
Session 2 – Older Workers	-0.887	32	0.01
Session 2 – Callows	-0.696	16	0.01
Session 3	-0.674	64	0.01
Colony 11			
Session 2 – Older Workers	-0.842	38	0.01
Session 2 – Callows	-0.768	30	0.01
Session 3	-0.546	63	0.01
Colony 12			
Session 2 – Older Workers	-0.758	22	0.01
Session 2 – Callows	-0.653	38	0.01
Session 3	-0.799	53	0.01

Table 10: The relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiment 3, sessions 2 and 3, calculated as r_s, Spearman's rank correlation coefficient, tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

A Closer Examination of Session 3 - Re-united Colonies

Experiment 3

The spatial organisation of brood-related tasks in session 3, when the original and subsidiary fractions of the colonies has been united is examined further. I calculate the Spearman's Rank Correlation Coefficient separately for the 'older' workers and 'callow' workers. The results are shown in Table 11, below.

Session 3 – Re-United Colonies				
Colony	Fraction of Colony Individuals were in in Sessions 1 and 2	r_s	n	Significance Level p<
Experiment 3				
9	Older	-0.720	20	0.01
9	Callow	-0.843	45	0.01
10	Older	-0.553	33	0.01
10	Callow	-0.737	31	0.01
11	Older	-0.856	35	0.01
11	Callow	0.082	28	NS
12	Older	-0.671	20	0.01
12	Callow	-0.886	33	0.01

Table 11 : The relationship between the frequency of performance of brood-related tasks and median distance of individuals from the colony centre for re-united colonies in experiment 3, calculated separately for individuals in the original and subsidiary fractions of the colonies during sessions 1 and 2, as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607). NS = non-significant.

There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative distance from the centre of the colony. This is the case for both subsets of individuals in session 3, in all the colonies except those in the 'callow' fraction of Colony 11. These results are discussed in section 4.3x.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the frequency at which callow individuals perform brood-related tasks and their median distance from the colony centre between session 1 (separate fractions) and session 3 (after unification) I perform the multiple comparison analysis detailed earlier in the chapter, comparing callows only. I also perform this analysis for older workers.

The results are shown in Table 11a, below.

z^{-}_{session} 1	z^{-} session 3	χ^2 session 1	Signif. Level_1 p<	χ^2 session 3	Signif. Level_3 $_p$p<	n_{mean1}	n_{mean} 3	t_s	Signif. level p<
Callows									
-1.190	-1.202	8.989	0.05	3.11	NS	36.8	33.3	-	-
Older workers									
-1.001	-0.925	2.538	NS	6.953	NS	30	24	-0.891	NS

Table 11a: Testing the relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiment 3, for combined colonies callow fractions, and combined older fractions separately, compared between sessions 1 and 3. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for both sessions compared, for the older workers. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the older workers in the experimental colonies in session 1 and the pooled *z* for all the older workers in experimental colonies in session 3 (where colonies exhibit a significant correlational relationship between the frequency at which individuals perform brood-related tasks and their median distance from the centre of the brood pile), shows that there is no significant difference between the colonies before the manipulation and after it.

There is sufficient evidence to reject the null hypothesis of homogeneity in the callow fractions of the colonies in experiment 3 during session 1. Therefore the colonies cannot be considered to be samples from a population exhibiting a common correlation.

I therefore use an alternative method to determine if there is any significant difference between the pooled callow fractions in experiment 3 in session 1 and session 3.

Correlation coefficients of the relationship between the frequency at which callows perform brood-related tasks in session 1, and the frequency at which they perform brood related tasks in session 3, are compared using Marascuilo's method, outlined in Zar (1984) p. 318. For the contrast I calculate the test statistic according to the equation below:

$$S = \frac{\left| \Sigma_i c_i z_i \right|}{SE}$$

$$SE = \sqrt{\Sigma\, c_i^2 \sigma_i^2{}_{zi}}$$

c_i is the contrast coefficient

The critical value for the test is:

$$S_\alpha = \sqrt{\chi^2_{\alpha,\,(k-1)}}$$

According to this method, the results from the comparison between the multiple correlation coefficients in session 1 and session 3, are given in Table 11b , below.

z, n, for correlation	-1.121 , 59
coefficent, session 1	- 1.516, 39
	-1.305, 31
	-0.781, 30
z, n for correlation	-1.231, 45
coefficient, session 3	-0.944, 31
	-1.403, 33
SE	0.023
$S_{\alpha 0.05}$	0.510
S	0.512
Signif p<	NS

Table 11b: Testing the relationship between the frequency of performance of brood-related tasks and the median distance of individuals from the colony centre for experiment 3, multiple comparisons using callow fractions compared between sessions 1 and 3. Calculated as according to the method in Zar (1984) using pooled correlation coefficients where colonies cannot be considered samples from a population exhibiting a common correlation among the variables.

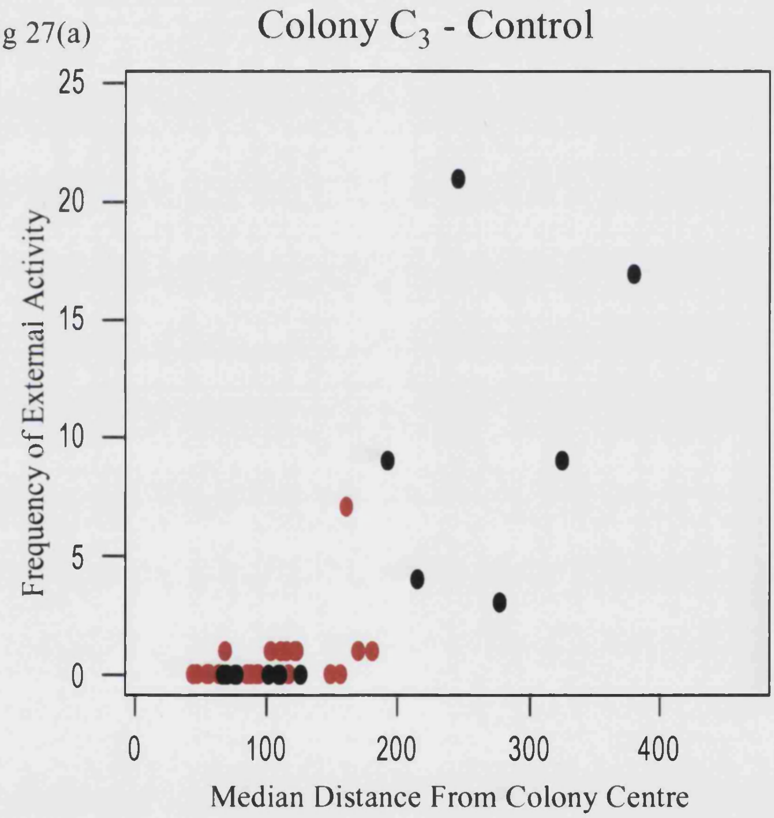
A multiple contrast among the correlation coefficients for the relationship between the frequency at which callows perform brood-related tasks and their median distance from the centre of the brood pile in experiment 3, shows that there is no significant difference between the colonies before the manipulation and after it.

ii. The spatial organisation of external activity

Experiment 3

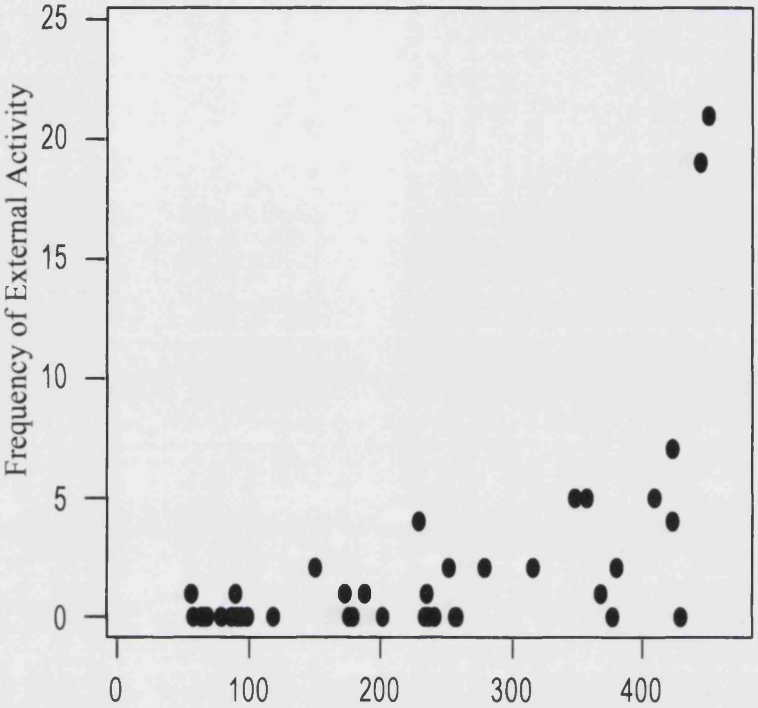
The graphs in Figure 27, below, show the relationship between the frequency at which individual workers perform external activity and their median distance from the colony centre, during session 1, in experiment 3. The Control colony, Colony C_3, and an typical example of an experimental colony, Colony 11, are shown.

Fig 27(a)



Colony 11 - An Example Experimental Colony
Original Fraction (Older Workers)

(b)



Subsidiary Fraction (Callow Workers)

(c)

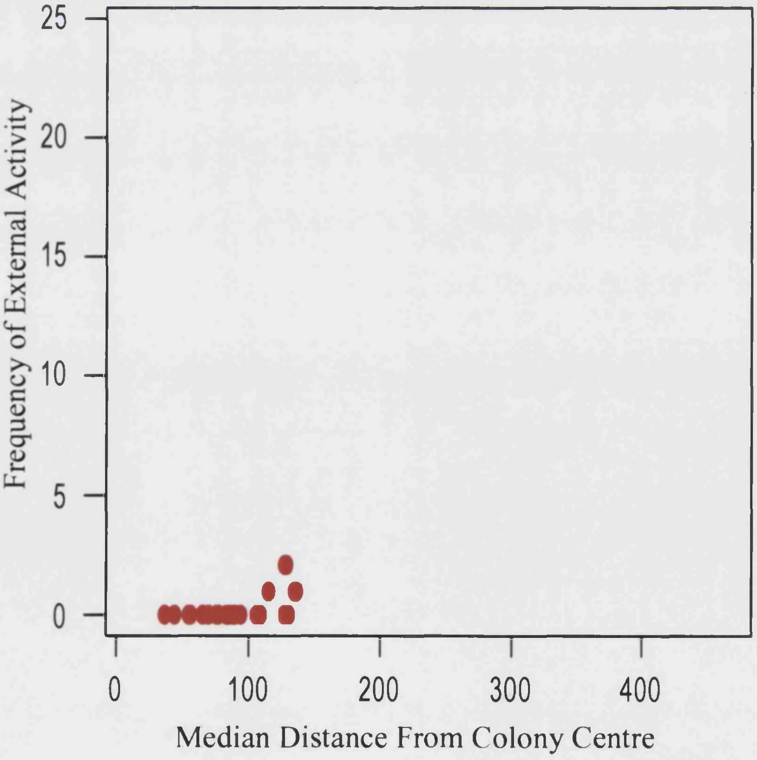


Figure 27 : (a) The relationship between the frequency of external activity and the median distance from the colony centre for Colony C_3, the Control, session 1; (b), Colony 11, an example experimental colony, older workers; (c) Colony 11, callow workers (shown in red).

In the Control colony (Colony C_3) callows, shown in red, tend to be located closer to centre of the brood pile, and exhibit a lower frequency of external activity than the older workers. Nevertheless, even at this very early stage in the life of individuals from the callow cohorts, there are those who already venture outside the nest.

When the frequency at which individuals perform external activity is logged, and plotted against their median distance from the colony centre, see Figures 28 (a) and (b), below, a linear regression model does not fit the data.

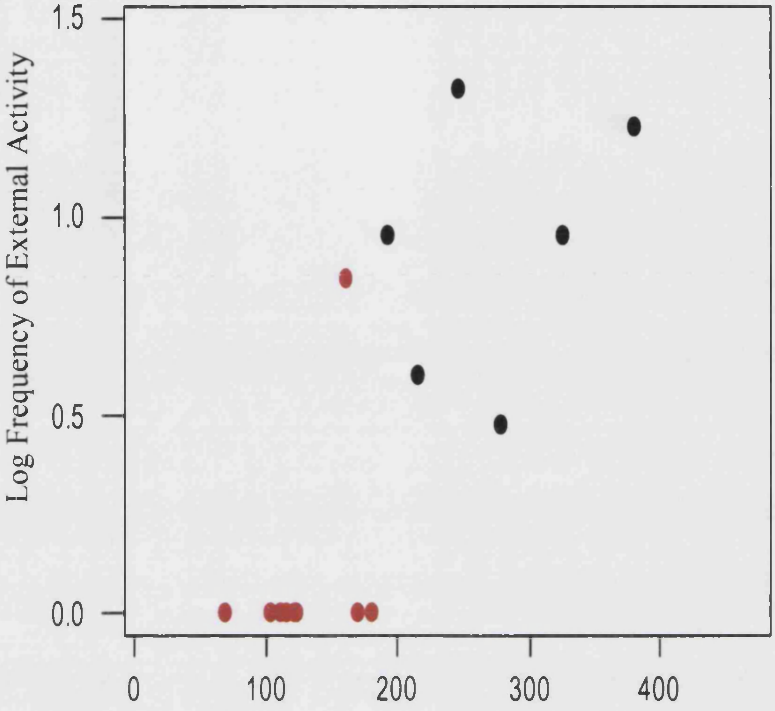
Control (Colony C_3):
log *f* external activity = - 0.417 + 0.00453 Median distance from colony centre
R^2 = 61.2%; df = 13; P = 0.063

Colony 11 - Older workers:
log *f* external activity = - 0.316 + 0.00256 Median distance from colony centre
R^2 = 55.5%; df = 17; P = 0.09

Colony 11 - Callow workers:
Insufficient numbers of individuals perform external activity to make this calculation valid.

Therefore the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre does not approximate an exponential decay.

Colony C_3 - Control
Logged Data



Colony 11 - An Example Experimental Colony
Original Fraction (Older Workers)
Logged Data

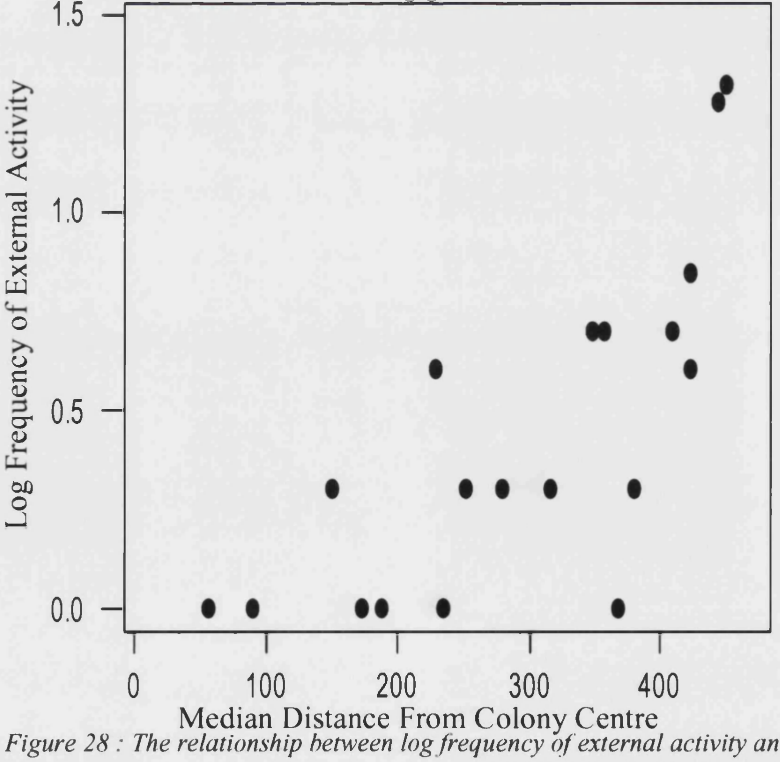


Figure 28 : The relationship between log frequency of external activity and the median distance from the colony centre for (a) Colony C_3 (the Control); and (b) Colony 11, an example experimental colony, 'older' fraction. Callows are shown in red.

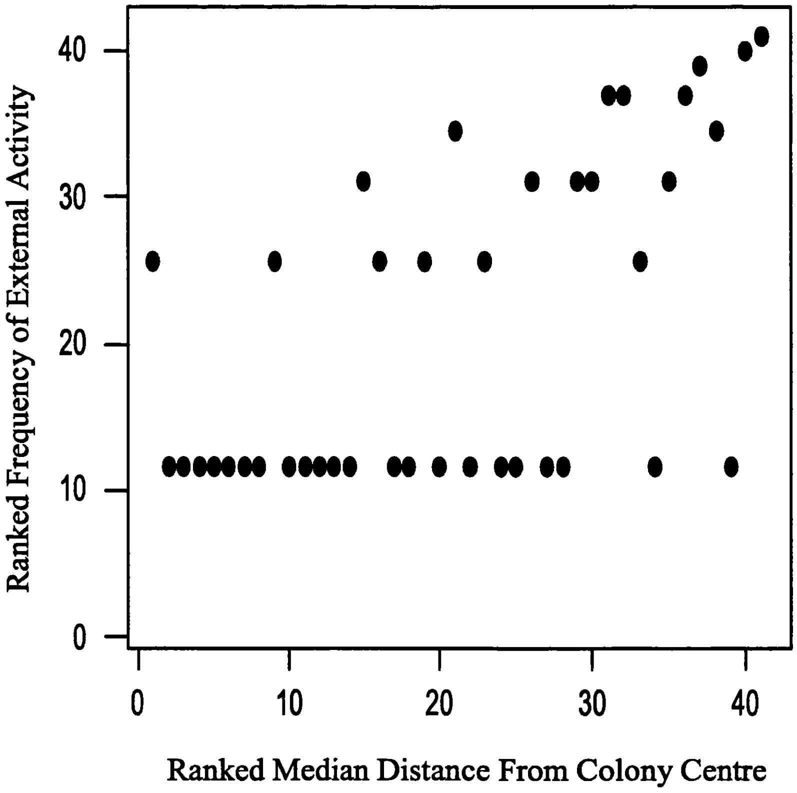
The frequency at which individuals perform external activity increases as their median distance from the brood pile increases for the Control colony, and for all the experimental colonies. Typical experimental plots are shown in Figures 27 (b) and (c), above.

The relationship between the frequency at which individuals perform external activity and their spatial position is further examined by carrying out correlation analyses, according to the procedure described at the beginning of this chapter. There is a significant, positive correlation between the relative frequency at which individuals perform external activity and their relative spatial position, for all the colonies during session 1 in experiment 3, see Table 12. Example plots of the relationship for the older workers and for the callows, are given below, Figure 29 (a) and (b), for a typical experimental colony in experiment 3 (Colony 11).

Session 1

Colony 11 - An Example Experimental Colony

Fig 29(a) Original Fraction (Older Workers)



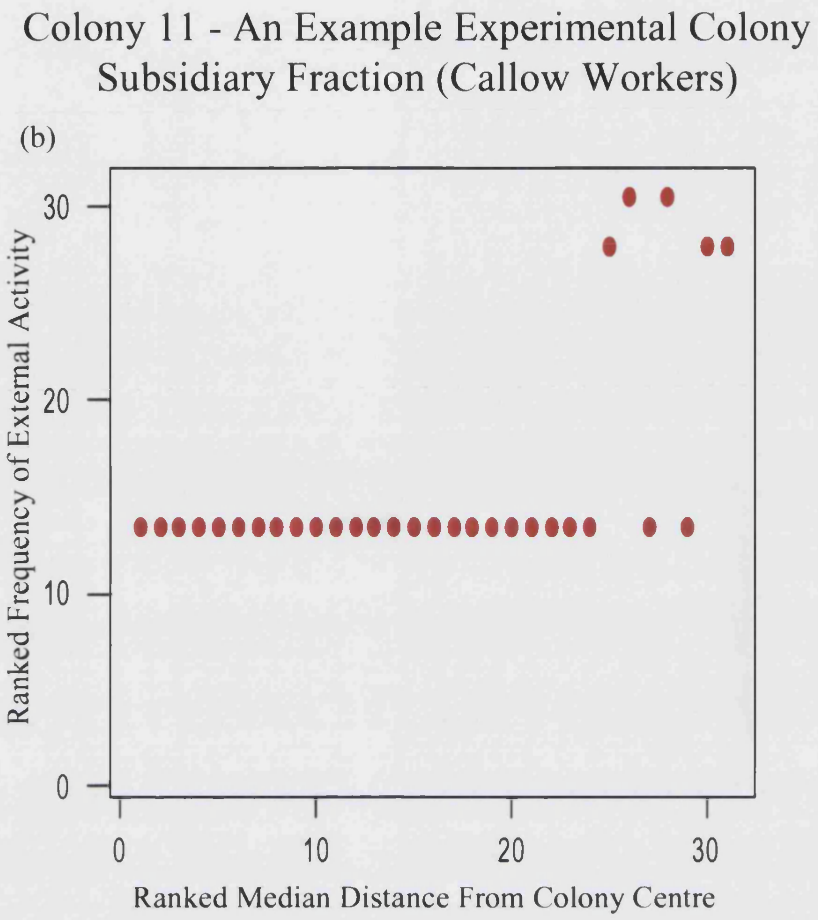


Figure 29: (a) The relationship between the ranked frequency of external activity and the ranked median distance from the colony centre in session 1, for Colony 11, an example experimental colony, older workers; (b) Colony 11, callow workers (shown in red).

Note, that for the data in Figure 29 (b), above, the correlation analysis shows that there is a positive, significant correlation between the relative frequency at which individuals perform external activity and their relative distance from the centre of the brood pile. There are essentially two clusters evident in Figure 29 (b): ants that have high ranked median distance from the colony centre as well as high ranked frequency of external activity, and ants that have low ranks on both scores. The correlation analysis is insensitive to these types of clusters, and this is noted.

Colony	Session	r_s	n	Significance Level p<
Experiment 3				
C_3	1	0.726	38	0.01
9 –Older Fraction	1	0.685	37	0.01
9 –Callow Fraction	1	0.240	59	0.01
10 –Older Fraction	1	0.339	33	0.01
10 –Callow Fraction	1	0.483	39	- No External Activity
11 –Older Fraction	1	0.592	41	0.01
11 –Callow Fraction	1	0.583	31	0.01
12 –Older Fraction	1	0.619	21	0.01
12 –Callow Fraction	1	0.506	30	0.01

Table 12: The relationship between the frequency of external activity and the median distance of individuals from the colony centre for experiment 3., session 1, calculated as r_s, *Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).*

What is the effect of uniting the 'Older' fractions with the 'Callow' fractions on the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre?

The graphs below show the relationship between the frequency at which individual workers perform external activity and their median position in the colony during:

session 2, which begins after 1 month has elapsed from the end of session 1, during which the two fractions of each colony are maintained separately.

session 3, when the two fractions of the colonies are united.

The Control colony (Colony C_3), and example plots of a typical experimental colony (Colony 11), are shown in Figures 30 (a-e), below.

Colony C_3 - Control

Fig 30(a)

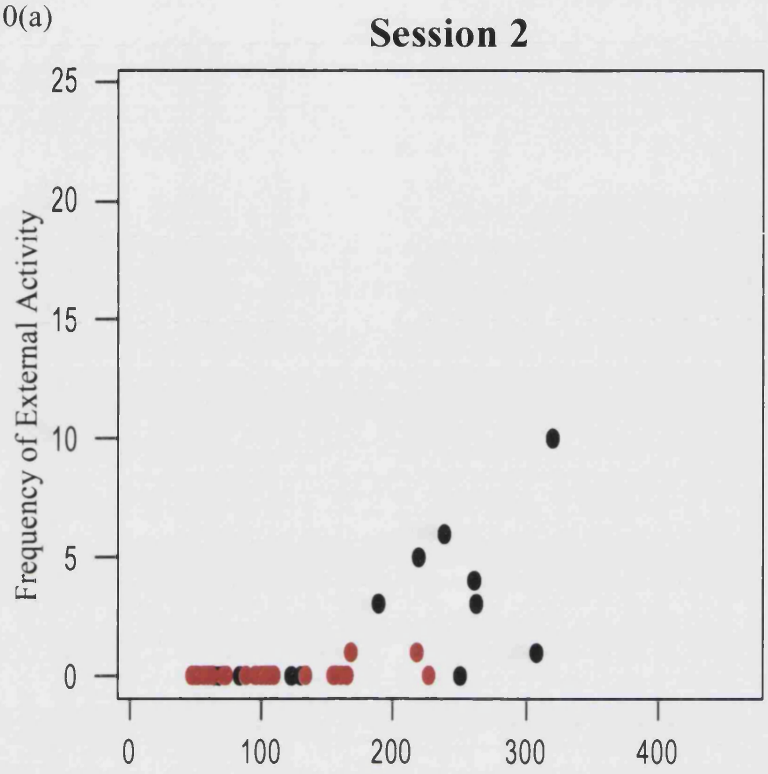
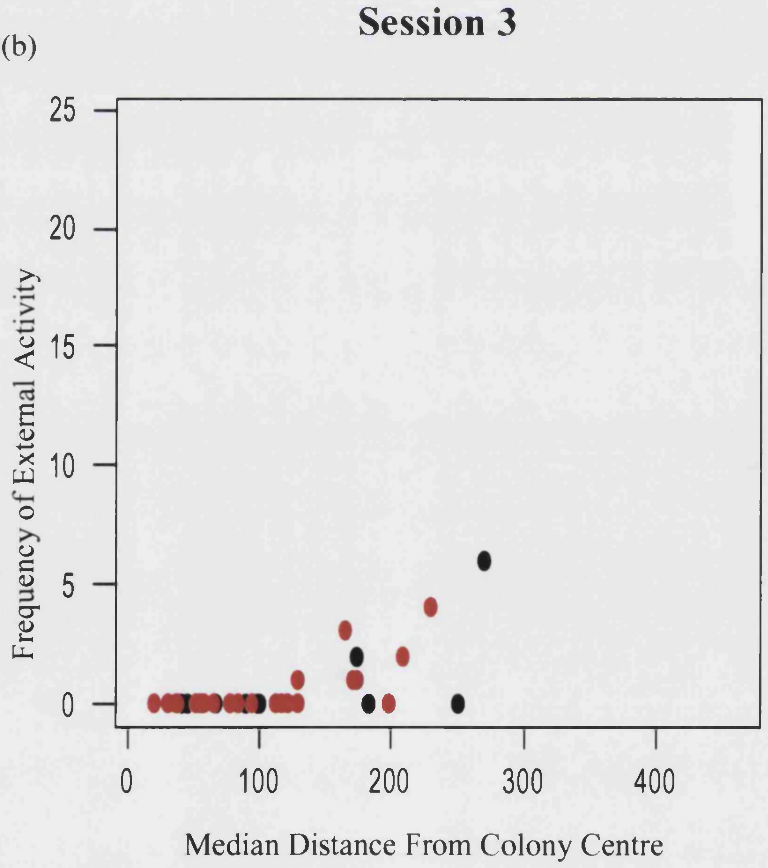


Fig 30(b)



Session 2

Fig 30(c) Colony 11 - An Example Experimental Colony
Original Fraction (Older Workers)

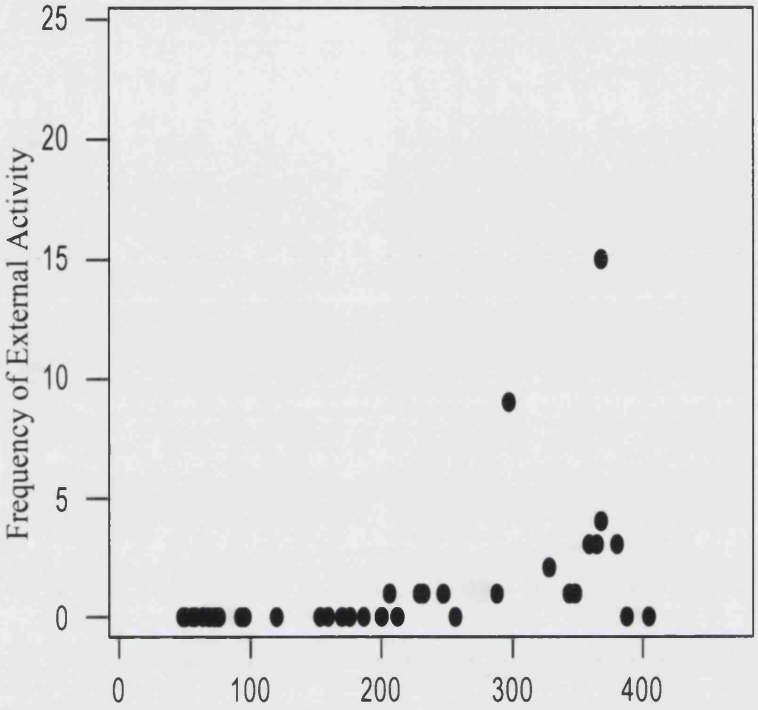
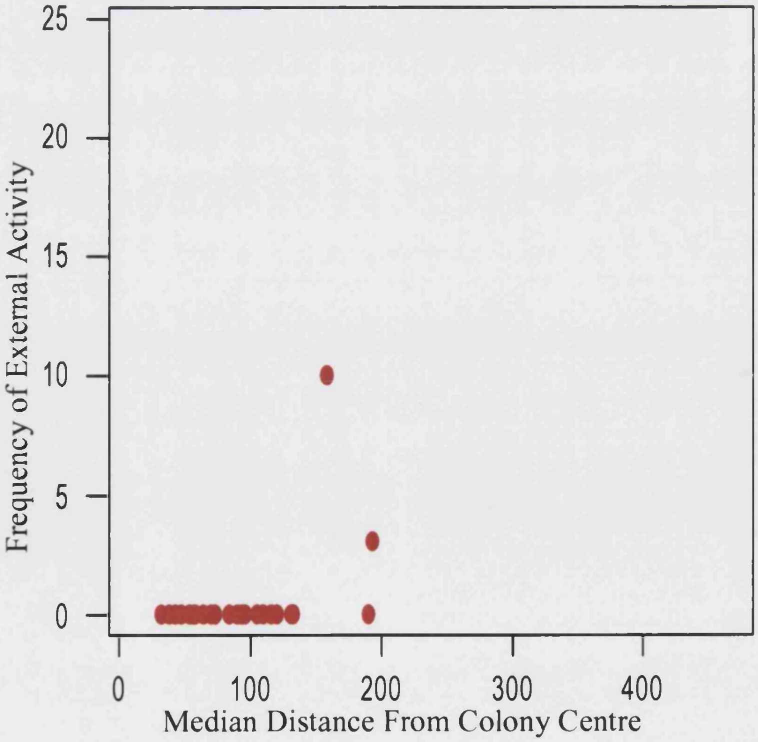
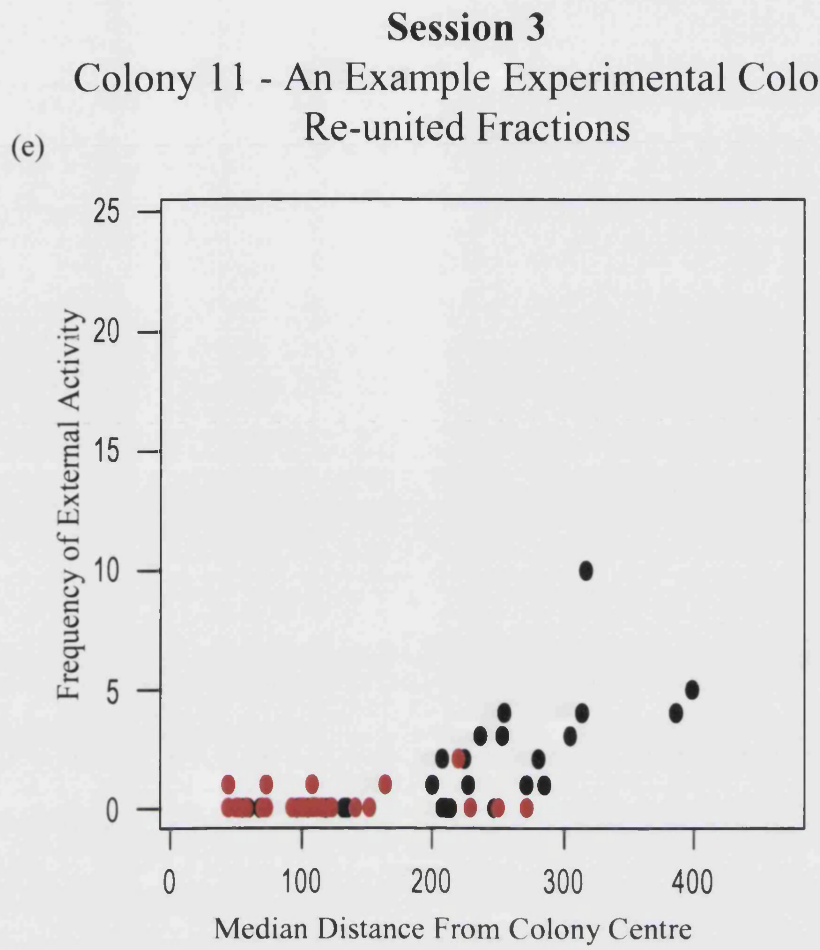


Fig 30(d) Colony 11 - An Example Experimental Colony
Subsidiary Fraction (Callow Workers)





Session 3
Colony 11 - An Example Experimental Colony
Re-united Fractions

(e)

Figure 30 : (a) The relationship between the frequency of external activity and the median distance from the colony centre for Colony C_3, the Control, session 2; (b) Colony C_3, session 3; (c) Colony 11, an example experimental colony, older workers, session 2; (d) Colony 11, callow workers (shown in red), session 2; (e) Colony 11, re-united fractions (callows shown in red), session 3.

The results of correlation analyses on these data are shown in Table 13, below, for experiment 3, sessions 2 (original and subsidiary fractions), and 3. There is a significant, negative correlation between the relative frequency at which individuals perform external activity and their median distance from the colony centre, for all the colonies in experiment 3, during sessions 2 (both fractions) and 3, except for the original (older) fraction of Colony 12 (discussed in section 4.3x). No external activity is recorded in the subsidiary (callow) fraction for sessions 1 and 2 for Colony 10, so no correlation coefficient can be calculated.

Colony/session	**r_s**	**n**	**Significance level p<**
Colony C_3			
Session 2	0.687	38	0.01
Session 3	0.617	35	0.01
Colony 9			
Session 2 – Older Workers	0.645	28	0.01
Session 2 – Callows	0.627	57	0.01
Session 3	0.562	65	0.01
Colony 10			
Session 2 – Older Workers	0.574	32	0.01
Session 2 – Callows	0.515	16	- No External Activity
Session 3	0.598	64	0.05
Colony 11			
Session 2 – Older Workers	0.694	38	0.01
Session 2 – Callows	0.416	30	0.01
Session 3	0.603	63	0.01
Colony 12			
Session 2 – Older Workers	0.235	22	NS
Session 2 – Callows	0.272	38	0.01
Session 3	0.487	53	0.01

Table 13 : The relationship between the ranked frequency of external activity and the ranked median of the distribution of distances from the colony centre, for experiment 3, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

A Closer Examination of Session 3 - Re-united Colonies

Experiment 3

The spatial organisation of external activity in session 3, when the original and subsidiary fractions of the colonies have been united, is examined further. I calculate the Spearman's rank correlation coefficient separately for the 'older' workers and for the 'callow' workers. The results are shown in Table 14, below.

Session 3 – Re-United Colonies				
Colony	Fraction of Colony Individuals were in in Sessions 1 and 2	r_s	n	Significance Level p<
Experiment 3				
9	Older	0.709	20	0.01
9	Callow	0.439	45	0.01
10	Older	0.708	33	0.01
10	Callow	0.510	31	0.01
11	Older	0.821	35	0.01
11	Callow	0.049	28	NS
12	Older	0.352	20	NS
12	Callow	0.564	33	0.01

Table 14 : The relationship between the frequency of external activity and the median distance of individuals from the colony centre for re-united colonies in experiment 3, calculated separately for individuals in the original and subsidiary fractions of the colonies during sessions 1 and 2, as r_s Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as $n > 10$ (Sokal and Rohlf, 1981 p.607). NS = non-significant.

There is a significant positive correlation between the relative frequency at which individuals perform external activity and their relative distance from the centre of the colony. This is the case for both subsets of individuals in session 3, in all the colonies except those in the 'callow' fraction of Colony 11, and those in the 'older' fraction of Colony 12. These results are discussed in section 4.3x.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the frequency at which callow individuals perform external activity and their median distance from the colony centre between session 1 (separate fractions) and session 3 (after unification) I perform the multiple comparison analysis detailed earlier in the chapter, comparing callows only. I also perform this analysis for older workers.

The results are shown in Table 14a, below.

z^{-}_{session} 1	z^{-} session 3	χ^2 session 1	Signif. Level_1 p<	χ^2 session 3	Signif. Level_3 $_{\text{p}}$p<	n_{mean1}	n_{mean} 3	t_s	Signif. level p<
Callows									
0.427	0.547	3.931	NS	0.502	NS	37.0	33.3	-1.918	NS
Older workers									
0.650	0.996	3.988	NS	1.450	NS	30.0	26.3	-1.224	0.01

Table 14a: Testing the relationship between the frequency of performance of external activity and the median distance of individuals from the colony centre for experiment 3, for combined colonies callow fractions, and combined older fractions separately, compared between sessions 1 and 3. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for both sessions compared, for the both the callow and the older workers. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the callow workers in the experimental colonies in session 1 and the pooled *z* for all the callow workers in experimental colonies in session 3 (where colonies exhibit a significant correlational relationship between the frequency at which individuals perform external activity and their median distance from the centre of the brood pile), shows that there is no significant difference between the colonies before the manipulation and after it. This is also the case for the experimental colonies

4.3 Discussion

An Investigation of the Spatial Organisation of Tasks

The spatial organisation of brood-related tasks and external activity has been investigated in colonies of *Leptothorax unifasciatus* (Sendova-Franks and Franks, 1993; 1994; 1995a; discussed in section 2.1ii.❸). Results showed that these tasks were highly organised spatially in this species. The number of times a worker performed brood-related tasks decreased linearly with the distance of her position from the centre of the colony. After a threshold distance from the centre of the colony, the frequency at which brood-related tasks were performed was zero. Conversely, it was shown that there was a threshold distance above which ants were very likely to go out of the nest (Sendova-Franks and Franks, 1995a). Workers re-adopted their relative spatial positions after emigration of the colony to a new nest-site. Individuals resumed their task, and the spatial organisation of the nest was re-established, a phenomenon known as *social resilience* (Sendova-Franks and Franks, 1994). The authors suggested that re-establishment of the relative spatial positions of the workers has implications for the role of learning in the maintenance of the division of labour.

This chapter investigates the spatial organisation of brood-related tasks and external activity (representative of two extremes of 'roles' within the colony, see section 2.2viii) in colonies of the ant *Leptothorax albipennis*. Crucially, the results show how the spatial organisation of these tasks in the colony is affected by sociotomy.

The results above are divided into the following sections:

4.1 An investigation of the spatial organisation of tasks in colonies with a manipulated task structure

    i. The spatial organisation of brood-related tasks
    ii. The spatial organisation of external activity

4.2 An investigation of the spatial organisation of tasks in colonies with a manipulated age structure

i. The spatial organisation of brood-related tasks
ii. The spatial organisation of external activity

The following discussion examines these results with reference to the questions posed in Chapter 3.

> What is the degree of division of labour within the colonies in terms of brood-related tasks and external activity?
> What is the degree of spatial organisation of brood related tasks, and of external activity, in colonies in experiments 1, 2 and 3?
> How is division of labour, and the spatial organisation of brood related tasks and external activity, affected by the manipulations carried out during the experiments? Specifically, these are the removal of those workers associated with external activity, and the colonies subsequent re-unification, in experiments 1 and 2; and the artificial construction of colonies consisting of 'callow' and 'older' workers, and their subsequent unification, in experiment 3.

For a summary of the methodology of all three experiments the reader is referred to Figure 9, Chapter 3.

Spatial Organisation of Brood-Related Tasks in Colonies with a Manipulated Task Structure

i. An investigation of the division of labour and the spatial organisation of brood-related tasks in un-manipulated colonies

To investigate the spatial organisation of brood-related tasks in colonies of *Leptothorax albipennis* in experiments 1and 2, I plotted the frequency at which each marked individual performs brood-related tasks in session 1, against the median of the distribution of distances of the individual from the colony centre during that session. Linear regressions were used to examine this relationship, using the logged frequency at which workers perform brood-related tasks, for some typical experimental colonies. Correlation analyses were carried out on all the colonies, according to the procedures described at the beginning of the chapter.

Experiment 1

There is an association between the location of an individual worker, in terms of her median distance from the colony centre, and the amount of brood-related tasks she performs. The results from session 1 show that the frequency at which an individual performs brood-related tasks decreases as her median distance from the centre of the brood pile increases. This is the case for the Control colony, and all four experimental colonies. Typical plots of this relationship are given in Figure 10 (a) and (b). The graphs also show that individuals whose median positions are above a threshold distance from the brood pile, do not perform brood-related tasks during the whole of session 1, or perform them at a very low frequency.

I investigated the relationship between the logged frequency at which individuals perform brood-related tasks and their median distance from the colony centre. Plots of this relationship are given in Figure 11 (a) and (b) for the Control colony, and experimental Colony 3, respectively, and both are a good approximation to a straight line. The results of linear regressions on these data show that the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre closely approximates an exponential decay. An individual with a median position halfway between the centre of the brood pile and the colony periphery, carries out brood-related tasks at half the log of the maximum frequency observed. The assumptions underlying linear regression are as follows:

For any value of x, y is normally distributed;

The magnitude of scatter of points about the line is constant throughout the length of the line (Kirkwood, 1988 p.63).

Because these assumptions are not met by the data, correlation analyses are carried out on the results from all the colonies as the main tool of analysis.

The relative frequency at which each individual performs brood-related tasks is significantly, negatively, correlated with her relative (median) distance from the colony centre (see Table 1). Thus, the division of labour with regard to brood-related tasks in the colonies is not discrete, but continuous.

Experiment 2

The results of experiment 2, during session 1, are very similar to those obtained in experiment 1. The frequency at which individuals perform brood-related tasks decreases as their median distance from the centre of the brood pile increases, Figure 12 (a). A plot of the relationship between the logged frequency at which individuals perform brood-related tasks, and their median distance from the colony centre, for a typical experimental colony, Figure 12 (b), is a good approximation to a straight line. I carried out linear regression, and the results fit the model well. Thus, the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre, is a good approximation to an exponential relationship. Correlation analyses, carried out on the data from all four experimental colonies and the Control (Colony C_2), show that the relationship between the relative variables is a significant negative correlation, as in experiment 1. There was, in fact, no difference between the two experiments up to this point, as in all cases the experimental colonies were un-manipulated. The Control colony in experiment 2, Colony C_2, had been treated differently, however, and this is discussed below.

The Control Colony, Colony C_2

Half of the brood of the Control colony in experiment 2 had been removed and replaced with stained, foreign, conspecific brood in order to control for the effects of putting stained, foreign conspecific brood in the subsidiary fractions of the experimental colonies in session 2. The Control colony, Colony C_2, shows the same pattern of spatially organised brood-related tasks during session 1, as the experimental colonies. Consequently, it can be concluded that replacing half of the colony's brood with stained, foreign, conspecific brood has no effect on the spatial organisation of brood-related tasks. However, and this must be noted for further discussion, the queen in this colony died during session 1 of the experiment. The death of the queen in this colony is unlikely to have been caused by the presence of stained, foreign brood. I do not conclude this from the results of the experimental colonies which were also provided with stained, foreign conspecific brood, as the queens were absent from these fractions. However, in three other *Leptothorax albipennis* colonies, in which stained foreign brood was placed on top of the nests, and subsequently adopted by the colonies, the queen did not die. I therefore conclude that the death of the queen in this colony resulted from 'natural causes'.

The purpose of the control was served. I established that the presence of stained, foreign, conspecific brood has no effect on the organisation of brood-related tasks. The remaining results from this control should be disregarded (they are given for the sake of completeness), as they may have been affected by the loss of the queen, or any subsequent senescence of this colony. As experiments 1 and 2 differed only in the adoption of stained, foreign, brood into the subsidiary fractions of the colonies in experiment 2, the Control from experiment 1 (Colony C_1), serves as an adequate control for the remaining variables in experiment 2. Colony C_1 was treated identically to the experimental colonies up to the beginning of session 2.

ii. An Investigation of the division of labour and the spatial organisation of external activity in un-manipulated colonies

I investigated the spatial organisation of external activity in colonies of

Leptothorax albipennis in experiments 1 and 2. The frequency at which each marked individual performs external activity in session 1, was plotted against the median of the distribution of distances of the individual from the colony centre during that session. It must be noted that this measure of spatial position could only be calculated for individuals that were inside the nest when at least some of the photographs were taken. Individuals that carried out external activity, but were not present inside the nest when any of the photographs were taken, could not be included in the analysis. However, such individuals are included in the analysis carried out in Chapter 8, where external activity is compared between pairs of sessions. Linear regression analyses, and correlation analyses were carried out as above.

Experiment 1

There is an association between the location of an individual worker, in terms of her distance from the colony centre, and the frequency at which she performs external activity. The results from session 1 show that individuals are much more likely to go outside the nest (or into the nest entrance) if they are situated at a relatively greater distance from the colony centre. Some individuals located closer to the centre of the brood pile do perform external activity, but they tend to do it at a lower frequency than those further out, and the majority of individuals closer to the centre of the brood pile do not perform any external activity during session 1, see Figure 18 (a) and (b).

I investigated the relationship between the logged frequency at which individuals perform external activity and their median distance from the colony centre. Plots of this relationship are given in Figure 19 (a) and (b) for the Control colony and an experimental colony, Colony 2, respectively. The results of linear regressions on these data show that the relationship between the frequency at which individuals perform external activity and their median distance from the colony centre closely approximates an exponential growth. Correlation analyses, performed on the data from all the colonies in experiment 1, show that the relative frequency at which individuals perform external activity is significantly, positively correlated with their relative (median) distance from the colony centre (see Table 5).

The division of labour, with respect to external activity, is also continuous. In fact, the division of labour with regard to these tasks appears to show greater continuity than in *Leptothorax unifasciatus* (Sendova-Franks and Franks, 1995a). In experiments on the latter species very few individuals ventured outside the nest if their median position in the colony was below a certain threshold, and if they did, they always exhibited very low frequencies of external activity. Although the trend in *Leptothorax albipennis* is similar, there are a small number of individuals whose median position in the colony is relatively close to the centre, that venture outside the nest at a similar frequency to some of the individuals at the extreme periphery of the nest.

Experiment 2

I carried out the same analysis on the results from experiment 2. Again, individuals are much more likely to go outside the nest if they are situated relatively further away from the centre of the brood pile. However, the relationship between the logged frequency at which individuals perform external activity and their median distance from the colony centre does not fit a linear model for the example experimental colony, Colony 2. I cannot therefore conclude that the frequency at which individuals perform external activity increases exponentially with their median distance from the colony centre for this colony. Examining the plot of this relationship, Figure 20 (a), shows that there are a small number of individuals, whose median position is relatively close to the colony centre, carrying out external activity at a relatively high frequency. However, correlation analyses still show that the relationship between the relative frequency at which individuals carry out external activity and their relative distance from the colony centre tends to be a significant, positive correlation (see Table 5). No such significant correlation is established for Colony 5, although the calculated p value is very close to the significance level, at 0.058.

Intriguingly, the Control colony for experiment 2 (Colony C_2), does not show a significant Spearman's rank correlation between the frequency at which individuals perform external activity and their median distance from the colony centre. A close examination of the data reveals that there is an extremely low level of external activity in this colony in this session. My hypothesis is that this is due to the colony's 'orphanage' state and the dominance interactions that occur within the colony after the death of the queen. However, this could also be due merely to the absence of a queen in this colony, and thus the lack of any centralised influence she may have on the organisation of external activity. This is relevant to the subsidiary fractions of the experimental colonies in session 3, experiments 1 and 2, which also lacked a queen, and the spatial organisation of their external activity, see session, 4.3iv., below. The behaviour of the queen, and her interactions with the workers, are analysed and discussed further in Chapter 5.

CONCLUSIONS

The following conclusions can be drawn:

Colonies of *Leptothorax albipennis* exhibit a continuous division of labour with regard to brood-related tasks and external activity.

The division of labour is spatially organised:

> The relative frequency at which individuals perform brood-related tasks is significantly, negatively correlated with their relative median distance from the colony centre.
>
> An exponential decay appears to be a good approximation in most cases.
>
> Individuals with median positions above a threshold distance from the brood pile, do not perform brood-related tasks, or perform them at a very low frequency.
>
> Individuals are much more likely to undertake external activity if they are situated relatively far away from the colony centre.
>
> The relative frequency at which individuals perform external activity is significantly, positively correlated with their relative median distance from the brood pile in 7 out of 8 of the colonies.
>
> Some individuals closer to the centre of the brood pile *do* perform external activity, but they tend to do it at a lower frequency than those further out, and the majority of individuals closer to the centre of the brood pile do not perform any external activity.

iii. How is the division of labour and the spatial organisation of brood-related tasks affected by the removal of individuals associated with external activity?

In the following section I discuss how the division of labour in the experimental colonies in experiments 1 and 2 is affected by the removal of individuals associated with external activity during session 2. A photographic and behavioural record of session 2 was kept in experiment 1, and this is examined. This was not necessary in experiment 2, as the experiments were identical up to the end of session 2. During session 3, the removed 'externals' were kept in a separate nest, which formed the 'subsidiary' fraction of the relevant colony. A photographic and behavioural record was kept of this 'subsidiary' fraction, as well as the 'original' fraction, of all the colonies.

The organisation of brood-related tasks cannot be examined in the subsidiary fractions of experiment 1 during session 3, as the nests did not contain any brood. In experiment 2 the subsidiary fractions were provided with stained, foreign, conspecific brood. Results are analysed in a similar way to 4.3i. and 4.3ii., above. The frequency at which each marked individual performs brood-related tasks in the relevant session is plotted against the median of the distribution of distances of the individual from the centre of the brood pile during that session. Correlation analyses were carried out on all the colonies according to the procedures described at the beginning of the chapter.

Experiment 1

The association between the location of an individual worker, in terms of her median distance from the colony centre, and the amount of brood-related tasks she performs, remains, during and after the removal of individuals associated with external activity (sessions 2 and 3 respectively). Figure 13 (a) and (b) are plots of the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre for the Control colony and a typical experimental colony (Colony 3), respectively, during session 2. The relationship is similar to that observed during session 1 for these colonies. The frequency at which an individual performs brood-related tasks decreases as her median distance from the centre of the brood pile increases. A close examination of the graphs in Figures 13 (a) and (b) show that, for both the Control and the experimental colony, the scatter of points is less spread out on the right hand side than is seen in the graphs of this relationship during session 1 (Figure 10 (a) and (b)). The 'threshold' distance from the centre of the brood pile, beyond which individuals perform little or no brood-related tasks, in fact, no longer exists to the same extent, and this is discussed below. However the overall pattern of the spatial organisation of brood-related tasks has been preserved. The relative frequency at which individuals perform brood-related tasks and their relative (median) distances from the colony centre are significantly, negatively correlated (see Table 2). When the frequency at which individuals perform brood-related tasks is logged, and plotted against their median distances from the colony centre, the resulting graph for experimental Colony 3, is a good approximation to a straight line, see Figure 16 (a). The exponential nature of the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the centre of the brood pile, is also preserved.

At the beginning of session 2, the Control colony (Colony C_1) was emigrated to a new nest, and the first third of the workers to leave the nest were removed. A week later, the fractions were re-united and the colony was photographed at the same rate as the experimental colonies, for the remainder of the session. The resulting pattern of the data is thus a consequence of this manipulation. My interpretation is that the colony takes a short period of time to 'adjust' to the manipulation and for its labour force to re-organise. Further evidence for this is provided by the plots of later sessions, where the right hand tail portion of the graph begins to spread out again in this colony, see Figure 14 (a).

In session 3, the organisation of brood-related tasks can only be examined in the original fraction of the colony, from which the individuals associated with external activity had been removed, as only these fractions had brood in their nests. Figure 14 (a) and (b) shows the resulting plots for the Control colony, and the example experimental colony, respectively. The Control colony has been discussed above. The relationship in the original fractions of the experimental colony is similar to that shown in Figure 13 (b). The frequency at which individuals perform brood-related tasks decreases with increasing distance from the centre of the brood pile. Correlation analyses show that there is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative median distance from the centre of the brood pile, for all the colonies except for Colony 4 (see Table 2). Colony 4 does show a negative correlation coefficient, but *n* is not sufficiently high to establish a significant correlation. Figure 16 (b), gives an example plot of the relationship when the frequency at which individuals perform brood-related tasks has been logged, and again indicates that the relationship is a good approximation to an exponential decay.

It is clear that the removal of workers associated with external activity from colonies of the ant *Leptothorax albipennis*, has little effect on the spatial organisation of brood-related tasks among the remaining individuals. My hypothesis is that it is these remaining individuals that were previously responsible for the majority of brood-related tasks before the 'externals' were removed. This is analysed and discussed further in Chapter 8, where I investigate individual performance of brood-related tasks. What is of considerable interest here, is the preservation of the profound spatial structure in the nest relating to these tasks, and minimum disruption to the relative performance of brood-related tasks in the colony, despite the removal of a large portion of the workforce.

Experiment 2

The association between the location of an individual worker, in terms of her median distance from the colony centre, and the frequency at which she performs brood-related tasks, tends to remain, after the removal of individuals associated with external activity in experiment 2. The treatment of the original fractions of the colonies was identical to that of the original fractions in experiment 1. In colonies containing sufficiently high numbers of individuals that have retained their marks to render the analysis valid (Colonies 7 and 8), the relationship between the variables is similar to that observed during session 1 for these colonies. Figure 17 (a) is a plot of the relationship between the frequency at which individuals perform brood-related tasks and their median distances from the colony centre for an example experimental colony (Colony 7). The frequency at which an individual performs brood-related tasks decreases as her median distance from the centre of the brood pile increases. Again, the right hand tail of the curve is less spread out, as was discussed for experiment 1. Correlation analyses show that there is a significant, negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative median distance from the colony centre (see Table 3), for Colonies 7 and 8. In Colonies 5 and 6, a high proportion of workers did not retain their marks and consequently could not be identified. Therefore there is insufficient data to examine the relationship.

Figure 17 (b) is a plot of the relationship between the frequency at which workers perform brood-related tasks and their median distance from the centre of the brood pile, for the subsidiary fraction of an example colony during session 3. The data show an astonishingly similar relationship to that seen in the original fraction of session 3, in both experiments 1 and 2, and to that seen in session 2 of experiment 1. My hypothesis is that these individuals, that previously performed brood-related tasks at a relatively low frequency, and were situated relatively far from the centre of the brood pile in the experimental colonies, now perform brood-related tasks and are situated close to the new pile of adopted brood. If this is the case, they have re-organised their labour to tend for the brood, and, moreover, they show the same pattern of spatial organisation as the other fraction of their colony. This has profound implications for task allocation in these colonies, and emphasises the role of spatial organisation in this process. An alternative hypothesis is that these individuals performed some brood-related tasks prior to their removal albeit at lower absolute frequencies, and continue to do this in the subsidiary fraction. This is examined and discussed further in Chapter 8. Importantly, there is no queen present in the subsidiary fractions of the colonies, and the results show that the absence of the queen does not affect the spatial organisation of brood-related tasks.

iv. How is the division of labour and the spatial organisation of individuals that perform external activity affected by the removal of individuals previously associated with external activity?

The following is a discussion of the spatial patterns of individuals that perform external activity in the colonies during and after the removal of the individuals previously associated with external activity (sessions 2 and 3, original fractions, respectively). The external activity of these removed workers, which subsequently constituted the 'subsidiary' fractions of each colony, was also examined. Results were analysed in a similar way to those above. The frequency at which each marked individual performs external activity in the relevant session is plotted against the median distance of the individual from the centre of the brood pile during that session. Correlation analyses are carried out according to the procedure described at the beginning of the chapter.

Experiment 1

I have shown that, in colonies which have not been manipulated (session 1), the frequency at which individuals perform external activity increases as their median distance from the centre of the brood pile increases. During session 2, when individuals carrying out external activity are being sequentially removed from the experimental colonies, no external activity is observed by individuals in the colony at the actual times that photographs were being taken. External activity *does* occur, but at very low frequencies, and no record was created as no activity was seen at the time the data was collected. This is also the case during session 3 for the original fractions of the colonies, from which individuals associated with external activity had been removed. In the Control colony, Colony C_1, external activity continues to occur, and an examination of the spatial organisation within the colony shows that the same pattern is observed in session 2, as was seen in session 1 of this, and the other colonies. Therefore the physical process of removing the individuals does not lead to any changes in the spatial organisation of external activity.

In the subsidiary fractions of the experimental colonies in session 3, which consist only of the removed individuals, a similar pattern of performance of external activity is observed to that seen in un-manipulated colonies during session 1. Individuals further away from the centre of the colony (taken as the centre of the brood pile in the original fraction of the relevant colony during session 1, because there is no brood in the subsidiary fractions) are more likely to perform external activity, see Figure 21 (b). The results of correlation analyses show that the relative frequency at which individuals perform external activity and their relative distance from the colony centre are significantly, positively correlated for three out of the four experimental colonies, see Table 6. Colony 4 still shows a positive correlation coefficient, but this is not significant. However, the calculated p value is between 0.05 and 0.1. A closer examination of the data for Colony 4 shows that one individual carried out external activity at an unusually high rate, skewing the results. If this individual is not considered, and the Spearman's rank correlation coefficient is re-calculated, a positive and significant correlation is established ($r_s = 0.528, p < 0.05, n = 18$).

Thus, the removal of the individuals associated with external activity has a profound effect on the subsequent organisation of external activity within the colony. However, when these removed individuals are placed in a new nest, they not only continue to perform external activity, but they exhibit the same spatial organisation within the colony as un-manipulated colonies. The absence of the queen and brood in these colonies has no effect on the spatial organisation of external activity. The individuals remaining in the colony from which the 'externals' have been removed exhibit a much lower level of external activity compared to the intact colony during session 1, although external activity does still occur.

During session 1 there is some degree of overlap between individuals that perform brood-related tasks and those that perform external activity – some individuals are capable of doing both as part of their normal task repertoire. These individuals are normally situated in the centre of the distribution of workers in the nest. There are two possible explanations for the occurrence of external activity, albeit at a low frequency, in the original fractions of the colonies during session 3. First, the individuals that carry out external activity in the original fractions of the colonies in session 3 of the experiment, are those individuals that carried out external activity in session 1, but were not removed. Second, the individuals that carry out external activity in the original fractions of the colonies in session 3 could be individuals that have not previously carried out external activity, and thus are demonstrating precocious external activity, as discussed in Chapter 2, section 2.2vi.❶. The former hypothesis is considered unlikely as the removal of individuals performing external activity in session 2 is very thorough, and the sampling procedure employed to remove them was designed to remove *all* individuals associated with external activity. The results are analysed and discussed further in Chapter 8, in the light of these hypotheses.

Experiment 2

No external activity is recorded during the photographic sessions for colonies 5, 6 and 7 in the original fractions during session 3, although it is observed that external activity does still occur, albeit at very low frequencies. External activity is recorded in the original fraction of session 3 for experimental Colony 8. However, although the correlation between the relative frequency at which individuals perform external activity and their relative distance from the colony centre is positive, it is not significant. A close examination of these data reveals that external activity still occurs only at very low frequencies during this session, with a maximum of three incidents of external activity for any one individual. Consequently it is difficult to establish a correlation between the variables.

The subsidiary fractions of the experimental colonies during session 3 contain the removed workers, and adopted brood. The familiar pattern of increasing frequency of external activity with increasing distance from the colony centre is established for these fractions, despite the fact that these workers have taken on brood-related tasks. There is a significant correlation between the relative frequency at which individuals perform external activity and their relative median distance from the centre of the adopted brood pile in Colonies 6, 7 and 8. There is insufficient data to establish a correlation in Colony 5 due to insufficient numbers of individuals that have retained their marks.

The spatial organisation of external activity in both fractions of the colonies during session 3 in experiment 2 is very similar to patterns observed in session 3 of experiment 1. I conclude that the adoption of brood by the subsidiary fractions of the colonies does not affect the patterns and spatial organisation of external activity in these colonies.

CONCLUSIONS

The following conclusions can be drawn:

Removal of workers associated with external activity from colonies of *Leptothorax albipennis* does not affect the spatial organisation of brood-related tasks among the remaining workers.

> The frequency at which brood-related tasks are performed by the remaining workers decreases with their increasing distance from the colony centre.
>
> The relative frequency at which individuals perform brood-related tasks is still significantly, negatively correlated with their relative median distance from the brood pile, for 5 out of 6 of the colonies.
>
> This relationship approximates well to an exponential decay.
>
> Thus there is minimum disruption to brood-related tasks in the colony despite the loss of a large proportion of the workforce.
>
> This has important consequences for colony survival.

Removal of workers previously associated with external activity in colonies of *Leptothorax albipennis* results in a drastic reduction in external activity among the remaining individuals, but some external activity does occur. There are two hypotheses to explain this:

> The individuals that carry out external activity after the removal of workers previously associated with external activity, undertook external activity prior to the removal of 'externals', but they were not removed from the colony, *and/or*
>
> The individuals that carry out external activity after the removal of workers previously associated with external activity are demonstrating precocious external activity.

Intriguingly, the removed workers are not only capable of carrying out brood-related tasks when placed in new nests with adopted brood, but they do so despite the absence of the queen.

There are two hypotheses to explain this:

> The individuals that carry out brood-related tasks in the subsidiary fractions also carried out brood-related tasks in the colonies before they were manipulated (session 1), but were still removed because they performed some external activity in session 2, *and/or*
>
> These individuals did not carry out brood-related tasks in the colonies before they were manipulated, and are consequently showing behavioural reversion to nurse-work.

Crucially, the familiar patterns of spatial organisation of brood-related tasks established in un-manipulated colonies, are also established in the colonies consisting only of removed workers, previously associated with external activity, and adopted foreign brood.

> The frequency at which individuals perform brood-related tasks in these colonies, is still significantly negatively correlated with their median distance from centre of the adopted brood pile for all colonies for which there is sufficient data.

The familiar pattern of spatial organisation of external activity observed in un-manipulated colonies is also established in the 'artificial' colonies consisting only of removed workers and adopted brood. This pattern is preserved despite the absence of the queen, and is still evident in colonies that have also taken on brood-related tasks.

> The frequency at which individuals perform external activity is still significantly, positively correlated with their median distance from the brood pile, for 6 out of 7 of the colonies.

v. How is the division of labour and the spatial organisation of brood-related tasks affected by the re-unification of the original and subsidiary fractions?

In the following section I will discuss how the division of labour with relation to brood-related tasks in the experimental colonies in experiments 1 and 2 is affected by re-unification of the original fractions of the colonies with the relevant subsidiary fractions at the beginning of session 4. The results are analysed in a similar way to the analysis above. Moreover I also performed a multiple analysis comparing the pooled results for experimental colonies between sessions 1 and 4 to determine if sociotomy affects the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the centre of the brood pile. The frequency at which each marked individual performs brood-related tasks in session 4 is plotted against the median of the distribution of distances of the individual from the centre of the brood pile in session 4. Correlation analyses are carried out on all the colonies according to the procedure at the beginning of the chapter.

Experiment 1

The association between the location of an individual worker, in terms of her median distance from the colony centre, and the amount of brood-related tasks she performs, remains, after the re-unification of individuals that were associated with external activity (the subsidiary fractions during session 3) and the original fractions of the colonies. Figure 15 (a) and (b) are plots of the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre for the Control colony and a typical experimental colony (Colony 3), respectively, during session 4. The relationship for the Control colony is similar to that observed during session 1. The frequency at which individuals perform brood-related tasks decreases as their median distance from the centre of the brood pile increases. The right hand side of the graph in Figure 15 (a) shows a scatter of points, forming a 'tail', in which individuals perform little or no brood-related tasks. This 'tail' is less apparent in sessions 2 or 3 for this colony and may be the result of the temporary removal of some individuals from the colony. The colony may take a period of time to 'recover' from this manipulation, as mentioned above. Moreover, the brood are likely to have reached a stage in this session where they require less care, associated with the time of year, and this may also have an effect on the re-appearance of this 'tail' of workers exhibiting low frequencies of brood-related tasks.

In Figure 16 (b) individuals that had been removed from the experimental colony, as they were associated with external activity, and thus formed the 'subsidiary' fraction of the colony during session 3, are shown in red. The pattern of the relationship is very similar to that observed in session 1, for this example experimental colony. I determine that the colonies can be considered samples from a population exhibiting a common correlation among the variables, in both session 1 and session 4. When I compare the correlational relationship between the variables for the pooled experimental colonies in session 1 and session 4, I determine that there is no significant difference between the relationships (see Table 2a). Again, the frequency at which individuals perform brood-related tasks in each colony decreases with increasing distance from the centre of the brood pile. When the frequency at which individuals perform brood-related tasks is logged and plotted against their median distance from the centre of the brood pile, the resulting relationship is a good approximation to a straight line, Figure 16 (c), indicating the relationship is closely approximated by an exponential decay curve.

The relative frequency at which individuals perform brood-related tasks and their relative (median) distances from the colony centre are significantly, negatively correlated (see Table 2), for all four experimental colonies and the Control.

Colonies continue to exhibit a division of labour with regard to brood-related tasks despite the fact that the colonies have been massively disrupted by the removal of workers associated with external activity, and then subsequently re-united with these removed workers. Moreover, the spatial organisation of brood-related tasks within the colony remains very similar to the pattern observed before the colonies were manipulated. This demonstrates not only the resilience of the colony to massive disruptions, such as the removal of an entire group of individuals associated with a particular role, but the tremendous importance of spatial organisation of tasks within the colony. This is analysed further in Chapter 8, and the implications of these results are discussed in Chapter 9.

A closer examination of Figure 15 (b) reveals that most of the individuals that were removed because they were associated with external activity (shown in red), are found in the right hand tail portion of the graph. They are relatively far from the centre of the brood pile and carry out little or no brood-related tasks. There are some individuals, however, that are located closer to the centre of the brood pile and exhibit a higher frequency of brood-related tasks. My hypothesis is that the individuals that are closer to the centre of the brood pile were also closer to the centre of the brood pile and carried out brood-related tasks in session 1, but were removed because they also carried out external activity, during session 2. The results are discussed further in Chapter 8, with reference to this hypothesis.

Table 4 shows Spearman's rank correlation coefficients for the experimental colonies in session 4, calculated separately for the individuals that were in the original fraction, and the individuals that were in the subsidiary fraction, during session 3. The relative frequency at which individuals perform brood-related tasks is significantly, negatively, correlated with their relative median distance from the centre of the brood pile, for both individuals that were in the original fractions, and those that were in the subsidiary fractions. This is the case for all the experimental colonies except those that were in the original fraction of Colony 1. These individuals still demonstrate a negative relationship between the two variables, but it was not significant. This is due to very low numbers of individuals that retained their marks in this portion of the colony. Thus, the spatial organisation seen in session 1 of the experiment is also evident in session 4, for both individuals that have been removed, and then replaced, and for individuals that had remained in the original fraction of the colony, as well as the two groups considered together.

Experiment 2

The overall pattern of association between the location of an individual worker and the frequency at which she performs brood-related tasks, also remains after the re-unification of the two colony fractions in experiment 2, when the subsidiary fractions were provided with brood. Figures 17 (c) and (d) show the relationship between the frequency at which individuals carry out brood-related tasks and their median distance from the centre of the brood pile in session 4, for two example experimental colonies in experiment 2 (Colonies 7 and 8, respectively). Both graphs show that the frequency at which individuals perform brood-related tasks decreases with increasing distance from the centre of the brood pile, the same as the pattern observed in session 1 of the experiment. However, the curve does show less spread of points in the right hand portion, and crucially, individuals that were in the subsidiary fraction of the colonies during session 3 (shown in red) are present throughout the colony, not just at the periphery of the distribution of workers. When I compare the correlational relationship between the variables for the pooled experimental colonies in session 1 and session 4, for the colonies in experiment 2, I determine that there is no significant difference between the relationships (see Table 2a). I also perform multiple comparisons between the correlational relationships between the frequency at which workers in session 4, experiment 1, perform brood-related activity, and these relationships for the colonies in experiment 2, to determine if the presence of brood in the subsidiary fractions of the colonies in experiment 2 had a significant effect on sociotomy, when fractions of the colonies were re-united (see Table 2b). I have already determined that the colonies in experiment 1, session 4, can be considered samples from a population exhibiting a common correlation. This is also the case for the colonies in experiment 2. When I compared the pooled correlation coefficients, I determined that there was no significant difference between them. I found the result was significant only at $0.05<p<0.1$. Therefore there is evidence at this level of significance that the presence of brood in the subsidiary fractions of the experimental colonies in experiment 2 has some effect on the relationship between the frequency at which individuals perform brood-related tasks. I discuss patterns of performance of brood-related tasks further in Chapter 8.

The relative frequency at which individuals perform brood-related tasks still tends to be significantly, negatively correlated with their relative median distance from the centre of the brood pile (Table 3). This is the case for Colonies 6, 7 and 8. There is insufficient data to calculate a correlation coefficient in Colony 5.

Again, the trend of decreasing frequency of performance of brood-related tasks with increasing distance from the centre of the brood pile is evident in these colonies. This still occurs when the removed workers are re-united with the colony. These removed workers had in fact established the same spatial organisation in a colony containing brood during session 3, and had to re-organise labour when they were re-united with the original fraction. This is analysed and discussed further in Chapter 8.

I carried out an analysis separately for individuals that were removed during session 2, and those that remained in the original fraction, in session 4, as for experiment 1. The results are shown in Table 4. There is a significant negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative distance from the centre of the colony, for both fractions in Colony 8, and for those individuals that were in the subsidiary fraction of Colony 6. This relationship is not a significant correlation for those individuals that were in the original or the subsidiary fractions in Colony 7. Figure 17 (c), the plot of the frequency at which individuals perform brood-related tasks against their median distance from the centre of the brood pile for Colony 7, is compared to Figure 17 (d), the corresponding plot for Colony 8. The general trend of decreasing frequency of brood-related tasks with increasing distance from the centre of the brood pile is still evident for those individuals that were in the subsidiary and original fractions for session 4, in Colony 7. The pattern is very similar to that observed for Colony 8, in Figure 17 (d), but a greater scatter of points in Colony 7 results in non-significant correlations. The general trend is the same for both colonies. There are insufficient numbers of individuals that have retained their marks to allow the calculation of correlation coefficients for Colony 5, or for those that were in the original fraction of Colony 6.

In the re-united colonies the overall spatial organisation of brood-related tasks is remarkably similar to that observed in the un-manipulated colonies. Even individuals that had been removed because they were associated with external activity, and had subsequently adopted and cared for foreign, conspecific brood, still demonstrate a general trend towards the same spatial organisation of brood-related tasks when re-united with the original fraction. However these individuals are more spread out in their spatial distribution than the corresponding individuals in the re-united colonies in experiment 1, which had no brood when they existed as separate fractions. In Chapter 8, I discuss whether individuals in the subsidiary fractions of the colonies in experiment 2 have changed their behavioural role to perform more brood-related tasks during session 3, and subsequently maintained this change after re-unification. I will also discuss whether individuals re-organise their division of labour with respect to brood related tasks when the individuals associated with external activity are removed, and subsequently re-organise their division of labour again when the colonies are re-united, whilst still maintaining the same overall spatial organisation throughout.

vi. How is the division of labour and the spatial organisation of individuals that perform external activity affected by the re-unification of the original and subsidiary fractions?

In the following section I will discuss how the spatial organisation of individuals that perform external activity in the experimental colonies in experiments 1 and 2 is affected by re-unification of the original fractions of the colonies with the relevant subsidiary fractions in session 4. Results are analysed in a similar way to the analysis above. The frequency at which each marked individual performs external activity in session 4 is plotted against the median of the distribution of distances of the individual from the centre of the brood pile in session 4. Correlation analyses are carried out according to the procedure described at the beginning of the chapter.

Experiment 1

The association between the location of a worker and her frequency of external activity remains, after re-unification of the two colony fractions. Figure 22 (a) is a graph of this relationship for the Control colony (Colony C_1). The frequency at which individuals perform external activity increases with increasing distance from the centre of the brood pile. The relationship between the relative frequency at which individuals perform external activity and their relative median distance from the centre of the brood pile is a significant, positive correlation (see Table 6). The spatial organisation of individuals that carry out external activity in this colony, is the same throughout sessions 1 to 4. Therefore, the physical act of removal of the workers does not affect this relationship.

Figure 22 (b) is the corresponding plot for Colony 2, a typical experimental colony. Individuals that were removed from this colony during session 2, and were thus in the subsidiary fraction of the colony in session 3, are shown in red. The graph shows the same familiar pattern of spatial organisation of individuals that carry out external activity. The frequency at which individuals carry out external activity increases as their median distance from the centre of the brood pile increases. This trend is true for all the experimental colonies and there is, in fact, a significant positive correlation between the two ranked variables for Colonies 1, 2 and 3, during session 4 (see Table 7). Multiple comparisons of pooled data for the experimental colonies in experiment 1 show that there is no significant difference between the correlational relationship between the variables in session 1 compared to session 4, I determine that there is no significant difference between the correlations (see Table 2a). Colony 4 does not show a significant relationship, although the general trend is similar. A closer examination of the data for this colony shows that the relationship is skewed by the relatively high performance of external activity by just three individuals in this colony. Individuals that were in the subsidiary fraction of Colony 2 during session 3, and thus were removed as 'externals' in the original colony, are seen to carry out almost all the external activity in the colony, Figure 22 (b). These individuals tend to be situated relatively further from the centre of the brood pile.

The individuals that remain in the original fraction show little external activity, and tend to be situated nearer to the brood pile.

The spatial organisation of external activity was examined separately for those workers that were in the original, and those that were in the subsidiary fractions of the colonies. There tends to be a significant, positive correlation between the relative frequency at which workers that were in the subsidiary fraction during session 3 perform external activity and their relative median position in the re-united colony (see Table 8). Colony 4 is an exception to this, and has been discussed above. There is no clear relationship between these variables during session 4 for those individuals that formed the original fraction in session 3. There is a significant, positive correlation between the relative frequency at which individuals perform external activity and their relative position in Colony 3, for workers that had been in the original fraction, but the relationship is not significant for these individuals in Colonies 1 or 2. There is insufficient data for this calculation for Colony 4. Therefore it can be concluded that although there is a tendency towards the same spatial structure for these individuals, the data indicates that generally the level of external activity exhibited by these individuals in session 4 is too low for a relationship to be established.

I conclude that the general trend of increasing frequency of external activity with increasing distance from the centre of the brood pile is preserved in session 4, despite the removal of workers associated with external activity, and the colonies subsequent re-unification. Again, this demonstrates the highly structured nature of work in these colonies. Whether particular individuals are conservative in their spatial organisation and task profiles with regard to external activity throughout the sessions of the experiment is analysed further and discussed in Chapter 8.

Experiment 2

I examined the relationship between the frequency at which individuals carry out external activity and their median distance from the centre of the brood pile for colonies in experiment 2, session 4. There is a significant, positive correlation between the relative frequency at which individuals perform external activity and their relative median distance from the colony centre during session 4 for all three colonies for which there was sufficient data (see Table 7). Thus, I conclude that the spatial organisation of individuals that perform external activity is very similar to that seen in the un-manipulated colonies during session 1. Multiple comparisons of pooled data for the experimental colonies in experiment 1 show that there is no significant difference between the pooled correlational relationships between the variables in session 1 compared session 4, I determine that there is no significant difference between the relationships (see Table 2a). The pattern of spatial organisation is also very similar to that seen during session 4 for the colonies in experiment 1, which did not have adopted brood in the subsidiary fractions in session 3.

The results from session 4 of experiment 2 are also analysed separately for the individuals that had been in the subsidiary fraction, and those that had been in the original fraction during session 3 (see Table 8). There is a positive, significant, correlation between the relative frequency at which individuals perform external activity and their relative spatial position for those that were in the subsidiary fraction for two out of the three colonies, for which there was sufficient data. Both the colonies for which there was sufficient data did not show a significant correlation between the variables among individuals that were in the original fraction. In the latter, very few individuals are involved in any external activity. Thus the pattern of spatial organisation of individuals that had been in the original or the subsidiary fractions, when examined separately, does not generally follow the established pattern, although crucially, when the fractions are considered together, the overall spatial organisation is preserved. This is discussed further in Chapter 8, which examines whether individuals alter their task profiles, and whether particular individuals are conservative in their spatial organisation within the colony despite the experimental manipulations.

CONCLUSIONS

The following conclusions can be drawn:

Removal of workers associated with external activity from colonies of *Leptothorax albipennis* into colonies without brood or a queen, and the subsequent re-unification of the fractions of the colonies, does not affect the overall spatial organisation of brood-related tasks in the colonies.

> The frequency at which individuals perform brood-related tasks decreases with increasing distance from the colony centre.
>
> The relative frequency at which individuals perform brood-related tasks is still significantly negatively correlated with their relative median distance from the brood pile, for 4 out of 4 of the re-united colonies.
>
> This relationship is closely approximated by an exponential decay.
>
> The relationship tends to be a significant, negative correlation for both the individuals that had been removed, and the individuals that had not been removed, considered separately.
>
> Thus, there is minimum disruption to the spatial organisation of brood-related tasks in the colony, despite the loss of a large proportion of the workforce, and its subsequent re-unification.
>
> This has important consequences for colony survival.

Removal of workers previously associated with external activity from colonies of *Leptothorax albipennis* into colonies without brood or a queen, and the subsequent re-unification of the fractions of the colonies, does not affect the overall spatial organisation of external activity in the colonies.

> The frequency at which individuals perform external activity increases with their increasing distance from the centre of the brood pile.

The relationship between the relative frequency at which individuals perform external activity and their relative median distance from the centre of the brood pile is a significant, positive, correlation for 3 out of 4 experimental colonies.

Individuals that are not removed from the original colonies continue to perform external activity at very low frequencies when the fractions of the colonies are re-united.

Individuals that are removed as 'externals' continue to show a similar pattern of spatial organisation of external activity after re-unification to that observed when they formed separate fractions with no brood.

The relationship between the relative frequency at which these individuals perform external activity in the re-united colonies and their relative distance from the centre of the brood pile is a significant positive correlation for 3 of the 4 colonies.

Removal of workers associated with external activity from colonies of *Leptothorax albipennis* into colonies with foreign brood, but no queen, and the subsequent re-unification of these fractions of the colonies with the original fractions, does not affect the overall spatial organisation of brood-related tasks.

The frequency at which brood-related tasks are performed by the workers decreases with increasing distance from the colony centre.

The relative frequency at which individuals perform brood-related tasks is still significantly negatively correlated with their relative median distance from the brood pile, for 3 out of 3 re-united colonies.

This relationship is closely approximated by an exponential decay.

Crucially, the relationship between the relative frequency at which individuals that were in the subsidiary fractions perform brood-related tasks and their median distance from the colony centre in the re-united colonies tends to be a significant, negative, correlation (3 out of 4 colonies). However, these individuals are present throughout the colonies, not just at the periphery of the distribution of workers, as they were in experiment 1.

Thus, there is minimum disruption to the spatial organisation of brood-related tasks in the colonies, despite the loss of a large proportion of the workforce, and its subsequent re-unification. In Chapter 8, I analyse the individual patterns of behaviour exhibited by workers, and discuss whether individuals in the subsidiary fractions of the colonies in experiment 2 have changed their behavioural role to perform more brood-related tasks, and subsequently maintained this change after re-unification.

Removal of workers associated with external activity from colonies of *Leptothorax albipennis* to form fractions with adopted foreign brood, but no queen, and the subsequent re-unification of the fractions of the colonies, does not affect the overall spatial organisation of external activity in the colonies.

The frequency at which individuals perform external activity increases with their increasing distance from the centre of the brood pile.

The relationship between the relative frequency at which individuals perform external activity and their relative median distance from the centre of the brood pile is a significant, positive, correlation for 3 out of 3 of the experimental colonies.

Individuals that were not removed from the original colonies continue to perform external activity at very low frequencies when the fractions of the colonies are re-united. The relationship between the relative frequency at which these individuals perform external activity in the re-united colonies and their relative median distance from the centre of the brood pile is not significantly correlated (in 2 out of 2 of the colonies), although it does still tend to be positive.

Individuals that have been removed as 'externals' continue to show a similar pattern of spatial organisation of external activity to that observed when they formed separate fractions with brood but no queen, when the fractions of the colonies are re-united. The relationship between the relative frequency at which these workers perform external activity and their relative median distance from the centre of the brood pile is a significant, positive, correlation for colonies for which there is sufficient data. Chapter 8 examines whether individuals alter their task profiles with respect to external activity in the subsidiary and original fractions of the colonies during session 3, and after re-unification in session 4.

Spatial Organisation of Brood-Related Tasks in Colonies with a Manipulated Age Structure

In experiment 3, single age cohort colonies, consisting only of callow workers and brood, were created from four experimental colonies. The remaining workers in each of these experimental colonies were consequently older workers (from previous annual age cohorts), brood, and a queen. The Control colony (Colony C_3) consisted of an un-manipulated colony, which was opened and marked at the same stage as the experimental colonies. At this point in Colony C_3's development callows were identified by their lighter pigmentation, and the results can be analysed separately for callows and older workers in this colony.

vii. An Investigation of the division of labour and the spatial organisation of brood-related tasks in artificially created single age cohort 'callow' colonies, and artificially created colonies consisting of 'older' workers - Experiment 3

I employed similar methods to those used to analyse the results from experiments 1 and 2 to investigate the division of labour and spatial organisation of brood-related tasks in both fractions of the four experimental colonies, and the Control colony in experiment 3. I plotted the frequency at which individuals perform brood-related tasks against the median of their distribution of distances from the colony centre during the three experimental sessions. In all the graphs in section 4.2i., callows are represented in red, whilst older individuals are represented in black, for both the experimental and Control colonies. The fractions of the colony were established and marked prior to the start of session 1, and no further manipulations were carried out for the duration of session 1, or for the duration of session 2 (which began a month after the end of session 1). After session 2 ended, the two fractions of each colony were united and session 3 began. The results are shown in section 4.2i. Linear regressions are used to examine the relationship between the logged frequency at which individuals perform brood-related tasks and their median distance from the centre of the brood pile, for some typical experimental colonies. Correlation analyses are carried out on all the colonies according to the procedures at the beginning of the chapter.

The familiar association between the location of an individual, in terms of her median distance from the colony centre, and the amount of brood-related tasks she performs is established for session 1. The frequency at which an individual performs brood-related tasks decreases as her median distance from the centre of the brood pile increases.

Figure 23 (a) is a plot of this relationship for the Control colony, callows are shown in red. Individuals with median positions above a threshold distance from the brood pile, do not perform brood-related tasks during the whole of session 1, or perform them at a very low frequency. These individuals tend to be older workers, and the callow workers in the Control colony tend to be located closer to the centre of the brood pile and perform brood-related tasks at a higher frequency. However, there is some overlap between the older and callow portions of the colony. Some of the older workers perform brood-related tasks with a relatively high frequency, and are situated relatively close to the centre of the brood pile. Conversely, some callow workers perform brood-related tasks at a relatively low frequency, and are relatively distant from the centre of the brood pile. According to age polyethism, individuals from different age cohorts perform different tasks in the colony. This is not the case in the un-manipulated Control colony, in which both older workers and callow workers perform brood-related tasks. However, there is a high degree of spatial organisation among brood-related tasks. There is a highly significant, negative, correlation between the relative frequency at which workers perform brood-related tasks and their relative median distance from the colony centre (see Table 9).

The relationship is very similar for all four experimental colonies, and typical plots are given in Figures 23 (b) and (c), separately for the 'older' and the 'callow' fractions of Colony 11. It can be clearly seen in Figure 23 (b), that the frequency at which the older individuals perform brood-related tasks decreases with increasing distance from the colony centre. As in the Control colony, there is a threshold distance from the colony centre beyond which individuals perform brood-related tasks at a frequency of zero, or very close to zero. Although there are individuals that exhibit high frequencies of brood-related tasks, the maximum frequency at which an individual carries out these tasks is lower than the maximum frequency seen in Figure 23 (c), the graph for the callows of this colony, which are in a separate nest. I compared the maximum frequency at which brood-related tasks are performed by the older workers in session 1 of the experimental colonies, with the callow workers. The distribution of maximum frequencies of performance of brood-related tasks is significantly lower in the older fractions compared to the callow fractions. There is no threshold distance beyond which individuals perform little or no brood-related tasks in the callow fraction, Figure 23 (c), and individuals are only situated relatively close to the centre of the brood pile. The patterns of the frequency at which individuals perform brood-related tasks and their spatial distribution, in the two separate fractions of Colony 11, are very similar to the pattern seen in the Control colony, if callows and older workers in the control are considered separately.

I investigated the relationship between the logged frequency at which individuals perform brood-related tasks and their median distance from the colony centre for the Control colony, and the two separate fractions of Colony 11, during session 1. Plots of these relationships are given in Figures 24 (a), (b) and (c), respectively, and all are a good approximation to a straight line. The results of linear regressions on these data show that the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre closely approximates an exponential decay. As a lot of the assumptions for regression analysis are not met, I also performed correlation analyses on the data for these, and the other colonies. The results are shown in Table 9. There is a highly significant, negative correlation between the relative frequency at which individuals perform brood-related tasks and their relative median distance from the colony centre, for both the 'callow' and 'older' fractions of all four experimental colonies.

The patterns of decreasing frequency of brood-related task performance with increasing distance from the centre of the brood pile, seen in the colonies in session 1, are preserved in session 2. This is the case for the Control colony, see

Figure 26 (a), and the experimental colonies, see typical plots in Figures 26 (c) and (d), 'older' and 'callow' fractions, respectively. However, some individuals in the callow fraction of Colony 11, are distributed slightly further away from the centre of the brood pile. This may be a result of changes associated with the callows growing older. If the latter is the case, these changes occur at a surprisingly young age. The time which elapses between the eclosion of these workers and the beginning of session 2 is a relatively small proportion of the total longevity of *L. albipennis* workers in the lab (which is 3.5 - 4 years (Ana Sendova-Franks, *pers comm.*)). What may be of greater importance is the experience of the callows, rather than their absolute age, and this is discussed with reference to their performance of external activity in section 4.3viii., below. However when I compare the absolute distribution of distances of the callows from the centre of the brood pile in session 1 compared to session 2, using Mann Whitney U tests (see Table 10a), there is no significant difference between sessions 1 and 2.

I conclude that the division of labour in these colonies is not based strictly on age as both older and younger individuals perform brood-related tasks. However, the maximum frequencies of brood-related task performance *are* observed among the callows. It has previously been shown, in colonies of *Leptothorax unifasciatus*, that in June, the younger generation tends to be located in the middle of the nest, whilst the older generation is spread out more evenly. Conversely, in September, the younger generation spreads out approximately evenly, whilst the older generation tends to located closer to the periphery of the nest (Sendova-Franks and Franks, 1995a).

I showed in sections 4.3i - vi., above, that colonies of *L. albipennis* exhibit strong spatial organisation with regard to brood-related tasks. These tasks are performed with decreasing frequency as the median distance of individuals from the colony centre increases. This spatial organisation still remains highly significant in colonies with no callow cohort, although a relatively large number of individuals perform brood-related tasks with a frequency of zero, see Figure 25 (a). Colonies consisting of a single age callow cohort and brood, but no queen, also demonstrate this highly significant spatial organisation with respect to brood-related tasks. Some of these callows perform brood-related tasks at a frequency of zero, but these are less numerous than in the 'older' fractions, see Figure 25 (b). It is clear, however, that the familiar pattern of spatial organisation of brood-related tasks is readily established among workers of the same age, despite their complete lack of previous experience of these (or any) tasks. This pattern is independent of the presence of the queen.

viii. An investigation of the division of labour and the spatial organisation of external activity in artificially created single age cohort 'callow' colonies, and artificially created colonies consisting of 'older' workers - Experiment 3

There is a similar association between the location of individual workers and the frequency at which they perform external activity in the Control colony in experiment 3, to the association established in the un-manipulated colonies during session 1 in experiments 1 and 2. Figure 27 (a) is a plot of the relationship between the frequency at which individuals perform external activity and the median of their distribution of distances from the centre of the brood pile for the Control colony in session 1. The frequency at which individuals perform external activity increases with increasing distance from the centre of the brood pile. Figure 27 (a) also shows that there is an overlap in the division of labour for external activity between callows and older workers. Some callows perform external activity, and some older workers do not. Callows tend to be situated relatively close to the brood pile, whereas older workers are distributed more evenly throughout the nest. The maximum frequency of external activity carried out by older workers is higher than the maximum frequency of external activity carried out by the callows. The relationship between the logged frequency at which individuals perform external activity and their median distance from the colony centre is investigated using linear regression analysis, see Figure 28 (a). The relationship does not fit a linear model well, and does not therefore approximate to an exponential growth curve. However, it should be noted that again, the data do not meet most of the assumptions for an analysis of this type, and moreover, the sample sizes are relatively small in this case. Correlation analysis shows that there is a highly significant, positive correlation between the relative frequency at which individuals in the Control colony perform external activity and their relative median distance from the centre of the brood pile (see Table 12).

The association between the frequency at which individuals perform external activity and their median distance from the colony centre also occurs in the experimental colonies, in both the 'older' and the 'callow' fractions. Figures 28 (b) and (c) are plots of this relationship for a typical experimental colony (Colony 11). It should be noted that there is much less external activity in the callow fraction of the colony than in the older fraction, although some does occur (an exception to this is Colony 10, discussed below). Individuals in the callow fraction are also only situated relatively close to the centre of the brood pile. In the older fraction, individuals are spread out throughout the nest. Figures 29 (a) and (b) show that a large number of individuals in both the older and the callow fractions of the colony do not carry out any external activity.

Linear regression analysis shows that the relationship between the logged frequency at which individuals perform external activity and their median distance from the colony centre does not approximate to a straight line for the older fraction, as for the Control colony. There is an insufficient amount of external activity in the callow fraction to test this relationship. Again, the results of linear regression analysis should be treated with some caution, due to the assumptions of this type of analysis and the relatively low sample size. There tends to be a highly significant, positive correlation between the relative frequency at which individuals perform external activity and their relative median distance from the centre of the colony (see Table 12). This is the case for both fractions of all the experimental colonies, except the callow fraction of Colony 10, for which no external activity is recorded during session 1.

Very similar patterns are found during session 2 of the experiment (see Figures 30 (a) Control; and (b) and (c) Older and callow fractions of Colony 11, respectively. However, in the callow fraction of Colony 11, the amount of external activity has increased, with some individuals performing external activity at a much higher frequency than they did in session 1. I analyse the absolute performance of external activity in session 1 and session 2 in Chapter 8. Individuals that perform external activity in the callow fractions are more widely spatially distributed in session 2, and this is also observed in the Control colony. This is attributed to the same 'juvenile' effect, which may be associated with experience, which is seen when examining the patterns of brood-related task performance in session 2.

The spatial patterns of organisation of external activity are the same in session 2 as in session 1. The frequency at which individuals perform external activity increases with their increasing distance from the colony centre. There is a highly significant, positive correlation between the relative frequency at which external activity is performed and relative median distance of individuals from the colony centre for the majority of the experimental colonies (see Table 13). The relationship between these variables is not a significant correlation for the callow fraction of Colony 10, for which I again recorded no external activity during session 2. The relationship is not a significant correlation for the older fraction of Colony 12. A closer examination of Colony 12 shows that some individuals at the periphery of the colony perform external activity with a frequency of zero, and one individual that was located in the centre of the colony, performs external activity with an unusually high frequency. This could be due to inter-colony variation, but it is clear that the spatial patterns which are generally observed for external activity are not always adhered to by every individual. Not every individual located near to the periphery of the colony exhibits external activity, and high performers are not exclusively located near the periphery. The role of individual variation from the general trends is discussed further in Chapter 8 in which I compare individual behaviour between sessions of the experiments.

I conclude that the division of labour in these colonies cannot be purely based on age, as both older and younger individuals perform external tasks. However, the maximum frequencies of external activity are among the older individuals. Callows tend to be situated closer to the brood pile, whereas older individuals are more spread out in their spatial distribution, and this was discussed with reference to brood-related tasks in section 4.3vii., above. Colonies of *L. albipennis* have a strong spatial organisation with regard to external activity. These tasks are performed with increasing frequency as the median distance of individuals from the colony centre increases. This spatial organisation still remains highly significant in colonies with no callow cohort. Colonies consisting of a single age callow cohort and brood, but no queen, also demonstrate this highly significant spatial organisation, but tend to exhibit less external activity than the older fractions. The spatial organisation of external activity tends to be preserved through session 2, although the frequency at which callow individuals perform external activity increases.

CONCLUSIONS

The following conclusions can be drawn:

The division of labour in colonies of *Leptothorax albipennis* is not strictly based on age: both older and callow individuals perform brood-related tasks and external activity. However:

The maximum frequencies of brood-related task performance are seen in callow individuals.

The maximum frequencies of external activity are seen in older individuals.

There is a strong spatial organisation with respect to tasks:

The frequency at which individuals perform brood-related tasks decreases with increasing distance from the brood pile. The relationship between relative frequency at which individuals perform brood-related tasks and their relative distance from the brood pile is a highly significant negative correlation.

The frequency at which individuals perform external activity increases with their increasing distance from the centre of the brood pile. The relationship between the relative frequency at which individuals perform external activity and their relative distance from the brood pile is a highly significant positive correlation.

Younger individuals tend to be located relatively closer to the brood pile, whereas older individuals are more spread out in their spatial distribution.

The removal of the callow cohort from colonies of *Leptothorax albipennis* does not affect the pattern of spatial organisation of brood-related tasks or external activity in the colonies.

Brood-related tasks are still carried out, although the maximum frequency of performance of brood-related tasks is significantly lower than that observed in the callow fraction. The relationship between the relative frequency at which individuals perform brood-related tasks and their relative distance from the brood pile is a highly significant negative correlation (4 out of 4 colonies). External activity is still carried out, and the relationship between the relative frequency at which individuals perform external activity and their relative distance from the brood pile is still a highly significant positive correlation (4 out of 4 colonies).

Colonies consisting of a single age callow cohort and brood, but no queen, show the same pattern of spatial organisation of brood-related tasks and external activity.

> The maximum frequency of performance of brood-related tasks is higher than in the older fraction. The relationship between the relative frequency at which brood-related tasks are performed and their relative distance from the centre of the brood pile is still a highly significant negative correlation (4 out of 4 colonies). External activity is still carried out, although at a lower frequency than in the older fractions. The relationship between the relative frequency at which external activity is performed and relative distance from the centre of the brood pile is still a highly significant positive correlation (3 out of 4 colonies – external activity was not recorded for one colony).

These patterns tend to be preserved throughout session 2, which begins a month after the end of session 1.

> The distribution of distances of callows from the centre of the brood pile is not significantly different in session 1 compared to session 2.

ix. An investigation of the division of labour and the spatial organisation of brood-related tasks after the unification of artificially created single age cohort 'callow' colonies and artificially created colonies consisting of 'older' workers - Experiment 3

I will discuss the division of labour and spatial organisation of brood-related tasks in the four experimental colonies after the 'callow' and 'older' fractions have been united, - session 3. Unlike the 'original' and 'subsidiary' fractions in experiments 1 and 2, the 'callow' and 'older' fractions in experiment 3 have never been part of the same colony when they are eclosed adults. The callows were removed as pupae and their only contact with older workers had been the 15 individuals placed in each nest to enable the pupae to eclose. These individuals were subsequently removed and were not re-united with the original fractions. I used multiple comparisons to determine the effect of re-uniting these fractions of the colonies after they have previously existed as separate fractions. I compared the relationship between the frequency at which individuals perform first, brood-related tasks, and second, external activity, with their median distance from the colony centre. I performed this analysis separately for pooled data from the callows only in session 1 compared to session 3, and then for the older workers in session 1 compared to session 3.

The familiar pattern of spatial organisation of brood-related tasks is established for the united experimental colonies. Figure 26 (e) is a plot of the relationship between the frequency at which individuals perform brood-related tasks and their median distance from the colony centre in a typical united colony, Colony 11. Callows are represented in red, older workers in black. The frequency at which individuals perform brood-related tasks decreases with increasing distance from the centre of the brood pile. Figure 26 (b) is a plot of the same variables for the Control colony (Colony C_3). This shows a similar overall pattern of performance of brood-related tasks. Correlation analyses show that the relationship between the relative frequency at which individuals perform brood-related tasks and their relative distance from the centre of the brood pile, is a highly significant, negative correlation for the Control, and for all four united experimental colonies (see Table 10).

I also calculated the correlation coefficients of this relationship for the callows and the older workers separately for the united experimental colonies (see Table 11). When tested, the correlations were all statistically highly significant, with the exception of the callows in Colony 11. A detailed examination of the red data points in Figure 26 (e) reveals that one individual in the callow portion of Colony 11, whose median distance from the brood pile is approximately half the maximum, performs brood-related tasks at a relatively high frequency, and this has skewed the results. This is further evidence that there are individuals that do not comply with the general trends. The pattern of spatial organisation of brood-related tasks does tend to be preserved in the united colonies. When I perform multiple comparisons using the previous method to determine the effect of the experimental manipulations on the relationship between the frequency at which callows perform brood-related tasks in session 1 compared to session 3, I do not find that all these colonies can be considered samples from a population exhibiting common a correlation coefficient. I therefore perform a different type of multiple comparison that can be utilised when populations are heterogenous. A multiple comparison shows that there is no significant difference between the correlation coefficients for the relationship for the callows in the experimental colonies before the manipulation (session 1 when they existed as separate colonies), and after the fractions of the colonies were united (session 3).

I performed multiple comparisons between the frequency at which individuals perform brood-related tasks in session 1 compared to session 3, for pooled experimental colonies considering only the older workers. I determined that there is no significant difference between the relationship in session 1 and session 3 (see Table 8a). Older workers maintain the same correlational relationship in session 1 compared to session 3 in the experimental colonies.

I also examined the absolute distribution of callows and older workers in the colonies. Callows tend to be located relatively close to the centre of the brood pile, whereas older workers tend to be located throughout the colony. Analyses using Mann Whitney U tests shows that the distribution of distances from the centre of the brood pile is significantly higher among the older workers compared to the callow workers during session 3, for two of the four experimental colonies (see Table 10b). There is, a great degree of overlap between the distributions of the callows and the older workers. Some older workers perform relatively high amounts of brood-related tasks and are relatively close to the centre of the brood pile, compared to other older individuals in the Control colony, see Figure 26 (b). My hypothesis is that these are the individuals that carry out brood-related tasks with a high frequency in sessions 1 and 2 when no callows are present, and they continue to do so despite the unification of the callows with the older workers in session 3. This is analysed and discussed further in Chapter 8, when I examine the behaviour of individuals compared between sessions of the experiment.

x. An investigation of the division of labour and the spatial organisation of external activity after the unification of artificially created single age cohort 'callow' colonies and artificially created colonies consisting of 'older' workers - Experiment 3

The spatial organisation of external activity has the same general pattern in the united experimental colonies, in session 3, as in sessions 1 and 2. The frequency at which individuals carry out external activity increases as their distance from the brood pile increases. Figures 30 (b) and (e) are plots of the relationship between the frequency at which individuals perform external activity and their median distance from the centre of the brood pile in the Control colony, and in Colony 11, respectively. The results of correlation analyses, performed according to the procedure at the beginning of this chapter, are given in Table 13, for all the colonies. The relationship between the relative frequency at which individuals perform external activity and their relative distance from the centre of the brood pile is a positive, significant correlation in all cases.

I also calculated correlation coefficients for this relationship separately for the callows, and for the older workers, during session 3, for all the experimental colonies (see Table 14). The relationship between the relative frequency at which callow individuals perform external activity and their median distance from the colony centre in the united colonies tends to be a significant, positive correlation. This was the case for all the callow fractions except for Colony 11. When I pooled the correlation coefficients and perform multiple analysis between the correlation between the frequency at which the callows perform external activity in session 1 compared to session 3, there is no significant difference (see Table 14a). Similarly, this relationship tends to be a significant, positive correlation for the older workers. However, the relationship is not a significant correlation for the older workers in Colony 12. However, Multiple correlations between pooled correlations for the experimental colonies that demonstrate a significant relationship (see Table 14a) show that there is no significant difference between the frequency at which older individuals perform external activity in session 1 compared to the frequency at which they perform external activity in session 3. The relationship between relative frequency at which individuals perform external activity and their relative distance from the colony centre is significant for the callows when they are in a nest with brood only, in Colony 11 sessions 1 and 2. The callows in Colony 11, during session 3, still tend to be situated closer to the brood pile than the older workers, but only perform external activity at very low frequencies, and thus no correlation is found with their distance from the brood pile. The older workers in Colony 12 did not show a significant spatial organisation of external activity in session 2, although they did in session 1. These results were skewed by one individual, situated relatively close to the brood pile, exhibiting an extremely high frequency of external activity.

I also examined the absolute distribution of the callows and the older workers in the united colonies. Again, callows tend to be situated closer to the centre of the brood pile, and older workers tend to be situated throughout the colony. There is, however, a great degree of overlap between the spatial distributions of the callows and the older workers. Moreover, some callow workers perform relatively high amounts of external activity. This is also true for some callows in the Control colony, see Figure 26 (b). This is a continuation of the pattern observed among the callows, in both the Control and the experimental colonies, during session 2. This is further evidence that some effect, associated with age, or experience, occurs at this juvenile stage.

CONCLUSIONS

The following conclusions can be drawn:

Strong spatial organisation with respect to tasks remains evident after the callows are united with the older fractions of colonies in *Leptothorax albipennis*:

The frequency at which individuals perform brood-related tasks decreases with their increasing distance from the colony centre. The relationship between the relative frequency at which individuals perform brood-related tasks and their relative distance from the centre of the brood pile is a highly significant, negative correlation (4 out of 4 colonies).

When callows and older individuals are considered separately, this relationship still tends to be significant for both the callows (3 out of 4 colonies) and the older workers (4 out of 4 colonies).

The frequency at which individuals perform external activity increases with their increasing distance from the centre of the brood pile. The relationship between the relative frequency at which individuals perform external activity and their relative distance from the colony centre is a significant positive correlation (4 out of 4 colonies).

When callows and older individuals are considered separately, this relationship still tends to be significant:

- for the callows, in colonies where there is a sufficiently high level of external activity;
- for the older workers (3 out of 4 colonies).

Callows tend to be located closer to the brood pile, whereas the older individuals are more spread out in their spatial distribution (for two of the four experimental colonies). However, there is a great degree of overlap between the spatial distributions of the callows and the older workers. Some callow workers perform external activity at relatively high frequencies, and this phenomenon, also present in the Control, is evidence of some 'juvenile effect', associated with age, or experience, at this early stage of life.

Chapter 5

An Investigation of Worker Interactions with the Queen, and Her Behaviour

5.1 An Investigation of Worker Interactions with the Queen

i. Colonies with a manipulated task structure

I investigate the degree of spatial organisation of the interactions of workers with the queen, in colonies of the ant *Leptothorax albipennis*. Interaction with the queen could include: grooming; trophallaxing with, or other types of contact with the queen by the workers. The performance of a queen-related task is established by contact between the mouthparts of a worker and the body of the queen, and is determined from the photographs taken during a particular experimental session. First, the frequency at which individuals had contact with the queen is determined during session 1, in which colonies are un-manipulated, for experiments 1 and 2. I analyse the results in a similar way to those for brood-related tasks, and external activity, in Chapter 4. The frequency at which individuals had contact with the queen is plotted against the median position of individuals, during that session.

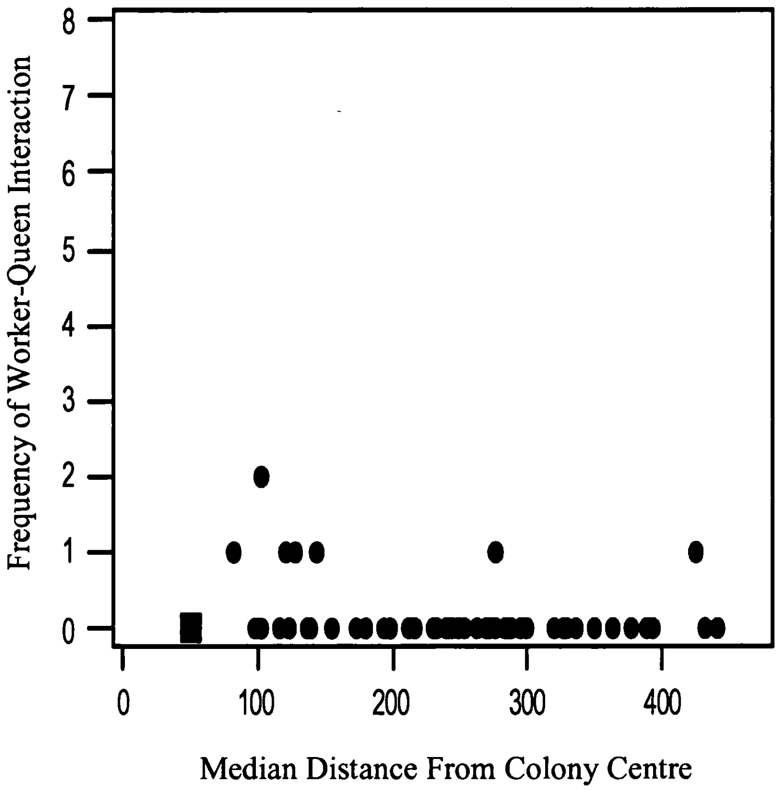
As before, the median position of each individual, including the queen, is calculated as the median of the individual's distribution of distances from the centre of the brood pile. The centre of the brood pile is calculated as the mean of the co-ordinates of the eggs and microlarvae, and is represented by the origin of each graph. The median of the distribution of distances of the the queen from the mean of the brood pile is represented on each of the graphs in Figures 31-35 as a blue square.

Experiment 1

The graphs below show the relationship between the frequency at which individual workers have contact with the queen and the median position of the workers in the colony during session 1, when colonies are un-manipulated, in experiment 1. The Control colony, Colony C_1, and a typical example of one of the experimental colonies, Colony 3, are shown.

Fig 31(a)

Colony C_1 - Control



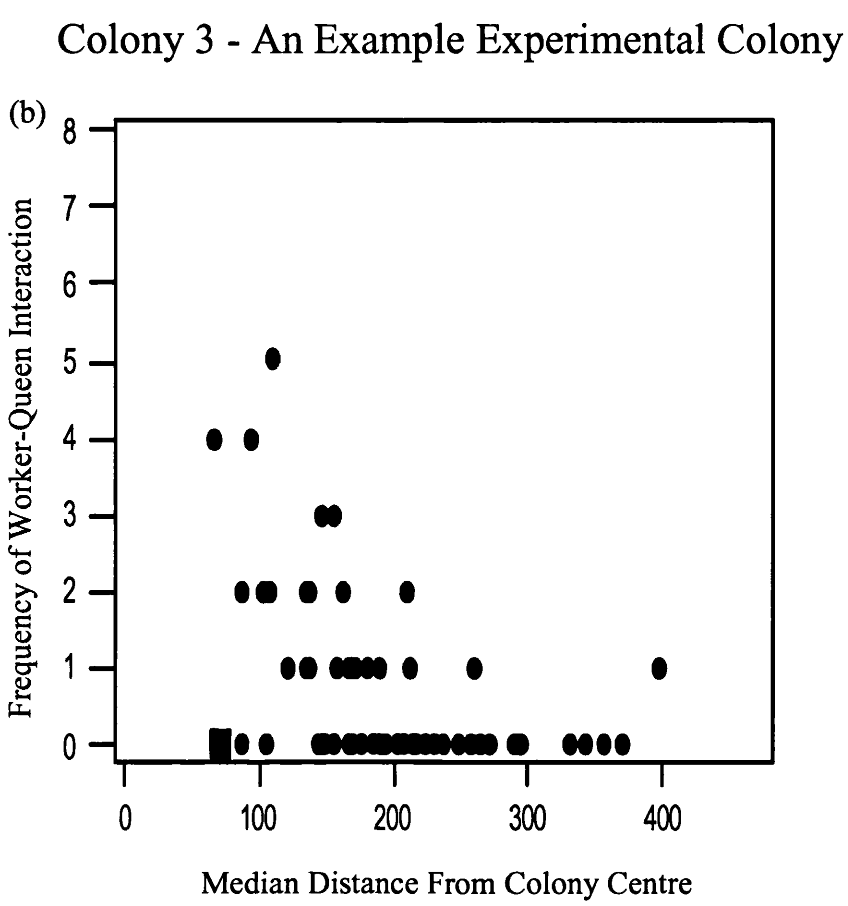


Figure 31: (a) The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre for Colony C_1, the Control, session 1; and (b), Colony 3, an example experimental colony. The position of the queen is represented by a blue square.

The frequency at which an individual worker has contact with the queen decreases as the worker's median distance from the brood pile increases, for the Control colony, and for all the experimental colonies. A typical experimental plot is shown in Figure 31 (b), above. Workers with a median position above a certain threshold distance from the brood pile have a frequency of contact with the queen of zero, or very close to zero. Colony 3 exhibits much more contact with the queen in session 1 than the Control colony, this is discussed in section 5.3i.

When the frequency of worker contact with the queen is logged, and plotted against median distance from the centre of the colony, the colonies typically show a close approximation to exponential decay, as in Figure 32, below.

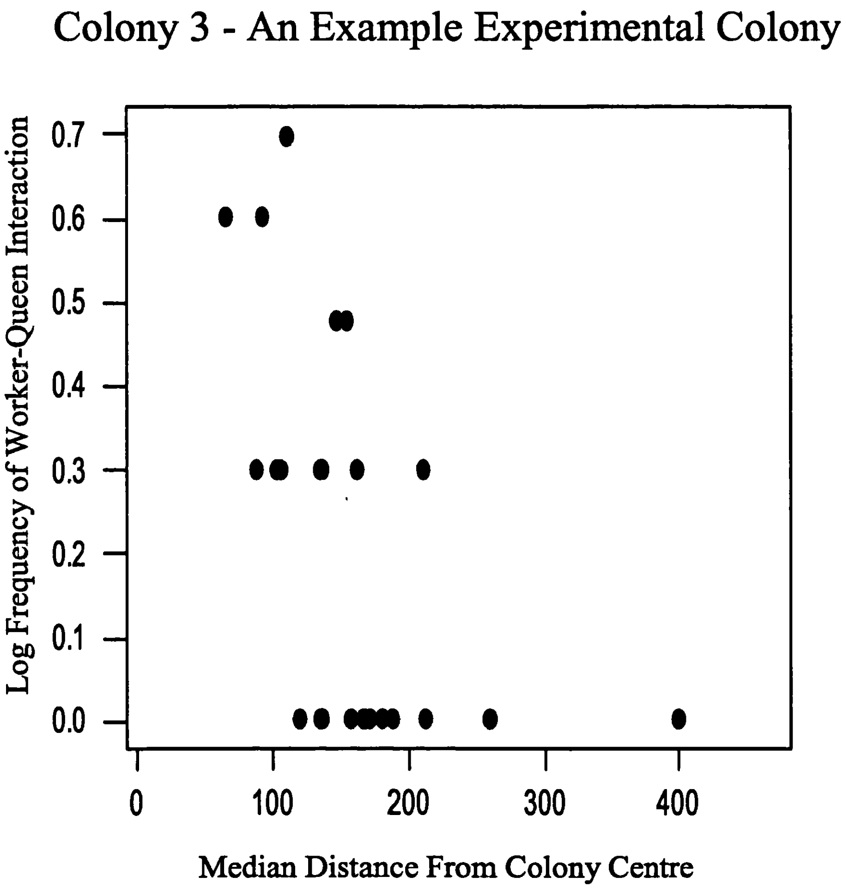


Figure 32 : The relationship between logged frequency at which workers have contact with the queen and their median distance from the colony centre for Colony 3, an example experimental colony.

Experiment 2

I analyse the relationship between the frequency at which individual workers have contact with the queen and their median position in the colony during session 1, when colonies are un-manipulated, in experiment 2, in the same way as experiment 1. No queen is present in the Control colony, Colony C_2, due to her death, discussed in section 4.3i.. The spatial pattern of queen-worker interactions is similar to that in the experimental colonies in experiment 1. The frequency at which individual interact with the queen decreases as their median distance from the colony centre increases, for all the experimental colonies.

The relationship between the frequency at which individuals have contact with the queen and their spatial position, is further examined by ranking individuals with respect to the frequency at which they interact with the queen, and their spatial position, and thus determining the Spearman's rank correlation coefficient, tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607). This shows that there is a significant negative correlation between the frequency at which individuals have contact with the queen and their spatial position, for all the colonies in experiments 1 and 2, for session 1, see Table 15, below.

Colony	**Session**	**r_s**	**n**	**Significance Level $p<$**
Experiment 1				
C_1	1	-0.264	58	0.05
1	1	-0.473	64	0.01
2	1	-0.609	68	0.01
3	1	-0.554	58	0.01
4	1	-0.648	52	0.01
Experiment 2				
C_2	No Queen Present			
5	1	-0.490	34	0.01
6	1	-0.670	25	0.01
7	1	-0.707	57	0.01
8	1	-0.550	72	0.01

Table 15 : The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre, for experiment 1 and experiment 2, calculated as r_s, the Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

How robust is the relationship between the frequency at which individuals interact with the queen and their median distance from the colony centre? I examine the spatial organisation of individuals contact with the queen in sessions 2 and 4 of experiment 1, and session 4 of experiment 2, to determine whether the relationship is preserved throughout the experiments despite the removal of a large proportion of workers from the experimental colonies, in session 2, and their re-unification in session 4. Furthermore, I examine the spatial organisation of contact with the queen in the original fractions of session 3. There is no queen present in the subsidiary fractions of the colonies (consisting of the removed workers).

The graphs below show the relationship between the frequency at which individuals interact with the queen and their median position in the colony, during:

session 2, when workers associated with external activity are being sequentially removed from the experimental colonies;

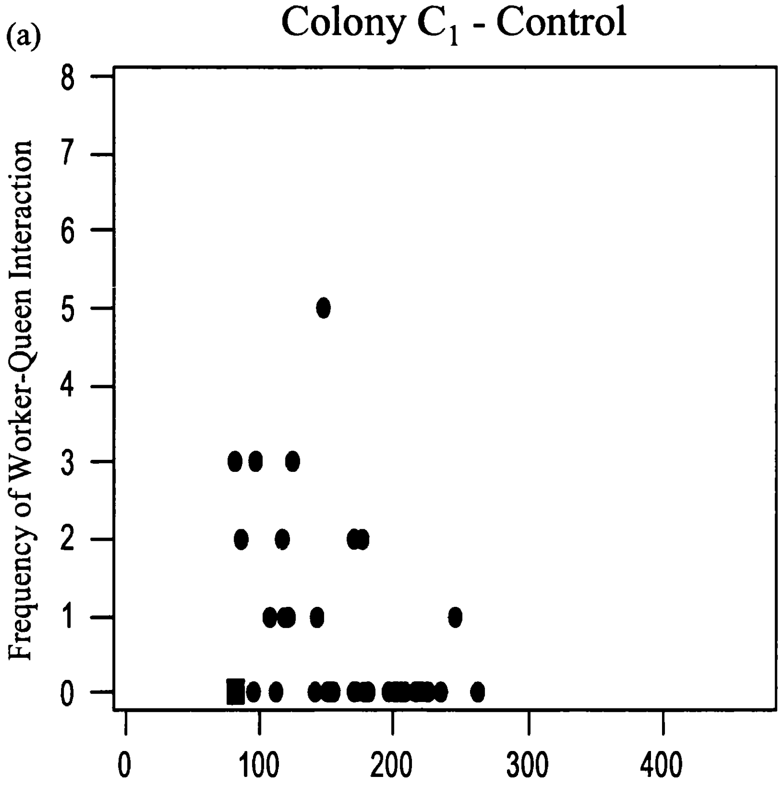
session 3, when the original fractions of the colony are maintained separately from the removed individuals in the subsidiary fractions;

session 4, when the two fractions of the colonies are re-united in experiment 1.

The Control colony, Colony C_1, and an example of one of the experimental colonies, Colony 3, are shown in Figures 33 and 34, below. Figure 35 shows the relationship for Colonies C_1, 3, and Colony 7, an example experimental colony from experiment 2. Individuals that were in the subsidiary fraction of Colony 3 during session 3 are shown in red, when the fractions of the colony are re-united in session 4, Figure 35 (b).

Session 2

Colony C_1 - Control



Colony 3 - An Example Experimental Colony

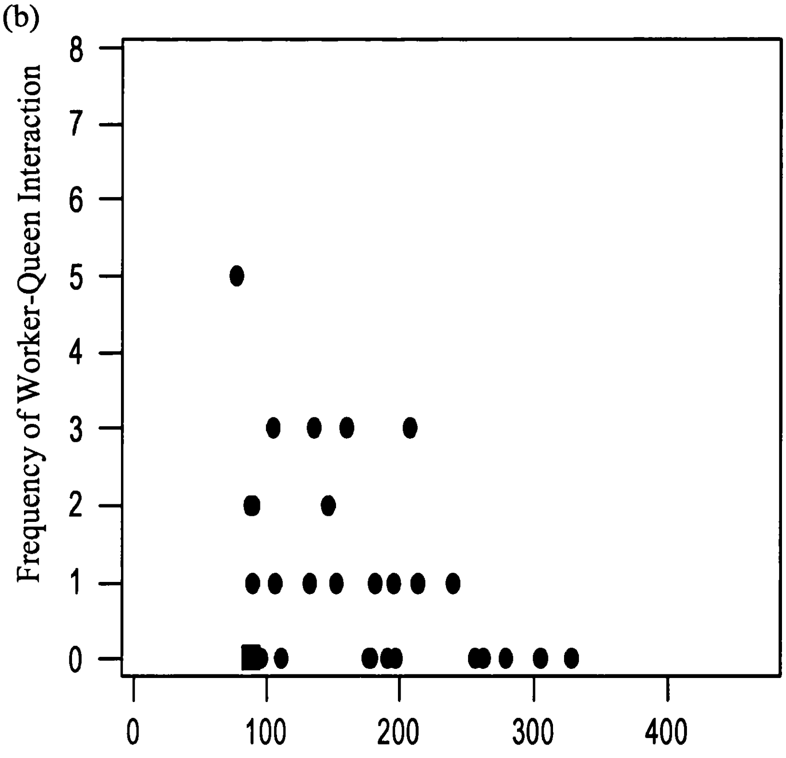
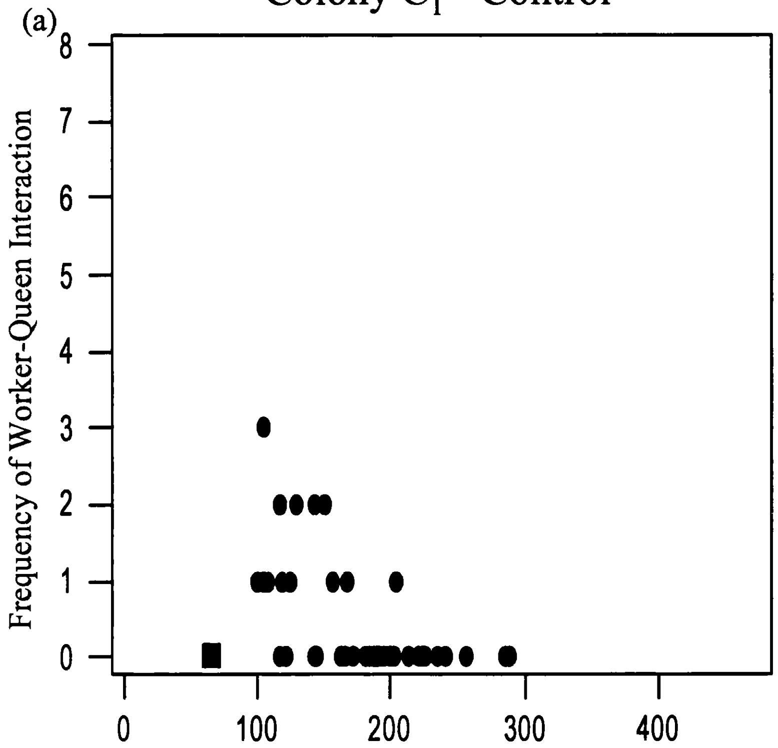


Figure 33: The relationship between frequency at which workers have contact with the queen and their median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, for session 2. The position of the queen is represented by a blue square

Session 3

Colony C_1 - Control



Colony 3 - An Example Experimental Colony

Original Fraction Only

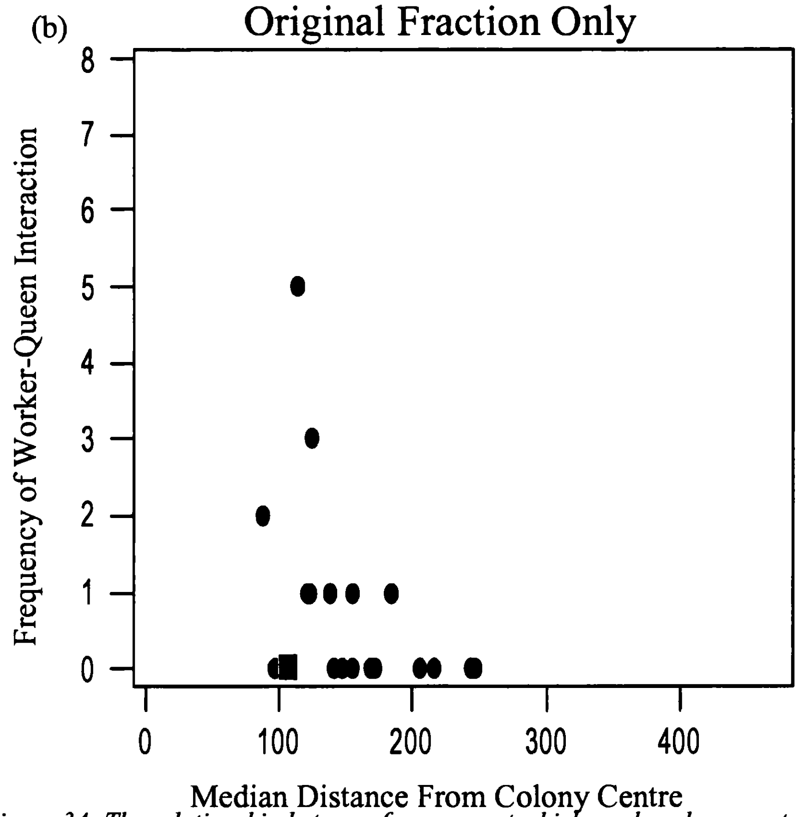
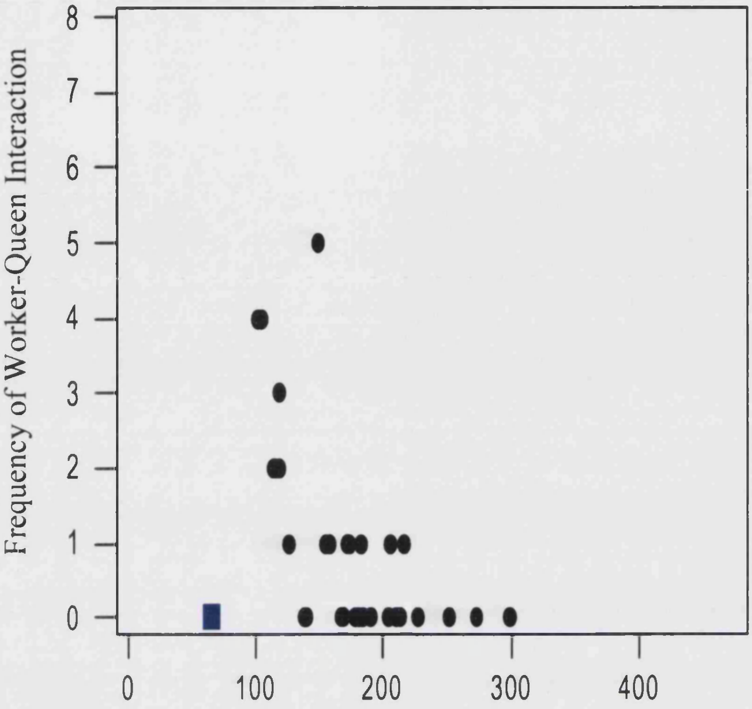


Figure 34: The relationship between frequency at which workers have contact with the queen and their median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, for session 3, original fraction. The position of the queen is represented by a blue square

Session 4

Fig 35(a)

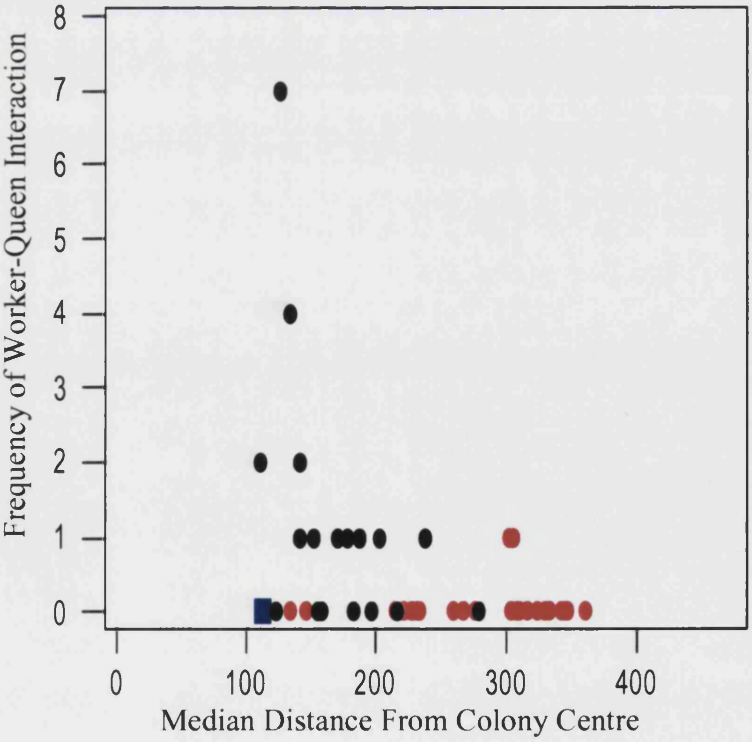
Colony C_1 - Control



Colony 3 - An Example Experimental Colony

Fig 35(b)

Re-united Fractions



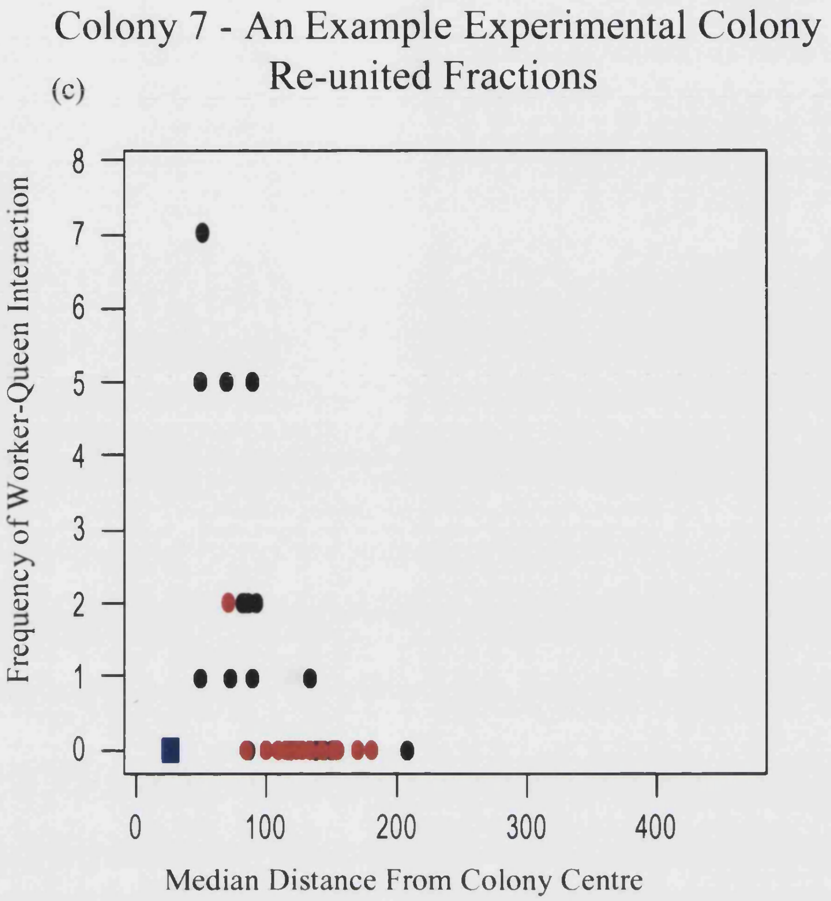


Figure 35: The relationship between frequency at which workers have contact with the queen and their median distance from the colony centre for (a) Colony C_1, the Control Colony; and (b) Colony 3, an example experimental colony, experiment 1; (c) Colony 7, an example experimental colony, experiment 2, for session 4. The position of the queen is represented by a blue square. Individuals that were removed from the experimental colonies during session 2, are indicated in red.

The Spearman's rank correlation coefficient is calculated for the relationship between the frequency at which workers interact with the queen and their median distance from the colony centre, for each colony in experiments 1 and 2, for sessions 2 (experiment 1 only), 3 (original fractions only), and 4. The statistic is tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and as a Spearman's rank correlation coefficient where n ≤ 10. There is a significant, negative correlation between the relative frequency at which individuals interact with the queen and their relative spatial position, for all the colonies in experiment 1, for session 2 and for session 4. During session 3 (original fraction) three out of four of the experimental colonies do not show a significant correlation, see Table 16, below. In experiment 2, all the sessions show significant results with the exception of Colony 6 during session 4, see Table 17, over.

Colony/session	r_s	df	Significance level p<
Colony C_1			
Session 2	-0.559	40	0.01
Session 3	-0.624	40	0.01
Session 4	-0.674	33	0.01
Colony 1			
Session 2	-0.637	23	0.01
Session 3 – original	-0.702	8	NS
Session 3 - subsidiary	-	-	-
Session 4	-0.740	30	0.01
Colony 2			
Session 2	-0.624	29	0.01
Session 3 – original	-0.244	19	NS
Session 3 – subsidiary	-	-	-
Session 4	-0.702	31	0.01
Colony 3			
Session 2	-0.527	27	0.01
Session 3 – original	-0.595	18	0.01
Session 3 – subsidiary	-	-	-
Session 4	-0.456	48	0.01
Colony 4			
Session 2	-0.413	18	0.05
Session 3 – original	-0.850	5	NS
Session 3 – subsidiary	-	-	-
Session 4	-0.810	22	0.01

Table 16 : The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre, for experiment 1, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where n ≤ 10.

Colony/session	r_s	df	Significance level p<
Colony C_2 – No Queen Present			
Colony 5			
Session 3 – original	-	0	-
Session 3 - subsidiary	-	-	-
Session 4	-	2	-
Colony 6			
Session 3 – original	-	1	-
Session 3 – subsidiary	-	-	-
Session 4	-0.464	7	NS
Colony 7			
Session 3 – original	-0.708	23	0.01
Session 3 – subsidiary	-	-	-
Session 4	-0.741	35	0.01
Colony 8			
Session 3 – original	-0.625	18	0.01
Session 3 – subsidiary	-	-	-
Session 4	-0.493	31	0.01

Table 17 : The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre, for experiment 2, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and as Spearman's rank correlation coefficient where n ≤ 10.

To determine any effect of experimental manipulations on the colonies I compare the correlational relationship between the frequency at which individuals have contact with the queen and their median distance from the centre from the brood pile in each experimental colony in session 4 with that of the Control colony in the same session. The results are shown in Table 17a, below.

Colony/Sess compared to control	$r_{s\,c}$	$r_{s\,e}$	n_c	n_e	z_c	z_e	t_s	Signif. level p<
Experiment 1								
Colony1/sess4	-0.674	-0.740	35	32	-0.818	-0.951	0.517	NS
Colony2sess 4	-0.674	-0.702	35	33	-0.818	-3.485	0.521	NS
Colony3/sess4	-0.674	-0.456	35	50	-0.818	-0.492	-1.142	NS
Colony4/sess4	-0.674	-0.810	35	24	-0.818	-1.127	1.100	NS
Experiment 2								
Colony5/sess4	-0.674	-	35	-	-0.818	-	-	-
Colony6sess 4	-0.674	-0.464	35	9	-0.818	-0.502	-0.709	NS
Colony7/sess4	-0.674	-0.741	35	37	-0.818	-0.953	0.547	NS
Colony8/sess4	-0.674	-0.493	35	33	-0.818	-0.540	-1.094	NS

Table 17a : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals interact with the queen in session 4 in each experimental colony and this relationship in the Control colony C_1, calculated as a Fisher's z-transform. $r_{s\,c}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of queen interaction and distance from the brood pile in the Control colony during session 4. $r_{s\,e}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of queen interaction and distance from the brood pile in each experimental colony during session 4. n_c= The number of individuals that retained their marks in the Control colony. n_e= The number of individuals that retained their marks in the experimental colony.

A Fisher's z-transform shows that there is no significant difference between the correlation of the Control colony, Colony C_1, and the experimental colonies in session 4. This applies to all the colonies which exhibit a significant correlation coefficient for the relationship between the frequency of interaction with the queen, and median distance from the brood pile.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the frequency at which individuals interact with the queen and their median distance from the colony centre between session 1 (un-manipulated colonies) and session 4 (after re-unification) I perform the multiple comparison analysis detailed in Chapter 4.

The results are shown in Table 17b, below.

z^{-} session 1	z^{-} session 4	χ^2 session 1	Signif. level$_1$ p<	χ^2 session 4	Signif. level$_4$ p<	n_{mean1}	$n_{mean\,4}$	t_s	Signif. level p<
Exp 1									
-0.650	-0.693	2.11	NS	7.42	NS	57.5	29.8	0.182	NS
Exp 2									
-0.709	-0.760	3.32	NS	2.69	NS	44	30	0.208	NS

Table 17b: Testing the relationship between the frequency interaction with the queen and the median distance of individuals from the colony centre for experiments 1 and 2, for combined colonies, compared between sessions 1 and 4. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for both sessions compared, and in both experiments. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 and the pooled *z* for all the experimental colonies in session 4 (where colonies exhibit a significant correlational relationship between the frequency at which individuals perform external activity and their median distance from the centre of the brood pile), shows that there is no significant difference between the colonies before the manipulation and after it.

ii.Colonies with a manipulated age structure

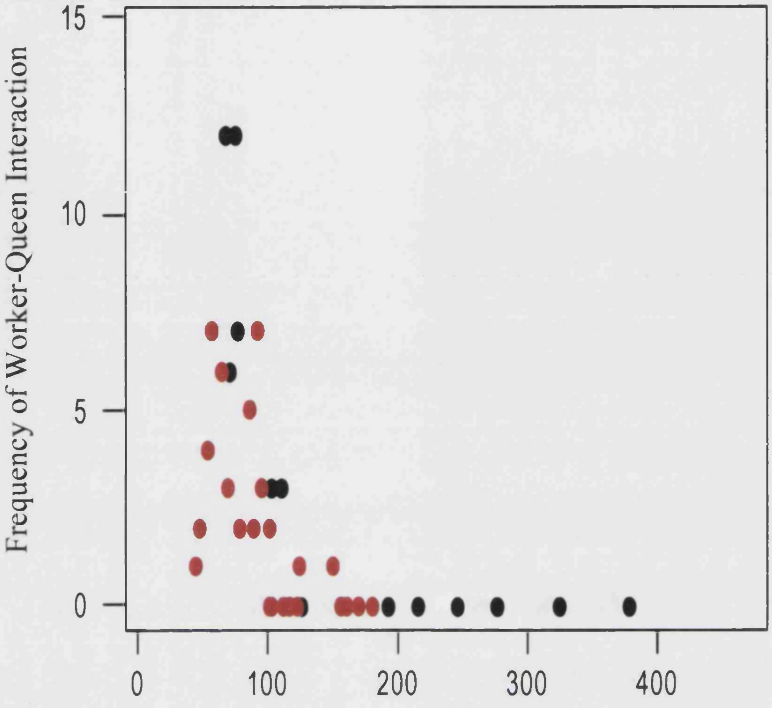
Experiment 3

I investigate the degree of spatial organisation of worker interactions with the queen, in colonies of the ant *Leptothorax albipennis* which have a manipulated age structure. The frequency at which individuals have contact with the queen is determined in the older fractions during session 1, the older fractions during session 2, and the combined fractions in session 3. The subsidiary fractions of the colonies, containing the callows in sessions 1 and 2, do not contain a queen. The performance of a queen-related task is established by contact between the mouth-parts of a worker and the queen, determined from a photograph taken during that session, as for experiments 1 and 2. As for brood-related tasks and external activity, in Chapter 4, I plot the frequency at which individuals interact with the queen against their median position during that session, determined from the photographic record.

As before, the median position of each individual, including the queen, is calculated as the median of its distribution of distances from the mean centre of the brood pile. The median of the distribution of distances of the queen from the mean centre of the brood pile is represented on each of the graphs in Figures 36 and 37, as a square, blue, symbol.

The graphs below show the relationship between the frequency at which individuals interact with the queen and their median position in the colony during session 1 in experiment 3. The Control colony, Colony C_3, and a typical experimental colony, Colony 9, are shown.

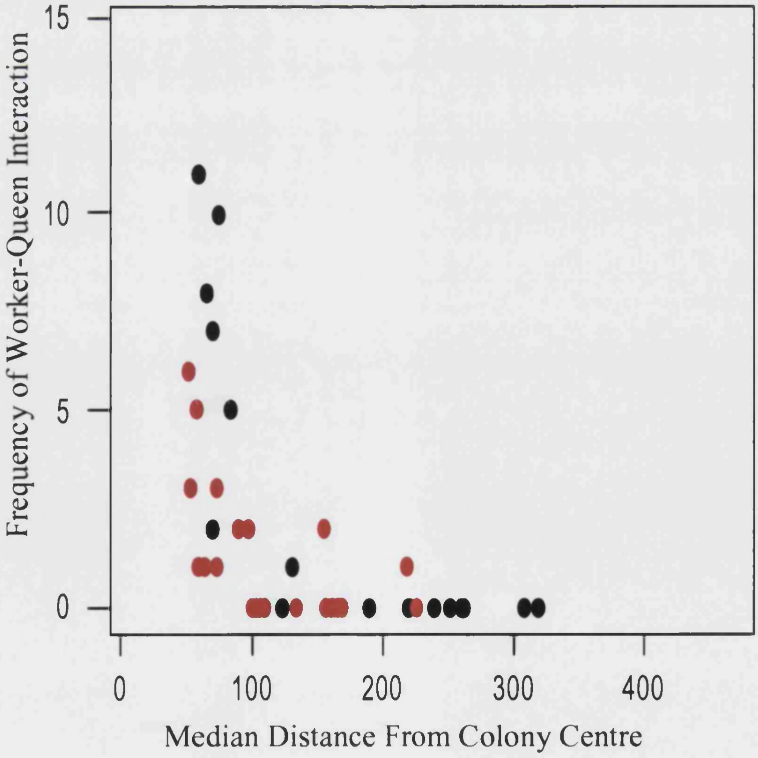
Fig 36(a)



Session 1

Colony C_3 - Control

Fig 36(b)



Session 2

Colony C_3 - Control

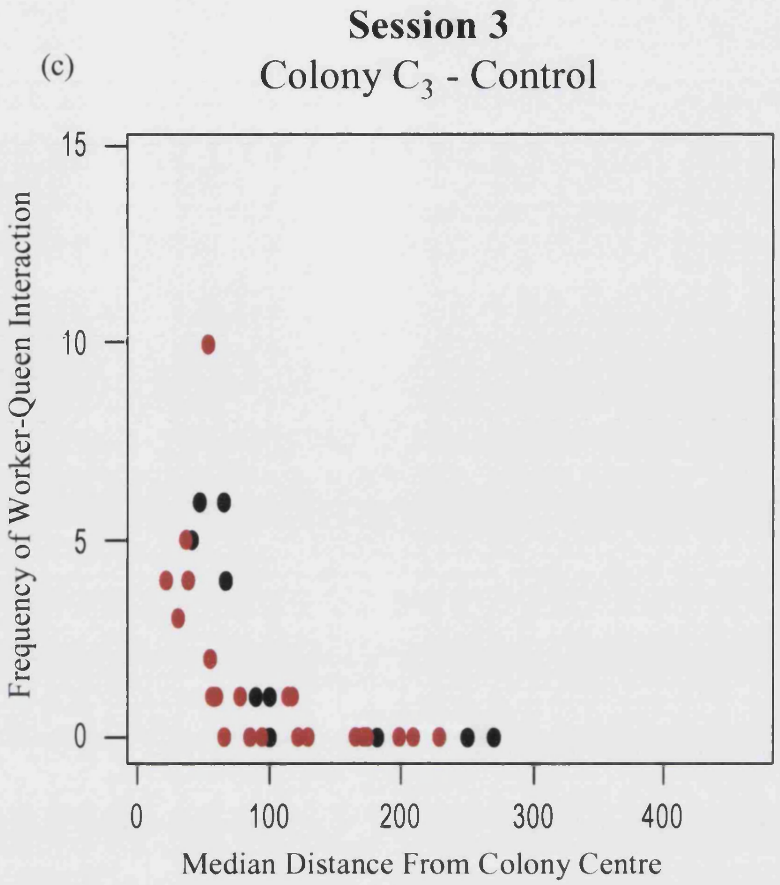
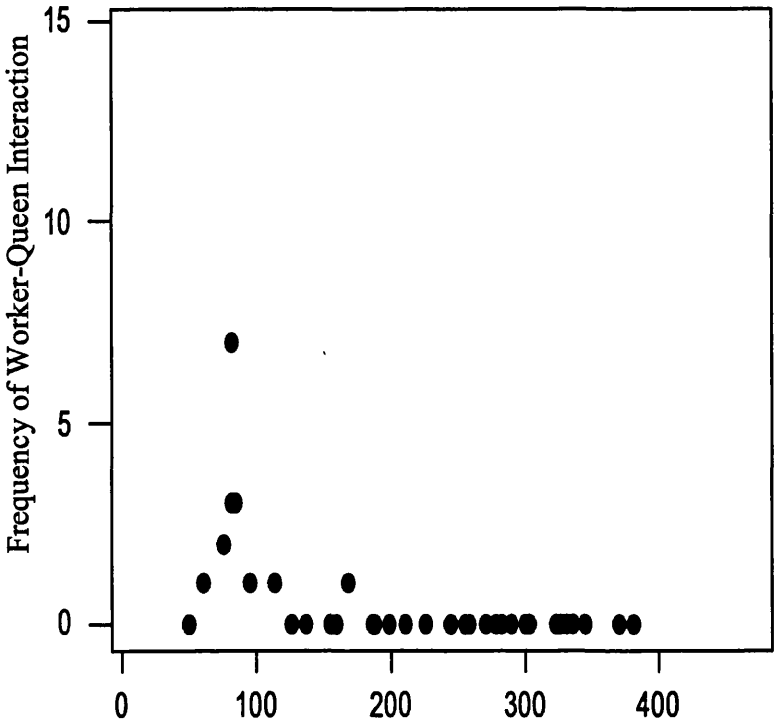


Figure 36: (a) The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre for Colony C_1, the Control, session 1; (b) session 2, and (c) session 3. Callows are shown in red.

Session 1

Colony 9 - An Example Experimental Colony

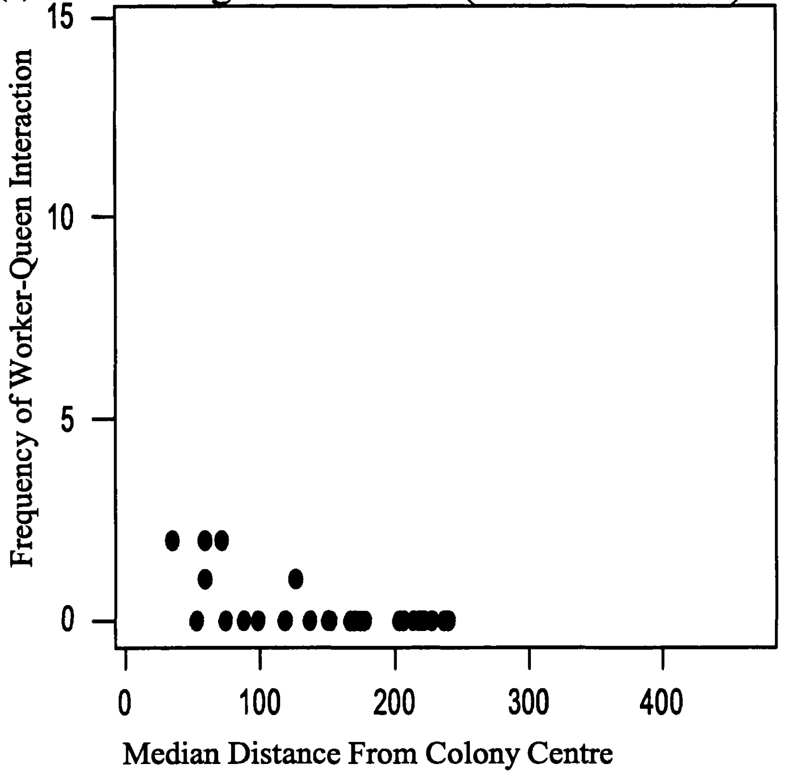
Fig 37(a) Original Fraction (Older Workers)

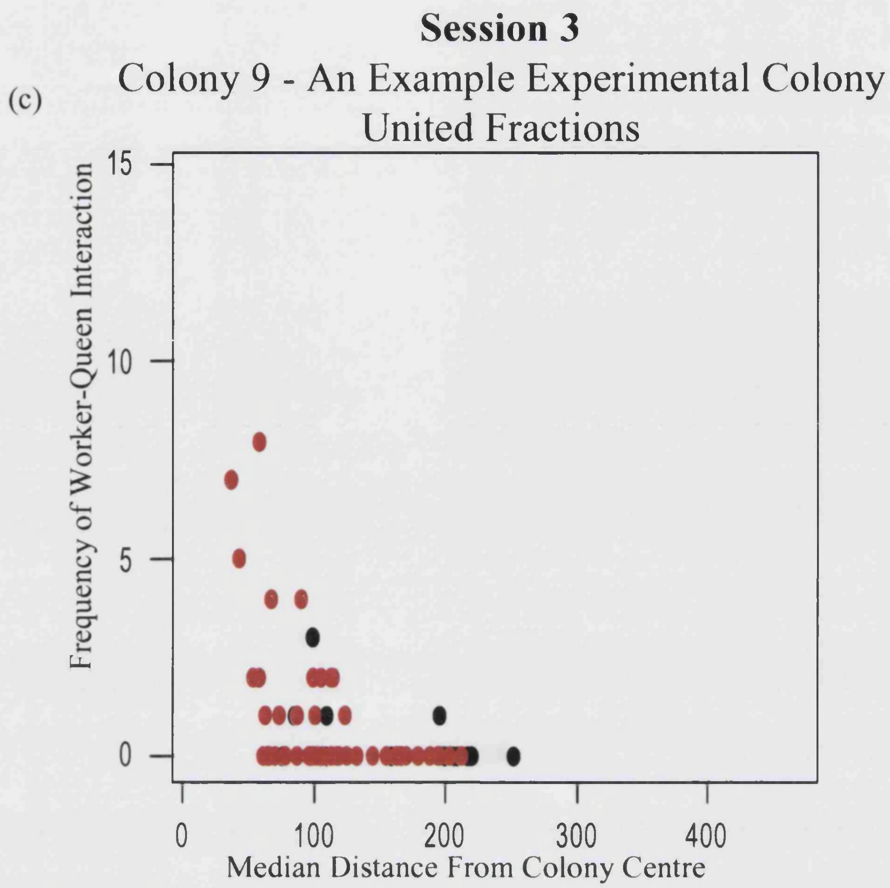


Session 2

Colony 9 - An Example Experimental Colony

Fig 37(b) Original Fraction (Older Workers)




Session 3

Colony 9 - An Example Experimental Colony

United Fractions

Figure 37: (a) The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre for Colony 9, an example experimental colony session 1 (older fraction only); (b) session 2 (older fraction only), and (c) session 3 (united fractions). Callows are shown in red.

The frequency at which individuals interact with the queen decreases as their median distance from the colony centre increases, for the Control colony, and for all the experimental colonies. Workers with a median position above a certain threshold distance from the brood pile have a frequency of contact with the queen of zero, or very close to zero.

The relationship between the frequency at which individuals interact with the queen and their position, is examined further by ranking individuals with respect to the frequency at which they contact the queen, and their position, and thus determining the Spearman's rank correlation coefficient, tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

There is a significant, negative correlation between the relative frequency at which individuals interact with the queen and their relative spatial position, for all the colonies in experiment 3, for all sessions, except for Colony 11, which does not show a significant relationship in session 3, see Table 18, below. The results are discussed in sections 5.3iv. and v.

Colony/session	r_s	n	Significance level $p<$
Colony C_3			
Session 1	-0.791	38	0.01
Session 2	-0.796	38	0.01
Session 3	-0.829	35	0.01
Colony 9			
Session 1 – Older Workers	-0.664	37	0.01
Session 1 – Callows	-	-	-
Session 2 – Older Workers	-0.568	28	0.01
Session 2 – Callows	-	-	-
Session 3	-0.534	65	0.01
Colony 10			
Session 1 – Older Workers	-0.718	33	0.01
Session 1 – Callows	-	-	-
Session 2 – Older Workers	-0.655	32	0.01
Session 2 – Callows	-	-	-
Session 3	-0.295	64	0.05
Colony 11			
Session 1 – Older Workers	-0.657	41	0.01
Session 1 – Callows	-	-	-
Session 2 – Older Workers	-0.723	38	0.01
Session 2 – Callows	-	-	-
Session 3	-0.193	63	NS
Colony 12			
Session 1 – Older Workers	-0.740	21	0.01
Session 1 – Callows	-	-	-
Session 2 – Older Workers	-0.575	22	0.01
Session 2 – Callows	-	-	-
Session 3	-0.417	53	0.01

Table 18 : The relationship between the frequency at which workers have contact with the queen and their median distance from the colony centre, for experiment 3, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as $n > 10$ (Sokal and Rohlf, 1981 p.607).

A Closer Examination of Session 3 - Re-united Colonies

Experiment 3

The spatial organisation of frequency of contacts with the queen in session 3, when the original and subsidiary fractions of the colonies has been united, is examined further. I calculate the Spearman's Rank Correlation Coefficient separately for the 'older' workers and 'callow' workers. The results are shown in Table 18a, below. I also calculated the correlation coefficient for this relationship for the callows, and the older workers separately in the Control Colony.

Session 3 - Re-United Colonies				
Colony	**Fraction of Colony Individuals were in Session 1 and 2 (not control)**	**r_s**	**N**	**Significant Level p<**
Expt 3				
Control	Older	-0.854	10	0.01
Control	Callow	-0.840	23	0.01
9	Older	-0.341	19	NS
9	Callow	-0.580	45	0.01
10	Older	-0.292	32	NS
10	Callow	-0.277	31	NS
11	Older	-0.295	34	NS
11	Callow	0.017	28	NS
12	Older	-0.613	14	0.05
12	Callow	-0.441	24	0.05

Table 18a : The relationship between the frequency of performance of queen contacts and median distance of individuals from the colony centre for re-united colonies in experiment 3, calculated separately for individuals in the original and subsidiary fractions of the colonies during sessions 1 and 2, as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607). NS = non-significant.

There is a significant negative correlation between the relative frequency at which individuals exhibit contact with the queen and their relative distance from the centre of the colony for the older workers of the Control Colony, and the Callow workers. This is the case for the older individuals in session 3, in one of the four experimental colonies. This is also the case for the callows in the united colonies in session 3 for two of the four experimental colonies. These results are discussed in section 5.3v.

To determine any effect of experimental manipulations on the colonies I compare the correlational relationship between the frequency at which callows have contact with the queen and their median distance from the centre from the brood pile in each experimental colony in session 3 with that of the callows in the Control colony in the same session. I also compare the relationship between the frequency at which older individuals have contact with the queen in session 3 with this relationship for the older individuals in the Control colony during this session. The results are shown in Table 18b, below.

Colony/Sess compared to control	$r_{s\,c}$	$r_{s\,e}$	n_c	n_e	z_c	z_e	t_s	**Signif. level p<**
Experiment 3								
Colony9/sess3 Callows	-0.840	-0.580	23	45	-0.221	-0.662	-0.957	NS
Colony10/sess3 Callows	-0.840	NS	23	-	-	-	-	-
Colony11/sess3 Callows	-0.840	NS	23	-	-	-	-	-
Colony12/sess3 Callows	-0.840	-0.441	23	24	-1.221	-0.473	-1.277	NS
Colony9/sess3 Older workers	-0.854	NS	10	-	-	-	-	-
Colony10/sess3 Older workers	-0.854	NS	10	-	-	-	-	-
Colony11/sess3 Older workers	-0.854	-	10	-	-	-	-	-
Colony12/sess3 Older workers	-0.854	-0.613	10	14	-1.271	-0.714	-0.498	NS

Table 18b : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals interact with the queen in session 3 in each experimental colony and this relationship in the Control colony C_3, calculated as a Fisher's z-transform. Comparisons are separate for callows and for older workers. $r_{s\,c}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of queen interaction and distance from the brood pile in the Control colony during session 3. $r_{s\,e}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of queen interaction and distance from the brood pile in each experimental colony during session 3. n_c= The number of individuals that retained their marks in the Control colony. n_e= The number of individuals that retained their marks in the experimental colony.

Fisher's z-transforms show that where significant correlational relationships are found between the frequency at which callows, or older workers, have contact with the queen in session 3, and their median distance from the colony centre, this relationship is no different from the relationship for the callows, or the older workers, in the Control during this session.

Multiple comparisons using pooled data

Because of the lack of the predominance of significant correlations for the relationship between frequency at which callows, or older workers, have contact with the queen, I did not perform multiple comparisons for these data.

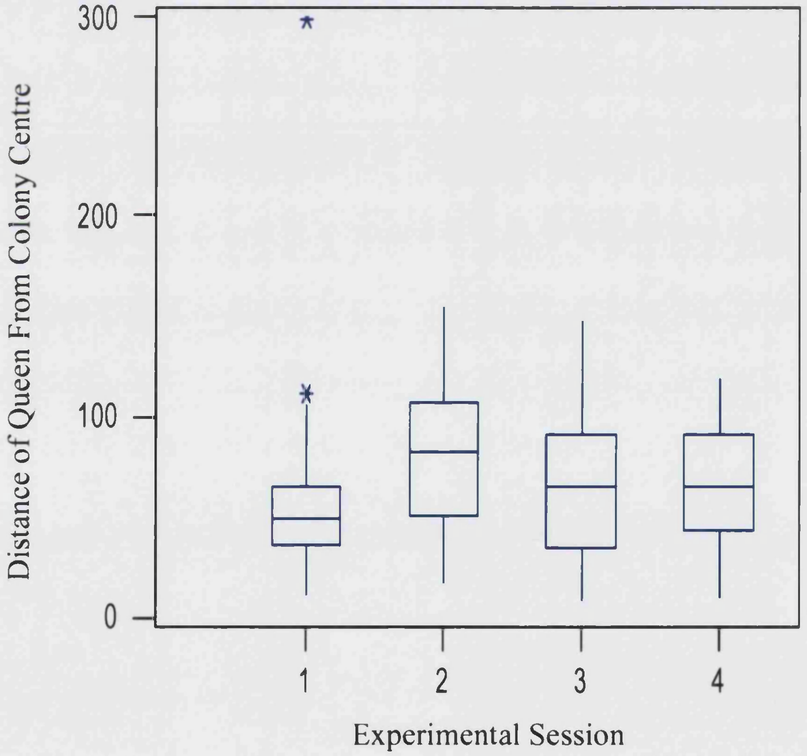
5.2. An Investigation of Queen Behaviour in Colonies with a Manipulated Task Structure

Experiment 1

I investigate the movements of the queen during experiment 1 by plotting the median and inter-quartile range of the distribution of her distance from the colony centre during each session of the experiment. Figures 38 (a) and (b), below, shows these results for for Colony C_1 (the Control colony), and Colony 3, a typical experimental colony, respectively.

Fig 38(a)

Colony C_1



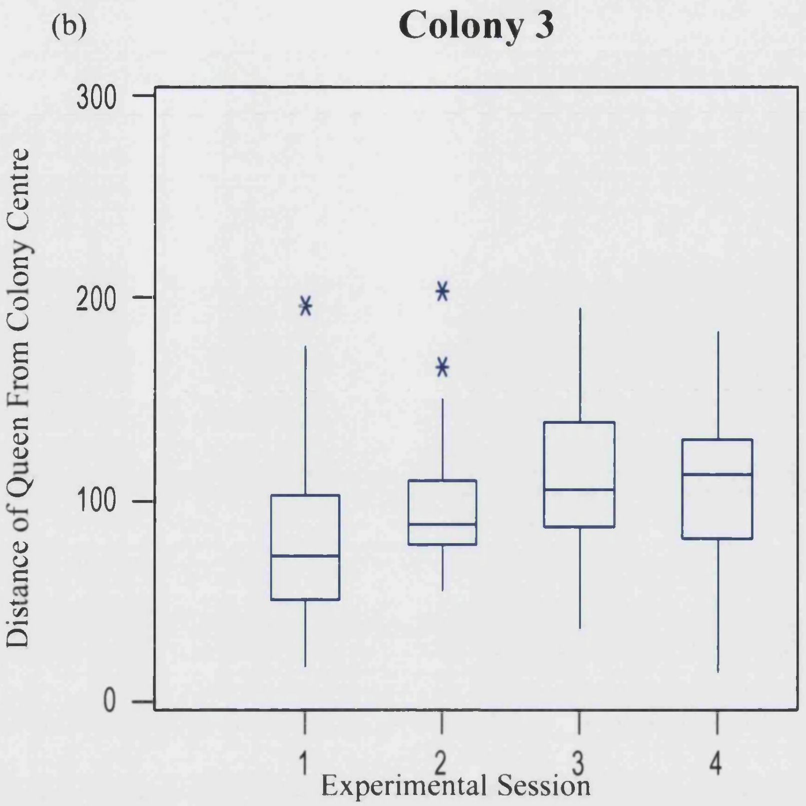


Figure 38 : The distance of the queen from the colony centre during each session of the experiment for (a) Colony C_1,the Control and (b) Colony 3, an example experimental colony.

Mann-Whitney U Tests are used to investigate the spatial distribution of the queen in each session compared to session 1, and the results are given in Table 19, below. The results are discussed in section 5.4.

Colony / Sessions compared	η_1	η_2	W	Significance Level p<
Colony C_1				
1 and 2	49.14	81.66	2208	0.01
1 and 3	49.14	64.94	2426	NS
1 and 4	49.14	64.78	2479	0.01
Colony 1				
1 and 2	49.55	68.84	2359	0.01
1 and 3 (original fraction)	49.55	56.66	2276	0.01
1 and 4	49.55	60.25	2211	0.01
Colony 2				
1 and 2	46.07	35.65	3164	0.01
1 and 3 (original fraction)	46.07	53.55	2468	NS
1 and 4	46.07	76.59	2049	0.01
Colony 3				
1 and 2	71.74	87.61	2509	0.01
1 and 3 (original fraction)	71.74	105.07	2170	0.01
1 and 4	71.74	111.82	2280	0.01
Colony 4				
1 and 2	41.80	44.96	2839	NS
1 and 3 (original fraction)	41.80	50.42	2569	NS
1 and 4	41.80	72.78	2035	0.01

Table 19: A comparison of the spatial distribution of the queen's positions in session 1 of the experiment and the other experimental sessions using Mann-Whitney U Tests. η_1 *= median of the distribution of distances of the queen during session 1 of the experiment.* η_2 *= median of distribution of distances of the queen during the session of the experiment being compared to session 1.*

Colony / Sessions compared	$\eta_{CONTROL}$	η_{EXP}	**Significance Level p<**
Session $1_{CONTROL}$ / Session 1_{EXP}			
Colony 1	49.14	49.55	NS
Colony 2	49.14	46.07	NS
Colony 3	49.14	71.74	0.01
Colony 4	49.14	41.18	NS
Session $2_{CONTROL}$ / Session 2_{EXP}			
Colony 1	81.66	68.84	NS
Colony 2	81.66	35.65	0.01
Colony 3	81.66	87.61	0.05
Colony 4	81.66	44.96	0.01
Session $3_{CONTROL}$ / Session 3_{EXP} (original fraction)			
Colony 1	64.94	56.66	NS
Colony 2	64.94	53.55	NS
Colony 3	64.94	105.07	0.01
Colony 4	64.94	50.42	0.05
Session $4_{CONTROL}$ / Session 4_{EXP}			
Colony 1	64.78	60.25	NS
Colony 2	64.78	76.59	NS
Colony 3	64.78	111.82	0.01
Colony 4	64.78	72.78	NS

Table 20: A comparison of the spatial distribution of the queen's positions in the Control colony with each of the four experimental colonies using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median of the distribution of distances of the queen in the Control colony,* η_{EXP} *= median of distribution of distances of the queen in the experimental colony.*

Mann-Whitney U Tests are also used to investigate the spatial distribution of the queen in the Control colony and each of the four experimental colonies, and the results are given in Table 20, above. These results are also discussed in section 5.4.

5.3 Discussion

An Investigation of Worker Interactions with the Queen, and Her Behaviour

The results to be discussed in section 5.3, are divided into the following sections:

5.1 An investigation of worker interactions with the Queen

i. Colonies with a manipulated task structure

ii. Colonies with a manipulated age structure

In Chapter 4, I established that colonies of *Leptothorax albipennis* exhibit a strong spatial structure with regard to brood-related tasks and external activity, and that this structure is independent of the presence or absence of the queen, the brood, and some of the workers. Sendova-Franks and Franks (1995a) found that worker-queen contacts were most likely to occur closest to the centre of the colony, where the queen is normally situated, in the ant *Leptothorax unifasciatus*. The aim of this chapter is to investigate patterns of interaction with the queen in colonies of *Leptothorax albipennis*, and how these patterns are affected by the manipulations carried out on the colonies. In section 5.2, I investigated the reaction of the queen to the sociotomy carried out in experiments 1, 2 and 3, and this is discussed in section 5.4, below. First, in section 5.1 above, I investigated the spatial patterns of the interactions of the workers with the queen, in un-manipulated colonies, and examined whether these patterns were resilient to the removal of individuals associated with external activity, and to their subsequent re-instatement, in experiments 1 and 2. I also investigated how the individuals that had been removed and had consequently composed a fraction from which the queen was absent, reacted to the queen when they were re-instated.

In Chapter 4, I also investigated the behaviour of artificially created callow colonies, which had never, as eclosed adults, been in the presence of older workers (except the very small number that had helped them eclose and were subsequently removed), or a queen. I found that these colonies exhibited the same spatial patterns of brood-related tasks, and of external activity, as un-manipulated colonies. These patterns were preserved in colonies from which the callows had all been removed. In section 5.1, above, I examined the patterns of spatial organisation of worker-queen interactions in these colonies, from which the callows had been removed. I also examined how the callows, that had never been in the presence of a queen, interacted with her, when they were united with the older workers from the original colonies, in experiment 3

For a summary of the methodology of all three experiments the reader is referred to Figure 9, Chapter 3.

i. Spatial organisation of worker-queen interaction in colonies with a manipulated task structure

Experiments 1 and 2

The frequency at which workers interact with the queen decreases with their increasing distance from the centre of the brood pile in the Control colony, Colony C_1, and all the experimental colonies in experiments 1 and 2, during which they are un-manipulated. The relationship between the relative frequency at which workers interact with the queen and their relative distance from the centre of the brood pile is a highly significant, negative correlation, see Table 15. This relationship is also significant for the Control colony at $p < 0.05$. Figures 31 (a) and (b) are graphs of the relationship between frequency at which individuals interact with the queen and their median distance from the centre of the brood pile for the Control colony, and an typical experimental colony, Colony 3, respectively. Figure 31 (a) shows that the overall frequency at which workers interact with the queen is lower in this colony, than in the experimental colony. The lower amount of interaction with the queen could be attributed to the temporary removal of one third of the workers from this colony to test for the effect of physical removal. This could be a 'real' effect due to the temporary removal of workers, although this is unlikely as the general pattern of interaction between workers and the queen is unaffected. Alternatively, this could simply be a reflection of the decrease in *n* for the one week that one third of the workers are missing. This is again considered unlikely as there is no decrease of corresponding magnitude in the frequency of brood-related tasks in the Control compared to the experimental colonies, see Chapter 4, and any effect should be negligible due to the relatively small proportion of workers removed and the short duration of this removal. Therefore, I attribute the decrease in worker-queen interaction to inter-colony variation, rather than any effect due to temporary removal of one third of the workers.

Figures 31 (a) and (b) also show that the queen's median distance from the centre of the brood pile is the closest, or closest but one, of all the individuals in the undisturbed fractions of the colony. I investigate in section 5.4 whether the queen's median position changes throughout the experiment. In the undisturbed colonies however, the pattern of worker interactions with the queen is very similar to that observed for colonies of *Leptothorax unifasciatus* (Sendova-Franks and Franks, 1995a). Workers show a higher frequency of interaction with the queen when their median position is relatively close to where her median position is located, near the centre of the brood pile. Workers with a median position above a certain threshold distance from the brood pile have a frequency of contact with the queen of zero, or very close to zero. This is attributed to the lack of overlap of their zones of movement with that of the queen. The relationship between frequency at which individuals interact with the queen and their median distance from the colony centre closely approximates an exponential decay, see Figure 32.

ii. How is worker-queen interaction affected by the removal of individuals associated with external activity?

Experiments 1 and 2

I examined the spatial pattern of worker-queen interactions in the colonies during the removal of individuals associated with external activity in experiment 1 (session 2), and during session 3, for experiments 1 and 2, when removed individuals existed as a separate fraction.

During session 2, when individuals associated with external activity were being removed, the spatial pattern of worker-queen interaction remains the same as that observed during session 1, when the fractions were un-manipulated. The relationship between the relative frequency at which individual workers interact with the queen and their relative distance from the centre of the brood pile is a significant, negative correlation for all four experimental colonies, and the Control, see Table 16. Figures 33 (a) and (b) show the spatial organisation of worker-queen interaction for the Control colony, and a typical experimental colony, Colony 3, during session 2. The patterns shown are very similar. I discuss whether the position of the queen has altered in the experimental colonies in section 5.4.

The relationship between the relative frequency at which individuals interact with the queen and their relative distance from the colony centre in the original fractions of the colonies in experiments 1and 2, remains a significant, negative correlation for all colonies for which there is a sufficiently high value of *n*. Colonies 5 and 6 do not have sufficient numbers of individuals that have retained their marks to allow the calculation of the Spearman's rank correlation coefficient. Colonies 1 and 4 have sufficient numbers to render the calculation valid, but there are insufficient numbers of individuals remaining in this fraction of the colony for a significant pattern to be established. In Colony 2, *n* is sufficiently high to establish a pattern, but the relationship, although negative, is not significant. A closer examination of these data indicates relatively low absolute frequencies of worker-queen interaction in this fraction of the colony. A maximum of two incidents of interaction with the queen are recorded for any one individual. This is attributed to inter-colony variation, as the pattern is not observed in the other colonies, which were treated similarly. Figures 34 (a) and (b) show the spatial organisation of worker-queen interactions in the Control colony, and for an example experimental colony, Colony 3, during session 3, original fraction. The patterns are very similar. Thus, the removal of the individuals associated with external activity does not affect the spatial organisation of worker-queen interaction, provided there are a sufficiently high number of individuals remaining in that fraction of the colony.

iii. How is worker-queen interaction affected by the subsequent re-unification of the original and subsidiary fractions of the colonies?

The behaviour of the re-united colonies in session 4 is discussed separately for experiments 1 and 2. Any difference in the patterns of worker-queen interaction between colonies from the two experiments is attributed to the presence of brood in the subsidiary fractions of the colonies in experiment 2, or, conversely, the absence of brood in these fractions of the colonies in experiment 1.

Experiment 1

The frequency at which individuals in the Control colony, Colony C_1, interact with the queen decreases with their increasing distance from the centre of the brood pile, see Figure 35 (a). This relationship is very similar to the pattern observed during the other sessions for this colony. The relationship between the relative frequency at which individuals interact with the queen and their relative distance from the centre of the brood pile during session 4 remains a highly significant, negative correlation, see Table 16. The absolute frequency at which individuals interact with the queen is very similar to these frequencies in other sessions. I therefore conclude that the pattern of workers interactions with the queen is not affected by the temporary, physical removal of workers from the colony, or seasonal changes in task demand or spatial organisation in the colony.

The frequency at which workers interact with the queen also decreases with their increasing distance from the centre of the brood pile, for all four experimental colonies during session 4. The relationship is shown for an example experimental colony, Colony 3, in Figure 35 (b). The relationship between ranked variables is a significant, negative correlation, as it was for these colonies in session 1, see Table 16. Moreover I also compared the relationship between the frequency at which individuals in each of the experimental colonies had contact with the queen in session 4, when colonies were re-united, with this relationship in the Control colony during session 4. There was no significant difference between the correlational relationship of the individuals in each of the experimental colonies with the Control colony in this session (see Table 17a). Individuals that were removed from the experimental colonies, and thus composed the subsidiary fraction of each colony during session 3, are shown in red in Figure 35 (b). It can be seen that these individuals interact with the queen at relatively low absolute frequencies. However, this must also have been the case prior to their removal, as the overall spatial pattern is still observed. Consequently, the removal of individuals associated with external activity into colonies with no brood or queen, and the subsequent re-unification of the two fractions of the colonies, does not affect the spatial organisation of interactions between the workers and the queen. This phenomenon is shown to be robust. There is no significant difference between the relationship between the frequency at which individuals have contact with the queen in session 1, and in session 4, when I pool the experimental colony data and perform multiple comparisons (see Table 17b).

Experiment 2

I examined the spatial organisation of worker-queen interactions observed in colonies from which individuals associated with external activity were removed and placed in a nest with brood, but no queen. For colonies that contained a sufficiently high number of individuals that had retained their marks in session 4, the relationship between the relative frequency at which workers interact with the queen, and their relative distance from the centre of the brood pile, remains highly statistically significant, see Table 17. The overall spatial pattern of interaction with the queen in session 4 is shown in Figure 35 (c), for an example experimental colony. It can be seen that the individuals that were in the subsidiary fraction of the colony during session 3, shown in red, have a relatively low absolute frequency of interaction with the queen. The overall relationship between the frequency at which workers in each of the experimental colonies have contact with the queen is not, however, significantly different from that of the Control colony when compared to session 4 (see Table 17a). Moreover, when I perform multiple comparisons using the pooled data from the experimental colonies with sufficient numbers of individuals that have retained their marks, there is no significant difference between the correlational relationship in session 1, before manipulation, and session 4 (see Table 17b).

It may be concluded that the spatial organisation of worker interaction with the queen is not affected by the reinstatement of individuals previously associated with external activity, which had been removed into a separate nest. The presence or absence of brood, and the absence of the queen, in the subsidiary fractions, has no effect on patterns of worker-queen interaction in session 4, when the subsidiary fractions are re-united with the original fractions. This is, perhaps, not unexpected as these workers interacted less with the queen in the original, un-manipulated colonies, as they were found further away from the centre of the brood pile, see above, and Chapter 7. I hypothesise that worker-queen interaction may be affected by removing a proportion of the workers, and subsequently re-uniting them with the original fraction, if the removed workers have been situated closer to the queen, and therefore interacted with her at higher absolute frequencies. This is discussed below, with reference to the results from experiment 3. It is particularly important to examine the effects of queen absence on colony interactions as the loss of the queen in the field may lead to an increase in dominance interactions among the remaining workers. The outcome of dominance interactions has been hypothesised to be a possible mechanism of task allocation in ants (Powell and Tschinkel, 1999, section 2.4ii.). The evidence presented above indicates that the absence of the queen from nests containing a proportion of the workers, and then re-unification of these workers with the colony and queen, does not affect patterns of worker-queen interaction. The influence of these results on possible mechanisms of task allocation involving dominance interactions is discussed further in Chapter 9.

iv. An investigation of worker-queen interaction in colonies from which the callow cohort has been removed.

Experiment 3

The frequency at which individuals interact with the queen decreases with their increasing distance from the centre of the brood pile in the Control colony, Colony C_3, which was un-manipulated. The relationship between the relative frequency at which workers interact with the queen and their relative distance from the centre of the brood pile is a highly significant, negative correlation, see Table 18. Figures 36 (a), (b) and (c) are graphs of the relationship between the frequency at which individuals interact with the queen and their median distance from the centre of the brood pile for the Control colony, during sessions 1, 2 and 3 respectively. Callows are shown in red, older workers in black. It can be seen in all three sessions that the frequency at which individuals interact with the queen is not determined by age for the duration of the experiment. Neither is the pattern affected by seasonal changes.

In the experimental colonies from which the callows, as pupae, had been removed, the relationship between the frequency at which individuals interact with the queen and their distance from the centre of the brood pile is similar during sessions 1 and 2 of the experiment. The frequency at which workers interact with the queen decreases with their increasing distance from the centre of the brood pile. The relationship between the relative frequency at which workers interact with the queen and their relative distance from the centre of the brood pile is a highly significant, negative correlation, see Table 18. Figure 37 (a) and (b) are graphs of the spatial distribution of interaction with the queen in the 'older' fraction of a typical experimental colony, Colony 9, during sessions 1 and 2 of the experiment, respectively. Although the overall spatial patterns of interaction are preserved throughout the duration of these sessions, the maximum frequency of interaction observed is lower during session 2. This phenomenon is not observed in the Control colony, and cannot therefore be a reflection of seasonal changes in the colony. This strongly suggests that the reduction in the absolute frequency of worker-queen interaction in session 2 is due to the absence of callow workers. One might expect there to be an increased amount of interaction between the workers and the queen in colonies from which the pupae have been removed, associated with the decreased number of 'obstacles' between the queen and the worker, however this is not the case. The decrease in interaction may be due to the increased space in the colonies resulting from the removal of pupae. The time lag in the response of the colony to manipulations is noted.

v. How is worker-queen interaction affected by the unification of the callow cohorts with the original colonies?

Experiment 3

The spatial pattern of interaction between the workers and the queen tends to be preserved in the experimental colonies during session 3, when the callows, which have never before (as adults) been in the presence of the queen, or older workers, are united with their original colonies. The relationship between the relative frequency at which individuals interact with the queen and their relative distance from the centre of the brood pile is a significant, negative correlation for all the colonies in session 3, see Table 18, except for Colony 11, which is discussed below. I analysed the data further by analysing the relationship between the frequency at which callows, and older workers, separately, had contact with the queen during session 3, and their relative distance from the centre of the brood pile (see Table 18a). The results show that there is some disruption to the patterns observed, when older and callow fractions are considered separately. I conclude that the unification of callows and older workers in session 3 has the effect of altering the relationship between frequency of interaction with the queen and median distance of individuals from the centre of the brood pile. Despite this, the patterns tend to be preserved when analysed for older workers and callows together, see above.

I carried out comparisons between the relationships for frequency of interaction with the queen and distance from the colony centre for the experimental colonies, callows and older workers considered separately, compared to the callows or older workers, as appropriate, in the Control colony during session 3, for fractions where I did find the relationship to be significant. The results (see Table 18b), show that, where significant patterns are found for the callows, or older workers in the experimental colonies, these relationships are not significantly different from the Control.. Therefore I conclude that there is evidence that the relationships between frequency of queen contacts and distance of individuals from the brood pile are preserved among the fractions considered separately, but that there is some disruption to these patterns when the colonies are united. I do not analyse multiple comparisons between the pooled correlations for this relationship in the experimental colonies compared to the Control, due to the lack of predominance of significant patters when the results are analysed separately for callows and older workers.

Figure 37 (c) is a graph of the relationship between the frequency at which workers interact with the queen and their median distance from the centre of the brood pile for Colony 9, a typical experimental colony. The graph shows that callows, shown in red, tend to exhibit a higher frequency of interaction with the queen than the older workers. The median of the callows distribution of distances form the centre of the brood pile during this session, tends to be lower. Thus they tend to be situated closer to the centre of the brood pile. As I have already determined in section 5.3iv., above, that age does not determine the frequency of interaction with the queen, it seems reasonable to attribute the patterns of interaction seen in the experimental colonies in session 3 to the spatial distribution of workers. The sorting procedure whereby workers establish these spatial positions is discussed further in Chapter 9. It is important to note that the overall pattern of interactions of callows with the queen is very similar to that observed for the callows in the un-manipulated Control colony, despite their inexperience of her presence as adults.

Colony 11 does not show a significant correlation between the relative frequency at which workers interact with the queen and their median distance from the centre of the brood pile in session 3. A closer examination of these data reveals that the spatial pattern of interaction is still preserved. The absolute number of interactions is very low, with a maximum of three contacts with the queen by any one worker. This may be attributed to inter-colony variation.

5.4 An investigation of queen behaviour

In the following section I will discuss the results from section 5.2, in which I investigated the reaction of the queen to the sociotomy carried out in experiment 1.

> 5.2 An Investigation of queen behaviour in colonies with a manipulated task structure.

First, I investigated the movements of the queen in each of the un-manipulated colonies during session 1 of the experiment, by determining the median, and inter-quartile range (IQR) of her distribution of distances from the centre of the brood pile. The IQR corresponds to the Spatial Fidelity Zone (SFZ) as determined in Sendova-Franks and Franks (1995a). I then investigated the effect that the removal of individuals associated with external activity has on the queen's median position and SFZ, by comparing the data between session 1 of the experiments and sessions 2 and 3, using Mann-Whitney U tests, see Table 19. Figures 38 (a) and (b) are graphs of the SFZ of the queen for each of the four sessions for the Control colony, Colony C_1, and for a typical experimental colony, Colony 3, respectively. The median distance of the queen from the centre of the brood pile is higher in sessions 2-4 than in session 1 in the Control colony. The SFZ of the queen is also larger in sessions 2-4, compared to session 1, in this colony. This could be a reaction to seasonal changes, or a reflection of disturbance to the behaviour of the queen resulting from the temporary removal of one third of the workers at the beginning of session 2. When tested statistically, the distribution of distances of the queen from the centre of the brood pile increases significantly in session 2, compared to session 1, in the Control colony. The median distance of the queen from the centre of the brood pile (η_1 in Table 19) is much greater in session 2 compared to session 1. This is attributed to the temporary effect on the queen's behaviour induced by the removal of one third of the workers. The trend is not significant in session 3 compared to session 1, and this, again, indicates that although there is some disturbance to the behaviour of the colony, this disturbance is temporary. The distribution of the queen's distances from the centre of the brood pile is significantly different in session 4 compared to session 1 in the Control colony, see Table 19, although the median of the distributions in session 4 is very similar to that observed in session 3. It can, therefore, be concluded that the range of distances of the queen, and hence her SFZ, has increased. This is indicated in Figure 38 (a). I attribute this to seasonal changes.

The results from each of the experimental colonies shows that the distribution of distances of the queen changes significantly in session 4 compared to session 1. In every case, the median position of the queen is further away from the brood pile in session 4. As there is also a significant difference between the distribution of the queen's distances from the brood pile in session 1 compared to session 4 in the Control colony, this may be a reflection of seasonal changes, rather than any reaction of the queen to sociotomy. Note that the Mann-Whitney U tests do not discriminate between changes in the queen's median position, and changes in her SFZ. There appear to be no other consistent patterns of change in the spatial distribution of the queens in the other sessions of the experiment when they are compared to session 1. Thus changes in the spatial distribution of the queen can be attributed to seasonal changes in the colony rather than a reflection of the increased space in the colony resulting from the removal of individuals associated with external activity.

I compare the spatial distribution of the queen in the Control colony and each of the four experimental colonies, for each session, see Table 20. There is some tendency towards intra-colony patterns of the spatial distribution of the queen. In an individual colony, the queen's distribution is either significantly different from that of the Control throughout the experiment, or not significantly different from the Control. This trend is not consistent for Colony 4. Thus it can be concluded that the spatial distribution of the queen is influenced by inter-colony variation, rather than by sociotomy.

Similar analyses were not carried out on the results from experiments 2 and 3, as it was not considered that this would provide any more useful insights, as the reaction of the queen to sociotomy is minimal.

CONCLUSIONS

The following conclusions can be drawn:

The frequency at which workers interact with the queen decreases with increasing distance of the workers from the centre of the brood pile, where the queen is normally situated.

The temporary removal of one third of the workers may induce a temporary decrease in the frequency of worker-queen interactions.

The removal of individuals associated with external activity does not tend to affect the spatial organisation of worker-queen interactions.

The subsequent re-unification of the original fractions of the colonies with the removed individuals, does not affect the spatial organisation of worker-queen interactions.

This is independent of the presence/absence of brood in the subsidiary fractions of the colonies.

These results have implication for orphaned colonies in the field, and for mechanisms of task allocation that may depend on dominance interactions, discussed further in Chapter 9.

The frequency at which workers interact with the queen is not dependent on worker age, but may be determined rather by worker spatial position in the nest.

The frequency at which workers interact with the queen decreases with increasing distance of the workers from the centre of the brood pile, where the queen is normally situated, in colonies from which callows have been removed.

The maximum frequency of worker-queen interactions is lower in colonies from which the callows have been removed compared to un-manipulated colonies. This may be a reflection of increased space in the nest resulting from the absence of callows.

Patterns of worker-queen interactions remain unchanged by the re-instatement of callows, even though the callows have never before been in the presence of the queen as eclosed workers.

Callows that have never been in the presence of the queen as eclosed workers tend to interact with her at a higher frequency than older workers, but show similar interaction frequencies to the callows in un-manipulated colonies.

The Spatial Distribution of the Queen

The distribution of distances of the queen from the Control colony centre increases temporarily due to the disturbance resulting from the temporary removal of one third of workers.
This trend is reflected in the experimental colonies

There is some trend towards increasing SFZ through the course of experiment 1 for the queen. I attribute this to seasonal changes.

There are no consistent patterns in the queen's distribution in the experimental colonies resulting from sociotomy.

The spatial distribution of the queen is influenced by inter-colony variation.

Chapter 6

An Investigation of Patterns of Interaction Among Workers

6.1 An Investigation of Patterns of Interaction Among Workers

i. Colonies with an manipulated task structure

To investigate the degree of spatial organisation of grooming, trophallaxing, or other types of contact among workers, in colonies of the ant *Leptothorax albipennis,* I determine the frequency at which individual workers interact with other workers during each session, for experiments 1 and 2. Interaction with another worker is defined as contact between the mouth-parts of one worker and any part of another worker, determined from the photographs taken during the session.

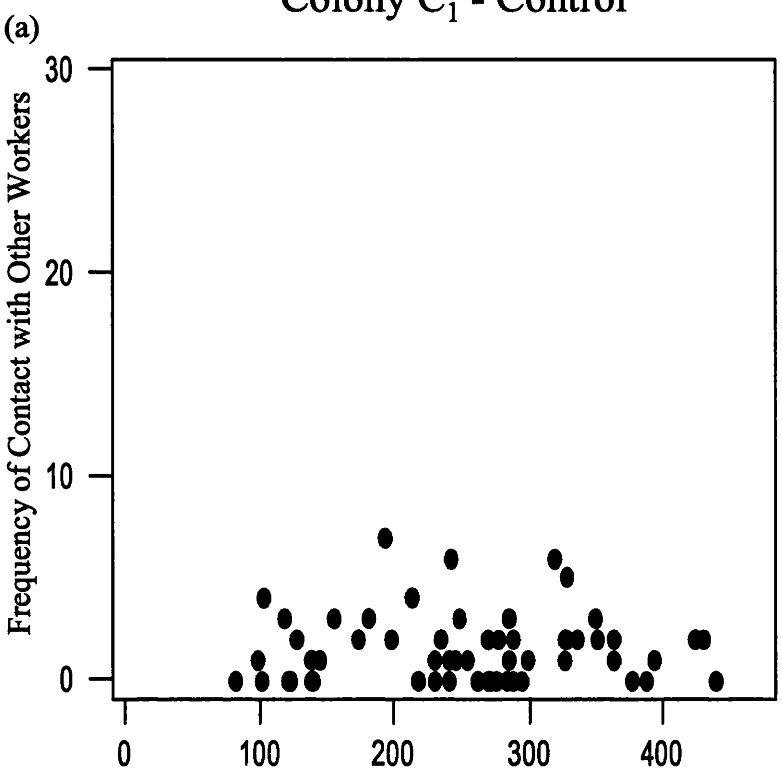
Individuals are ranked, as before, according to the frequency at which they perform these actions, and with respect to the median of the distribution of their distances from the colony centre during each session. I calculate the Spearman's rank correlation coefficient for the relationship between these two rankings. In all cases where $n > 10$, the relationship is tested as an ordinary product-moment correlation coefficient (Sokal and Rohlf, 1981 p.607). Where $n \leq 10$, the Spearman's rank correlation coefficient is calculated according to the following formula:

$$\boxed{r_s = 1 - \frac{6 \sum d^2}{n (n^2 - 1)}}$$

There appears to be no clear relationship between the frequency at which individuals interact with other workers and their spatial position. Figures 39 (a) and (b) demonstrate this for (a) Colony C_1, the Control colony in experiment 1, and Colony 3, a typical experimental colony. The complete analysis shows that there is no clear relationship in colonies in either experiment 1 or experiment 2, see Tables 21 and 22, below.

Session 1

Colony C_1 - Control



Colony 3 - An Example Experimental Colony

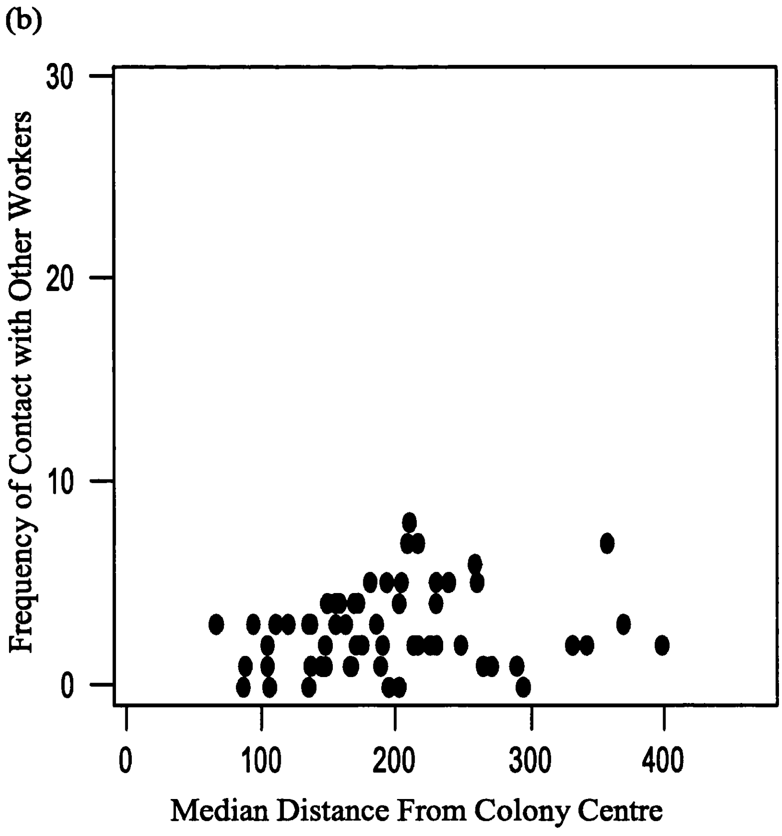


Figure 39: The relationship between the frequency at which an individual interacts with other workers and her median distance from the colony centre for (a) Colony C_1, the Control colony; and (b) Colony 3, an example experimental colony, for session 1.

Experiment 1

Colony/session	r_s	n	Significance level p<
Colony C_1			
Session 1	0.090	58	NS
Session 2	0.245	40	NS
Session 3	-0.313	40	0.05
Session 4	-0.502	33	0.01
Colony 1			
Session 1	-0.473	64	0.01
Session 2	-0.374	23	NS
Session 3 – original	-0.381	8	NS
Session 3 - subsidiary	-0.819	24	0.01
Session 4	-0.441	30	0.05
Colony 2			
Session 1	-0.410	68	0.01
Session 2	-0.438	29	0.05
Session 3 – original	-0.016	19	NS
Session 3 – subsidiary	-0.923	17	0.01
Session 4	-0.720	31	0.01
Colony 3			
Session 1	0.207	58	NS
Session 2	-0.089	27	NS
Session 3 – original	-0.132	18	NS
Session 3 – subsidiary	-0.402	36	0.05
Session 4	0.018	48	NS
Colony 4			
Session 1	0.190	52	NS
Session 2	-0.087	18	NS
Session 3 – original	-0.825	5	NS
Session 3 – subsidiary	-0.259	19	NS
Session 4	0.341	22	NS

Table 21: The relationship between the frequency at which individuals interact with nest mates and their median distances from the colony centre, for experiment 1, calculated as r_s, the Spearman's rank correlation coefficient where $n < 10$, as an ordinary product-moment correlation coefficient where $n \leq 10$ (Sokal and Rohlf, 1981 p.607).

Experiment 2

Colony/session	r_s	n	Significance level p<
Colony C_2			
Session 1	-0.444	17	NS
Session 3	0.081	28	NS
Session 4	-	2	-
Colony 5			
Session 1	0.135	34	NS
Session 3 – original	-	0	-
Session 3 - subsidiary	-	1	-
Session 4	-	2	-
Colony 6			
Session 1	-0.438	25	0.05
Session 3 – original	-	1	-
Session 3 – subsidiary	-0.614	6	NS
Session 4	-0.607	7	NS
Colony 7			
Session 1	-0.528	57	0.01
Session 3 – original	-0.661	23	0.01
Session 3 – subsidiary	0.181	19	NS
Session 4	-0.635	35	0.01
Colony 8			
Session 1	-0.277	72	0.05
Session 3 – original	0.224	18	NS
Session 3 – subsidiary	0.030	19	NS
Session 4	-0.282	31	NS

Table 22 : The relationship between the frequency at which individuals interact with nest mates and their median distance from the colony centre, for experiment 2, calculated as r_s, the Spearman's rank correlation coefficient where n < 10, as an ordinary product-moment correlation coefficient where n ≤ 10 (Sokal and Rohlf, 1981 p.607).

It should be noted that where there is a significant correlation between the relative frequency at which individuals interact and their relative distance from the centre of the brood pile, the relationship is a negative correlation.

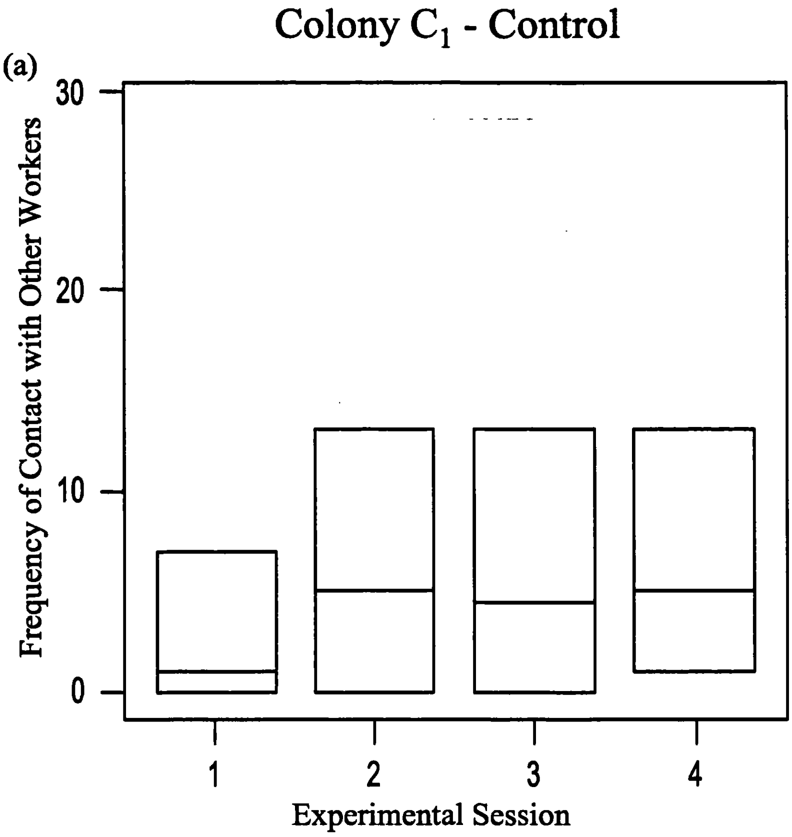
I determine that significant negative correlations sometimes occur between the frequency at which individuals interact with other individuals and their median distance from the centre of the brood pile in experiments 1 and 2. This is discussed further later in Chapter 6. Where significant patterns do occur, I determine any effect of experimental manipulations on the colonies by comparing the correlational relationship between the frequency at which individuals interact with other individuals and their median distance from the centre from the brood pile in each relevant experimental colony in session 4 with that of the Control colony in the same session. The results are shown in Table 22a, below.

Colony/Sess compared to control	$r_{s\,c}$	$r_{s\,e}$	n_c	n_e	z_c	z_e	t_s	**Signif. level $p<0.05$**
Experiment 1								
Colony1/sess4	-0.502	-0.441	33	30	-0.552	-0.473	-0.296	NS
Colony2sess 4	-0.502	-0.720	33	31	-0.552	-0.908	1.354	NS
Colony3/sess4	-0.502	NS	-	-	-	-	-	-
Colony4/sess4	-0.502	NS	-	-	-	-	-	-
Experiment 2								
Colony5/sess4	-0.502	-	33	2	-	-	-	-
Colony6sess 4	-0.502	NS	-	-	-	-	-	-
Colony7/sess4	-0.502	-0.635	33	35	-0.552	-0.750	0.778	NS
Colony8/sess4	-0.502	NS	-	-	-	-	-	-

Table 22a : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals interact with the other individuals in session 4 in each experimental colony and this relationship in the Control colony C_1, calculated as a Fisher's z-transform. $r_{s\,c}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of worker interaction and distance from the brood pile in the Control colony during session 4. $r_{s\,e}$ = the Spearman's Rank Correlation Coefficient for the relationship between frequency of worker interaction and distance from the brood pile in each experimental colony during session 4. n_c= The number of individuals that retained their marks in the Control colony. n_e= The number of individuals that retained their marks in the experimental colony.

Fisher's z-transforms shows that there is no significant difference between the correlation of the Control colony, Colony C_1, and the experimental colonies in session 4. This applies to all colonies where I determine that there is a significant negative correlation between the frequency at which workers interact and their median distance from the centre of the brood pile .

To determine if the absolute frequency of worker-worker interaction varies between the sessions of the experiment, I plot graphs of the median and range of the total number of worker-worker contacts for each session of experiment 1 for the Colony C_1, the Control, and an example of one of the experimental colonies, Colony 3, see Figure 40 (a) and (b), below.



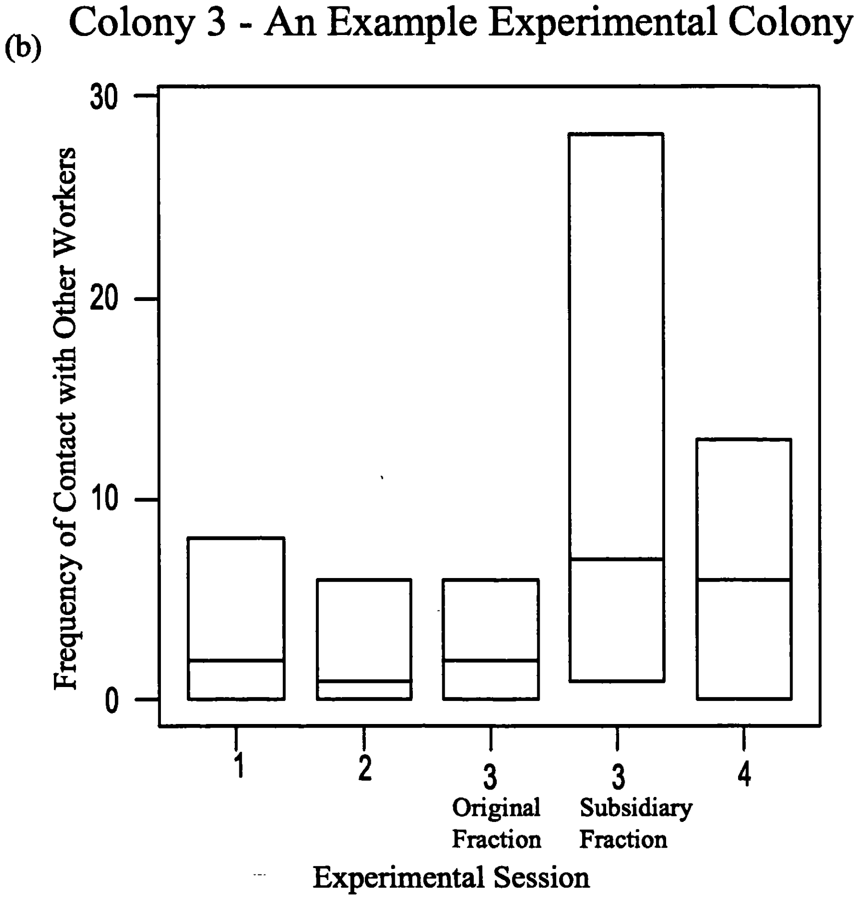


Figure 40: The frequency at which individuals interact with other workers for (a) Colony C_1, the Control colony; and (b) Colony 3, an example experimental colony, for each session of experiment 1. The median and range are shown for each session.

Figure 40 (a) shows that the median frequency at which workers interact with other workers is higher in sessions 2, 3 and 4, in the Control colony, compared to session 1. The variation in the number of interactions between workers is also greater in these sessions. In the example experimental colony shown in Figure 40 (b) (Colony 3), the median frequency of worker-worker interaction is dramatically higher in the subsidiary fraction of session 3, and decreases slightly in session 4. The range in the number of worker-worker interactions is also dramatically higher in the subsidiary fraction of session 3 than in session 1. The range decreases in session 4, but is still greater than that observed in session 1.

The results of statistical analyses using Mann-Whitney U tests to compare the absolute amount of interaction among workers in the colonies in sessions 2, 3 and 4, compared to session 1, are given in Table 23 (experiment 1), and Table 24 (experiment 2).

I also compare the absolute frequency of worker-worker interaction in each session of the experiment between the Control and each experimental colony, for experiments 1 and 2, using Mann-Whitney U tests. I use the Control colony for experiment 1 (Colony C1) for the comparisons in experiment 2, as well as for experiment 1 (Chapter 4). The results for experiments 1 and 2 are shown in Tables 25 and 26 respectively, below. The results are discussed in section 6.2i.

Colony / Sessions Compared	η_1	η_2	**W**	**Significance Level p <**
Colony C_1				
1 and 2	1.0	5.0	2255.0	0.01
1 and 3	1.0	4.5	2085.5	0.01
1 and 4	1.0	5.0	1890.0	0.01
Colony 1				
1 and 2	1.0	1.0	2909.5	NS
1 and 3 (original fraction)	1.0	5.0	2112.5	0.01
1 and 3 (subsidiary fraction)	1.0	10.0	2215.0	0.01
1 and 4	1.0	7.5	2236.0	0.01
Colony 2				
1 and 2	2.0	2.0	3202.5	NS
1 and 3 (original fraction)	2.0	1.0	3109.0	NS
1 and 3 (subsidiary fraction)	2.0	5.0	2655.0	0.01
1 and 4	2.0	5.0	2817.5	0.01
Colony 3				
1 and 2	2.0	1.0	2737.5	0.05
1 and 3 (original fraction)	2.0	2.0	2288.0	NS
1 and 3 (subsidiary fraction)	2.0	7.0	2068.0	0.01
1 and 4	2.0	6.0	2427.5	0.01
Colony 4				
1 and 2	3.0	0.5	2140.0	0.01
1 and 3 (original fraction)	3.0	1.0	1557.0	NS
1 and 3 (subsidiary fraction)	3.0	5.5	1566.0	0.01
1 and 4	3.0	4.0	1795.0	NS

Table 23: A comparison of the distribution of the number of worker-worker interactions for workers in session 1 of experiment 1 and the other sessions, for each colony, using Mann-Whitney U Tests. η_1 *= median of the distribution of the number of worker-worker interactions during session 1 of the experiment.* η_2 *= median of the distribution of the number of worker-worker interactions during session 3.*

Colony / Sessions Compared	η_1	η_2	W	Significance Level p <
Colony C_2				
1 and 3	2.0	5.0	215.0	0.01
1 and 4	2.0	3.0	270.0	0.05
Colony 5				
1 and 3 (original fraction)	-	-	-	-
1 and 3 (subsidiary fraction)	-	-	-	-
1 and 4	1.5	6.0	596.5	0.05
Colony 6				
1 and 3 (original fraction)	-	-	-	-
1 and 3 (subsidiary fraction)	3.0	4.0	375.0	NS
1 and 4	3.0	4.0	383.0	NS
Colony 7				
1 and 3 (original fraction)	3.0	5.0	2057.0	0.01
1 and 3 (subsidiary fraction)	3.0	1.0	2463.0	0.01
1 and 4	3.0	4.0	2482.0	NS
Colony 8				
1 and 3 (original fraction)	4.0	3.0	3603.5	0.01
1 and 3 (subsidiary fraction)	4.0	2.0	3769.0	0.01
1 and 4	4.0	2.0	4373.5	0.01

Table 24: A comparison of the number of worker-worker interactions for workers in session 1 of experiment 2 and the other sessions, for each colony, using Mann-Whitney U Tests. η_1 *= median of the distribution of the number of worker-worker interactions during session 1 of the experiment.* η_2 *= median of the distribution of the number of worker-worker interactions during session 3.*

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1						
C_1 vs Colony 1	58	64	1.0	1.0	3694.5	NS
C_1 vs Colony 2	58	68	1.0	2.0	3204.5	0.05
C_1 vs Colony 3	58	58	1.0	2.0	2747.5	0.01
C_1 vs Colony 4	58	52	1.0	3.0	2551.0	0.01
Session 2						
C_1 vs Colony 1	40	23	5.0	1.0	1691.0	0.01
C_1 vs Colony 2	40	29	5.0	2.0	1735.5	0.01
C_1 vs Colony 3	40	27	5.0	1.0	1765.5	0.01
C_1 vs Colony 4	40	18	5.0	0.5	1489.5	0.01
Session 3 – Original Fraction						
C_1 vs Colony 1	40	8	4.5	5.0	985.5	NS
C_1 vs Colony 2	40	19	4.5	1.0	1448.0	0.01
C_1 vs Colony 3	40	18	4.5	2.0	1368.0	0.01
C_1 vs Colony 4	40	5	4.5	1.0	984.0	0.05
Session 3 – Subsidiary Fraction						
C_1 vs Colony 1	40	24	4.5	10.0	1080.5	0.01
C_1 vs Colony 2	40	17	4.5	5.0	1140.5	NS
C_1 vs Colony 3	40	36	4.5	7.0	1334.0	0.05
C_1 vs Colony 4	40	19	4.5	5.0	1120.5	NS
Session 4						
C_1 vs Colony 1	33	30	5.0	7.5	954.0	NS
C_1 vs Colony 2	33	31	5.0	5.0	1146.5	NS
C_1 vs Colony 3	33	48	5.0	6.0	1346.0	NS
C_1 vs Colony 4	33	22	5.0	4.0	1013.0	NS

Table 25: A comparison of the number of worker-worker interactions in each session of the experimental colonies with the Control, using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median of the distribution of the number of worker-worker interactions during the relevant session of the Control..* η_{EXP} *= median of the distribution of the number of worker-worker interactions during the relevant session of the experimental colony being considered.*

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1						
C_1 vs Colony 5	58	34	1.0	1.5	2551.5	NS
C_1 vs Colony 6	58	25	1.0	3.0	2142.5	0.01
C_1 vs Colony 7	58	57	1.0	3.0	2548.0	0.01
C_1 vs Colony 8	58	72	1.0	4.0	2302.5	0.01
Session 3 – Original Fraction						
C_1 vs Colony 5	40	0	4.5	-	-	-
C_1 vs Colony 6	40	1	4.5	-	-	-
C_1 vs Colony 7	40	23	4.5	5.0	1214.5	NS
C_1 vs Colony 8	40	18	4.5	3.0	1338.0	0.01
Session 3 – Subsidiary Fraction						
C_1 vs Colony 5	40	1	4.5	-	-	-
C_1 vs Colony 6	40	6	4.5	4.0	962.5	NS
C_1 vs Colony 7	40	19	4.5	1.0	1460.0	0.01
C_1 vs Colony 8	40	19	4.5	2.0	1428.0	0.01
Session 4						
C_1 vs Colony 5	33	2	5.0	-	-	-
C_1 vs Colony 6	33	7	5.0	4.0	711.0	NS
C_1 vs Colony 7	33	35	5.0	4.0	1312.0	0.05
C_1 vs Colony 8	33	31	5.0	2.0	1437.5	0.01

Table 26: A comparison of the number of worker-worker interactions in each session of the experimental colonies with the Control, using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median of the distribution of the number of worker-worker interactions during the relevant session of the Control..* η_{EXP} *= median of the distribution of the number of worker-worker interactions during the relevant session of the experimental colony being considered.*

ii. Colonies with a manipulated age structure

Experiment 3

To investigate the spatial organisation of interactions between workers in artificially constructed single age cohort, 'all young' colonies, and colonies from which the callows have been removed, I carry out a similar analysis using the data from experiment 3. I also investigate the organisation of worker interactions in the united colonies (session 3). Interactions between workers are defined in the same way as for experiments 1 and 2.

There is a significant, negative correlation between the relative frequency at which workers interact with other workers and their spatial position in the nest, in all sessions of the Colony C_3, the Control. There is no clear consistent relationship between these variables for the experimental colonies, see Table 27, below. This is discussed further in section 6.2ii.

Colony/session	r_s	n	Significance level p<
Colony C_3			
Session 1	-0.336	38	0.01
Session 2	-0.720	38	0.01
Session 3	-0.832	35	0.01
Colony 9			
Session 1 – Older Workers	0.011	37	NS
Session 1 – Callows	0.021	59	NS
Session 2 – Older Workers	-0.310	28	NS
Session 2 – Callows	-0.362	57	0.01
Session 3	-0.329	65	0.01
Colony 10			
Session 1 – Older Workers	-0.352	33	0.05
Session 1 – Callows	-0.220	39	NS
Session 2 – Older Workers	-0.469	32	0.01
Session 2 – Callows	-0.366	16	NS
Session 3	-0.586	64	0.01
Colony 11			
Session 1 – Older Workers	-0.303	41	NS
Session 1 – Callows	-0.102	31	NS
Session 2 – Older Workers	-0.091	38	NS
Session 2 – Callows	-0.080	30	NS
Session 3	-0.170	63	NS
Colony 12			
Session 1 – Older Workers	-0.107	21	NS
Session 1 – Callows	-0.242	38	NS
Session 2 – Older Workers	-0.195	22	NS
Session 2 – Callows	-0.437	38	0.01
Session 3	-0.056	53	NS

Table 27 : The relationship between the frequency of interactions with nest mates and the median of the distribution of distances from the colony centre, for experiment 3, calculated as Spearman's rank correlation coefficient, r_s and tested as an ordinary product-moment correlation coefficient as in all cases n > 10 (Sokal and Rohlf, 1981 p.607).

A closer examination of the relationship between the frequency at which individuals interact and their median distance from the colony centre is given in Table 27a, below. I calculate the Spearman's rank correlation coefficient separately for older, and for callow, individuals, in session 3. There is a significant, negative correlation between the frequency at which older individuals have contact with other individuals in session 3 compared to their distance from the colony centre in the Control colony. This is also case for three of the four experimental colonies. When this relationship is examined for the callows, separately, the Control colony demonstrates a negative, significant correlation. This is the case for two of the four experimental colonies.

Session 3 – Re-United Colonies				
Colony	**Fraction of Colony Individuals were in in Sessions 1 and 2**	**r_s**	**n**	**Significance Level p<**
Experiment 3				
Control	Older	-0.841	10	0.01
Control	Callows	-0.799	23	0.01
9	Older	-0.574	19	0.01
9	Callow	-0.364	45	0.05
10	Older	-0.800	32	0.01
10	Callow	-0.372	31	0.05
11	Older	-0.295	34	NS
11	Callow	0.017	28	NS
12	Older	-0.613	14	0.05
12	Callow	0.030	24	NS

Table 27a : The relationship between the frequency at which individuals interact and their median distance from the colony centre for callow and older workers separately in session 3, experiment 3. Spearman's rank correlation coefficient, r_s ,tested as an ordinary product-moment correlation coefficient as n ≥ 10 (Sokal and Rohlf, 1981 p.607).

I determine that significant negative correlations sometimes occur between the frequency at which individuals interact with other individuals and their median distance from the centre of the brood pile in experiment 3. This is discussed further later in Chapter 6. Where significant patterns do occur, I compare the correlational relationship between the frequency at which callows interact with other individuals and their median distance from the centre from the brood pile in each experimental colony in session 3 with that of the callows in the Control colony in the same session.

I also compare the relationship between the frequency at which older individuals interact with other individuals in session 3 with this relationship for the older individuals in the Control colony during this session. The results are shown in Table 27b, below.

Colony/Sess compared to control	$r_{s\,c}$	$r_{s\,e}$	n_c	n_e	z_c	z_e	t_s	**Signif. level p<**
Experiment 3								
Colony9/sess3 Callows	-0.799	-0.364	23	45	-1.096	-0.381	-2.632	0.01
Colony10/sess3 Callows	-0.799	-0.372	23	31	-1.096	-0.391	-2.408	0.05
Colony11/sess3 Callows	-0.799	0.017	23	28	-	-	-	-
Colony12/sess3 Callows	-0.799	0.030	23	24	-	-	-	-
Colony9/sess3 Older workers	-0.841	-0.574	10	19	-1.225	-0.653	-1.262	NS
Colony10/sess3 Older workers	-0.841	-0.800	10	32	-1.225	-1.099	-0.299	NS
Colony11/sess3 Older workers	-0.841	-0.295	10	34	-1.225	-	-	-
Colony12/sess3 Older workers	-0.841	-0.613	10	14	-1.225	-0.714	-1.057	NS

Table 27b : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals interact with other workers in session 3 in each experimental colony and this relationship in the Control colony C_3, *calculated as a Fisher's z-transform. Comparisons are separate for callows and for older workers.* $r_{s\,c}$ *= the Spearman's Rank Correlation Coefficient for the relationship between frequency of worker interaction and distance from the brood pile in the Control colony during session 3.* $r_{s\,e}$ *= the Spearman's Rank Correlation Coefficient for the relationship between frequency of worker interaction and distance from the brood pile in each experimental colony during session 3.* n_c*= The number of individuals that retained their marks in the Control colony.* n_e*= The number of individuals that retained their marks in the experimental colony.*

Fisher's z-transforms show that there is a significant difference between the frequency at which callow workers interact and their median distance from the centre of the brood pile in session 3 compared to the callows in the Control colony. This is discussed further in section 6.2.ii. There is no significant difference between the frequency at which the older workers interact and their median distance from the centre of the brood pile in session 3 and the older workers in the Control colony in the same session. I did not perform multiple correlations for these data due to the predominance of non-significant correlations in the relationships between frequency at which individuals interact and their median distance from the colony centre.

I carry out statistical analysis, using Mann-Whitney U tests, as for experiments 1 and 2. I compare the level of interactions among workers in the older and in the callow fractions during session 1 with the level in the older or callow fractions in the united colony in session 3. I also analyse this relationship considering all the workers together. The results are shown in Table 28, and are discussed in section 6.2ii.

Colony / Sessions Compared	η_1	η_2	W	Significance Level p <
Colony C_3				
1 and 3 (older workers)	4.5	2.0	115.0	NS
1 and 3 (callow workers)	3.0	2.0	591.5	NS
1 and 3 (united)	4.0	2.0	1211.0	NS
Colony 9				
1 and 3 (older workers)	2.0	3.0	321.0	NS
1 and 3 (callow workers)	3.0	2.0	2134.5	NS
1 and 3	3.0	3.0	4072.0	NS
Colony 10				
1 and 3 (older workers)	5.0	2.0	1423.5	0.01
1 and 3 (callow workers)	2.0	2.0	990.0	NS
1 and 3 (united)	4.0	2.0	4787.0	0.01
Colony 11				
1 and 3 (older workers)	3.0	2.0	1346.0	0.05
1 and 3 (callow workers)	3.0	3.0	778.0	NS
1 and 3 (united)	3.0	2.0	4137.5	NS
Colony 12				
1 and 3 (older workers)	2.0	0.0	278.5	0.01
1 and 3 (callow workers)	1.5	1.0	665.0	NS
1 and 3 (united)	2.0	1.5	1614.5	NS

Table 28: Comparison of the number of worker-worker interactions for workers in session 1 of the experiment and the other experimental sessions using Mann-Whitney U Tests. η_1 = median of the distribution of the number of worker-worker interactions during session 1 of the experiment. η_2 = median of the distribution of the number of worker-worker interactions during session 3.

Mann-Whitney U tests are also used to compare the level of interactions among workers in each session of the experiment with the level of interactions among workers for that session of the Control. The results of this analysis are shown in Table 29, below, and are discussed in section 6.2ii.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1 – Older Fraction						
C_3 vs Colony 9	38	37	3.0	2.0	1689.0	0.01
C_3 vs Colony 10	38	33	3.0	5.0	998.0	0.01
C_3 vs Colony 11	38	41	3.0	3.0	1587.5	NS
C_3 vs Colony 12	38	21	3.0	2.0	1274.0	0.05
Session 1 – Callow Fraction						
C_3 vs Colony 9	38	59	3.0	3.0	2011.0	NS
C_3 vs Colony 10	38	39	3.0	2.0	1662.0	NS
C_3 vs Colony 11	38	31	3.0	2.0	1476.5	NS
C_3 vs Colony 12	38	30	3.0	1.5	1552.5	0.01
Session 2 – Older Fraction						
C_3 vs Colony 9	38	28	2.0	2.5	1250.0	NS
C_3 vs Colony 10	38	32	2.0	2.0	1378.5	NS
C_3 vs Colony 11	38	38	2.0	2.0	1540.5	NS
C_3 vs Colony 12	38	22	2.0	6.0	958.5	0.01
Session 2 – Callow Fraction						
C_3 vs Colony 9	38	57	2.0	2.0	1990.5	NS
C_3 vs Colony 10	38	16	2.0	1.0	1156.0	0.05
C_3 vs Colony 11	38	30	2.0	2.0	1260.5	NS
C_3 vs Colony 12	38	38	2.0	2.5	1398.0	0.05
Session 3 – United Fractions						
C_3 vs Colony 9	35	65	2.0	3.0	1676.0	NS
C_3 vs Colony 10	35	64	2.0	2.0	1769.5	NS
C_3 vs Colony 11	35	63	2.0	2.0	1677.0	NS
C_3 vs Colony 12	35	53	2.0	2.0	1690.0	NS

Table 29: Comparison of the number of worker-worker interactions for workers in each session of the experimental colonies with the Control, using Mann-Whitney U Tests. $\eta_{CONTROL}$ = *median of the distribution of the number of worker-worker interactions during the relevant session of the Control..* η_{EXP} = *median of the distribution of the number of worker-worker interactions during the relevant session of the experimental colony being considered.*

6.2 Discussion

An Investigation of Patterns of Interaction Among Workers

The results above are divided into the following sections:

6.1 An investigation of patterns of interaction among workers

i. Colonies with an manipulated task structure

ii. Colonies with a manipulated age structure

The following discussion examines the results with reference to the questions posed in Chapter 3:

> How does the pattern of interactions among the workers change as a consequence of the sociotomy of the colonies?
>
> How are any patterns of interactions among the workers affected by the absence of the queen (and the brood) in fractions of the colony?
>
> What are the implications of any patterns of interactions for processes of task allocation, in the presence, and in the absence of the queen?

i. An investigation of patterns of interactions among workers in colonies with a manipulated task structure

Experiment 1

Figure 39 (a) shows the relationship between the frequency at which individual workers in the Control colony interact with their nest-mates, and their spatial position in the nest, during session 1, when the colony is un-manipulated. Figure 39 (b) is the corresponding graph for a typical experimental colony, Colony 3. The relationship between the relative frequency at which individuals interact with their nest-mates and their relative median distance from the centre of the brood pile is not a significant Spearman's rank correlation for either of these colonies. A full analysis of this relationship for all the colonies in the experiment, in all four sessions, is given in Table 21, and is discussed below.

There is some tendency for the relative frequency at which individual workers interact with their nest-mates to be significantly negatively correlated with the median of their relative distance from the centre of the brood pile. This is the case in some sessions for both the Control colony (specifically sessions 3 and 4) and experimental colonies 1, 2 and 3. The relationship is not consistent for particular sessions for all experimental colonies. During session 1, the Control colony, and two of the four experimental colonies do not show a significant correlation between the relative frequency of interaction among workers and relative spatial position. The other two experimental colonies, however, do exhibit a significant negative pattern for these variables. All the colonies are un-manipulated during session 1, so the occurrence or absence of a pattern must be the result of inter-colony variation.

There is no significant spatial pattern of interaction among the workers in all four experimental colonies in the original fractions of the colonies during session 3, after the individuals associated with external activity had been removed. This is also the case for three out of four experimental colonies during session 2, when the externals were being removed. The Control colony did not show a significant relationship between the relative frequency of interactions among the workers and their relative spatial position during session 2, at the beginning of which one third of the workers were removed for one week. However, the relationship between the relative frequency at which workers interact and their relative distance from the centre of the brood pile is a significant, negative correlation for the Control colony during session 3. Therefore, I conclude that the removal of workers from the colony tends to disrupt the spatial pattern of interactions among workers if the pattern existed when the colony was un-manipulated. If there was no spatial pattern of interactions among workers in the un-manipulated colony, there remains no spatial pattern during and after the removal of individuals associated with external activity.

The subsidiary fractions of the colonies during session 3, which consist only of removed externals, and from which both the queen and brood are absent, tend to show a significant negative correlation between the relative frequency at which workers interact and their spatial position. This relationship is also significant for the Control colony during session 3, during which the colony is un-manipulated.

The Control colony in session 4 exhibits a highly significant, negative correlation between the relative frequency at which workers interact and their relative spatial position. This is also the case for two of the four experimental colonies (1 and 2). Colonies 3 and 4 do not show a significant correlation between these variables. I conclude that those experimental colonies that demonstrate a significant spatial pattern of interactions among workers whilst the colony is un-manipulated during session 1, also demonstrate this relationship among removed individuals associated with external activity in the separated colony fractions. This relationship is also maintained in these colonies when these fractions are re-united with the original workers. There is no significant difference between the correlational relationship between the frequency at which workers interact with other workers and their median distance from the brood pile, in these experimental colonies during session 4, and this relationship for the Control colony (see Table 22a). This is evidence that the correlational relationship, when it does occur, is not significantly different from the relationship when it occurs in an un-manipulated colony.

The trends in the existence of a spatial pattern of interactions among workers are not consistent among colonies, and therefore it is not meaningful to perform multiple comparisons between the experimental colonies. However, there are some patterns which are consistent within colonies. Therefore I conclude that these patterns are a result of inter-colony variation. I hypothesise that these patterns only occur in colonies which exhibit a high level of interaction amongst workers. There is some evidence that spatial patterns of interactions among workers may be influenced by seasonal trends, and consequent changes in task demand which occur during sociogenesis. The Control colony demonstrates significant spatial patterns of interactions in sessions 3 and 4, but not in sessions 1 and 2. This could also be the result of seasonal increase in the level of worker interactions, and the subsequent establishment of spatial patterns. I investigated the hypothesis that activity increases across the season by comparing the absolute frequency of interaction among workers in the Control colony in each session compared to session 1, see Figure 40 (a). The results of statistical analysis on these data using Mann-Whitney U tests, are given in Table 23. I carried out the same analysis on the data from the experimental colonies, and the results are discussed below. The results for the Control colony show that the distribution in the frequency at which workers interact is, indeed, significantly higher in sessions 2, 3 and 4 than in session 1. Consequently there is evidence that the intensity of interactions among workers does increase as a result of seasonal changes in task demand.

I investigated the effect of sociotomy on the experimental colonies in experiment 1 by comparing the absolute frequency of interaction among workers in each session of the experiment, with the corresponding session in the Control colony (Table 25). I also compared the frequency of interaction among workers for each experimental colony, in each experimental session compared to session 1 (Table 23). The intensity of interaction among workers in the experimental colonies does not follow the same seasonal patterns as those observed in the Control colony. The Control colony demonstrates an increase in the intensity of interaction between the workers in session 2, compared to session 1, and this increase is maintained for the duration of the experiment. In the experimental colonies, the general trends observed (there is some variation) are:

Session 1: the intensity of interaction among workers tends to be higher in the experimental colonies compared to the Control (in 3 out of 4 colonies), see Table 25.

Session 2: The intensity of interaction among workers is significantly lower in the experimental colonies than in the Control (Table 25). When the intensity of interaction in these experimental colonies during session 2 is compared to session 1, two of the colonies show a significantly lower intensity of this activity than they did in session 1 (Colonies 3 and 4). The intensity of interactions in session 2 in the other two colonies (Colonies 1 and 2) is not significantly different (Table 23). In support of my hypothesis - that a spatial pattern is only evident in colonies which exhibit a high intensity of interaction among workers; the colony which exhibits the highest median number of worker-worker interactions (Colony 2) during session 2, is the only colony that demonstrates a significant spatial pattern of worker interactions (Table 21).

Session 3:

Original Fraction: the experimental colonies tend to show a significantly lower intensity of interaction among the workers in the original fraction of session 3, compared to the Control (in 3 out of 4 colonies), see Table 25. These same three experimental colonies do not show significantly different intensities of interaction among the workers compared to that determined during session 1 in the relevant colony (Table 23). Colony 1, which does not exhibit a significantly different intensity of interaction from that of the Control (Table 25), does show a significantly higher intensity of worker interaction compared to that observed for this colony during session 2 (Table 23). This colony does not demonstrate a significant spatial pattern in the organisation of these interactions (Table 21), but this may be due to insignificant numbers of individuals that have retained their marks in this colony to establish a significant pattern ($n = 8$, $r_s = - 0.381$).

Subsidiary Fraction: all four experimental colonies exhibit a significantly higher intensity of interaction among the workers than they did during session 1 (Table 23). However, only two of the four colonies (Colonies 1 and 3) demonstrate a significantly higher intensity than the Control colony (Table 25). Both these colonies demonstrate a significant spatial pattern of worker interactions (Table 21).

Session 4: the intensity of interaction among the workers in all four experimental colonies does not differ significantly from that of the Control colony

(Table 25). The median number of interactions for individual workers is higher in all these colonies compared to session 1 of the relevant colony, and this is significant in three of the experimental colonies (Table 23). These colonies tend not to demonstrate significant spatial pattern of worker interactions (Colonies 3 and 4, Table 21). Colony 4 does not show a significant spatial pattern in any of the sessions, and the establishment of a spatial pattern is therefore affected by a factor other than the absolute intensity of interactions. This appears not to be associated with colony size (see Table 1, Appendix B).

CONCLUSIONS

I conclude that sociotomy disrupts the intensity of interaction among the workers in colonies of *Leptothorax albipennis*. There is some variation in the intensity of interaction observed in colonies prior to any manipulations on the colonies. After the re-unification of the fractions of the experimental colonies in session 4, there is no difference between the intensity of interaction observed in these colonies compared to the Control. Consequently the seasonal increase in the intensity of interaction tends to be preserved. The removal of individuals associated with external activity from the experimental colonies results in a decrease in the intensity of interaction among the remaining workers. The removed workers demonstrate a higher intensity of interaction than that observed among the remaining workers in the original fraction, and may also interact with other workers at a higher frequency than in un-manipulated colonies.

In un-manipulated colonies, there is a negative correlation between the relative frequency at which individuals interact with other workers, and their relative distance from the centre of the brood pile. Individuals associated with external activity, therefore, do not exhibit an innately higher intensity of interaction. The resulting higher intensity of interaction established in the subsidiary fractions must, therefore, be a consequence of sociotomy. An explanation for this could be that externals, when they constitute a colony fractions on their own, demonstrate a higher intensity of interaction resulting from the physical absence of obstacles to interaction, in the form of brood. The effect of brood in the subsidiary fractions of the colonies in experiment 2 is examined below. The hypothesis that externals demonstrate higher intensities of interaction when they constitute a separate fraction has implications for possible mechanisms of task allocation in these colonies.

There is evidence that the establishment of spatial patterns of organisation of interaction among workers is linked to the occurrence of a high intensity of interaction in the colonies (the statistic used to compare the absolute intensity of interaction takes into account the distribution in the frequency of worker interactions, as well as the median level). More over , the relationship between the frequency at which individuals interact with other individuals and their median distance from the centre of the brood pile, is not significantly different, when it does occur, in manipulated colonies after they have been re-united, compared to the Control colony. There appears to be no clear relationship between the size of the worker population and the intensity of interaction among the workers (see Table 1, Appendix B). The implications of these results for mechanisms of task allocation are discussed further in Chapter 9.

Experiment 2

I hypothesised above that the absence of brood in the subsidiary fractions of the colonies in experiment 1 may lead to continued relatively high frequencies of interaction among the 'external' workers. In experiment 2, these subsidiary fractions were provided with stained foreign brood, which was subsequently adopted by these fractions. The Control colony for experiment 2 (Colony C_2) was not used for subsequent comparisons of the data due to the death of the queen in this colony, discussed in Chapter 4. However, the results are given for this colony for the sake of completeness, and indicate similar trends in the organisation of spatial patterns of worker interactions to those observed in Colony C_1, where *n* is sufficiently high to render the analysis valid. The analysis is not valid for colonies 5 and 6 as these colonies did not contain sufficient numbers of individuals that retained their marks. The analysis therefore focuses on Colonies 7 and 8.

The subsidiary fractions of Colonies 7 and 8 during session 3, did not show the same negative correlation between the relative frequency at which individuals interact with other workers, and their relative spatial position, as that which tends to be observed in this session in experiment 1 (see Table 22). I established above, that there is evidence that such spatial patterns of interaction among workers are established only when the intensity (distribution in the frequency) of worker interactions is relatively high. Is the absence of patterns in the subsidiary fractions of Colonies 7 and 8 during session 3 linked to low intensities of interaction in these fractions? An analysis using Mann-Whitney U tests to compare the intensity of interaction among workers in these fractions compared to their distribution during session 1, indicates that the intensity of interaction is lower in the subsidiary fractions during session 3 (Table 24). I have shown, above, however, that the intensity of interaction among workers tends to increase throughout the course of the experiment in un-manipulated colonies. Therefore I also compared the intensity of interaction among workers to that of the Control colony (Colony C_1) in session 3, using Mann-Whitney U tests (Table 26). The intensity of interaction is significantly lower among workers in the subsidiary fractions of Colonies 7 and 8 during session 3, compared to the Control colony during session 3.

These results are evidence that the presence of brood in the subsidiary fractions of the colonies during session 3 results in a lower intensity of interaction among the workers. Consequently, spatial patterns of organisation of interaction among the workers are not established in these fractions. The reduction in the intensity of interaction cannot simply be a result of a decrease in the number of individuals in the nest, as this is not seen in the subsidiary fractions of the experimental colonies of experiment 1, during session 3, and there is no clear relationship between the intensity of interaction and colony size. The reduction could be related to the presence of the brood in terms of them forming physical obstacles to interaction, or temporal obstacles if workers spend time performing brood-related tasks and less time interacting with each other.

The results for Colonies 7 and 8 during session 4 are not entirely consistent, and again, inter-colony variation may be involved. Colony 7 does demonstrate a significant spatial pattern of interaction among the workers (Table 22), however, Colony 8 does not. There is no significant difference between the relationship between the frequency at which individuals interact in session 4 of the experimental colonies and the Control colony in the same session (see Table22a). This comparison is not performed for colonies where no significant correlational relationships between the variables were determined. This may be explained by the higher intensity of interaction among workers in session 4 compared to session 1 for Colony 7 (Table 24). The intensity of worker-worker interaction is not significantly higher in session 4 of Colony 8 compared to session 1 (Table 24). The intensity of interaction among workers in Colonies 7 and 8 during session 4 is significantly lower than that of the Control colony during session 4 (Table 26). There is evidence, therefore, that the decrease in the intensity of interaction observed in the subsidiary fraction of these colonies, during session 3, is maintained when these fractions are re-united with their original fractions. This may be linked to resilience of changes associated with experienced by externals during session 3. The resilience of changes that occur as a result of experience are analysed further in Chapter 8.

CONCLUSIONS

The presence of brood in colonies consisting of removed 'externals' results in significantly lower intensities of interaction among workers compared to un-manipulated colonies. This trend is maintained after these fractions have been re-united with the original workers. Conversely, the absence of brood in these colonies results in higher intensities of interaction among workers compared to these colonies when they are un-manipulated during session 1, and even compared to the Control during the corresponding session in some colonies. An explanation for these results is that external individuals show a higher intensity of interaction when they are not physically (or temporally, in terms of the time spent performing brood-related tasks) impeded by the presence of brood. This decrease in the intensity of interaction amongst the external workers provided with brood, may continue when these workers are re-united with the original fractions of their colonies. The resilience of any change in behaviour among these individuals is analysed and discussed further in Chapter 8.

The intensity of interactions among workers is influenced by sociotomy, and is independent of the presence or absence of the queen. These results have implications for possible mechanisms of task allocation involving dominance interactions (see Chapter 2), and are discussed further in Chapter 9.

ii. An investigation of patterns of interaction among workers in colonies with a manipulated age structure

Experiment 3

Table 27 shows that there is a spatial pattern of worker interactions in the Control colony in experiment 3. There is a highly significant, negative correlation between the relative frequency at which an individual interacts with other workers, and relative median distance from the colony centre, for all sessions of this experiment. This pattern is also found in some of the experimental colonies during some sessions of the experiment, but there appears to be no consistency to the patterns in particular sessions. Thus, sociotomy can disrupt the spatial patterns of interaction between workers.

I performed a more detailed analysis of the relationship between the frequency at which individuals interacted in session 3 of the experiment and their distance from the centre of the brood pile. I calculated the correlation between the relative frequency at which individuals interacted with other individuals in session 3 and their relative distance from the centre of the brood pile, separately for the callows and the older workers, in the Control colony, and for all four experimental colonies. The results are shown in Table 27a. It can be seen that both the callows and the older workers separately, in the Control colony, exhibit a significant correlation for this relationship. Moreover, there is a significant, negative correlation for this relationship for the older workers in the experimental colonies for three of the four experimental colonies, and for the callow workers in two of the four experimental colonies. This is evidence that there is some tendency for individual fractions of the colony to exhibit patterns between the frequency at which they interact with other individuals and their median distance from the centre of the brood pile. The frequency of interaction decreases as distance from the centre of the brood pile increases.

I examined this relationship further by comparing the correlational relationships between frequency of interaction and distance from the centre of the brood pile, for each of the fractions which exhibited a significant correlation for this relationship, to this relationship for the Control colony, considering callows, and older workers separately. The results are shown in Table 27b. The results show that there is a significant difference between the relationship between the frequency at which the callows interact in session 3, and the frequency at which the callows in the Control colony interact in session 3. Consequently I conclude that the existence of the callows as separate fractions in session 1 and 2, results in an alteration of their behaviour in session 3, when they are united with older workers, compared to callows which have been in an un-manipulated colony in sessions 1 and 2. The effect of separation from eclosion, on the behaviour of the callows is examined further in Chapters 7 and 8. There is no significant difference between the frequency at which older workers interact in session 3 and the frequency at which the older workers in the Control colony interact. The existence of the older workers in separate fractions does not have a significant effect on the frequency at which they interact compared to older workers which were in an un-manipulated colony in sessions 1 and 2.

It is clear that there is a considerable amount of variation in patterns within colonies. To further investigate this, and the effect of sociotomy on the experimental colonies, I carried out statistical analysis comparing the intensity of interaction among workers in sessions 1 and 3 during the experiment. I carried out this analysis separately for the older workers, and for the callows, which existed as separate fractions in session 1. I also performed this analysis for the combined results from older and callow workers compared between sessions 1 and 3 (Table 28). There is no significant difference in the intensity of interaction between the older workers in sessions 1 and 3, or the callow workers in sessions 1 and 3, for the un-manipulated Control colony. Thus the intensity of interaction between the workers does not change through the course of the experiment in the un-manipulated colony.

There is a trend towards a decrease in the intensity of interaction among the older workers in the experimental colonies in session 3 compared to session 1. There is no significant change in the level of interaction among the callows in any of the experimental colonies between these sessions (Table 28), despite the absence of the queen, and the inexperience of the workers. The intensity of interaction among the workers is compared for each session to the relevant session of the Control colony (Table 29). There are no consistent patterns, although there is no significant difference between the intensity of interaction among the workers in the experimental colonies and the Control when the older and callow workers are considered together in the united colonies in session 3 (Table 29).

The results in section 6.2ii., above, show that the intensity of interaction among the removed workers increases when they are in a colony fraction on their own, and decreases if brood is present in these fractions. The decrease in the intensity of interaction that occurs in the latter tends to be maintained when the fractions of the colonies are re-united with their original fractions. I hypothesised above, that the decrease in the intensity of interaction by these individuals is associated with a change resulting from the presence of brood, and that this change is maintained to some degree when the colony fractions are re-united. In experiment 3, there is some tendency for the older fractions of the colony to exhibit a decrease in the intensity of their interactions during session 3, when the fractions of the colonies are re-united, compared to session 1 when they exist as fractions on their own (with brood). If one considers the 'older' fractions in session 1, experiment 3, to be equivalent to the subsidiary fractions of the colonies during session 3, experiment 2, it can be hypothesised that they exhibit a decrease in the intensity of their interactions. Comparisons between the intensity of interactions in these 'older' fractions and the Control during session 1, experiment 3, do show that there is a decrease for two of the four experimental colonies, and again, this may be a result of some change in behaviour associated with the presence of brood. Inter-colony variation does play a role, however, and this may explain why these results are not consistent for the other two experimental colonies for this comparison. The results from experiment 2 have shown that the decrease in the intensity of interaction exhibited in subsidiary fractions consisting of removed workers and brood tends to be maintained when the colonies are re-united. Thus, the decrease in the intensity of interaction of the older workers in the united colonies in session 3, experiment 3, compared to session 1, could be explained by the maintenance, and indeed, in some cases, extension, of the tendency to decrease the intensity of interaction exhibited in session 1. I conclude that there is an association between previous experience and subsequent behaviour. The role of experience in determining the individual behaviour of workers is analysed and discussed further in Chapter 8.

CONCLUSIONS

In the un-manipulated Control colony there is a significant negative correlation between the relative frequency at which workers interact with other workers, and their relative median distance from the centre of the brood pile. This pattern is preserved for the course of the experiment, and is similar to the pattern observed in the Control colony from experiment 1 during the last two sessions of the experiment. As experiment 3 was carried out slightly later in the season, these patterns are consistent with the results from the Control colony in experiment 1.

The sociotomy carried out on these colonies can disrupt the spatial patterns of interactions between workers. The intensity of interaction among the older workers tends to decrease in session 3 compared to session 1, and there is evidence that this is a result of their experience of decreased intensity of interaction during session 1. This is consistent with the results from experiment 2 in which the intensity of worker-worker interaction decreases in the subsidiary fractions during session 3, and this decrease is maintained during session 4. I hypothesise that both these changes are associated with a change in behaviour connected with the presence of brood, and this is analysed further in Chapter 8. There is no significant change in the level of interactions among the callows in any of the experimental colonies between these sessions, and I therefore hypothesise that the callow fractions do not exhibit similar changes in behaviour resulting from the presence of brood in experiment 3.

Chapter 7

An Investigation of the Resilience of Spatial Organisation within the Colonies

In Chapter 4, I investigated the spatial organisation of brood-related tasks and external activity in colonies of *Leptothorax albipennis*, and how this organisation was affected by sociotomy. The aim of the present chapter is to determine the degree of resilience of colony spatial organisation, in terms of the relative positions of individually recognisable workers.

Analysis is carried out as follows:

I calculate the median position of each marked individual, relative to the centre of the brood pile, as in Chapter 4.

Comparative data files of the median positions of workers that have at least one recorded position for each session to be compared, are created for each pair of sessions to be compared.

The relationship between the spatial position of an individual in session 1, and her spatial position in the session compared to session 1, is investigated by plotting graphs of these variables for the Control, and a typical experimental colony.

The centre of the brood pile is represented by the origin of each graph.

In the analysis for experiments 1 and 2, individuals that were in the subsidiary fractions of the colonies during session 3 (experiments 1 and 2) are represented in red, individuals that were in the original fractions, in black.

In the analysis for experiment 3, callow individuals are represented in red, older individuals in black.

In each plot the chronologically later session is represented on the y axis.

Individuals are ranked with respect to their median position for each session to be compared.

Correlation analyses are carried out for all the colonies according to the procedures at the beginning of Chapter 4.

Fisher's z-transforms are used for comparisons of the correlation coefficients for the Control and the experimental colonies.

7.1 An Investigation of the Resilience of Spatial Organisation in Colonies with a Manipulated Task Structure

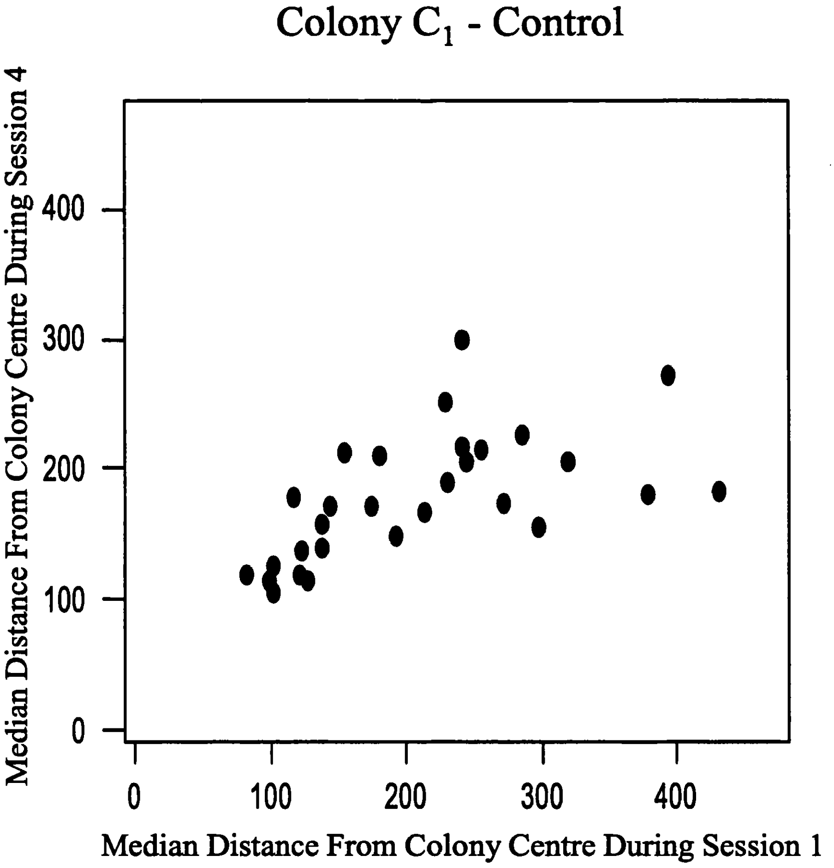
I compare the spatial position of individually marked workers in each experimental session with their position in the un-manipulated colony during session 1.

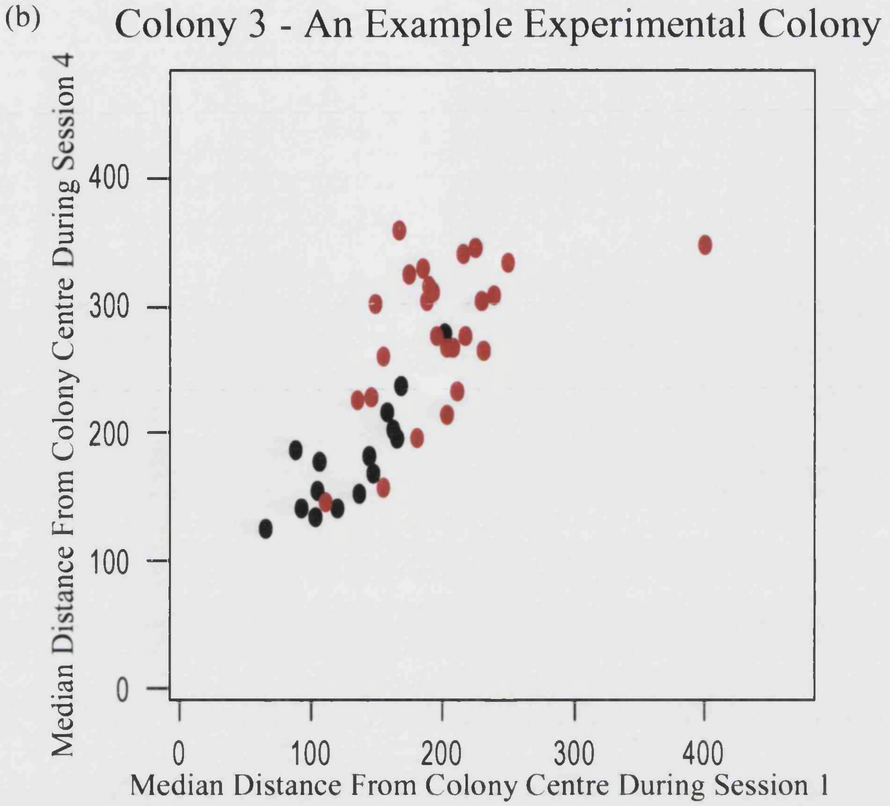
Experiment 1

Session 1 (Un-manipulated) and Session 4 (Re-united)

The graphs below show the relationship between the median position of individually marked workers in the colony during session 1 and their median position during session 4, in experiment 1. The Control colony (Colony C_1), and a typical experimental colony (Colony 3), are shown.

Fig 41(a)





(b) Colony 3 - An Example Experimental Colony

Figure 41 : (a) The relationship between the median distance from the colony centre in session 1, when the colonies remain un-manipulated and session 4, when the two fractions of the experimental colonies are re-united, for Colony C_1, the Control and (b) Colony 3, an example experimental colony, experiment 1.

The relationship between the relative position of individuals in session 1 and session 4 is further examined by carrying out correlation analyses according to the procedure described at the beginning of Chapter 4. The results are shown in Table 30, below. The relative median distance of an individual from the centre of the brood pile during session 1 is significantly, positively correlated with her relative median distance from the centre of the brood pile in session 4. This is the case for both the Control (Colony C_1), and experimental colonies 1, 2 and 3. Colony 4 is bordering on significance *(p = 0.08)*. These results are discussed in section 7.3i.

The spatial organisation of individuals is also compared between session 1 and session 2, during which individuals associated with external activity are being removed, and to the appropriate fraction of session 3, see table 30. Example plots of these comparisons are given in Figures 42 and 43, and are discussed in section 7.3i.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_1			
Session 1 / Session 4	0.710	29	0.01
Session 1 / Session 2	0.725	36	0.01
Session 1 / Session 3	0.728	36	0.01
Colony 1			
Session 1 / Session 4	0.632	26	0.01
Session 1 / Session 2	0.741	20	0.01
Session 1 / Session 3 (Orig)	0.029	6	NS
Session 1 / Session 3 (Subsid)	0.656	22	0.01
Colony 2			
Session 1 / Session 4	0.539	30	0.01
Session 1 / Session 2	0.567	27	0.01
Session 1 / Session 3 (Orig)	0.162	16	NS
Session / Session 3 (Subsid)	0.450	30	0.01
Colony 3			
Session 1 / Session 4	0.781	41	0.01
Session 1 / Session 2	0.813	23	0.01
Session 1 / Session 3 (Orig)	0.743	15	0.01
Session 1/ Session 3 (Subsid)	0.675	29	0.01
Colony 4			
Session 1 / Session 4	0.391	21	$0.05 < p < 0.1$
Session 1 / Session 2	0.691	18	0.01
Session 1 / Session 3 (Orig)	0.000	5	NS
Session 1 / Session 3 (Subsid)	0.522	17	0.05

Table 30 : The relationship between individual's spatial positions compared between sessions. Spearman's rank correlation coefficient, r_s ,tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

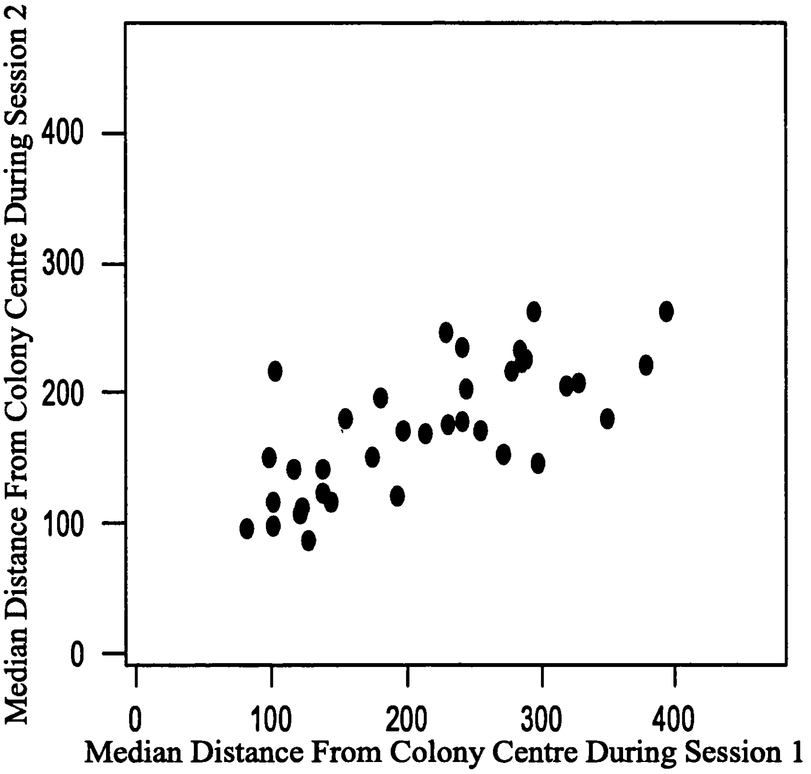
A Fisher's z-transform shows that there is no significant difference between the correlation of the Control colony and each of the experimental colonies, for the comparison between session 1 and session 4, see Table 31, below.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	**Significance level P<**
C_1 and 1	0.710	0.632	29	26	0.887	0.745	0.498	NS
C_1 and 2	0.710	0.539	29	30	0.887	0.603	1.035	NS
C_1 and 3	0.710	0.781	29	41	0.887	1.048	-0.632	NS
C_1 and 4	0.710	0.392	29	21	0.887	0.414	1.543	NS

Table 31 : A comparison between the Spearman's rank correlation coefficients of relative positions of individuals in session 1 and relative positions in session 4 with that of Control colony C_1, calculated as a Fisher's z-transform.

Colony C_1 - Control

(a) **Session 1 (Un-manipulated) and Session 2**



(b) **Session 1 (Un-manipulated) and Session 3**

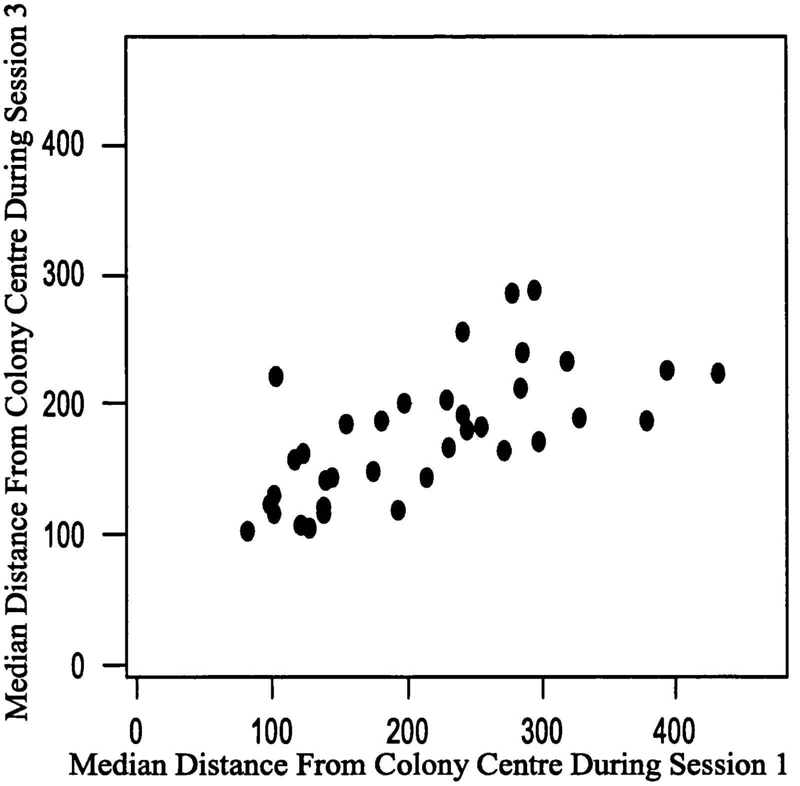


Figure 42 : (a) The relationship between the median distance from the colony centre in session 1 and session 2 and (b) session 1 and session 3, for Colony C_1, the Control.

Colony 3 - An Example Experimental Colony

Fig 43(a)

Session 1 (Un-manipulated) and Session 2

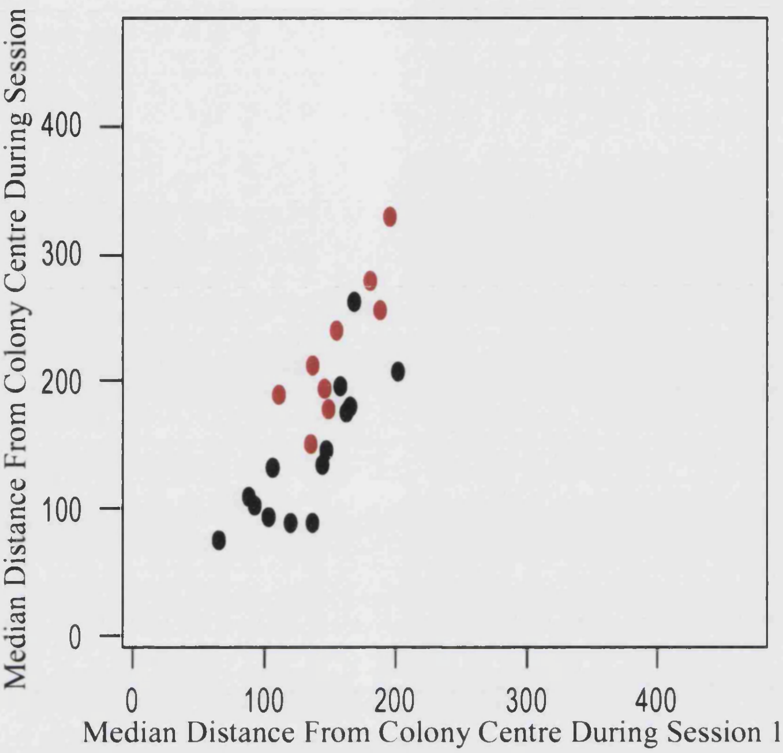
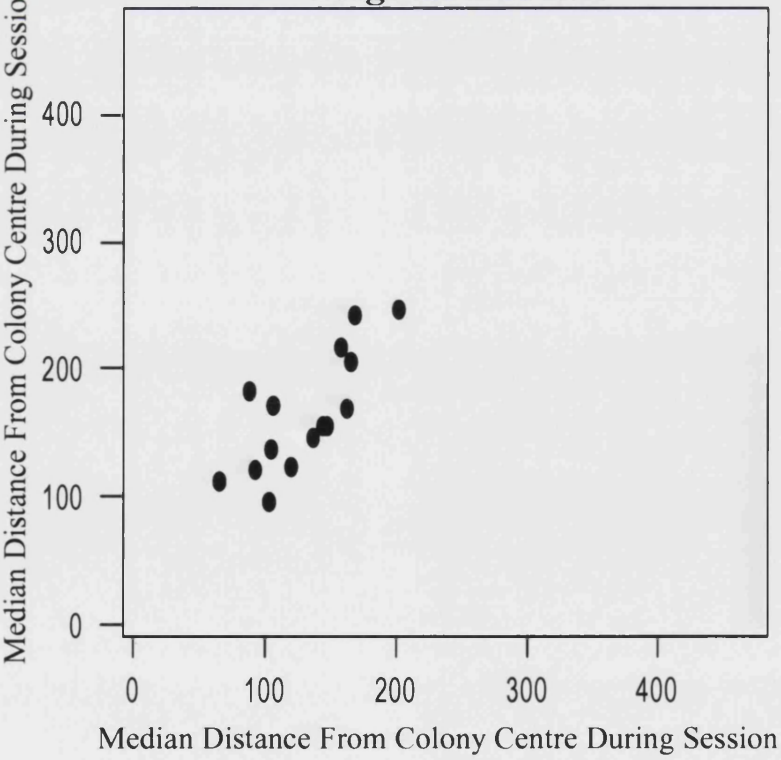


Fig 43(b)

Session 1 (Un-manipulated) and Session 3 -Original fraction



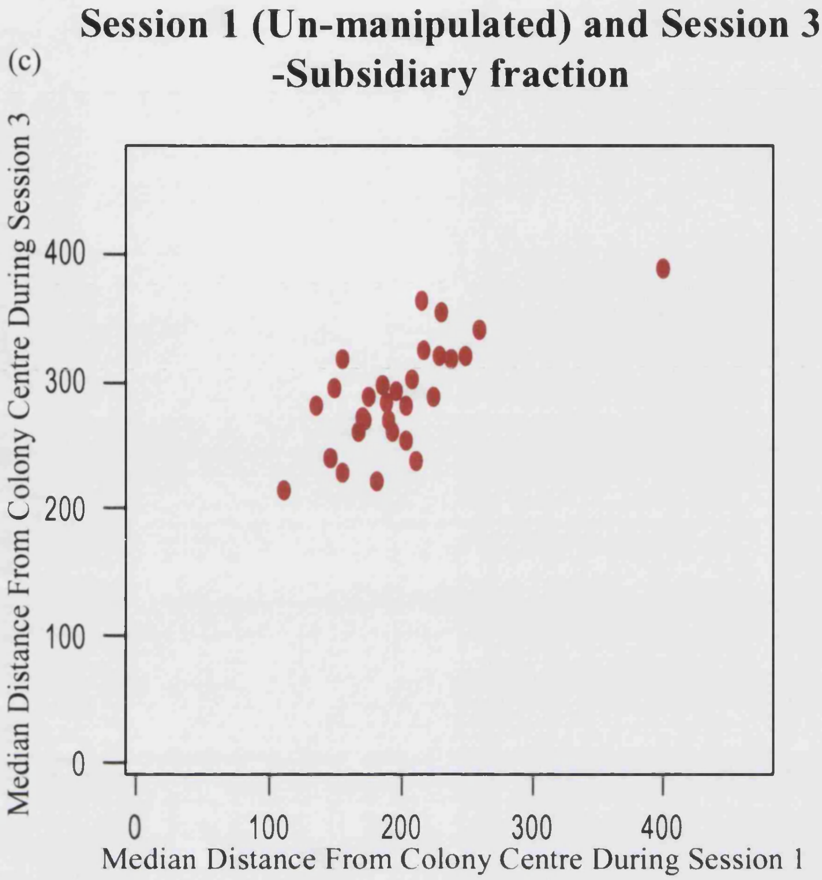


Figure 43 : (a) The relationship between the median distance from the colony centre in session 1 and session 2, (b) session 1 and session 3, original fraction and (c) session 1 and session 3, subsidiary fraction, for Colony 3, an example experimental colony.

Experiment 2

I carry out the same correlation analyses on the results from experiment 2. The relative median distance of individuals from centre of the brood pile in session 1 is significantly, positively correlated with their relative position in session 4, when the fractions were re-united for the Control colony., and for experimental colonies 7 and 8. This is also the case for the relationship between the relative median distance of individuals from the brood pile in session 1 compared to session 3 for the Control (Colony C_2), and in both the original and subsidiary fractions of experimental colonies 7 and 8. There are insufficient numbers of individuals that had retained their marks in Colonies 5 and 6, and correlations cannot be calculated, except for a comparison of position in session 1 with session 4 for Colony 6, which does not give a significant result. This is discussed further in section 7.3.i.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_2			
Session 1 / Session 4	0.635	14	0.05
Session 1 / Session 3	0.518	15	0.05
Colony 5			
Session 1 / Session 4	-	2	-
Session 1 / Session 3 (Orig)	-	0	-
Session 1 / Session 3 (Subsid)	-	1	-
Colony 6			
Session 1 / Session 4	0.286	7	NS
Session 1 / Session 3 (Orig)	-	1	-
Session / Session 3 (Subsid)	-	4	-
Colony 7			
Session 1 / Session 4	0.540	35	0.01
Session 1 / Session 3 (Orig)	0.667	23	0.01
Session 1/ Session 3 (Subsid)	0.645	19	0.01
Colony 8			
Session 1 / Session 4	0.614	27	0.01
Session 1 / Session 3 (Orig)	0.546	15	0.05
Session 1 / Session 3 (Subsid)	0.600	17	0.05

Table 32 : The relationship between individual's spatial positions compared between sessions. Spearman's rank correlation coefficient, r_s, tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

A Fisher's z-transform shows that there is no significant difference between the correlation for the Control colony (Colony C_2), and each of the experimental colonies 6, 7 and 8, for the comparison between session 1 and session 4, see Table 33, below.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	**Significance level P <**
C_2 and 6	0.635	0.286	14	7	0.750	0.294	0.780	NS
C_2 and 7	0.635	0.540	14	35	0.750	0.604	0.417	NS
C_2 and 8	0.635	0.614	14	27	0.750	0.715	0.095	NS

Table 33 : A comparison between the Spearman's rank correlation coefficients of relative positions of individuals in session 1 and their relative positions in session 4 with that of Control (Colony C_2) calculated as a Fisher's z-transform

The queen in the Control colony (Colony C_2) died, and this has been discussed in Chapter 4. Again, I use Colony C_1 as an adequate control for experiment 2. Consequently, I compare the correlation coefficients of the relationship between the relative median position of individuals in sessions 1 and 4, for experiment 2, with that of the Control colony from experiment 1 (Colony C_1). There is also no significant difference between the correlation for this Control colony and each of the experimental colonies 6, 7 and 8 in the comparison between sessions 1 and session 4, se Table 34, below.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	**Significance level P<**
C_1 and 6	0.710	0.286	29	7	0.887	0.294	1.104	NS
C_1 and 7	0.710	0.540	29	35	0.887	0.604	1.072	NS
C_1 and 8	0.710	0.614	29	27	0.887	0.715	0.607	NS

Table 34 (b) : A comparison between the Spearman's rank correlation coefficients of relative positions of individuals in session 1 and relative positions in session 4 with that of Control (Colony C_1) calculated as a Fisher's z-transform

The graphs below are plots of the relationship between the median position of the workers in Colony 7 (an example experimental colony) during session 1 compared to their median position in:

session 3, original fractions, from which individuals associated with eternal activity have been removed;

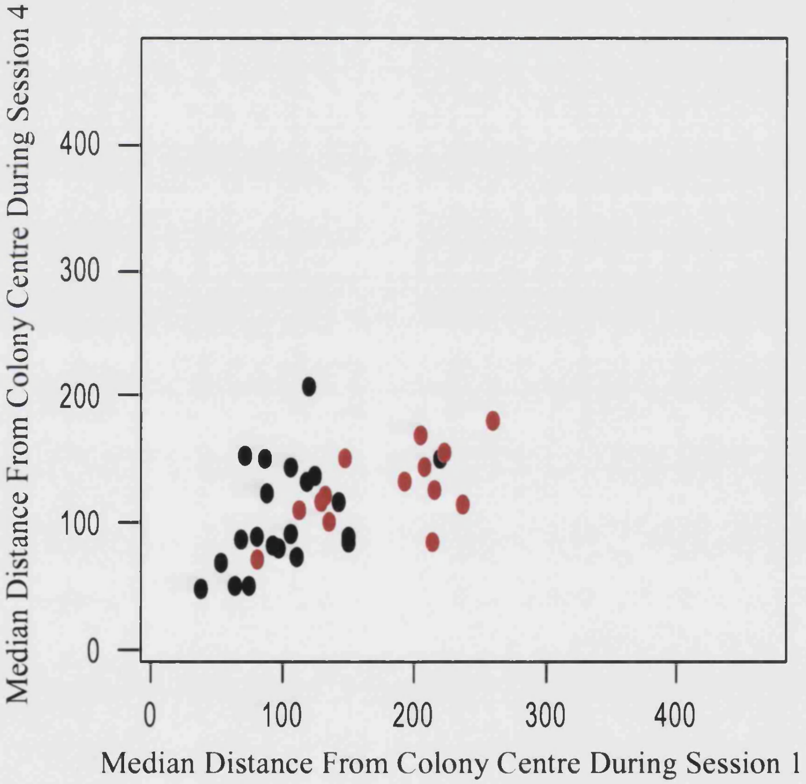
session 3, subsidiary fractions, in which removed ‘externals’ are maintained in a separate nest containing stained, foreign, conspecific brood.

session 4, when the two fractions of the colonies are re-united.

Colony 7 - An Example Experimental Colony

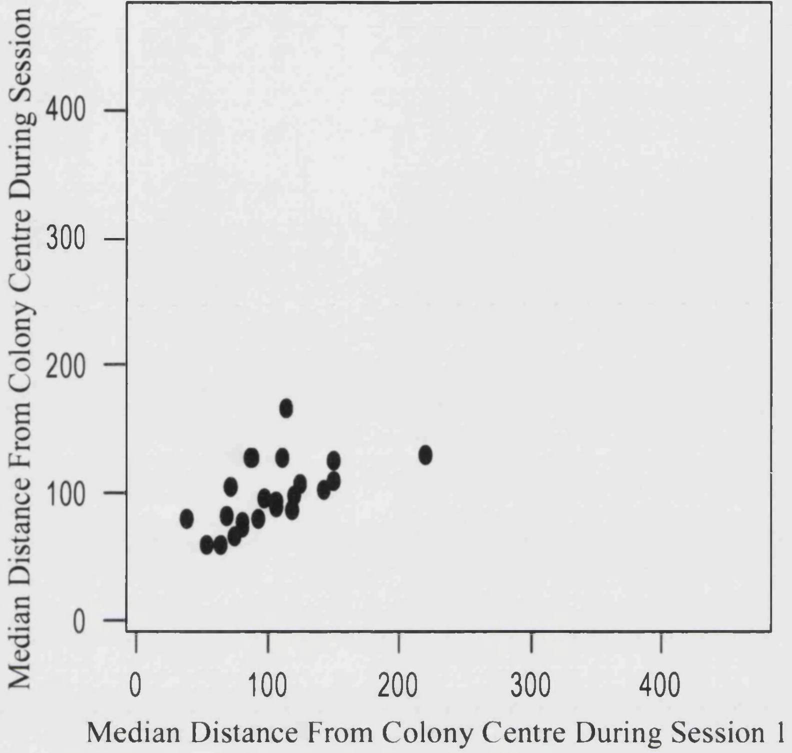
Session 1 (Un-manipulated) and Session 4 (Re-united)

Fig 44(a)



(b)

Session 1 (Un-manipulated) and Session 3 -Original fraction



(c)

Session 1 (Un-manipulated) and Session 3 -Subsidiary fraction

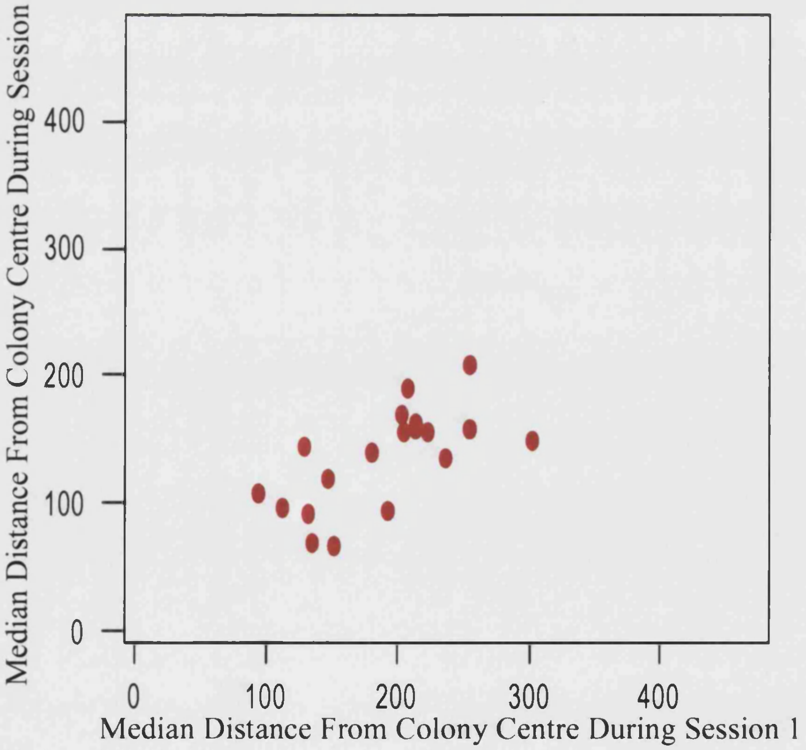


Figure 44 : (a) The relationship between the median distance from the colony centre in session 1, and session 2, (b) session 1 and session 3, original fraction and (c) session 1 and session 3, subsidiary fraction , for Colony 7, an example experimental colony.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the ranked median distance from the centre of the brood pile in session 1 (un-manipulated colonies) compared to session 4 (after re-unification) I perform multiple comparison analysis. Using the method outlined in Chapter 4 I determine whether the colonies can be considered to be samples from a population exhibiting a common correlation between the ranked median distance from the centre of the brood pile in session 1 compared to session 4. I then compare the pooled *z* from the experimental colonies to *z* calculated for the Control colony for this relationship.

The results are shown in Table 35c, below.

$\bar{z}_{exp}$ session 1 and 4	χ^2_{exp} session 1and 4	Signif. Level_{exp} session1&4 P<	$z_{control}$	$n_{exp\ session\ 1\&4}$	$n_{control}$	t_s	Signif. level p<
Exp 1							
0.832	3.36	NS	0.887	29.3	29.0	-0.198	NS
Exp 2							
0.652	0.169	NS	0.887	28.0	29.0	-0.840	NS

Table 35c: Testing the relationship between the ranked median distance of individuals from the centre of the brood pile in session 1 compared to session 4 for combined colonies, compared to this relationship in the Control colony. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the experimental colonies for in both experiments. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 compared to session 4, with *z* calculated for this relationship for the Control colony shows that there is no significant difference between this relationship for the experimental colonies and the Control colony.

7.2 An Investigation of the Resilience of Spatial Organisation in Colonies with a Manipulated Age Structure

Experiment 3

I analyse the results from experiment 3 in a similar way to those of experiments 1 and 2. I investigate the relationship between the relative distance of individuals from the centre of the brood pile in the 'older' (original), and in the 'callow' (subsidiary) fractions during session 1 with their relative median distance from the centre of the brood pile in the relevant fraction during session 2; and to overall relative position in the united colonies in session 3. The results are shown in Table 35, below.

Colony/Sessions Compared	r_s	n	Significance Level P<
Colony C_3			
Session 1 / Session 3	0.768	33	0.01
Session 1 / Session 2	0.719	34	0.01
Colony 9			
Session 1 / Session 3	0.685	64	0.01
Session 1 / Session 2 (Older Fraction)	0.785	26	0.01
Session 1 / Session 2 (Callow Fraction)	0.763	57	0.01
Colony 10			
Session 1 / Session 3	0.555	63	0.01
Session 1 / Session 2 (Older Fraction)	0.833	31	0.01
Session 1 / Session 2 (Callow Fraction)	0.691	16	0.01
Colony 11			
Session 1 / Session 3	0.858	62	0.01
Session 1 / Session 2 (Older Fraction)	0.850	36	0.01
Session 1 / Session 2 (Callow Fraction)	0.802	29	0.01
Colony 12			
Session 1 / Session 3	0.556	38	0.01
Session 1 / Session 2 (Older Fraction)	0.568	15	0.01
Session 1 / Session 2 (Callow Fraction)	0.575	27	0.01

Table 35 : The relationship between individual's spatial positions compared between sessions, Spearman's rank correlation coefficient, r_s ,tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

There is a significant, positive correlation between the relative distance of an individual from the centre of the brood pile in session 1 and her relative position in session 2, in the Control, and in both the older, and the callow fractions of the experimental colonies. The relationship between the relative position of an individual in session 1 and their relative position in session 3 is also a significant, positive correlation, for the Control, and the experimental colonies when older and callow individuals are considered together.

A Fisher's z-transform shows that there is no significant difference between the correlation of the Control colony for the comparison of spatial positions between sessions 1 and 3, and each of the experimental colonies (callows and older workers considered together, see Table 36, below).

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	**Significance level P<**
C_3 and 9	0.768	0.685	33	64	1.015	0.838	0.794	NS
C_3 and 10	0.768	0.555	33	63	1.015	0.626	1.743	NS
C_3 and 11	0.768	0.858	33	62	1.015	1.286	-1.205	NS
C_3 and 12	0.768	0.556	33	38	1.015	0.627	1.561	NS

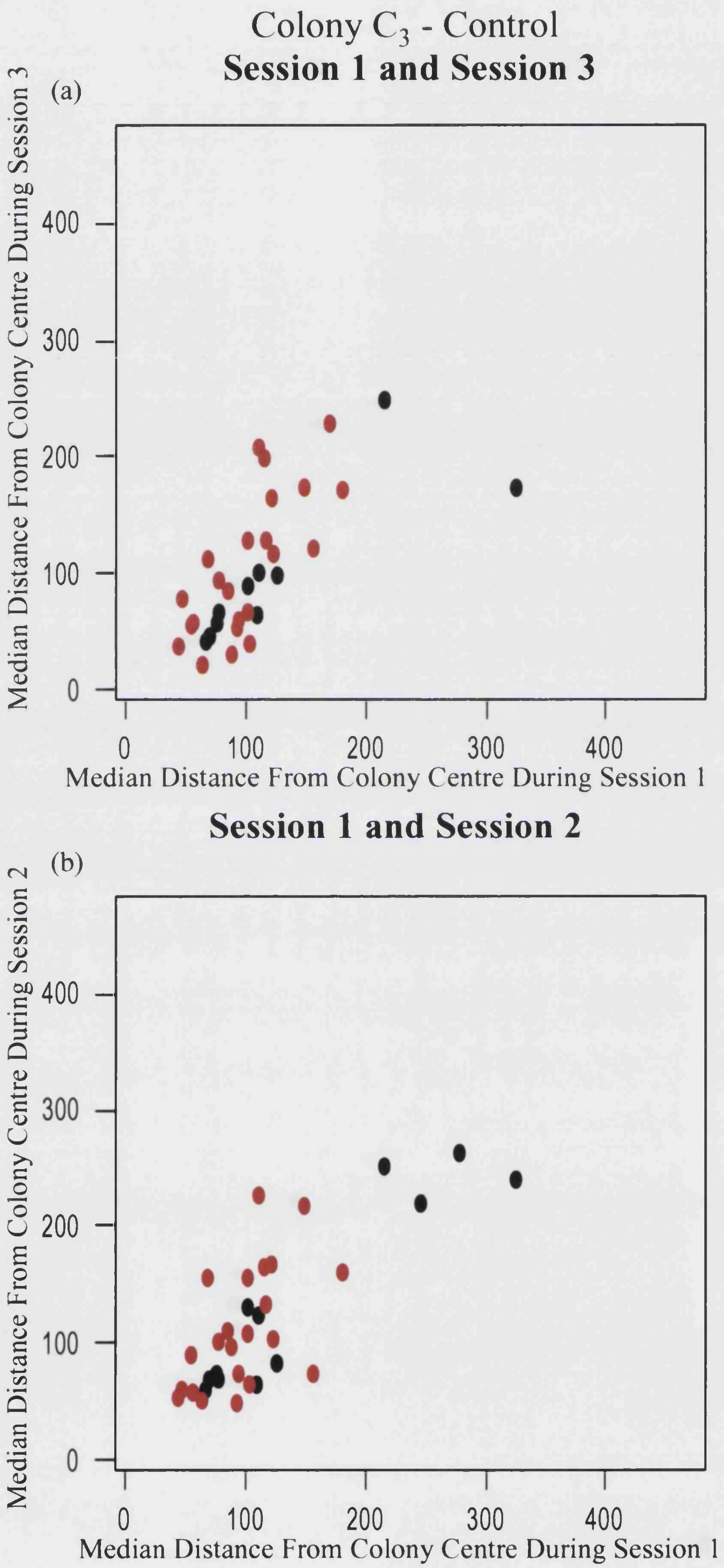
Table 36 : A comparison between the Spearman's rank correlation coefficients of relative positions of individuals in session 1 and relative positions in session 3 with that of Control colony C_3, calculated as a Fisher's z-transform.

A closer examination of the comparison between sessions 1 and 3 is given in Table 37, below. I calculate the Spearman's rank correlation coefficient separately for older, and for callow, individuals. There is a significant, positive correlation between the relative spatial position of callow individuals in session 1 compared to their relative position in session 3. This is the case for the Control colony, and for all four experimental colonies. There is a significant, positive correlation between the relative spatial position of older individuals in session 1, and their position in session 3 for the Control colony. This is also the case for experimental colonies 9, 10 and 11. These results are discussed in section 7.3ii.

Colony/Sessions Compared	**r_s**	**n**	**Significance level p<**
Colony C_3			
Session 1 / Session 3 (Older Fraction)	0.939	10	0.01
Session 1 / Session 3 (Callow Fraction)	0.722	23	0.01
Colony 9			
Session 1 / Session 3 (Older Fraction)	0.840	19	0.01
Session 1 / Session 3 (Callow Fraction)	0.738	45	0.01
Colony 10			
Session 1 / Session 3 (Older Fraction)	0.661	32	0.01
Session 1 / Session 3 (Callow Fraction)	0.555	31	0.01
Colony 11			
Session 1 / Session 3 (Older Fraction)	0.858	34	0.01
Session 1 / Session 3 (Callow Fraction)	0.808	28	0.01
Colony 12			
Session 1 / Session 3 (Older Fraction)	0.348	14	NS
Session 1 / Session 3 (Callow Fraction)	0.629	24	0.01

Table 37 : The relationship between individual's spatial positions compared between sessions separately for older and callow individuals. Spearman's rank correlation coefficient, r_s ,tested as an ordinary product-moment correlation coefficient as $n > 10$ (Sokal and Rohlf, 1981 p.607).

The graphs below show the relationship between the median position of the workers in the colony during session 1 compared to their median position in other sessions for the Control in experiment 3 (Colony C_3).



Colony C_3 - Control

Session 1 and Session 3

Session 1 and Session 2

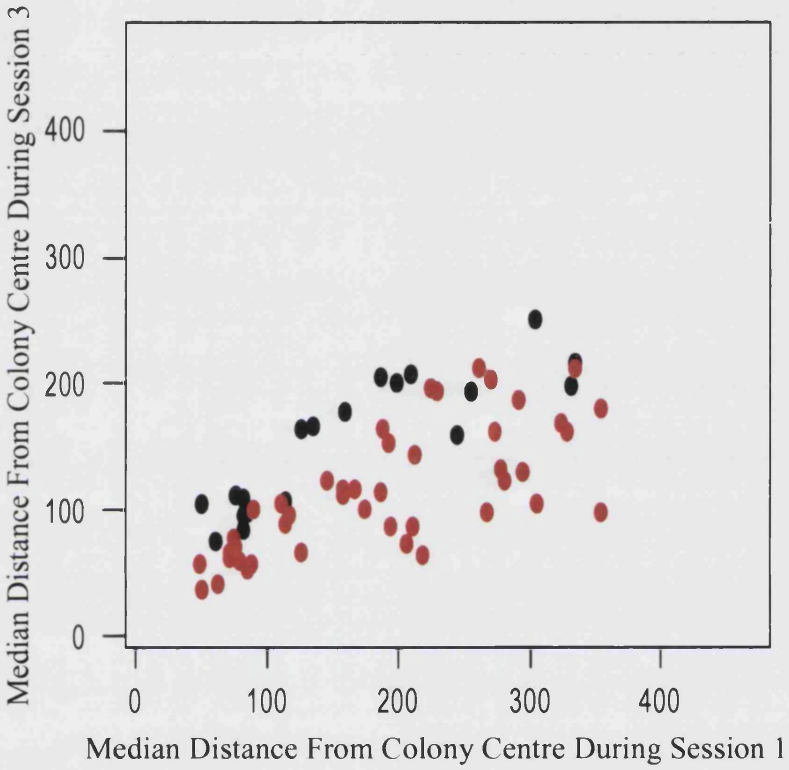
Figure 45 : (a) The relationship between the median distance from the colony centre in session 1 and session 3; and (b) session 1 and session 2, for Colony C_3, the Control, experiment 3. Callows are shown in red.

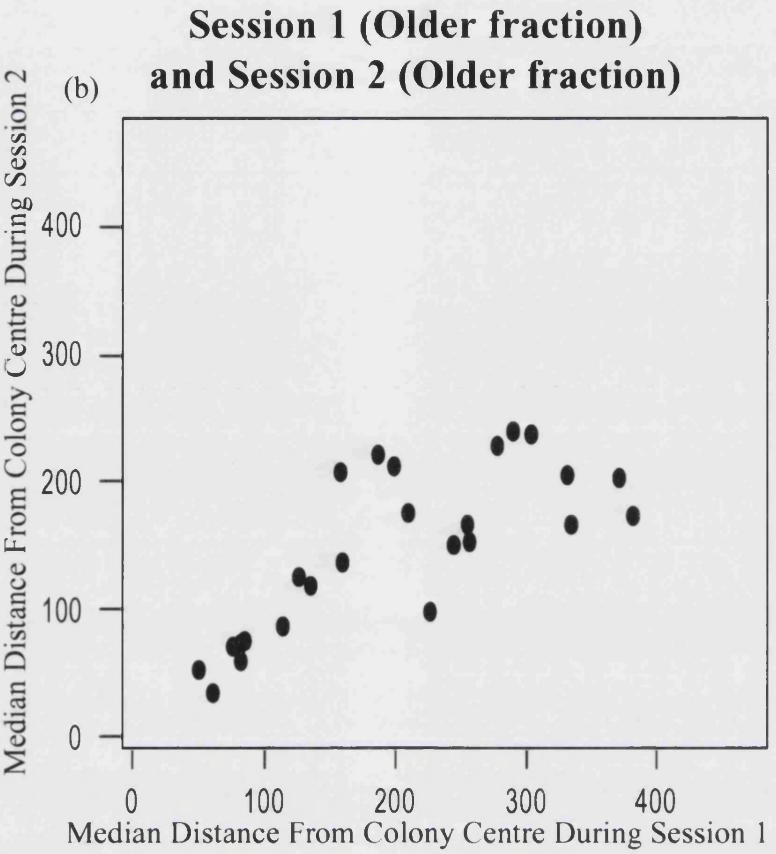
Colony 9 - An Example Experimental Colony

The graphs below show typical plots of the relationship between the median position of the workers in the colony during session 1 compared to their median position in other sessions, for Colony 9.

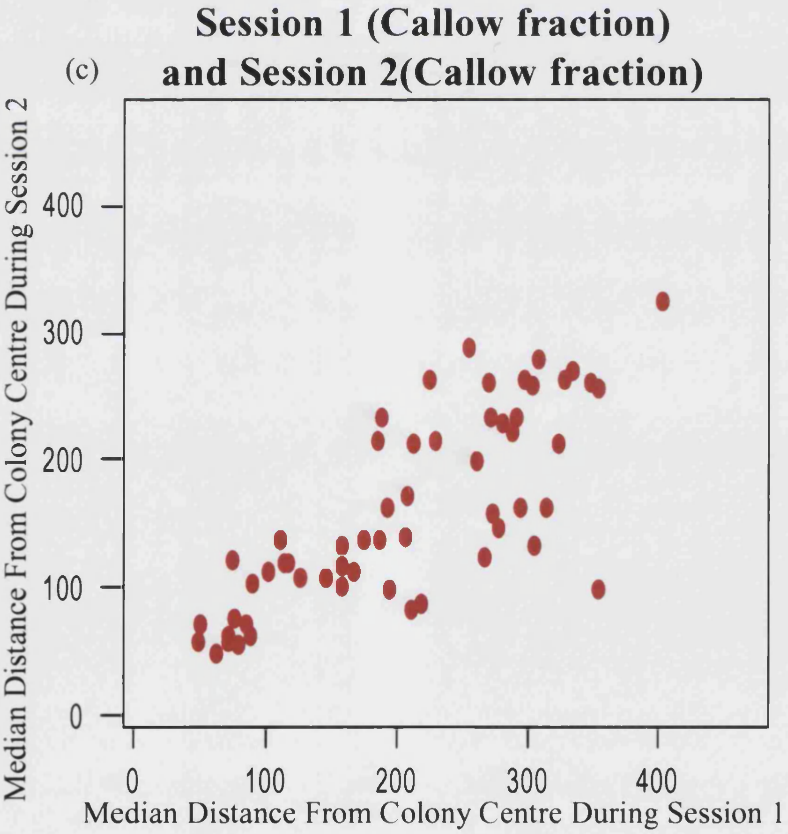
Fig 46(a)

Session 1 (Callow and Older fractions) and Session 3 (United fractions)





Session 1 (Older fraction) and Session 2 (Older fraction)



Session 1 (Callow fraction) and Session 2(Callow fraction)

Figure 46 : (a) The relationship between the median distance from the colony centre in session 1 and session 3; (b) session 1 and session 2,(older workers); and (c) session 1 and session 2 (callow workers), for Colony 9, an example experimental colony. Callows are shown in red.

Multiple comparisons using pooled data

To determine if sociotomy has a significant effect on the relationship between the ranked median distance from the centre of the brood pile in session 1 (un-manipulated colonies) compared to session 4 (after re-unification) I perform multiple comparison analysis for the older workers and the callow workers separately. Using the method outlined earlier in Chapter 7. The results are shown in Table 37a, below.

z^{-}_{exp} session 1 and 3	χ^2_{exp} session 1and 3	Signif. Level$_{exp}$ session1&3 P<	$z_{control}$	$n_{exp\ session\ 1\&3}$	$n_{control}$	t_s	Signif. level p<
Callows							
0.869	3.850	NS	0.912	29.0	23.0	-0.144	NS
Older workers							
1.085	3.990	NS	1.730	25.3	10.0	-0.489	NS

Table 37a: Testing the relationship between the ranked median distance of individuals from the centre of the brood pile in session 1 compared to session 3 for combined experimental colonies, taking the callows and the older workers separately, compared to this relationship in the Control colony. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the experimental colonies for in both fractions. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 compared to session 4 for the callows and the older workers separately, with *z* calculated for this relationship for the Control colony separately, shows that there is no significant difference between this relationship for the experimental colonies and the Control colony for either fractions.

7.3 Discussion
An Investigation of the Resilience of Spatial Organisation Within the Colonies

I have shown that brood-related tasks and external activity are spatially organised in colonies of *Leptothorax albipennis* (Chapter 4). I analysed the results from experiments 1, 2, and 3, with respect to the relationship between the frequency at which these tasks are performed by the workers in each colony and the median distance of these workers from the centre of the brood pile. Brood-related tasks and external activity are spatially organised in un-manipulated colonies (session 1 of experiments 1 and 2). The frequency at which an individual performs brood-related tasks decreases as her median distance from the brood pile increases, and there is a threshold distance from the brood pile above which individuals perform brood-related tasks at a frequency of zero, or very close to zero. The frequency at which an individual carries out external activity generally increases as her median distance from the brood pile increases.

The spatial organisation of brood-related tasks is shown to withstand the removal of workers associated with external activity in experiments 1 and 2. Levels of external activity in these colonies are generally too low after the removal of these individuals for a spatial pattern to be established (session 3, original fractions) for these tasks. The same pattern of spatial organisation of external activity observed in the un-manipulated colonies is evident among the removed workers when they are placed in a separate nest. This occurs despite the absence of the queen, and any brood (session 3, subsidiary fractions, experiments 1 and 2). The pattern of spatial organisation of brood-related tasks observed in un-manipulated colonies is also evident in these fractions of the colony when the nests do contain brood (session 3, subsidiary fractions, experiment 2). The familiar spatial patterns of both brood-related tasks and external activity, are evident when the removed individuals are re-united with the original fractions of the colonies (session 4, experiments 1 and 2).

The same spatial patterns with respect to brood-related tasks and to external activity are observed in artificially created, single age-cohort colonies of *Leptothorax albipennis*, consisting only of callow workers and brood. These patterns are also evident in colonies from which all these callows have been removed. When these two fractions are united, the spatial patterns of both brood-related tasks and external activity are still evident.

In sections 7.1 and 7.2, above, I examined the spatial organisation in these colonies further in order to investigate the importance of spatial organisation in the maintenance of division of labour in this species. The aim of the chapter is to establish whether individual workers show fidelity to their relative spatial position in the colonies despite sociotomy. I compared the relative spatial position (measured as median distance from the centre of the brood pile) of individual workers throughout the experiments. In the following section, I discuss whether the relative spatial positions of individuals are conserved in the Control colonies, which were un-manipulated except for the temporary removal of workers to test for the effect of physical removal. I also discuss whether the relative spatial positions of individuals in the experimental colonies are resilient to sociotomy.

The results above are divided into the following sections:

7.1 An investigation of the resilience of spatial organisation in colonies with a manipulated task structure

7.2 An investigation of the resilience of spatial organisation in colonies with a manipulated age structure

For a summary of the methodology of all three experiments the reader is referred to Figure 9, Chapter 3.

i. An Investigation of the Resilience of Spatial Organisation in Colonies with a Manipulated Task Structure

To investigate whether individual workers are faithful to their relative spatial positions in colonies of *Leptothorax albipennis* during experiments 1 and 2, I carried out correlation analyses (according to the procedure described in Chapter 4). Using these methods I examined the relationship between the relative distance of individuals from the centre of the brood pile during each experimental session compared to their relative position during session 1. Experimental colonies are compared to the Control using Fisher's z-transforms: the correlation coefficient for the relationship between the relative spatial position of individuals during session 1 and session 4 for the Control colony is compared to the correlation coefficient for the same relationship for each of the experimental colonies. I also performed multiple comparisons of the pooled data using the pooled correlation between relative distance of individuals from the centre of the brood pile in session 1 compared to session 4, compared to this relationship for the Control colony. Graphs of the relationship between the spatial positions of individuals during each session compared to session 1 are used to investigate the absolute spatial distribution of individuals compared between sessions.

Experiment 1

There is a significant association between the position of an individual in session 1 and its position in session 4 for the Control colony. Colony C_1 controls for the effect of the temporary physical removal of individuals. Figure 41 (a) is a plot of the relationship between the median distance of individuals from the centre of the brood pile in the Control colony during session 1 against their median distance from the centre of the brood pile during session 4. There is a positive relationship between these two variables. I carried out correlation analyses on the relationship between the relative distance of individuals from the centre of the brood pile during session 1 compared their relative position during session 4. The results are shown in Table 30. There is a highly significant, positive correlation between the relative positions of individual workers during session 1 compared to their relative position in session 4. I conclude that the median distance of an individual worker from the centre of the brood pile, relative to other workers, does not change significantly over the length of experiment 1, despite the temporary physical removal of one third of the population when the colony is emigrated. Any change in spatial organisation observed in the experimental colonies is due solely to the effect of the experimental manipulations, and not simply to the effect of physically removing some of the workers.

Another important point arises from this analysis. It is noted that when the removed workers were returned to the Control colony, after a short period of time, the colony is not subsequently emigrated. The workers are simply placed in the foraging arena of the nest. Sendova-Franks and Franks (1994) showed that workers in colonies of *Leptothorax unifasciatus* establish the same relative positions (and behavioural roles) in the colony after the colony emigrates to an entirely new nest site, a phenomenon known as *social resilience*. The results from the Control colony (Colony C_1), discussed here, show that *Leptothorax albipennis* workers will still re-establish their relative spatial positions when placed in the foraging arena of the nest after temporarily being removed from it during an emigration event. I conclude that it is not necessary for colonies to be emigrated in order for spatial order to be re-established.

The association between the relative position of individuals during session 1 and their relative position during session 4 is also seen in the experimental colonies, despite sociotomy. Figure 41 (b) is a plot of the median distance of individuals from the centre of the brood pile during session 1, where experimental colonies were un-manipulated, compared to their median distance from the centre of the brood pile during session 4, for an typical experimental colony (Colony 3). Recall that at the beginning of session 4 each colony had been re-united after a period of being maintained as two separate fractions, consisting of workers that had been associated with external activity and the remaining workers. There is a positive relationship between the relative positions of individuals during session 1 and their relative positions during session 4. This relationship is a highly significant, positive correlation for experimental colonies 1, 2 and 3 (see Table 30).

The *p* value for the relationship for Colony 4 is 0.08, and a closer examination of the data reveals that it is skewed by a number of individuals whose median positions are relatively further away from the centre of the brood pile in session 4, compared to session 1. Of the four experimental colonies, Colony 4 has the smallest number of individuals that have retained their marks, and I attribute the lack of significance of the correlation coefficient for this colony to the small sample size. Moreover, the proportion of individuals removed from this colony because they were associated with external activity is much greater in this colony than the other experimental colonies (see Backen *et al.*, 2000, Colony 4 ≡ Colony 6 in this paper). The removal of a relatively high proportion of individuals may have affected the subsequent tendency of individuals to retain their relative spatial organisation after the re-unification of the colony fractions.

Examining Figure 41 (b) reveals that there is a slight tendency for the median distance of individuals from the colony centre to have increased in session 4 compared to session 1, for all the workers in Colony 3, except one, which is situated much further out than the other workers. This increase in the median distance from the centre of the brood pile is also observed for some individuals in experimental Colonies 2 and 4, but not for Colony 1. Sendova-Franks and Franks (1995a) showed that the median size of SFZ increased after hibernation to a peak in May, after which it contracted again towards hibernation, in colonies of *Leptothorax unifasciatus*. Thus the increase in worker's median distances from the centre of the brood pile seen in most of the experimental colonies could reflect a seasonal increase in the size of the individuals' SFZs. However, this trend is not observed for the Control colony, and cannot therefore be attributed to seasonal effects, but must be due to the manipulations carried out on the experimental colonies. My hypothesis is that any expansion in the size of the individuals' SFZs, and consequent increase in their median distance from the colony centre, occurs as a result of the increased space available in the nest during sessions 2 and 3, resulting from the absence of a proportion of the population. This expansion is subsequently retained after the re-unification of the colonies in session 4. The degree of expansion by individuals would be affected by the amount of space in the nest after sociotomy, and would consequently be affected by population size and the proportion of individuals removed from the colony during session 2. This could explain the inter-colony variation observed for this phenomenon.

Fisher's z-transforms were used to compare the correlation coefficient for the relationship between the relative position of individuals in session 1 compared to session 4 for the Control colony and the correlation coefficient for this relationship for each of the experimental colonies. The results are shown in Table 31. There is no significant difference between the correlation coefficient for this relationship in the Control compared to any of the experimental colonies. In fact, only Colony 3 has a higher correlation coefficient than the Control for this relationship, although this was not significantly different from the Control. It is important to note that the Fisher's z-transform shows that there is no significant difference between the correlation coefficient for the relationship between the relative position of individuals in session 1 compared to session 4 for the Control and the correlation coefficient for this relationship in Colony 4. Colony 4 is thus considered to show some degree of spatial fidelity. I also performed multiple comparisons, first determining that the colonies can be considered to be samples from a population exhibiting a common correlation coefficient (see Table 35c). There is no significant difference between the correlation for the relationship between session 1 and 4 for the pooled experimental colonies compared to this relationship for the Control colony.

Individuals that were removed from Colony 3 during session 2, and thus formed the 'subsidiary' fraction during session 3, before being re-united with the other individuals in session 4, are shown in red in Figure 41 (b). It has already been seen in Chapter 4, that these individuals tend to be situated further away from the colony centre than the individuals that were not removed in session 2. What is of particular interest here is that individuals re-adopt the same relative spatial positions whether they are situated relatively far or relatively close to the centre of the brood pile, and whether or not they have been removed from the colony. Again, this emphasises the point, that the colony need not be emigrated during re-unification for individuals to show spatial fidelity. This would seem logical for those individuals situated nearer the periphery of the nest, as it would be relatively easy for them to re-adopt these positions, but it also applies to those individuals situated further towards the centre. There must, therefore, be a very efficient 'sorting' procedure to enable these individuals to re-adopt their positions relative to other individuals in the nest. This is discussed further in Chapter 9. Moreover, the overlap in spatial organisation between the individuals that had been removed, and those that were not, is clearly visible in Figure 41 (b). This confirms the effectiveness of the removal procedure, as not only individuals that were relatively far from the centre of the brood pile, and therefore likely to perform external activity with a relatively high frequency, were removed. The removal procedure also removed individuals that were located closer to the centre of the brood pile, and therefore performed external activity at a relatively low frequency, because of the strong spatial organisation of these tasks demonstrated in Chapter 4. This has implications for the flexibility of task performance by individuals that carry out external activity in the original fraction during session 3. They are unlikely to have performed external activity previously otherwise they would have been removed. This is analysed and discussed further in Chapter 8.

I also examined the association between the position of an individual during session 1 and its position during other sessions of the experiment. Figures 42 (a) and (b) are plots of this relationship for session 1 compared to session 2, and session 1 compared to session 3, respectively, for the Control. The association between the spatial position of an individual in session 1 and both session 2, and session 3, is maintained. Correlation analyses of the relationship between the relative distance from the centre of the brood pile of an individual in session 1, compared to session 2, and to session 3, show that there is a positive, significant correlation between these variables for the Control colony for all session comparisons (see Table 30). Thus, the relative positions of individuals are not affected by the physical removal of some individuals from the colony, or by seasonal effects.

Figures 43 (a), (b) and (c), are plots of the relationship between the median distance from the centre of the brood pile of individuals during session 1 compared to session 2, for session 1 compared to session 3 (original fraction), or to session 3 (subsidiary fraction), respectively, for Colony 3. The positive association between the position of the individuals during each session is seen in each case. I examined the relationship between individual's relative spatial position in session 1, compared to the each of the other sessions, for all the session comparisons for each of the experimental colonies (see Table 30). The relative spatial positions of individuals during session 1 of all four experimental colonies is highly significantly correlated with their relative spatial positions during session 2, when individuals associated with external activity were being removed. The relative position of an individual tends not to be significantly correlated between session 1 and session 3, original fraction. I think this is a result of the low numbers of individuals that have retained their marks in colonies 1 and 4. Colony 3, which has a higher number of marked individuals in sessions 3, original fraction, does show that the relationship between relative position during these two sessions is a significant, positive correlation (see also the results for session 3, original fraction, experiment 2). However, the relationship is not a significant correlation for Colony 2. A closer examination of the relationship between the spatial position of individuals during session 1, compared to their positions in the original fractions during session 3 for Colony 2, reveals the data is skewed by two individuals that move relatively further away from the centre of the brood pile during session 3, compared to session 1. I conclude that individuals may move, in terms of their relative positions in the colony, when the workers associated with external activity are removed. The results discussed above strongly suggest that individuals may expand outwards, in terms of their absolute position, to fill the space left in the colony after a number of individuals have been removed.

There is an association between the spatial position of an individual during session 1 and spatial position in the subsidiary fraction during session 3. The results in Table 30 show that relative positions of individuals in session 1 are significantly correlated with their spatial positions in the subsidiary fractions during session 3, for all the experimental colonies. No queen or items of brood are present in these fractions, and yet individuals still maintain the same relative spatial positions as they did in the original colony. As the frequency at which individuals perform external activity increases with their median distance from the colony centre, see Chapter 4, the individuals that are removed first from the colonies in session 2, are likely to be further away from the brood pile, than those that are removed towards the end of session 2. Thus one could expect the relative positions of these individuals to be reversed in the subsidiary fraction and the individuals that are removed first into the subsidiary colony to move further towards its centre, as the nest is empty. Conversely, individuals still maintain the same relative positions. Again, this emphasises the efficiency of the 'sorting' procedure (see Chapter 9).

Experiment 2

The results from experiment 2 are analysed in the same way as the results above. Very similar patterns can be seen in Figures 44 (a), (b) and (c), which are plots of the median distance of individuals from the colony centre during session 1 compared to their positions during session 4, and to their positions in session 3 (original and subsidiary fractions, respectively), for an example experimental colony (Colony 7). Note that individuals show high fidelity to their absolute positions. Individuals that are removed as 'externals' (represented in red) tend to be located relatively further away from the brood pile compared to individuals that are not removed. Intriguingly, the median distance of the former individuals in the subsidiary fraction remains relatively far away from the centre of the brood pile during session 3, compared to individuals in the original fraction. This is clearly visible when Figures 44 (b) and (c) are compared to Figure 44 (a). One possible explanation for this phenomenon is that some information from the environment (for example nest geometry or other possible templates (see section 2.4i.)) may be used to establish the absolute positions of individual in the nest. This does not imply that these cues are used to establish the same relative position in the nest.

Colonies 5 and 6 do not contain sufficiently high numbers of individuals that have retained their marks for correlation coefficients to be calculated. A Spearman's rank correlation coefficient is calculated for session 1 compared to session 4, for Colony 6, but it is not a significant result probably due to the small number of individuals that have retained their marks ($n= 7$).

The relative spatial position of an individual during session 1 is significantly, positively correlated with relative spatial position during session 3 (original fraction), session 3 (subsidiary fraction), and session 4, for colonies 7 and 8. The significant correlation shown between the relative positions of workers in session 1 and the original fraction of session 3 for these colonies confirms the view given above, that any non-significance in this relationship for colonies in experiment 1 may be due to insufficient data, or variation in the proportions of the population in the two fractions. I conclude that the presence of brood in the subsidiary fractions of session 3 does not affect spatial fidelity in these colonies, or the spatial fidelity seen when these fractions are re-united with the original fractions of the colonies.

Individuals in the Control colony, experiment 2, (Colony C_2), still exhibit spatial fidelity to their relative positions during session 1 compared to session 4, and between sessions 1 and 3, despite the death of the queen. Moreover, Fisher's z-transforms show that there is no significant difference between the correlation coefficient of the relationship for Colony C_2 when session 1 is compared to session 4, and the correlation coefficients of the corresponding relationships for Colonies 6, 7 and 8 (see Table 33). Fisher's z-transforms also show there is no significant difference between the correlation coefficient for the relationship between relative spatial position in session 1 and session 4, for the Control colony for experiment 1 (Colony C_1), and the correlation coefficients for this relationship for the experimental colonies. Colony C_1 is used in preference to Colony C_2 due to the latter's orphanage state. No significant differences are found between the correlation coefficients for the relationship of the Control colony and Colony 6, despite the fact that Colony 6 does not show a significant correlation. This implies Colony 6 has some degree of spatial fidelity despite the very low numbers of individuals that retained their marks. I performed multiple correlation analysis on these data, showing that there is no significant difference between the relationship comparing the relative distance of individuals from the colony centre in session 1 and session 4, for pooled correlation coefficients from the three significant experimental colonies, and this relationship in the Control colony (see Table 35c).

CONCLUSIONS

The following conclusions can be drawn:

Individuals in colonies of *Leptothorax albipennis* re-adopt the same relative spatial positions despite the removal of workers associated with external activity.

Individuals associated with external activity, removed from the colonies, re-adopt the same relative spatial positions when placed in a new nest, as those they occupied in the original colony, irrespective of the order in which they are removed. The resilience of the relative spatial positions occupied by workers is independent of the presence of the queen, the presence of all of the workers, and still occurs with or without the brood. .

I conclude that the presence of brood in the subsidiary fractions of session 3 does not affect spatial fidelity in these colonies.

When individuals that were removed into the subsidiary fractions of the colonies are re-united with the original fractions they re-adopt the same relative spatial positions as they occupied in the colonies prior to sociotomy.

It is not necessary for the colony to emigrate in order for spatial organisation to be re-established.

I conclude that the presence of brood in the subsidiary fractions during session 3 does not affect spatial organisation when these fractions are re-united with the original fractions of the colonies.

A very efficient '**sorting**' mechanism must be operating. The sorting procedure must be able to function independently of the presence of the queen, the brood, and a large number of the workers.

ii. An Investigation of the Resilience of Spatial Organisation in Colonies with a Manipulated Age Structure

Experiment 3

I carried out a similar analysis for the results from experiment 3, in which the age structure of the experimental colonies was manipulated. The Control colony (Colony C_3), remained un-manipulated, but individuals were marked at a stage in the development of the colony where callow individuals could still be recognised due to their lighter pigmentation. I ranked individuals in each colony with respect to their median distance from the centre of the brood pile during each of sessions 1, 2 and 3. The rankings are compared between sessions 1 and 2, separately for the 'callow' and 'older' fractions of each colony, and sessions 1 and 3, where the relative positions of individuals in the separate fractions of session 1 are together compared to their relative positions in the united colony in session 3. The Spearman's rank correlation coefficients for these relationships are shown in Table 35, and are discussed below, separately for each comparison.

There is highly significant, positive correlation between the relative position of individuals in session 1 compared to session 2, for the Control colony (Colony C_3). This analysis included both the callows, and the older workers in the Control colony. Figure 45 (b) is a plot of the relationship between the absolute positions of individuals during session 1 compared to session 2 (callows are shown in red, older workers in black). This graph shows that callows tend to be situated nearer to the centre of the brood pile in both sessions, whereas older workers are distributed throughout the nest in both sessions. Similar results are found when the positions of individuals are compared between sessions 1 and 3, see Figure 45 (a). There is also a highly significant, positive correlation between the relative positions of individuals in session 1 compared to session 3, when callows and older workers are considered together. The spatial distribution of callow and older workers in the nest is very similar in this comparison.

I also carried out correlation analyses on the relationship between relative spatial position during session 1 compared to session 3, separately for callows and older workers, for the Control colony (see Table 37). The relationship is a highly significant, positive correlation, in the case of both the callows and the older workers. I conclude, therefore, that there is a strong spatial structure in un-manipulated colonies of this species, as seen in the Control colony for experiment 1 (Colony C_1), and the experimental colonies in session 1 of both experiments 1 and 2. The results from Colony C_3, also concur with the results from an analysis of the behavioural roles exhibited by individuals in this colony (discussed in Chapter 4). Older workers are not limited behaviourally just to external activity, or spatially to the periphery of the nest, as might be expected by predictions from age polyethism. It has been seen in Chapter 4 that older individuals sometimes carry out brood-related tasks, and that they are sometimes located near the brood pile. Moreover, the results from the current analysis show that older individuals are not only distributed throughout the nest, but that individual workers exhibit resilience in their spatial organisation, and remain in the same relative positions, at least for the duration of the experiment. Callow workers tend to be found closer to the centre of the brood pile, and individual callow workers also exhibit spatial fidelity over the course of this experiment.

I analysed the relationship between the relative spatial positions of individuals in session 1 compared to session 2 (older and callow fractions) using the same procedure for all four experimental colonies. There is a highly significant, positive correlation between the relative positions of individuals in this comparison for both the ‘callow’ and ‘older’ fractions of each colony. As demonstrated in Chapter 4, these fractions of the colonies exhibit a strong spatial organisation with respect to brood-related tasks, and to external activity, and this spatial organisation is strongly conserved between sessions 1 and 2, during which colonies were not manipulated further. The results here show that individual workers remain in the same positions relative to each other throughout these sessions. This phenomenon of spatial fidelity occurs in both colonies containing no callow workers, and also in colonies consisting only of callow workers and some brood, with no queen, and no older workers. These callows have had no experience of ever having been part of a colony consisting of these components (apart from the fifteen older workers placed in the nest to help the pupae eclose, which were removed soon afterwards).

I also examined the absolute positions of workers in session 1 compared to session 2, for an example experimental colony, by plotting the relationship between median distance from the colony centre compared between these two sessions for the 'older' and 'callow' fractions, see Figures 46 (b) and (c) respectively. Figure 46 (c) shows that there is a difference in the spatial distribution of individuals in the 'callow' fraction of the colony compared to the Control colony. Although they exhibit spatial fidelity, and spatial organisation of brood-related tasks, as well as external activity, individuals in the callow fraction are not limited spatially to the centre of the nest, as they are in the Control colony, Figure 45 (b). In fact, the absolute spatial positions of individuals in the callow fraction vary from relatively near to the centre of the brood pile, to the periphery of the nest. This is an important result. It shows individuals with no prior experience of being in a nest with older individuals, will distribute themselves spatially into the available nest space, in a similar way that individuals in un-manipulated colonies distribute themselves. Spatial positions cannot therefore be a function of age. Moreover, the spatial organisation of these individuals is resilient over time, in the same way that spatial organisation of workers in un-manipulated colonies is resilient over time. Task allocation, with respect to brood-related tasks, and external activity, and the spatial organisation and fidelity seen in these artificially created single age cohort colonies, arises therefore

> In the absence of the queen, or any effect she might have on the colony by physical or chemical means;
> In the absence of any older workers in the colony; and,
> In the absence of experience or previous learning about any tasks or spatial positions.

The importance of this result is fundamental to the discussion of task allocation, and is discussed further in Chapter 9.

I also examined the relationship between the spatial organisation of the workers when the 'older' and 'callow' fractions existed separately in session 1 with their spatial organisation when the fractions were united in session 3. Table 35 shows that the relationship between the relative spatial positions of individuals in session 1 compared to session 3, is a highly significant, positive correlation, when callows and older workers are considered together. Fisher's z-transforms, see Table 36, show that there is no significant difference between the correlation coefficient for this relationship in each experimental colony compared to the correlation coefficient for the same comparison in the Control colony. The correlation coefficients for this relationship are pooled for the experimental colonies, and I performed multiple comparisons between the pooled value and the correlation coefficient for the relationship in the Control colony. I performed this analysis separately for the callows and for the older workers. The results, in Table 37a, show that there is no significant difference between the relationship between relative distance from the colony centre in the pooled experimental colonies compared between sessions 1 and 4, and this relationship for the Control colony. Thus, there is no difference in the extent of spatial fidelity of all the workers in the experimental colonies, compared to an un-manipulated colony, despite the existence of these colonies as two separate fractions up to session 3 of the experiment. I also examined this relationship considering callows and older workers separately. The results from the correlation analyses are shown in Table 37. The relative spatial positions of callow workers during session 1 are significantly, positively correlated to their relative positions during session 3, in all four experimental colonies. The relationship is also a significant, positive correlation for the older workers in all the experimental colonies except Colony 12. This may be due to the low number of workers that have retained their marks in this colony.

I examined the absolute spatial distribution of workers in the united experimental colonies compared to their spatial distribution during session 1. This relationship differs from the spatial distribution of workers in the un-manipulated Control colony. Figure 46 (a) is a plot of the relationship between the absolute median distance of individuals from the colony centre during session 3 compared to their positions in the separate fractions during session 1, in Colony 9. The 'callow' cohort remains distributed throughout the nest, despite being re-united with the older workers. In the Control colony, Figure 45 (a), callows are found only near the centre of the brood pile. This confirms that spatial organisation of workers in the nests is not a function of age, but is a function rather of experience, and subsequent spatial fidelity.

CONCLUSIONS

The following conclusions can be drawn:

Workers in un-manipulated colonies of *Leptothorax albipennis* show resilience in terms of their relative spatial organisation over time. Callows in un-manipulated colonies tend to be located relatively near to the centre of the brood pile, whereas older workers are distributed throughout the nest. The latter observation strongly suggests that spatial position is not a function of age.

Workers in colonies from which all the callows have been removed show the same overall spatial organisation as that seen in un-manipulated colonies.

Individuals are distributed spatially throughout the nest.

The relative spatial positions of individuals are resilient over time.

Workers in artificially created colonies consisting only of completely inexperienced callows from a single age cohort, and brood, but no queen, or any older workers, exhibit the same overall spatial organisation as that seen in un-manipulated colonies.

Individuals are distributed spatially throughout the nest.

The relative positions of individuals are resilient over time.

Therefore the spatial positions adopted by workers is not a function of age, as all individuals are from the same age cohort.

The spatial organisation and fidelity seen in these artificially created single age cohort colonies arises, therefore, without the presence of the queen, or any influence she may have on the colony by physical or chemical means; without the presence of any older workers in the colony; and without any previous experience or previous learning of any tasks or spatial positions.

When the 'callow' fractions of the colonies are united with their relevant 'older' fractions, workers remain faithful to the relative positions they occupied in the separate fractions.

> The absolute spatial distribution of callows remains - callows are distributed throughout the nest.

Spatial organisation of workers in the nests is not a function of age, but is rather a function of experience, and subsequent spatial fidelity.

Chapter 8

An Investigation of the Flexibility of Individual Performance of Brood-related Tasks and External Activity within the Colonies

8.1 An Investigation of the Flexibility of Individual Performance of Tasks in Colonies with a Manipulated Task Structure

In Chapters 4 and 7, I investigated the resiliency of the spatial distribution of tasks, and workers that perform them, inside the nest, in colonies of *Leptothorax albipennis.* The aim of the following chapter is to compare the resilience of relative task profiles of individuals to sociotomy. I compare the frequency at which brood-related tasks, or external activity, are performed by each individual between sessions of both experiments 1 and 2, for every individually marked individual. Only individuals that have retained their marks throughout both sessions to be compared, are included in the analysis.

i. The flexibility of individual performance of brood-related tasks

Analysis is carried out as follows:

The frequency at which each marked individual performs brood-related tasks during each experimental session is determined from the photographic record for that session.

Data files are created for each pair of sessions to be compared. The data files consist of the total frequency at which brood-related tasks are performed by all individuals that have retained their marks in each of the two sessions.

Individuals are only included in the analysis if they:

i. Have at least one recorded position in the nest (even if the frequency at which they perform brood-related tasks is zero, in any particular session).

ii. Are individually recognisable in both sessions to be compared.

The relationship between the frequency at which individuals perform brood-related tasks in session 1, compared to the frequency at which they perform brood-related tasks in the session being compared to, is investigated by plotting graphs of these variables for the Control, and for typical experimental colonies.

In the analysis individuals that were in the subsidiary fractions of the colonies during session 3 are represented in red, individuals that were in the original fractions are represented in black (experiments 1 and 2).

In each plot the chronologically later session is represented on the y axis.

It should be noted points may overlap on the graphs and this accounts for any discrepancy between the number of points on each plot, and the corresponding '*n*' in the results tables.

Individuals are ranked with respect to the frequency at which they perform brood-related tasks in each session to be compared.

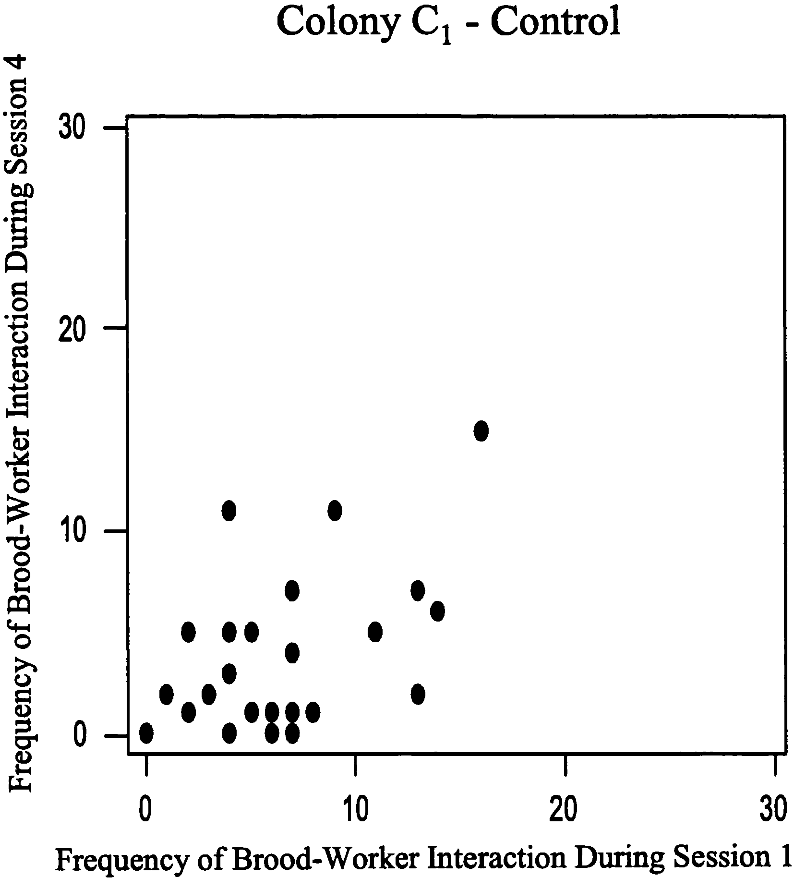
Correlation analyses are carried out for all the colonies according to the procedures described at the beginning of Chapter 4.

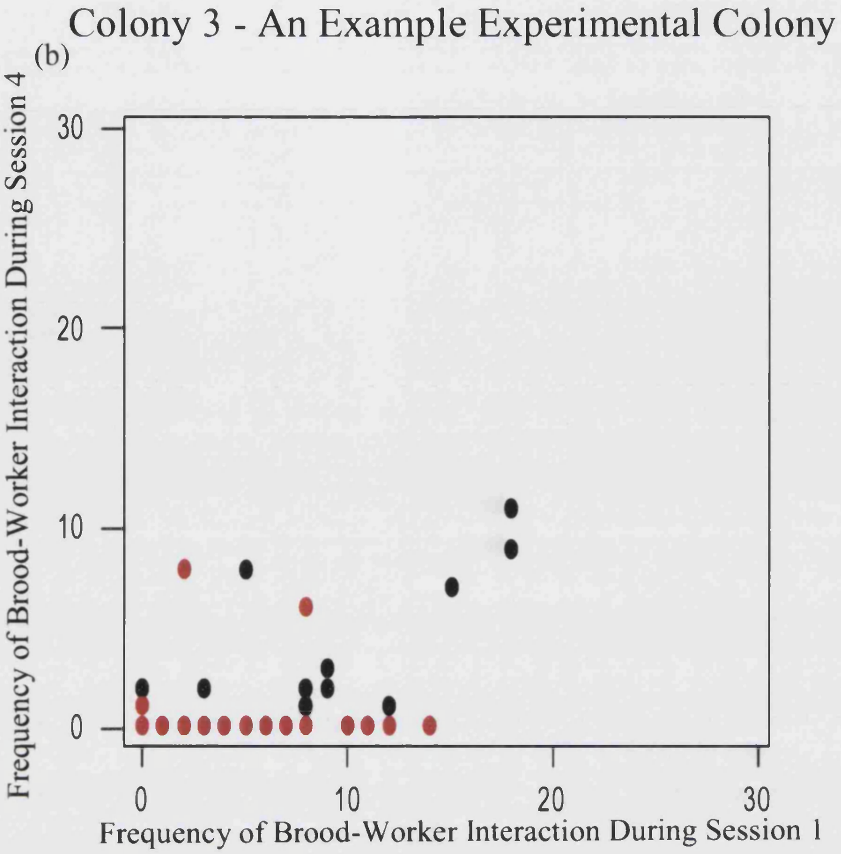
Experiment 1

Session 1 (Un-manipulated) and Session 4 (Re-united)

The graphs below show the relationship between the frequency at which workers perform brood-related tasks in the colony during session 1 and the frequency at which they perform brood-related tasks during session 4, in experiment 1. The Control colony (Colony C_1), and a typical experimental colony (Colony 3), are shown.

Fig 47(a)





Colony 3 - An Example Experimental Colony

(b)

Figure 47 : (a) The relationship between the frequency of brood-worker interaction in session 1, when the colonies remain un-manipulated and session 4, when the two fractions of the experimental colonies are re-united, for Colony C_1, the Control; and (b) Colony 3, an example experimental colony, experiment 1.

The relationship between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4 is further examined by carrying out correlation analyses according to the procedure described at the beginning of Chapter 4. The results are shown in Table 38, below. There is a significant, positive correlation between the relative frequency at which individuals perform brood-related tasks during session 1 and the relative frequency at which they perform brood-related tasks during session 4 for the Control colony (Colony C_1). This is not the case for experimental colonies 1, 3 and 4. There is a significant correlation at $p < 0.05$, for Colony 2. These results are discussed in section 8.3i.

I also compare the resilience of individual brood-related task performance between session 1 and session 2 (during which individuals associated with external activity are being removed), and session 3 (original fraction), see Table 38. Plots of these comparisons are given for the Control colony, and for a typical experimental colony (see Figures 48 and 49, respectively). As there is no brood present in the subsidiary fractions of session 3, there can be no comparison of the resilience of brood-related task performance involving these fractions.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_1			
Session 1 / Session 4	0.469	29	0.01
Session 1 / Session 2	0.668	36	0.01
Session 1 / Session 3	0.602	36	0.01
Colony 1			
Session 1 / Session 4	0.286	26	NS
Session 1 / Session 2	0.314	20	NS
Session 1 / Session 3 (Orig)	-0.814	6	NS
Session 1 / Session 3 (Subsid)	-	-	-
Colony 2			
Session 1 / Session 4	0.424	30	0.05
Session 1 / Session 2	0.544	27	0.01
Session 1 / Session 3 (Orig)	0.114	17	NS
Session / Session 3 (Subsid)	-	-	-
Colony 3			
Session 1 / Session 4	0.156	41	NS
Session 1 / Session 2	0.287	23	NS
Session 1 / Session 3 (Orig)	0.319	15	NS
Session 1/ Session 3 (Subsid)	-	-	-
Colony 4			
Session 1 / Session 4	0.342	21	NS
Session 1 / Session 2	0.718	18	0.01
Session 1 / Session 3 (Orig)	0.500	5	NS
Session 1 / Session 3 (Subsid)	-	-	-

Table 38: The relationship between the frequency at which individuals perform brood-related tasks compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where n ≤ 10. '-' = no brood present during session 3.

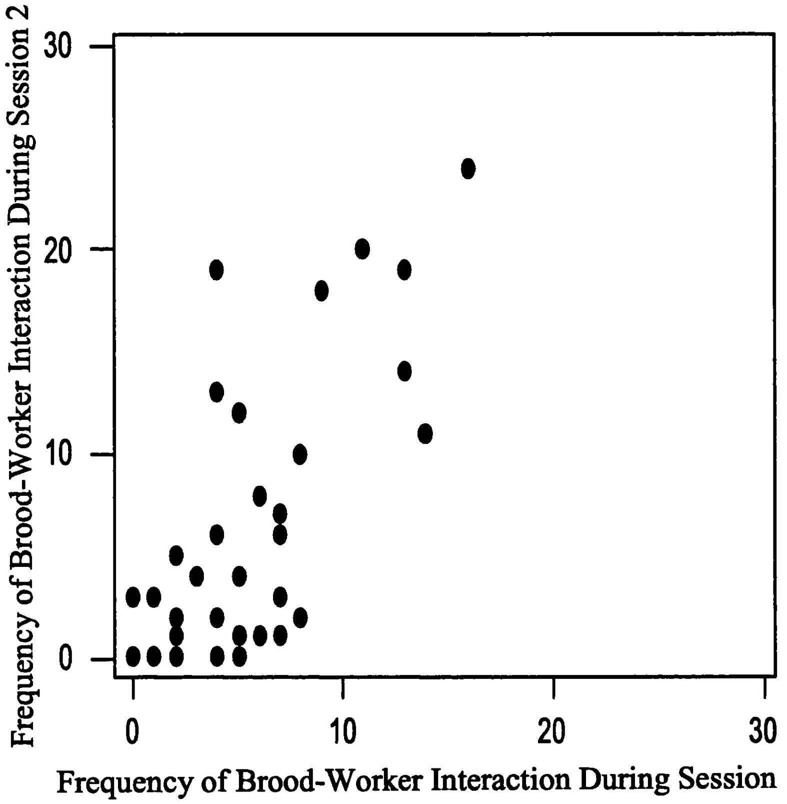
A closer examination of the comparison of the resiliency of individual brood-related task performance between sessions 1 and 4 is given in Table 39, below. Correlation analyses are performed separately for individuals that were in the original fractions of each colony, and those that were in the subsidiary fractions during session 3. These results show that there is no correlation between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4, for individuals that were in the original fractions during session 3, or for individuals that were in the subsidiary fractions. An exception to this is Colony 3, which shows a significant, negative correlation between the variables ($p < 0.05$). These results are discussed in section 8.3i.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony 1			
Session 1 / Session 4 (Original Fraction)	-0.543	6	NS
Session 1 / Session 4 (Subsidiary Fraction)	0.167	20	NS
Colony 2			
Session 1 / Session 4 (Original Fraction)	0.419	17	NS
Session 1 / Session 4 (Subsidiary Fraction)	-0.049	13	NS
Colony 3			
Session 1 / Session 4 (Original Fraction)	0.406	15	NS
Session 1 / Session 4 (Subsidiary Fraction)	-0.389	26	0.05
Colony 4			
Session 1 / Session 4 (Original Fraction)	-	4	-
Session 1 / Session 4 (Subsidiary Fraction)	0.375	17	NS

Table 39 : The relationship between the frequency at which individuals perform brood-related tasks compared between sessions 1 and 4, calculated separately for those individuals that were in the original and those that were in the subsidiary fraction of the colony during session 3. Calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$. '-'= n is insufficient to calculate statistic.

Colony C_1 - Control

(a) **Session 1 (Un-manipulated) and Session 2**



(b) **Session 1 (Un-manipulated) and Session 3**

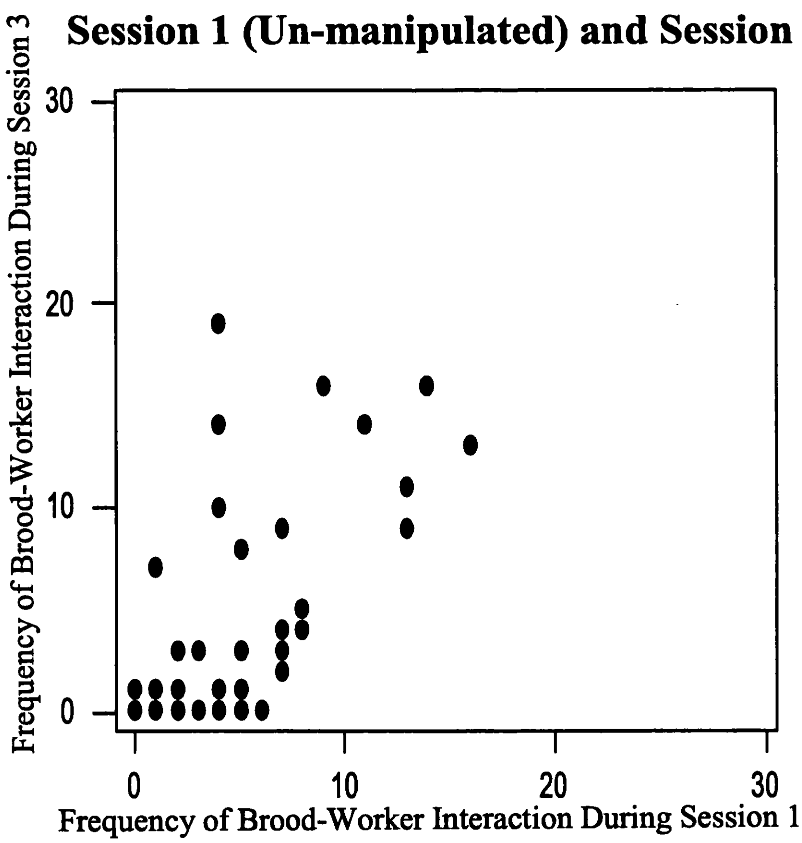
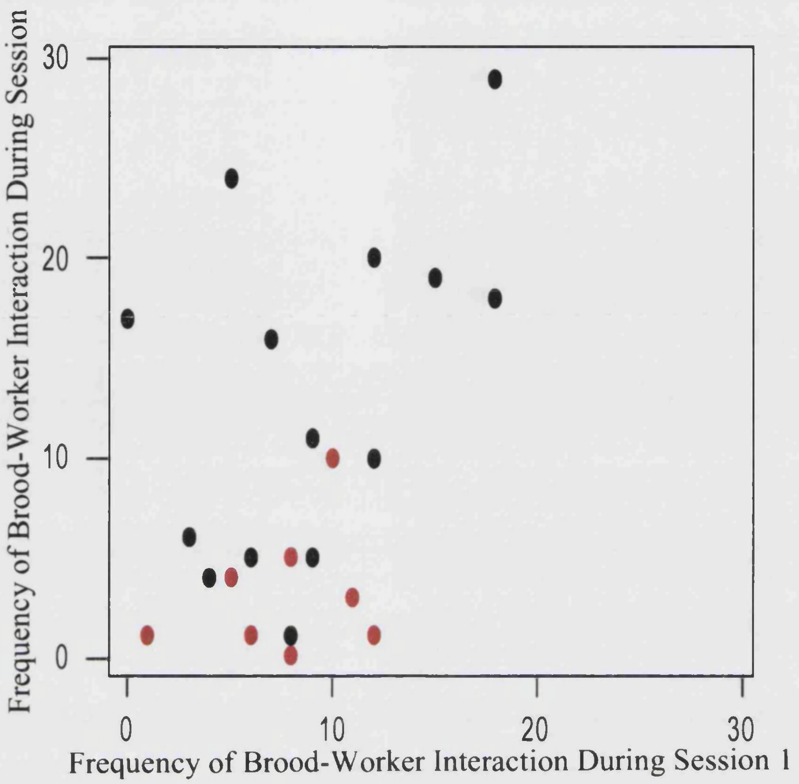


Figure 48 : (a) The relationship between the frequency of brood-worker interaction in session 1 and session 2; and (b) session 1 and session 3, for Colony C_1, the Control.

Colony 3 - An Example Experimental Colony

(a) **Session 1 (Un-manipulated) and Session 2**



(b) **Session 1 (Un-manipulated) and Session 3 -Original fraction**

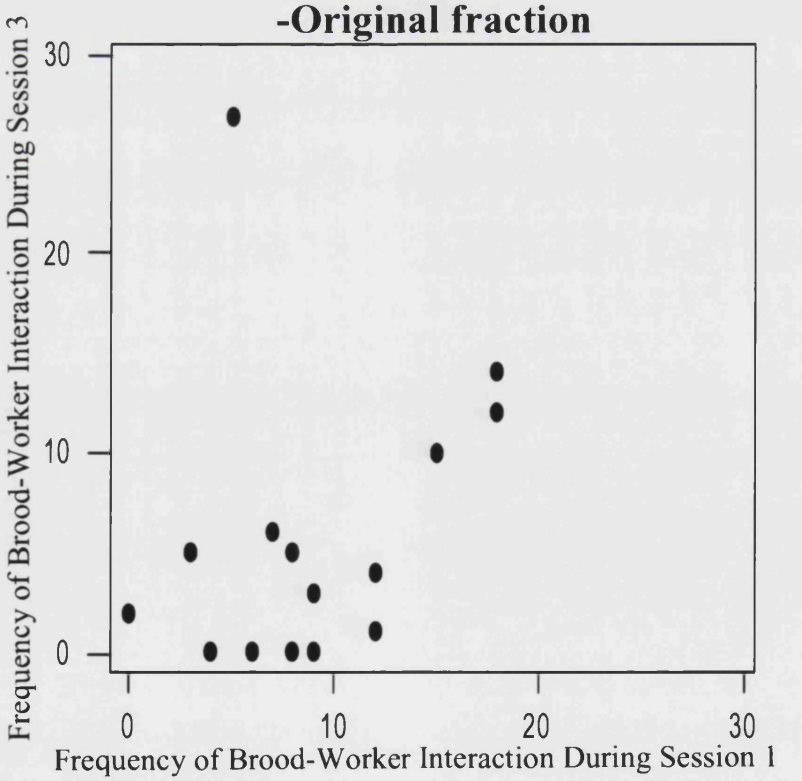


Figure 49 : (a) The relationship between the frequency of brood-worker interaction in session 1 and session 2; and (b) session 1 and session 3, original fraction, for Colony 3, an example experimental colony.

I carry out further analysis on the results of experiment 1 to determine if sociotomy affects the absolute frequency at which individuals carry out brood-related tasks. I compared the distribution of the frequency at which brood-related tasks are performed in each colony during each session of the experiment (excluding session 3 (subsidiary fraction) as there was no brood present in this fraction) with the distribution of the absolute frequency of brood-related tasks performed in session 1 using Mann-Whitney U tests (see Table 40, below).

Colonies / Sessions Compared	**N $_1$**	**N $_2$**	**η_1**	**η_2**	**W**	**Significance Level p <**
Colony C_1						
1 and 2	58	40	3.0	3.5	2774.0	NS
1 and 3	58	40	3.0	2.5	2920.0	NS
1 and 4	58	33	3.0	2.0	2773.0	NS
Colony 1						
1 and 2	64	23	3.5	6.0	2690.5	NS
1 and 3 (original fraction)	64	8	3.5	9.0	2214.5	0.05
1 and 3 (subsidiary fraction)	No Brood Present					
1 and 4	64	30	3.5	0.0	3313.5	0.05
Colony 2						
1 and 2	68	29	4.0	10.0	2806.0	0.01
1 and 3 (original fraction)	68	19	4.0	3.0	2965.5	NS
1 and 3 (subsidiary fraction)	No Brood Present					
1 and 4	68	31	4.0	1.0	3529.5	NS
Colony 3						
1 and 2	58	27	4.5	6.0	2295.0	NS
1 and 3 (original fraction)	58	18	4.5	4.5	2212.5	NS
1 and 3 (subsidiary fraction)	No Brood Present					
1 and 4	58	48	4.5	0.0	3753.0	0.01
Colony 4						
1 and 2	52	18	2.5	5.0	1753.5	NS
1 and 3 (original fraction)	52	5	2.5	4.0	1483.0	NS
1 and 3 (subsidiary fraction)	No Brood Present					
1 and 4	52	22	2.5	1.0	2116.0	0.05

Table 40 : A comparison of the distribution of the frequency at which brood-related tasks are performed by colonies in session 1 and the other sessions, experiment 1, using Mann-Whitney U Tests. η_1 *= median frequency of brood-related tasks performed during session 1 of the experiment.* η_2 *= median frequency of brood-related tasks performed during the session being compared to.*

I also compare the distribution of the frequency at which brood-related tasks are performed during each session for the experimental colonies with the relevant session of the Control colony (see Table 41). The results are discussed in section 8.3i.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	**Significance Level p<**
Session 1						
C_1 vs Colony 1	58	64	3.0	3.5	3365.5	NS
C_1 vs Colony 2	58	68	3.0	4.0	3614.5	NS
C_1 vs Colony 3	58	58	3.0	4.5	3156.5	NS
C_1 vs Colony 4	58	52	3.0	2.5	3190.5	NS
Session 2						
C_1 vs Colony 1	40	23	3.5	6.0	1204.5	NS
C_1 vs Colony 2	40	29	3.5	10.0	1143.5	0.01
C_1 vs Colony 3	40	27	3.5	6.0	1223.0	NS
C_1 vs Colony 4	40	18	3.5	5.0	1143.5	NS
Session 3 – Original Fraction						
C_1 vs Colony 1	40	8	2.5	9.0	903.5	0.05
C_1 vs Colony 2	40	19	2.5	3.0	1162.5	NS
C_1 vs Colony 3	40	18	2.5	4.5	1124.0	NS
C_1 vs Colony 4	40	5	2.5	4.0	897.5	NS
Session 3 – Subsidiary Fraction						
C_1 vs Colony 1	40	24	No Brood Present			
C_1 vs Colony 2	40	17				
C_1 vs Colony 3	40	36				
C_1 vs Colony 4	40	19				
Session 4						
C_1 vs Colony 1	33	30	2.0	0.0	1137.5	NS
C_1 vs Colony 2	33	31	2.0	1.0	1103.0	NS
C_1 vs Colony 3	33	48	2.0	0.0	1634.5	0.01
C_1 vs Colony 4	33	22	2.0	1.0	998.0	NS

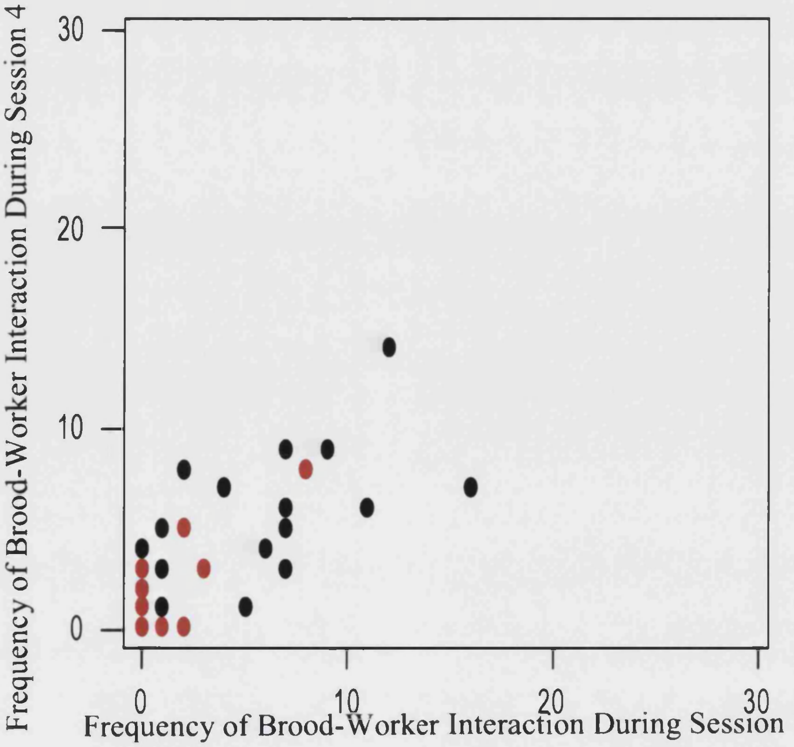
Table 41 : A comparison of the distribution of the frequency at which brood-related tasks are performed in each session of the experimental colonies with the relevant session of the Control colony, using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median frequency of brood-related tasks performed in the relevant session of the Control.* η_{EXP} *= median frequency of brood-related tasks performed in the relevant session of the experimental colony being considered.*

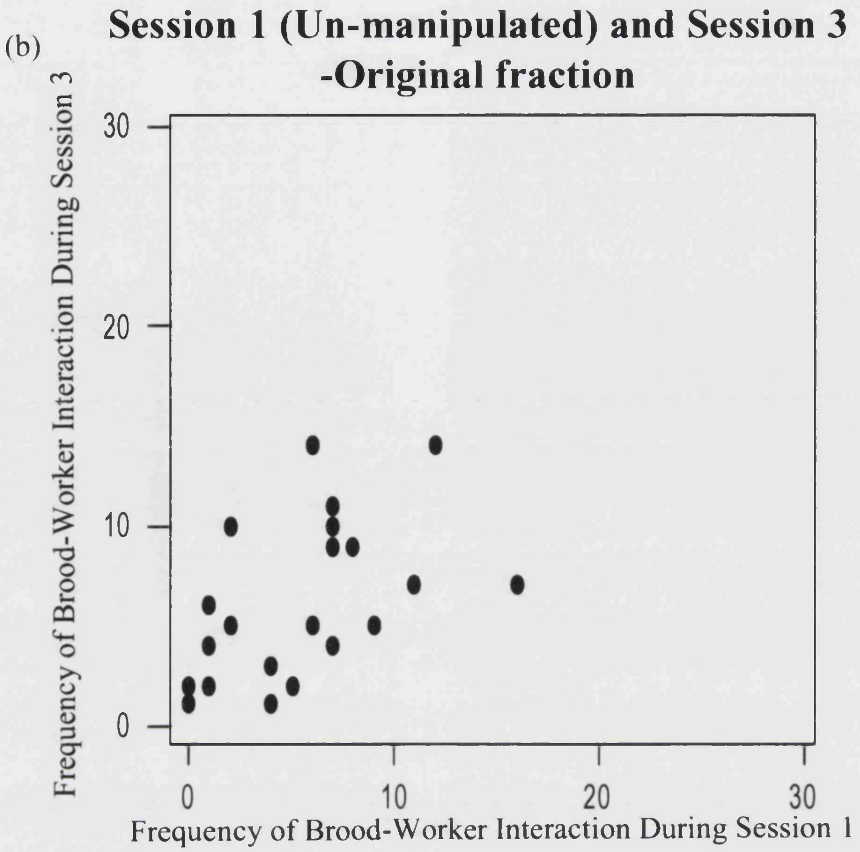
Experiment 2

I carry out the same statistical analysis as in section 8.1i, above, on the results from experiment 2 (see Table 42). There is no correlation between the relative frequency at which individuals perform brood-related tasks during session 1 compared to session 4, or to session 3, for the Control colony in which the queen died (Colony C_2). Colony C_2 has been discussed in section 4.3i. The results of the analysis in this chapter are consequently discussed with respect to the results from the Control colony from experiment 1 (Colony C_1). There is a significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4, and also, to the relevant fraction of session 3, for Colonies 7 and 8. Example plots of these comparisons are given for Colony 7 (see Figure 50, below). There is insufficient data, due to the number of individuals that have not retained their marks, in Colonies 5 and 6, and correlations cannot be calculated, except for a comparison of the resiliency of individual brood-related task performance in session 1 with session 4 for Colony 6, which did not give a significant result. This is discussed further in section 8.3i.

Colony 7 - An Example Experimental Colony

Fig 50(a) **Session 1 (Un-manipulated) and Session 4 (Re-united)**




(b) Session 1 (Un-manipulated) and Session 3 -Original fraction

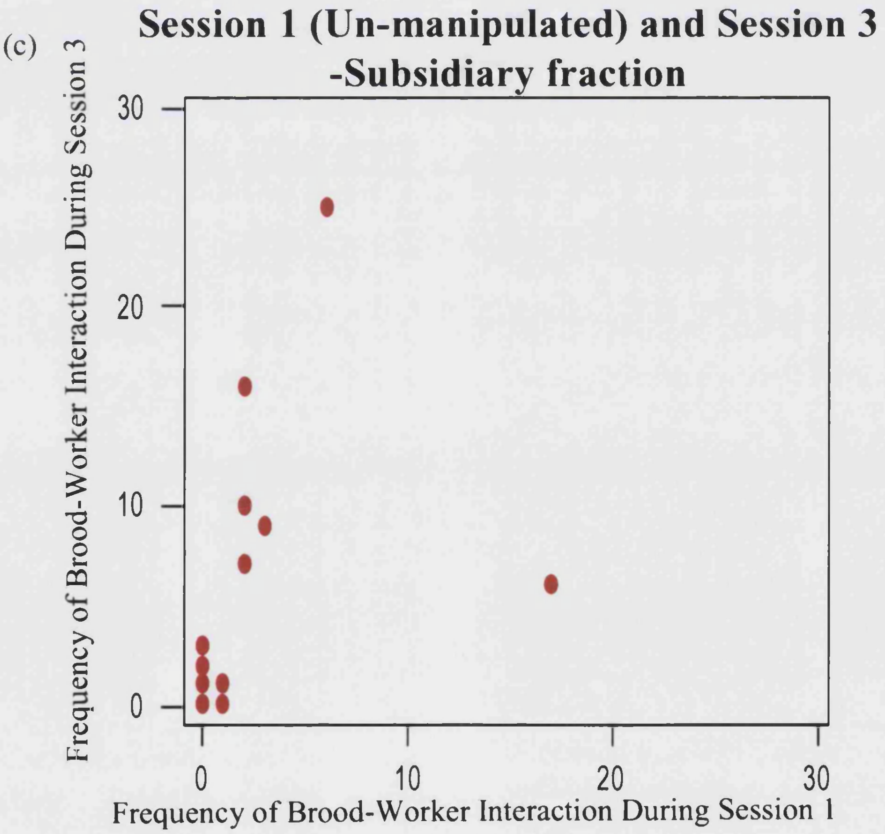

(c) Session 1 (Un-manipulated) and Session 3 -Subsidiary fraction

Figure 50 : The relationship between the frequency of brood-worker interaction in (a) session 1 and session 4; (b) session 1 and session 3, original fraction; and,(c) session 1 and session 3, subsidiary fraction, for Colony 7, an example experimental colony. Individuals were in the subsidiary fraction of the colony during session 3 are shown in red

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_2			
Session 1 / Session 4	0.520	14	NS
Session 1 / Session 3	-0.026	16	NS
Colony 5			
Session 1 / Session 4		2	
Session 1 / Session 3 (Original Fraction)		1	
Session 1 / Session 3 (Subsidiary Fraction)	-	-	-
Colony 6			
Session 1 / Session 4	0.241	7	NS
Session 1 / Session 3 (Original Fraction)	-	1	-
Session / Session 3 (Subsidiary Fraction)	-	4	-
Colony 7			
Session 1 / Session 4	0.666	35	0.01
Session 1 / Session 3 (Original Fraction)	0.678	23	0.01
Session 1/ Session 3 (Subsidiary Fraction)	0.682	19	0.01
Colony 8			
Session 1 / Session 4	0.634	27	0.01
Session 1 / Session 3 (Original Fraction)	0.601	16	0.05
Session 1 / Session 3 Subsidiary Fraction	0.749	17	0.01

Table 42 : The relationship between the frequency at which individuals perform brood-related tasks compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where n > 10 (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where n ≤ 10.

A Fisher's z-transform shows that there is no significant difference between the correlation of the Control colony, and the experimental colonies 6, 7 and 8, for the comparison between session 1 and session 4, see Table 43, below.

Colonies Compared	r_s	r_{s2}	n_1	n_2	z_1	z_2	t_s	**Significance level P<**
C_1 and 6	0.469	0.241	29	7	0.509	0.246	0.490	NS
C_1 and 7	0.469	0.666	29	35	0.509	0.804	-1.116	NS
C_1 and 8	0.469	0.634	29	27	0.509	0.748	-0.845	NS

Table 43 : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals perform brood-related tasks in session 1 and session 4 with that of Control colony C_1, calculated as a Fisher's z-transform.

A closer examination of the comparison of the resiliency of individual brood-related task performance between sessions 1 and 4, is given in Table 44, below. I carry out correlation analyses separately for individuals that were in the original fractions of each colony, and for those that were in the subsidiary fractions, during session 3. For the experimental colonies for which there is sufficient data to calculate a correlation coefficient, the results show that there is a significant, positive correlation between the relative frequency at which individuals that were in the original fractions perform brood-related tasks in session 1 compared to session 4 (Colonies 7 and 8). There is no correlation between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4 for individuals that were in the subsidiary fractions of the colonies during session 3, for Colonies 6, 7 and 8. These results are discussed in section 8.3i.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony 5			
Session 1 / Session 4 (Original Fraction)	-	0	-
Session 1 / Session 4 (Subsidiary Fraction)	-	2	-
Colony 6			
Session 1 / Session 4 (Original Fraction)	-	0	-
Session 1 / Session 4 (Subsidiary Fraction)	0.241	7	NS
Colony 7			
Session 1 / Session 4 (Original Fraction)	0.679	20	0.01
Session 1 / Session 4 (Subsidiary Fraction)	0.285	15	NS
Colony 8			
Session 1 / Session 4 (Original Fraction)	0.699	14	0.01
Session 1 / Session 4 (Subsidiary Fraction)	0.383	13	NS

Table 44: The relationship between the frequency at which individuals perform brood-related tasks in session 1 and session 4, calculated separately for individuals that were n the original and the subsidiary fraction of the colony during session 3. Calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

I carry out further analysis on the results of experiment 2 to determine if sociotomy affects the absolute frequency at which individuals carry out brood-related tasks. I compare the distribution of the frequency of performance of brood-related tasks in each colony during each session of the experiment with the distribution of the frequency of performance of brood-related tasks during session 1, using Mann-Whitney U tests (see Table 45, below).

Colonies / Sessions Compared	N_1	N_2	η_1	η_2	W	**Significance Level p<**
Colony C_1						
1 and 3	58	40	3.0	2.5	2920.0	NS
1 and 4	58	33	3.0	2.0	2773.0	NS
Colony 5						
1 and 3 (original fraction)	34	0	-	-	-	-
1 and 3 (subsidiary fraction)	34	1	-	-	-	-
1 and 4	34	2	1.0	2.0	613.5	NS
Colony 6						
1 and 3 (original fraction)	25	1	-	-	-	-
1 and 3 (subsidiary fraction)	25	6	1.0	5.0	359.0	0.05
1 and 4	25	7	1.0	2.0	379.0	NS
Colony 7						
1 and 3 (original fraction)	57	23	1.0	5.0	1991.0	0.01
1 and 3 (subsidiary fraction)	57	19	1.0	1.0	2097.0	NS
1 and 4	57	35	1.0	3.0	2413.5	NS
Colony 8						
1 and 3 (original fraction)	72	18	2.0	8.5	2895.0	0.01
1 and 3 (subsidiary fraction)	72	19	2.0	2.0	3214.0	NS
1 and 4	72	31	2.0	4.0	3565.0	NS

Table 45 : A comparison of the distribution of the frequency of performance of brood-related tasks in session 1 of experiment 2 and the other sessions, for each colony, using Mann-Whitney U Tests. η_1 *= median frequency of brood-related tasks during session 1 of the experiment.* η_2 *= median frequency of brood-related tasks performed during the session to which session 1 is being compared..*

I also compare the distribution of the frequency of brood-related tasks performance during each session for the experimental colonies with the relevant session of the Control colony (see Table 46). The results are discussed in section 8.3i.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1						
C_1 vs Colony 5	58	34	3.0	1.0	3179.0	0.01
C_1 vs Colony 6	58	25	3.0	1.0	2687.0	0.05
C_1 vs Colony 7	58	57	3.0	1.0	3789.5	0.05
C_1 vs Colony 8	58	72	3.0	2.0	4137.0	NS
Session 3 – Original Fraction						
C_1 vs Colony 5	40	0	2.5	-	-	-
C_1 vs Colony 6	40	1	2.5	-	-	-
C_1 vs Colony 7	40	23	2.5	5.0	1164.0	NS
C_1 vs Colony 8	40	18	2.5	8.5	1037.0	0.05
Session 3 – Subsidiary Fraction						
C_1 vs Colony 5	40	1	2.5	-	-	-
C_1 vs Colony 6	40	6	2.5	5.0	907.0	NS
C_1 vs Colony 7	40	19	2.5	1.0	1209.0	NS
C_1 vs Colony 8	40	19	2.5	2.0	1184.0	NS
Session 4						
C_1 vs Colony 5	33	2	2.0	2.0	596.5	NS
C_1 vs Colony 6	33	7	2.0	2.0	664.5	NS
C_1 vs Colony 7	33	35	2.0	3.0	1083.5	NS
C_1 vs Colony 8	33	31	2.0	4.0	1027.0	NS

Table 46 : A comparison of the distribution of the frequency at which brood-related tasks are performed in each session of the experimental colonies with the relevant session of the Control colony (note that Colony C_1 is used), using Mann-Whitney U Tests. $\eta_{CONTROL}$ = median frequency of performance of brood-related tasks in the relevant session of the Control.. η_{EXP} = median frequency of performance of brood-related tasks in the relevant session of the experimental colony being considered.

Multiple comparisons using pooled data

I determine that there is a significant relationship between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 4 for colonies in which sufficient numbers of indivduals have retained their marks in experiment 2. To determine if sociotomy has a significant effect on the relationship between the ranked frequency at which individuals perform brood-related tasks in session 1 (un-manipulated colonies) compared to session 4 (after re-unification) I perform multiple comparison analysis. Using the method outlined in Chapter 7, I determine whether the colonies can be considered to be samples from a population exhibiting a common correlation between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 4. I then compare the pooled *z* from the experimental colonies to *z* calculated for the Control colony for this relationship. The results are shown in Table 46a, below

$\bar{z}_{exp}$ session 1 and 4	**χ^2 exp session 1and 4**	**Signif. Level$_{exp}$ session1&4 P<**	**$z_{control}$**	**$n_{exp\ session\ 1\&4}$**	**$n_{control}$**	**t_s**	**Signif. level p<**
Exp 2							
0.780	0.042	NS	0.509	28.0	29.0	0.967	NS

Table 46a: Testing the relationship between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 4 for combined colonies in experiment 2, compared to this relationship in the Control colony. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the experimental colonies for in experiment 2. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 compared to session 4, with *z* calculated for this relationship for the Control colony shows that there is no significant difference between this relationship for the experimental colonies and the Control colony in experiment 2.

ii. The flexibility of individual performance of external activity

I investigate the effect of sociotomy on individual performance of external activity in experiments 1 and 2. Similar methods of analysis are used as in section 8.1i., above.

The frequency at which each individual performs external activity during each experimental session is determined from the behavioural record for that session.

Data files are created for each pair of sessions to be compared. The data files consist of the total frequency at which external activity is performed by particular individuals in each of the two sessions.

Individuals are included in the analysis if they:

i.either, have at least one recorded position in the nest (even if the frequency at which they perform external activity is zero, in any particular session), or:

ii. have been recorded as performing external activity but do not have a recorded position in the nest.

Individuals are only included in the analysis if they are individually recognisable throughout both sessions to be compared.

The relationship between the frequency at which external activity is performed by particular individuals in session 1, and the frequency at which they perform external activity in the session to which session 1 is being compared, is investigated by plotting graphs of these variables for the Control, and for example experimental colonies.

In the analysis individuals that were in the subsidiary fractions of the colonies during session 3 are represented in red, individuals that were in the original fraction, in black (experiments 1 and 2).

In each plot the chronologically later session is represented on the y axis.

It should be noted that points may overlap on the graphs and this accounts for any discrepancy between the number of points on each plot, and the corresponding '*n*' in the statistical tables.

Individuals are ranked with respect to the frequency at which they perform external activity in each session to be compared.

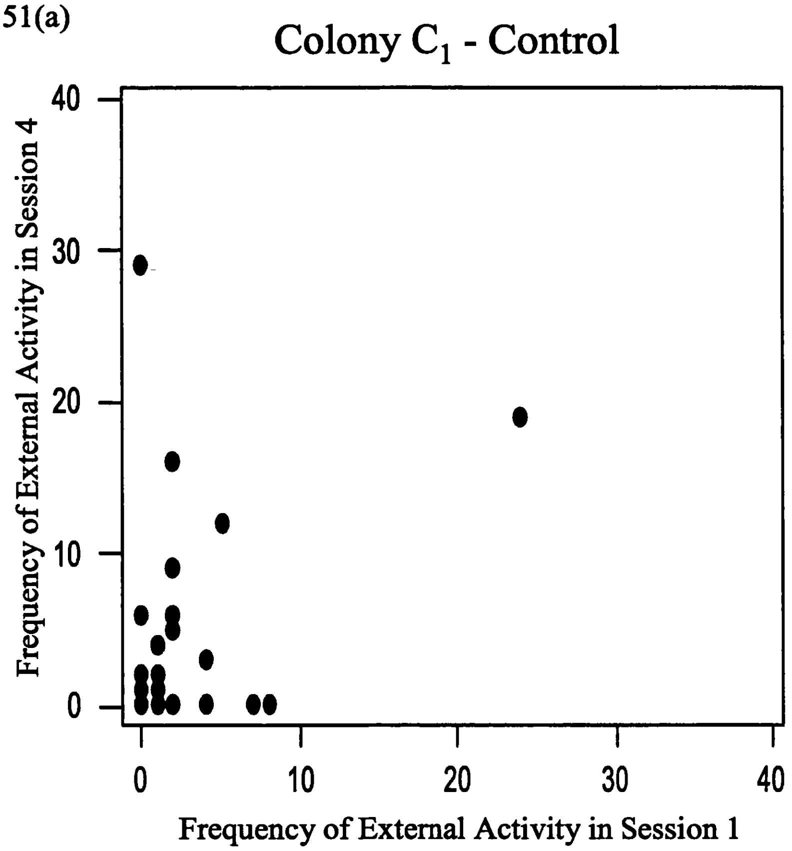
Correlation analyses are carried out for all the colonies according to the procedures described at the beginning of Chapter 4.

Experiment 1

Session 1 (Un-manipulated) and Session 4 (Re-united)

The graphs below show the relationship between the frequency at which workers perform external activity during session 1 and the frequency at which they perform external activity during session 4, in experiment 1. The Control colony (Colony C_1), and an example of one of the experimental colonies (Colony 3), are shown.

Fig 51(a)



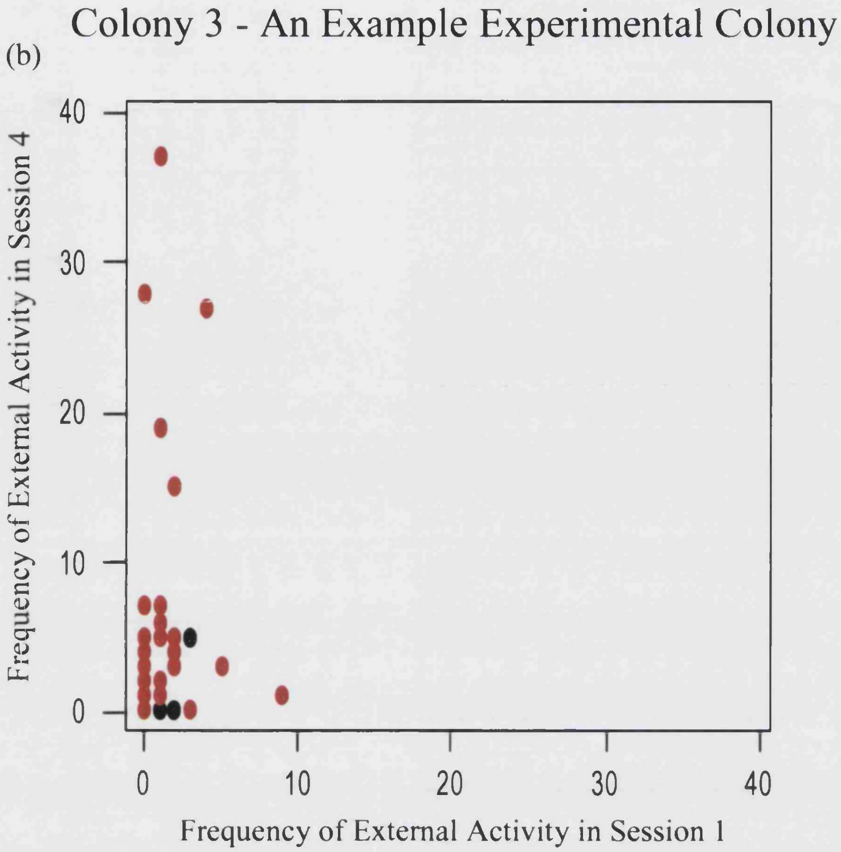


Figure 51 : The relationship between the frequency at which individuals perform external activity in (a) session 1, when the colonies remain un-manipulated and session 4, when the two fractions of the experimental colonies are re-united, for Colony C_1, the Control; and (b) Colony 3, an example experimental colony, experiment 1.

The relationship between the relative frequency at which individuals perform external activity in session 1 compared to session 4 is further examined by carrying out correlation analyses according to the procedure described at the beginning of Chapter 4. The results are shown in Table 47. There is a significant, positive correlation between the relative frequency at which individuals perform external activity during session 1 and the relative frequency at which they perform external activity during session 4 for the Control colony (Colony C_1). There is a significant, positive correlation between the relative frequency at which individuals perform external activity in session 1 compared to session 4 for Colonies 1 and 3. This relationship is not a significant correlation for Colonies 2 and 4. These results are discussed in section 8.3ii.

I also compare the resilience of individual performance of external activity between session 1 and session 2 (during which individuals associated with external activity are being removed), and to the relevant fraction of session 3 (original or subsidiary) , see Table 47, below. Example plots of these comparisons are also given for the Control colony, and for an example of one of the experimental colonies (see Figures 52 and 53, respectively).

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_1			
Session 1 / Session 4	0.390	35	0.05
Session 1 / Session 2	0.286	51	0.05
Session 1 / Session 3	0.391	46	0.01
Colony 1			
Session 1 / Session 4	0.492	44	0.01
Session 1 / Session 2	No External Activity in Session 2		
Session 1 / Session 3 (Orig)	No External Activity in Session 3 (Original Fraction)		
Session 1 / Session 3 (Subsid)	0.279	59	0.05
Colony 2			
Session 1 / Session 4	0.173	38	NS
Session 1 / Session 2	No External Activity in Session 2		
Session 1 / Session 3 (Orig)	No External Activity in Session 3 (Original Fraction)		
Session / Session 3 (Subsid)	0.170	28	NS
Colony 3			
Session 1 / Session 4	0.402	56	0.01
Session 1 / Session 2	0.115	60	NS
Session 1 / Session 3 (Orig)	0.118	18	NS
Session 1/ Session 3 (Subsid)	0.210	51	NS
Colony 4			
Session 1 / Session 4	0.148	32	NS
Session 1 / Session 2	No External Activity in Session 2		
Session 1 / Session 3 (Orig)	0.486	6	NS
Session 1 / Session 3 (Subsid)	0.139	47	NS

Table 47: The relationship between individuals' frequency of external activity compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

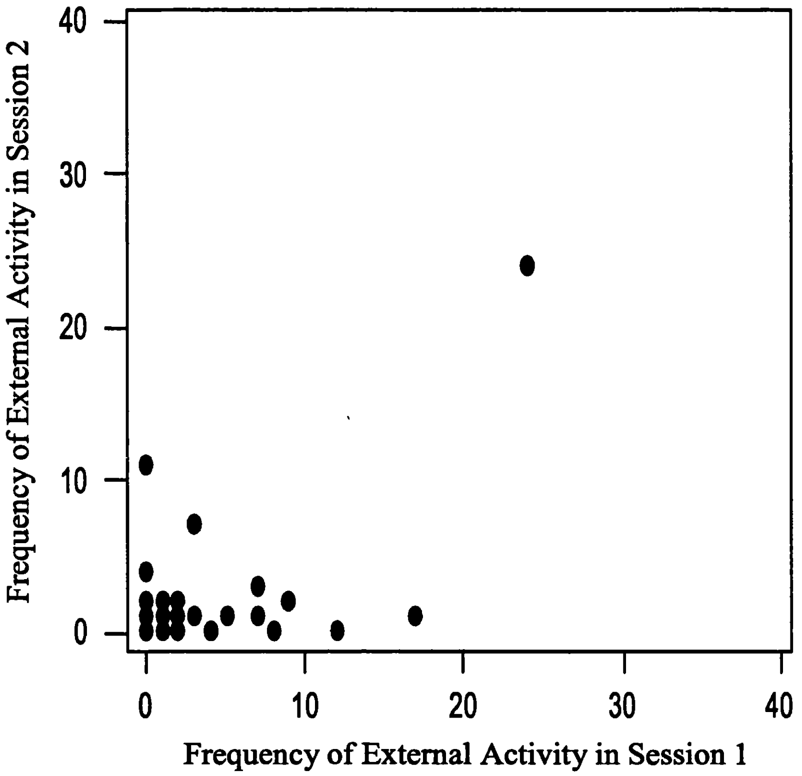
A closer examination of the comparison of the resiliency of individual performance of external activity between sessions 1 and 4 is given in Table 48, below. I carry out correlation analyses separately for individuals that were in the original fraction of each colony, and those that were in the subsidiary fractions during session 3. These results show that there is no correlation between the relative frequency at which individuals perform external activity in session 1 compared to session 4 either for individuals that were in the original, or for those that were in the subsidiary fractions, in Colonies 2 and 4. There is a significant, positive correlation between the relative frequency at which individuals perform external activity in sessions 1 and 4 for those individuals that were in the subsidiary fractions of Colonies 1 and 3, during session 3. There is no correlation for the comparison for individuals that were in the original fractions of these colonies. These results are discussed in section 8.3ii.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony 1			
Session 1 / Session 4 (Original Fraction)	0.536	7	NS
Session 1 / Session 4 (Subsidiary Fraction)	0.463	37	0.01
Colony 2			
Session 1 / Session 4 (Original Fraction)	0.401	19	NS
Session 1 / Session 4 (Subsidiary Fraction)	0.103	19	NS
Colony 3			
Session 1 / Session 4 (Original Fraction)	0.372	18	NS
Session 1 / Session 4 (Subsidiary Fraction)	0.327	40	0.05
Colony 4			
Session 1 / Session 4 (Original Fraction)	-0.029	6	NS
Session 1 / Session 4 (Subsidiary Fraction)	0.137	28	NS

Table 48 : The relationship between individuals' frequency of external activity compared between session 1 and session 4, calculated separately for individuals that were in the original and the subsidiary fraction of the colony during session 3, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

Colony C_1 - Control

(a) **Session 1 (Un-manipulated) and Session 2**



(b) **Session 1 (Un-manipulated) and Session 3**

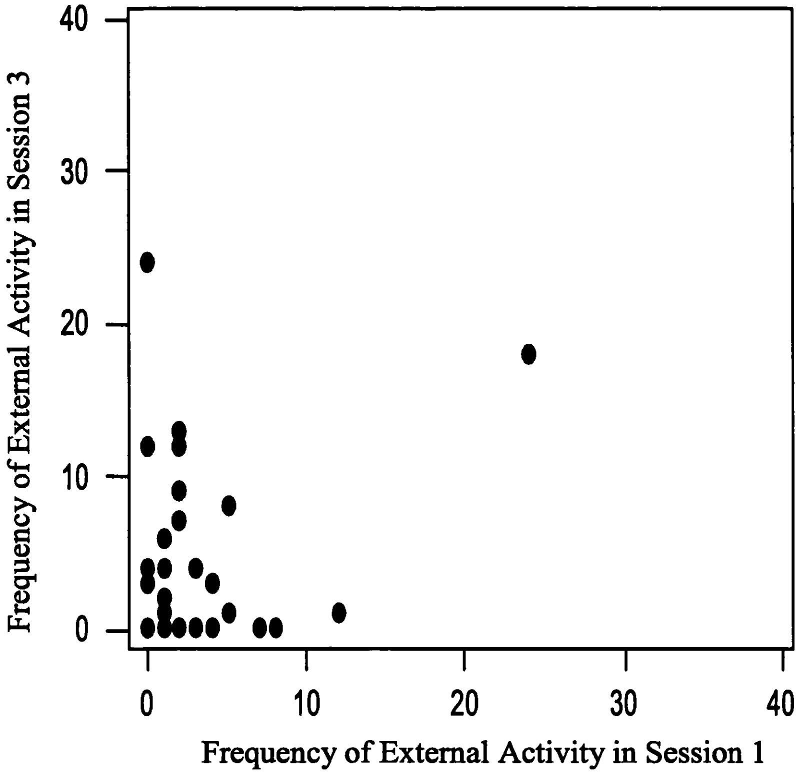


Figure 52 : (a) The relationship between the frequency at which individuals perform external activity in session 1 and session 2; and (b) session 1 and session 3, for Colony C_1*, the Control.*

Colony 3 - An Example Experimental Colony

Fig 53(a) **Session 1 (Un-manipulated) and Session 2**

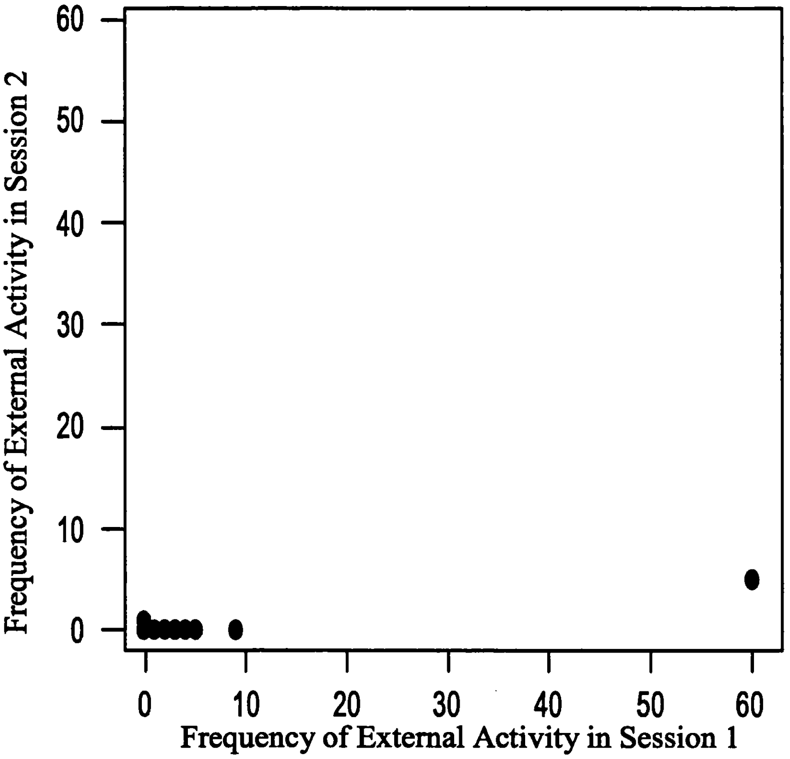
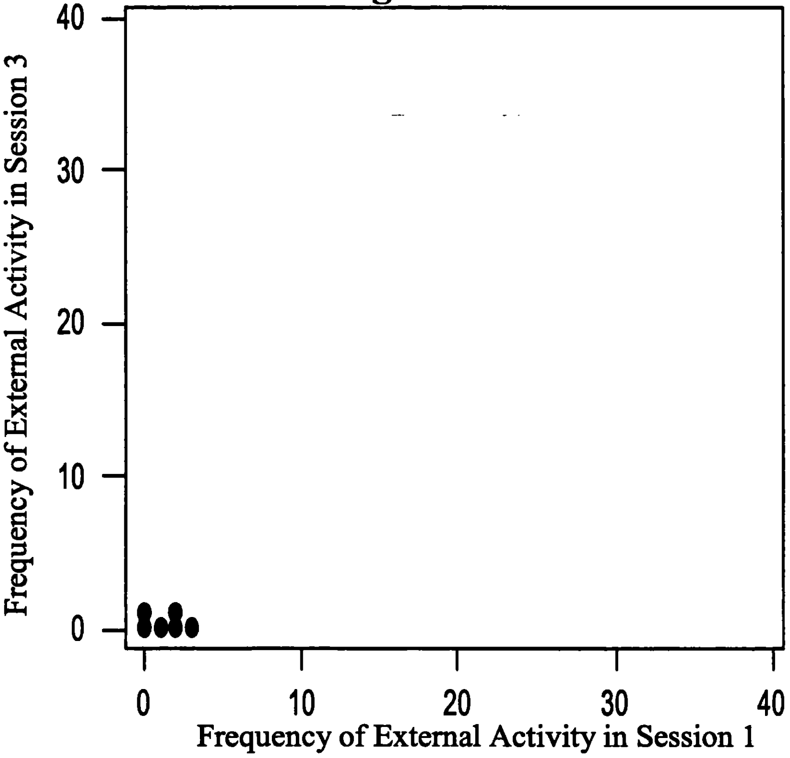


Fig 53(b) **Session 1 (Un-manipulated) and Session 3 -Original fraction**



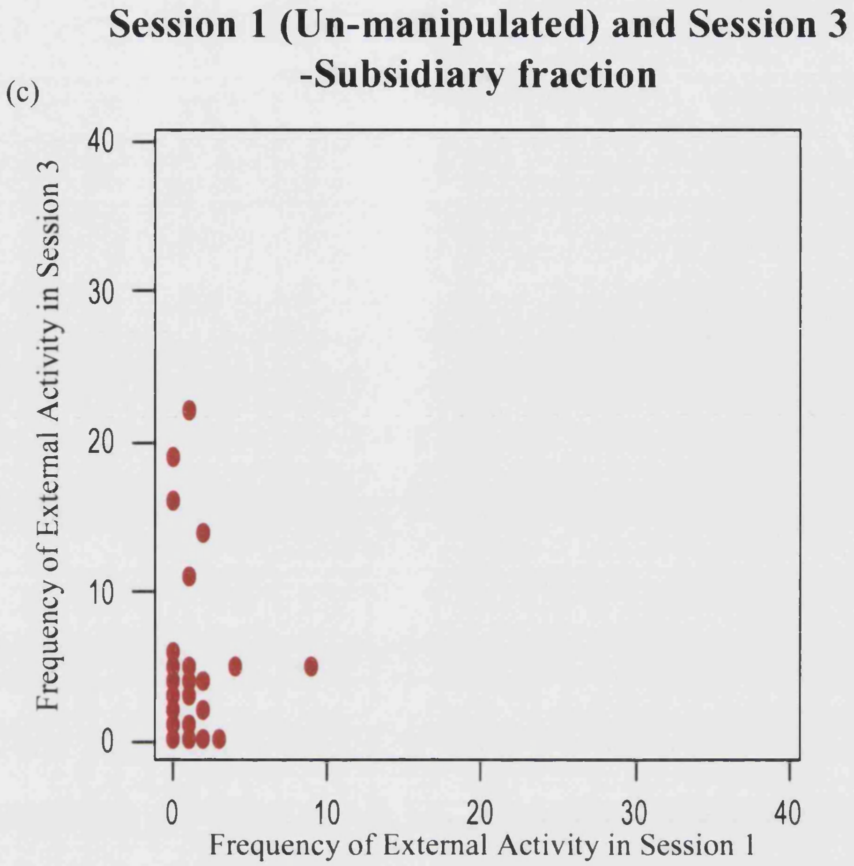


Figure 53 : (a) The relationship between the frequency at which individuals perform external activity in session 1 and session 2; (b) session 1 and session 3, original fraction; (c) session 1 and session 3, subsidiary fraction; for Colony 3, an example experimental colony. Individuals were in the subsidiary fraction of the colony during session 3 are shown in red.

I carry out further analysis of the results from experiment 1 to determine if sociotomy affects the absolute frequency at which individuals perform external activity. I compare the distribution of the frequency at which external activity is performed in each colony during each session of the experiment with the distribution of the frequency at which external activity is performed during session 1, using Mann-Whitney U tests (see Table 49, below).

Colonies / Sessions Compared	N_1	N_2	η_1	η_2	W	Significance Level p <
Colony C_1						
1 and 2	72	50	1.0	0.0	5077.5	0.01
1 and 3	72	48	1.0	0.0	4693.0	NS
1 and 4	72	37	1.0	0.0	4263.0	0.05
Colony 1						
1 and 2	No External Activity During Session 2					
1 and 3 (original fraction)	77	11	2.0	0.0	3646.0	0.01
1 and 3 (subsidiary fraction)	77	53	2.0	2.0	4803.0	NS
1 and 4	77	47	2.0	1.0	4952.0	NS
Colony 2						
1 and 2	No External Activity During Session 2					
1 and 3 (original fraction)	No External Activity During Session 3 (original fraction)					
1 and 3 (subsidiary fraction)	92	30	1.0	1.0	5842.5	NS
1 and 4	92	38	1.0	0.0	6602.5	0.01
Colony 3						
1 and 2	100	29	0.0	0.0	7018.5	0.01*
1 and 3 (original fraction)	100	20	0.0	0.0	6403.0	0.01*
1 and 3 (subsidiary fraction)	100	52	0.0	1.0	6737.0	0.01
1 and 4	100	59	0.0	1.0	7314.5	0.01
Colony 4						
1 and 2	No External Activity During Session 2					
1 and 3 (original fraction)	66	6	2.0	0.0	2535.5	0.01
1 and 3 (subsidiary fraction)	66	49	2.0	1.0	3900.5	NS
1 and 4	66	32	2.0	1.0	3527.5	0.01

*Table 49 : A comparison of the distribution of the frequency at which external activity is performed in session 1 of experiment 1 and the other sessions, for each colony, using Mann-Whitney U Tests. η_1 = median frequency of external activity during session 1 of the experiment. η_2 = median frequency of external activity during the session to which session 1 is being compared. * = one-tailed test (null hypothesis η_1 is not greater than η_2, H_1 : η_1 is greater than η_2)*

I also compare the absolute frequency at which external activity is performed during each session for the experimental colonies with the relevant session of the Control colony (see Table 50). The results are discussed in section 8.3ii.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1						
C_1 vs Colony 1	72	77	1.0	2.0	5317.5	NS
C_1 vs Colony 2	72	92	1.0	1.0	6016.5	NS
C_1 vs Colony 3	72	100	1.0	0.0	7401.0	0.01
C_1 vs Colony 4	72	66	1.0	2.0	4782.5	NS
Session 2						
C_1 vs Colony 1	50	No Ext Act	0.0	-	-	-
C_1 vs Colony 2	50	No Ext Act	0.0	-	-	-
C_1 vs Colony 3	50	29	0.0	0.0	2210.5	0.01
C_1 vs Colony 4	50	No Ext Act	0.0	-	-	-
Session 3 – Original Fraction						
C_1 vs Colony 1	48	11	0.0	0.0	1502.0	NS
C_1 vs Colony 2	48	No Ext Act	0.0	-	-	-
C_1 vs Colony 3	48	20	0.0	0.0	1824.0	0.01
C_1 vs Colony 4	48	6	0.0	0.0	1364.0	NS
Session 3 – Subsidiary Fraction						
C_1 vs Colony 1	48	53	0.0	2.0	2023.5	0.01
C_1 vs Colony 2	48	30	0.0	1.0	1800.5	NS
C_1 vs Colony 3	48	52	0.0	1.0	2176.5	NS
C_1 vs Colony 4	48	49	0.0	1.0	2038.5	0.05
Session 4						
C_1 vs Colony 1	37	47	0.0	1.0	1413.0	NS
C_1 vs Colony 2	37	38	0.0	0.0	1442.0	NS
C_1 vs Colony 3	37	59	0.0	1.0	1637.5	NS
C_1 vs Colony 4	37	32	0.0	1.0	1224.5	NS

Table 50 : A comparison of the distribution of the frequency at which external activity is performed in each session of the experimental colonies with the relevant session of the Control colony, using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median frequency of external activity in the relevant session of the Control..* η_{EXP} *= median frequency of external activity in the relevant session of the experimental colony being considered.*

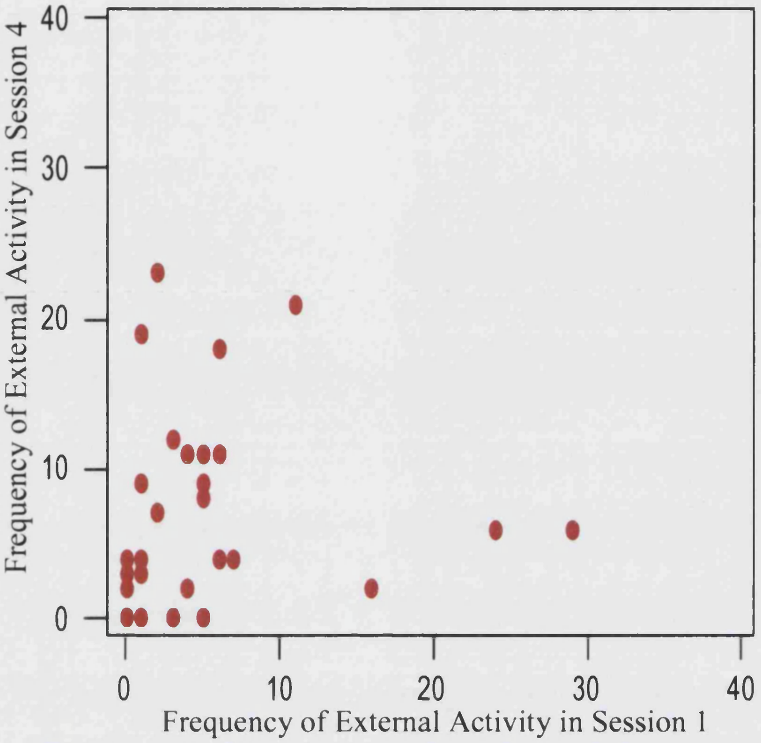
Experiment 2

I carry out the same statistical analysis as in section 8.1ii., above, on the results from experiment 2 (see Table 51). The relative frequency at which individuals perform external activity during session 1 is not correlated with the relative frequency at which they perform external activity during session 4 for the Control Colony (Colony C_2). However, it is correlated with the relative frequency of external activity during session 3 in this colony. Nevertheless, this Control colony is not used for comparisons in this Chapter (see section 4.3i.), and is given only for the sake of completeness. Colony C_1 is used for comparisons with the experimental colonies in experiment 2.

There is a significant, positive correlation between the relative frequency at which external activity is performed in session 1 compared to the relative frequency at which external activity is performed in session 4, for Colonies 6, 7 and 8 (See Table 51). Colonies 7 and 8 also show significant, positive correlations between the relative frequency at which external activity is performed in session 1 and in the subsidiary fraction of session 3. Colonies 5 and 6 do not show this significant correlation. Of the four experimental colonies, only Colony 8 shows any external activity during the photographic sessions in the original fraction during session 3, and the relative frequency at which these individuals perform external activity in this session is significantly correlated to the frequency at which they perform external activity in session 1. Plots of the comparison of the frequency at which individuals perform external activity in session 1 compared to session 4, and to the subsidiary fractions during session 3, are given for an example experimental colony (see Figure 54 (a) and (b), respectively). Also shown, Figure 54 (c), is a plot of the relationship between the frequency at which individuals perform external activity in session 1 compared to the original fraction of session 3 for the only colony for which external activity is recorded in the latter session (Colony 8).

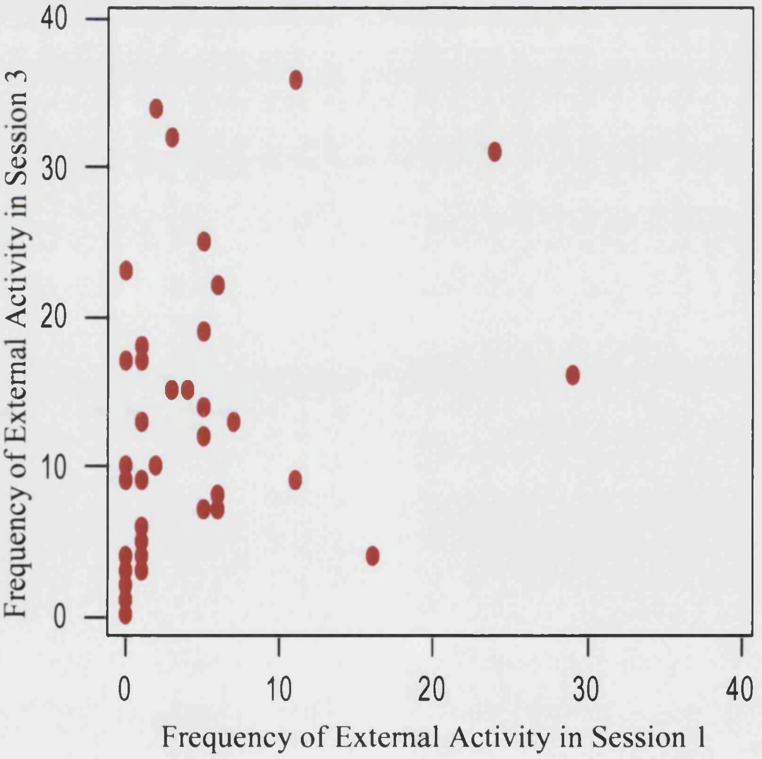
Colony 7 - An Example Experimental Colony

Fig 54(a)



Session 1 (Un-manipulated) and Session 4 (Re-united)

Fig 54(b)



Session 1 (Un-manipulated) and Session 3 (Subsidiary fraction)

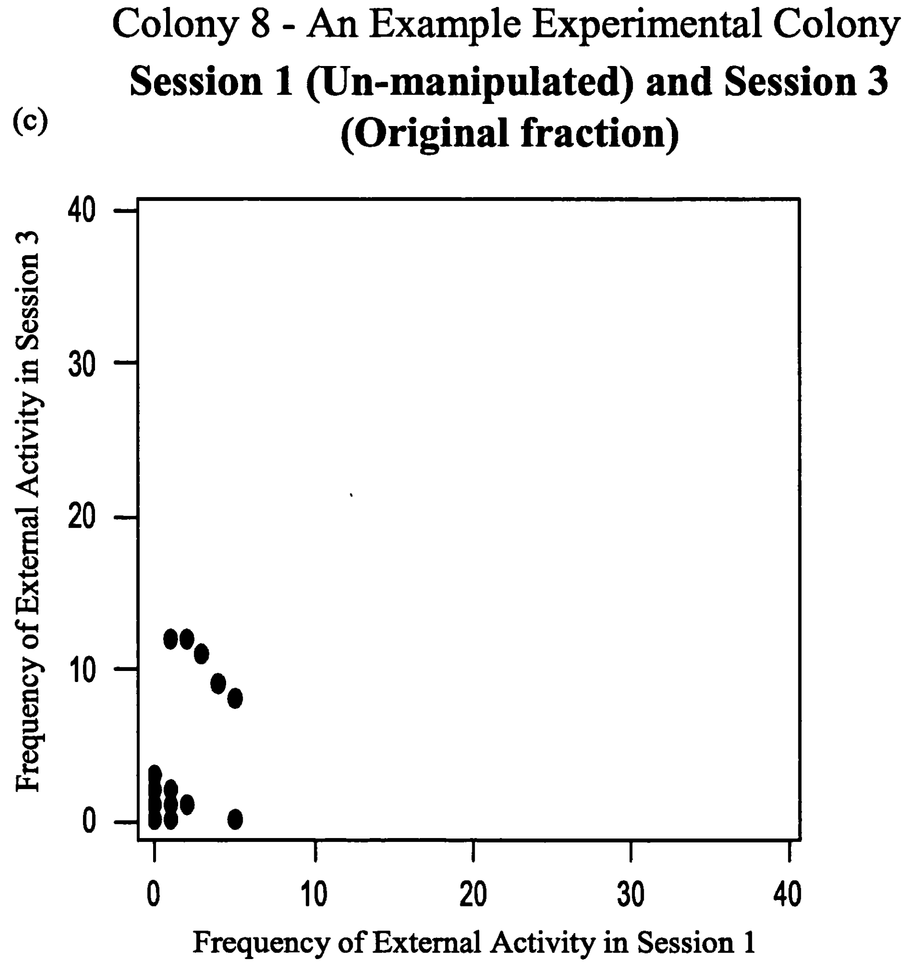


Figure 54 : The relationship between the frequency at which individuals perform external activity in (a) session 1 and session 4; (b) session 1 and session 3, subsidiary fraction, for Colony 7, an example experimental colony; (c) session 1 and session 3, original fraction, for Colony 8. Individuals that were in the subsidiary fraction of the colony during session 3 are shown in red.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony C_2			
Session 1 / Session 4	0.261	28	NS
Session 1 / Session 3	0.626	32	0.01
Colony 5			
Session 1 / Session 4	0.106	26	NS
Session 1 / Session 3 (Original Fraction)	No External Activity in Session 3 (Original Fraction)		
Session 1 / Session 3 (Subsidiary Fraction)	0.243	37	NS
Colony 6			
Session 1 / Session 4	0.650	12	0.05
Session 1 / Session 3 (Original Fraction)	No External Activity in Session 3 (Original Fraction)		
Session / Session 3 (Subsidiary Fraction)	0.439	18	NS
Colony 7			
Session 1 / Session 4	0.674	54	0.01
Session 1 / Session 3 (Original Fraction)	No External Activity in Session 3 (Original Fraction)		
Session 1/ Session 3 (Subsidiary Fraction)	0.561	44	0.01
Colony 8			
Session 1 / Session 4	0.572	55	0.01
Session 1 / Session 3 (Original Fraction)	0.537	23	0.01
Session 1 / Session 3 Subsidiary Fraction	0.316	43	0.05

Table 51 : The relationship between individuals' frequency of external activity compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

A Fisher's z-transform shows that there is no significant difference between the correlation of the Control colony (Colony C_1), and each of the experimental colonies 6, 7 and 8, for the comparison between sessions 1 and 4, see Table 52, below.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	Significance level P<
C_1 and 6	0.390	0.650	35	12	0.412	0.775	-0.963	NS
C_1 and 7	0.390	0.674	35	54	0.412	0.818	-1.801	NS
C_1 and 8	0.390	0.572	35	55	0.412	0.650	-1.062	NS

Table 52 : A comparison between the Spearman's rank correlation coefficients of frequency at which individuals perform external activity in session 1 and session 4 with that of Control colony C_1, calculated as a Fisher's z-transform.

A closer examination of the comparison between the resiliency of individual performance of external activity between sessions 1 and 4 is given in Table 53, below. I carry out correlation analyses separately for individuals that were in the original fractions of each colony, and for those that were in the subsidiary fractions of the colonies, during session 3. There is a significant, positive correlation between the relative frequency at which individuals that were in the subsidiary fractions perform external activity in session 1 compared to session 4, for Colonies 6,7 and 8. There is no correlation between these variables for individuals that were in the subsidiary fraction of Colony 5 during session 3. Of the individuals that were in the original fractions during session 3 in these colonies, only Colony 8 shows sufficient external activity during session 4 for it to be possible to establish a correlation. This relationship is a significant, positive correlation. These results are discussed in section 8.3ii.

Colony/Sessions Compared	r_s	n	Significance level p<
Colony 5			
Session 1 / Session 4 (Original Fraction)	-	0	-
Session 1 / Session 4 (Subsidiary Fraction)	0.106	26	NS
Colony 6			
Session 1 / Session 4 (Original Fraction)	-	1	-
Session 1 / Session 4 (Subsidiary Fraction)	0.615	11	0.05
Colony 7			
Session 1 / Session 4 (Original Fraction)	-	19	Insufficient external activity to establish correlation
Session 1 / Session 4 (Subsidiary Fraction)	0.428	35	0.01
Colony 8			
Session 1 / Session 4 (Original Fraction)	0.676	21	0.01
Session 1 / Session 4 (Subsidiary Fraction)	0.438	34	0.01

Table 53 : The relationship between individuals' frequency of external activity compared between session 1 and session 4, calculated separately for individuals that were in the original and the subsidiary fraction of the colony during session 3, as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient where $n > 10$ (Sokal and Rohlf, 1981 p.607), and Spearman's rank correlation coefficient where $n \leq 10$.

I carry out further analysis on the results of experiment 2 to determine if sociotomy affects the absolute frequency at which individuals perform external activity. I compare the distribution of the frequency of external activity in each colony during each session of the experiment with the same distribution during session 1, using Mann-Whitney U tests, see Table 54, below.

Colonies / Sessions Compared	N_1	N_2	η_1	η_2	W	Significance Level p <
Colony C_1						
1 and 3	72	48	1.0	0.0	4693.0	NS
1 and 4	72	37	1.0	0.0	4263.0	0.05
Colony 5						
1 and 3 (original fraction)	No External Activity During Session 3 (original fraction)					
1 and 3 (subsidiary fraction)	54	33	5.5	9.0	1987.5	0.01
1 and 4	54	25	5.5	9.0	1913.5	0.01
Colony 6						
1 and 3 (original fraction)	No External Activity During Session 3 (original fraction)					
1 and 3 (subsidiary fraction)	40	18	7.0	5.0	1247.5	NS
1 and 4	40	11	7.0	1.0	1126.5	0.05
Colony 7						
1 and 3 (original fraction)	No External Activity During Session 3 (original fraction)					
1 and 3 (subsidiary fraction)	69	40	1.0	10.0	2887.5	0.01
1 and 4	69	55	1.0	0.0	4233.5	NS
Colony 8						
1 and 3 (original fraction)	109	28	2.0	1.0	7778.0	NS
1 and 3 (subsidiary fraction)	109	42	2.0	6.0	7455.5	0.01
1 and 4	109	54	2.0	1.0	9260.0	NS

Table 54: A comparison of the distribution of the frequency of external activity in session 1 of experiment 1 and the other sessions, for each colony, using Mann-Whitney U Tests. η_1 *= median frequency of external activity during session 1 of the experiment.* η_2 *= median frequency of external activity during the session to which session 1 is being compared.*

I also compare the absolute frequency of external activity performed during each session for the experimental colonies with the relevant session of the Control colony (see Table 55). The results are discussed in section 8.3ii.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1						
C_1 vs Colony 5	72	54	1.0	5.5	3716.5	0.01
C_1 vs Colony 6	72	40	1.0	7.0	3326.5	0.01
C_1 vs Colony 7	72	69	1.0	1.0	5402.0	NS
C_1 vs Colony 8	72	109	1.0	2.0	6272.5	NS
Session 3 – Original Fraction						
C_1 vs Colony 5	48	No Ext Act	0.0	-	-	-
C_1 vs Colony 6	48	No Ext Act	0.0	-	-	-
C_1 vs Colony 7	48	No Ext Act	0.0	-	-	-
C_1 vs Colony 8	48	28	0.0	1.0	1789.5	NS
Session 3 – Subsidiary Fraction						
C_1 vs Colony 5	48	33	0.0	9.0	1393.0	0.01
C_1 vs Colony 6	48	18	0.0	5.0	1385.5	0.01
C_1 vs Colony 7	48	40	0.0	10.0	1453.0	0.01
C_1 vs Colony 8	48	42	0.0	6.0	1674.0	0.01
Session 4						
C_1 vs Colony 5	37	25	0.0	9.0	842.0	0.01
C_1 vs Colony 6	37	11	0.0	1.0	842.5	NS
C_1 vs Colony 7	37	55	0.0	0.0	1581.5	NS
C_1 vs Colony 8	37	54	0.0	1.0	1518.5	NS

Table 55 : A comparison of the distribution of the frequency at which external activity is performed in each session of the experimental colonies with the relevant session of the Control colony (note that Colony C_1 is used), using Mann-Whitney U Tests. $\eta_{CONTROL}$ = median frequency of external activity in the relevant session of the Control. η_{EXP} = median frequency of external activity in the relevant session of the experimental colony being considered.

Multiple comparisons using pooled data

I determine that there is a significant relationship between the ranked frequency at which individuals perform external activity in session 1 compared to session 4 for colonies in which sufficient numbers of individuals have retained their marks in experiment 2. To determine if sociotomy has a significant effect on the relationship between the ranked frequency at which individuals perform external activity in session 1 (un-manipulated colonies) compared to session 4 (after re-unification) I perform multiple comparison analysis. Using the method outlined in Chapter 7, I determine whether the colonies can be considered to be samples from a population exhibiting a common correlation between the ranked frequency at which individuals perform external activity in session 1 compared to session 4. I then compare the pooled *z* from the experimental colonies to *z* calculated for the Control colony for this relationship. The results are shown in Table 55a, below

z^{-}_{exp} session 1 and 4	χ^2_{exp} session 1and 4	Signif. Level_{exp} session1&4 P<	$z_{control}$	$n_{exp\ session\ 1\&4}$	$n_{control}$	t_s	Signif. level p<
Exp 2							
0.737	0.737	NS	0.412	37.3	35.0	1.320	NS

Table 55a: Testing the relationship between the ranked frequency at which individuals perform external activity in session 1 compared to session 4 for combined colonies in experiment 2, compared to this relationship in the Control colony. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the experimental colonies for in experiment 2. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the experimental colonies in session 1 compared to session 4, with *z* calculated for this relationship for the Control colony shows that there is no significant difference between this relationship for the experimental colonies and the Control colony in experiment 2.

8.2 An Investigation of the Flexibility of Individual Performance of Tasks in Colonies with a Manipulated Age Structure

Experiment 3

i. The flexibility of individual performance of brood-related tasks

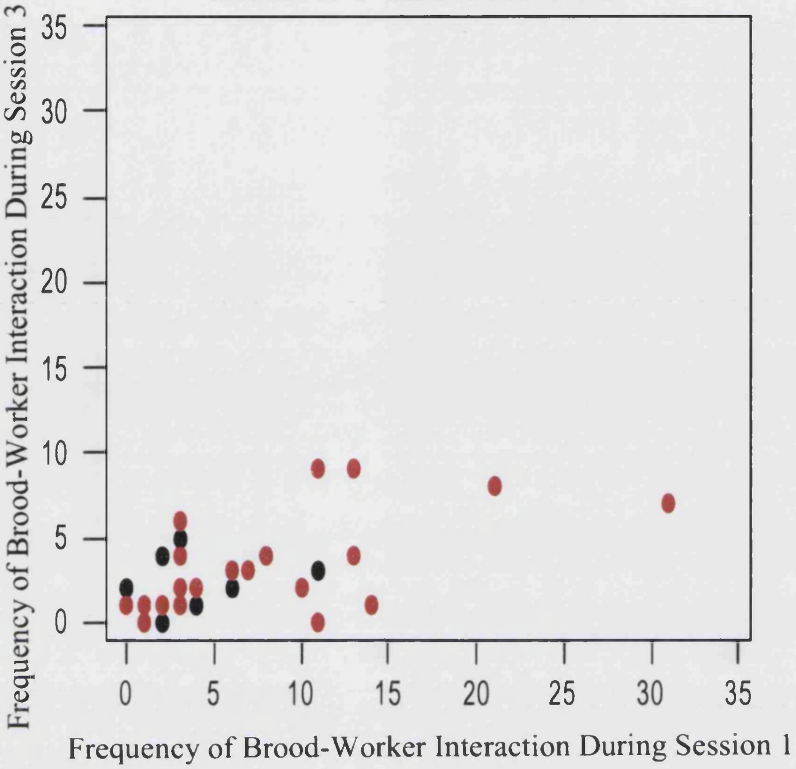
I carry out the same analysis as in section 8.1, above, using the data from experiment 3. I compare the frequency at which individuals carry out brood-related tasks during session 1 in both the 'older' (original) and 'callow' (subsidiary) fractions, to the frequency at which they carry out brood-related tasks in the relevant fraction of session 2; and to the frequency at which these individuals carry out brood-related tasks in the united colonies in session 3.

The relationship between the frequency at which workers perform brood-related tasks in the colony during session 1, compared to the frequency at which they perform brood-related tasks in the other sessions, for the Control colony in experiment 3 (Colony C_3), is shown in the graphs below. Individuals that were identified as callows when the colony was marked, just before the beginning of session 1, are shown in red.

Colony C_3 - Control

(a)

Session 1 and Session 3



(b)

Session 1 and Session 2

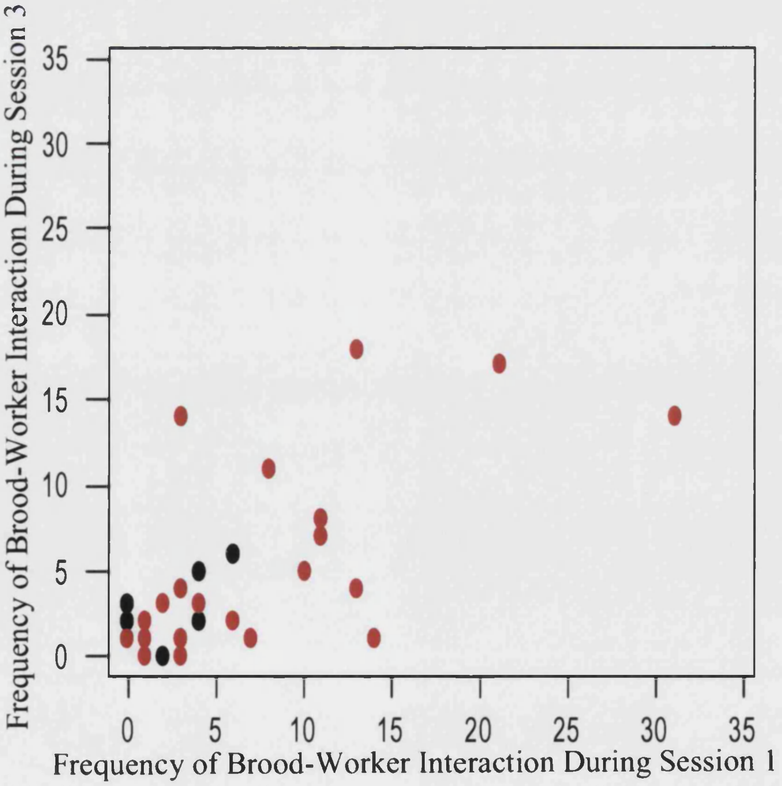
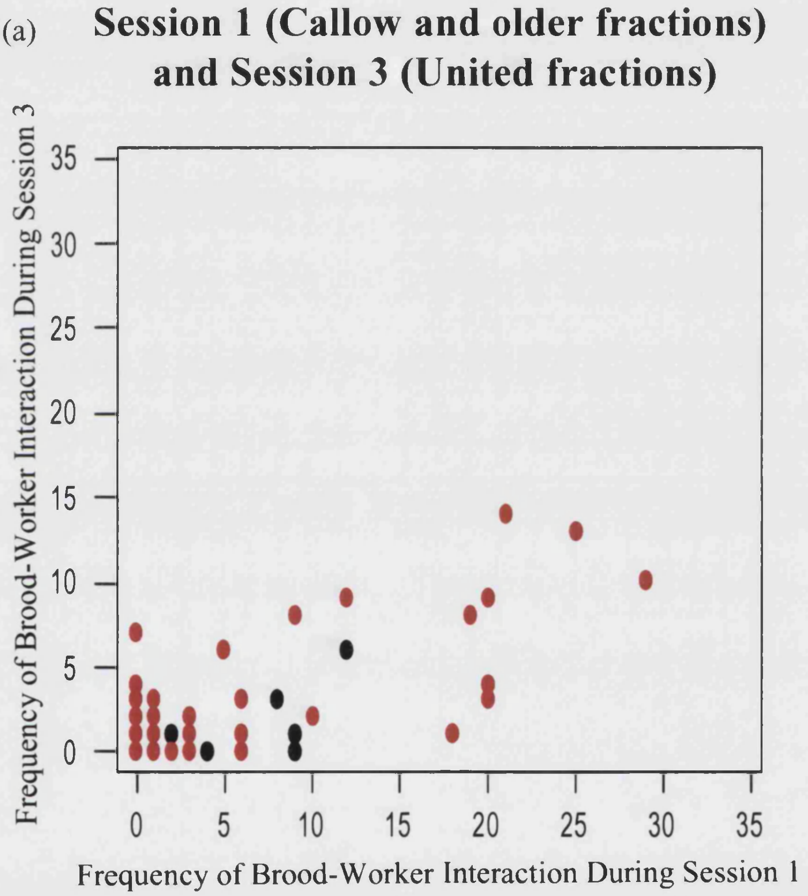


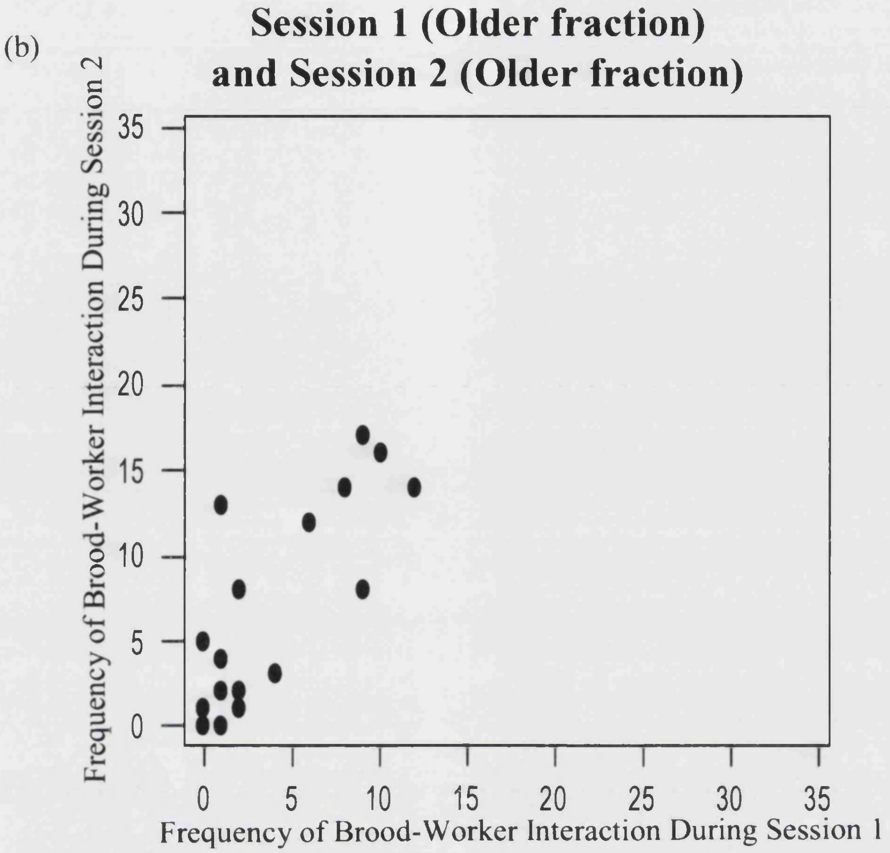
Figure 55 : The relationship between the frequency at which individuals perform brood-related tasks in (a) session 1 and session 3; and (b) session 1 and session 2, for Colony C_3, the Control, experiment 3. Callows are shown in red.

Colony 9 - An Example Experimental Colony

The graphs below show typical plots of the relationship between the frequency at which workers perform brood-related tasks in the colony during session 1 compared to the frequency at which they perform brood-related tasks in the other sessions, for a typical experimental colony (Colony 9). Individuals that were in the 'callow' (subsidiary) fraction of the colony during sessions 1 and 2 are shown in red.

Fig 56(a)





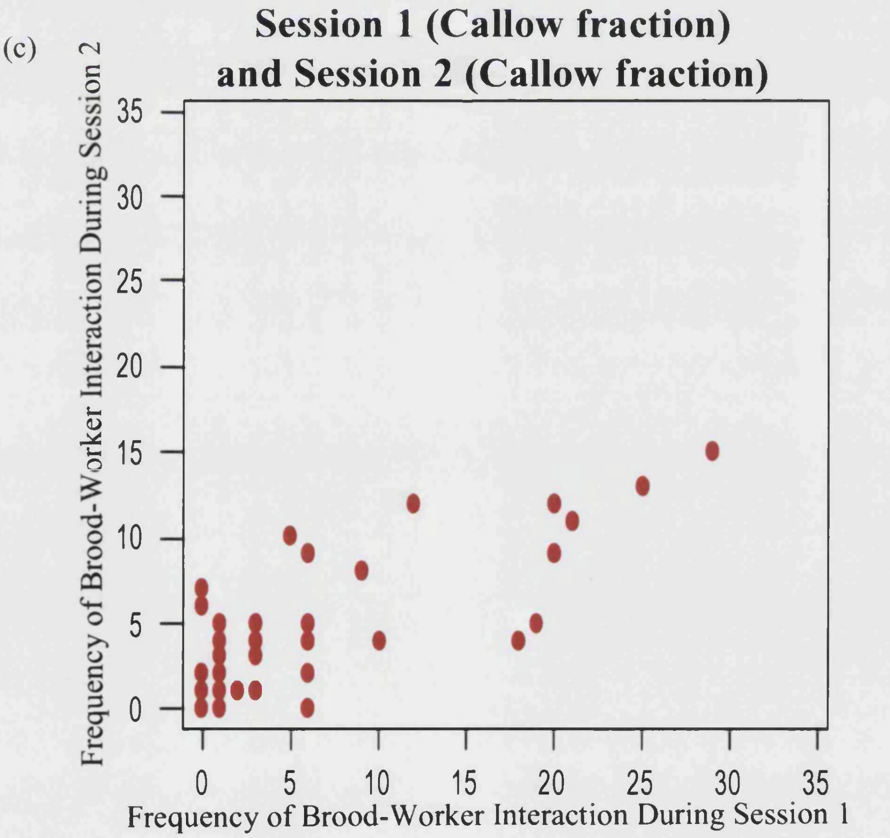


Figure 56 : The relationship between the frequency at which individuals perform brood-related tasks in (a) session 1 and session 3; (b) session 1 and session 2 (older workers; and (c) session 1 and session 2 (callow workers), for Colony 9, a typical experimental colony. Callows are shown in red.

I carry out the same statistical analysis on these results as in section 8.1i, above. I investigate the relationship between the frequency at which individuals perform brood-related tasks in session 1 compared to the other sessions by calculating the Spearman's rank correlation coefficient according to the methods described at the beginning of this chapter. There is a significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in session 1 and the relative frequency at which they perform brood-related tasks in both sessions 2 and 3, for the Control (Colony C_3), and for all four experimental colonies, see Table 56, below.

Colony/Sessions Compared	r_s	n	Significance Level P<
Colony C_3			
Session 1 / Session 3	0.571	33	0.01
Session 1 / Session 2	0.607	34	0.01
Colony 9			
Session 1 / Session 3	0.549	64	0.01
Session 1 / Session 2 (Older Fraction)	0.804	26	0.01
Session 1 / Session 2 (Callow Fraction)	0.715	57	0.01
Colony 10			
Session 1 / Session 3	0.524	63	0.01
Session 1 / Session 2 (Older Fraction)	0.797	31	0.01
Session 1 / Session 2 (Callow Fraction)	0.852	16	0.01
Colony 11			
Session 1 / Session 3	0.413	62	0.01
Session 1 / Session 2 (Older Fraction)	0.732	36	0.01
Session 1 / Session 2 (Callow Fraction)	0.859	29	0.01
Colony 12			
Session 1 / Session 3	0.588	38	0.01
Session 1 / Session 2 (Older Fraction)	0.715	15	0.01
Session 1 / Session 2 (Callow Fraction)	0.742	27	0.01

Table 56 : The relationship between individuals' frequency of performance of brood-related tasks compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

A Fisher's z-transform shows that there is no significant difference between the correlation coefficient for the Control colony, and each of the experimental colonies, for the relationship between the relative frequency at which brood-related tasks are performed in session 1 and session 3, see Table 57, below.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	Significance level P<
C_3 and 9	0.571	0.549	33	64	0.649	0.617	0.144	NS
C_3 and 10	0.571	0.524	33	63	0.649	0.582	0.300	NS
C_3 and 11	0.571	0.413	33	62	0.649	0.439	0.936	NS
C_3 and 12	0.571	0.588	33	38	0.649	0.675	-0.103	NS

Table 57 : A comparison between the Spearman's rank correlation coefficients of the relationship between the frequency at which individuals perform brood-related tasks in session 1 and session 3 with that of Control colony C_3, calculated as a Fisher's z-transform

I carry out correlation analyses separately for individuals that were in the original fraction of each colony, and for those that were in the subsidiary fractions of the colonies, during session 1. There is a significant, positive correlation between the relative frequency at which individuals that were in the subsidiary (callow) fractions of the colonies perform brood-related tasks in session 1 compared to session 3 for all the experimental colonies, except for Colony 11, which does not show a significant correlation, see Table 58, below. This relationship is a significant, positive correlation for individuals that were in the original (older) fraction during sessions 1 and 2 in all the experimental colonies. These results are discussed in section 8.3iii.

Colony/Sessions Compared	r_s	n	Significance level p<
Control Colony			
Session 1 / Session 3 (Older Fraction)	0.240	10	NS
Session 1 / Session 3 (Callow Fraction)	0.641	23	0.01
Colony 9			
Session 1 / Session 3 (Older Fraction)	0.675	19	0.01
Session 1 / Session 3 (Callow Fraction)	0.566	45	0.01
Colony 10			
Session 1 / Session 3 (Older Fraction)	0.610	32	0.01
Session 1 / Session 3 (Callow Fraction)	0.480	31	0.01
Colony 11			
Session 1 / Session 3 (Older Fraction)	0.698	34	0.01
Session 1 / Session 3 (Callow Fraction)	-0.058	28	NS
Colony 12			
Session 1 / Session 3 (Older Fraction)	0.749	14	0.01
Session 1 / Session 3 (Callow Fraction)	0.710	24	0.01

Table 58 : The relationship between individuals frequency of performance of brood-related tasks compared between sessions calculated separately for those individuals that were in the subsidiary fractions during session 1, and those that were in the original fraction, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

I carry out further analysis on the results of experiment 3 to determine if sociotomy affects the absolute frequency at which individuals perform brood-related tasks. I compare the frequency at which brood-related tasks are performed during session 2 of the experiment with the frequency at which brood-related tasks are performed in the relevant fraction during session 1 using Mann-Whitney U tests (see Table 59, below). I also compare the frequency at which brood-related tasks are performed during the original (older) fraction during session 1 and the frequency at which the older workers in the united colonies in session 3 perform brood-related tasks. I carry out this analysis for the subsidiary (callow) fraction during session 1 compared to the callow workers in the united colonies during session 3.

Colonies / Sessions Compared	N_1	N_2	η_1	η_2	W	**Significance Level p <**
Colony C_3						
1 and 2	38	38	3.0	2.5	1525.5	NS
1 and 3	38	35	3.0	2.0	1561.5	NS
Colony 9						
1 and 2 (older fraction)	37	28	1.0	1.0	1128.5	NS
1 and 2 (callow fraction)	59	57	1.0	2.0	3365.0	NS
1 and 3 (older workers)	37	19	1.0	0.0	1118.0	NS
1 and 3 (callow workers)	59	45	1.0	1.0	3058.0	NS
Colony 10						
1 and 2 (older fraction)	33	32	3.0	7.5	936.0	0.05
1 and 2 (callow fraction)	39	16	8.0	3.0	1203.5	0.05
1 and 3 (older workers)	33	33	3.0	1.0	1245.0	NS
1 and 3 (callow workers)	39	31	8.0	2.0	1648.0	0.01
Colony 11						
1 and 2 (older fraction)	41	38	0.0	0.0	1635.0	NS
1 and 2 (callow fraction)	31	30	3.0	4.5	944.0	NS
1 and 3 (older workers)	41	35	0.0	0.0	1649.0	NS
1 and 3 (callow workers)	31	28	3.0	2.0	1030.0	NS
Colony 12						
1 and 2 (older fraction)	21	22	1.0	1.5	449.5	NS
1 and 2 (callow fraction)	30	38	1.0	3.0	952.5	NS
1 and 3 (older workers)	21	20	1.0	0.0	516.5	0.05
1 and 3 (callow workers	30	33	1.0	4.0	807.5	0.05

Table 59 : A comparison of the distribution of the frequency at which brood-related tasks are performed in session 1 and session 2 (experiment 3), calculated separately for each fraction, for each colony, using Mann-Whitney U Tests. Comparisons of the distribution of the frequency at which brood-related tasks are performed by older workers, and by callow workers, in the relevant fraction of session 1 compared to session 3. η_1 *= median frequency at which brood-related tasks are performed during session 1 of the experiment.* η_2 *= median frequency at which brood-related tasks are performed during the session to which session 1 is being compared.*

I also compare the frequency at which brood-related tasks are performed during each session for the experimental colonies with the relevant session of the Control colony (see Table 60). The results are discussed in section 8.3iii.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1 – Older Fraction						
C_3 vs Colony 9	38	37	3.0	1.0	1765.5	0.01
C_3 vs Colony 10	38	33	3.0	3.0	1392.0	NS
C_3 vs Colony 11	38	41	3.0	0.0	1795.5	0.01
C_3 vs Colony 12	38	21	3.0	1.0	1265.5	0.05
Session 1 – Callow Fraction						
C_3 vs Colony 9	38	59	3.0	1.0	2170.0	0.05
C_3 vs Colony 10	38	39	3.0	8.0	1224.5	0.01
C_3 vs Colony 11	38	31	3.0	3.0	1260.0	NS
C_3 vs Colony 12	38	30	3.0	1.0	1460.0	NS
Session 2 – Older Fraction						
C_3 vs Colony 9	38	28	2.5	1.0	1348.0	NS
C_3 vs Colony 10	38	32	2.5	7.5	1144.0	0.05
C_3 vs Colony 11	38	38	2.5	0.0	1668.0	0.05
C_3 vs Colony 12	38	22	2.5	1.5	1226.0	NS
Session 2 – Callow Fraction						
C_3 vs Colony 9	38	57	2.5	2.0	1967.0	NS
C_3 vs Colony 10	38	16	2.5	3.0	1011.0	NS
C_3 vs Colony 11	38	30	2.5	4.5	1158.5	NS
C_3 vs Colony 12	38	38	2.5	3.0	1479.0	NS
Session 3 – United Fractions						
C_3 vs Colony 9	35	65	2.0	1.0	2030.5	NS
C_3 vs Colony 10	35	64	2.0	1.0	1805.5	NS
C_3 vs Colony 11	35	63	2.0	1.0	1900.5	NS
C_3 vs Colony 12	35	53	2.0	2.0	1546.0	NS

Table 60 : A comparison of the distribution of the frequency at which brood-related tasks are performed in each session of the experimental colonies with the relevant session of the Control colony, using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median frequency at which brood-related tasks are performed in the relevant session of the Control;* η_{EXP} *= median frequency at which brood-related tasks are performed in relevant session of the experimental colony.*

Multiple comparisons using pooled data

I determine that there is a significant relationship between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 3 for colonies in experiment 3. When I examine the relationships separately for the callow and the older workers (see Table 58) I find there is no significant correlation between the frequency at which the older workers perform brood-related tasks in session 3 and their median distance from the colony centre in the Control colony. This fractions consists of relatively low numbers of individuals that have retained their marks (n=10). Consequently I do not perform multiple comparisons for the older workers in the experimental colonies in session 3 for this relationship compared to the Control colony.

However, to determine if sociotomy has a significant effect on the relationship between the ranked frequency at which individuals perform brood-related tasks in session 1 (callow fractions separately) compared to session 3 (after unification) I perform multiple comparison analysis for the callows separately. Using the method outlined in Chapter 7, I determine whether the colonies can be considered to be samples from a population exhibiting a common correlation between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 3. I then compare the pooled z from the experimental colonies to z calculated for the Control colony, for the callows separately for this relationship. The results are shown in Table 60a, below:

$\bar{z}_{exp}$ session 1 and 3	χ^2_{exp} session 1and 3	Signif. Level$_{exp}$ session1&3 P<	$z_{control}$	$n_{exp\ session\ 1\&3}$	$n_{control}$	T_s	Signif. level p<
Callows							
0.662	1.623	NS	0.641	30.3	23	-0.071	NS

Table 60a: Testing the relationship between the ranked frequency at which callows perform brood-related tasks in session 1 compared to session 3 for combined colonies in experiment 3, calculated for the callows separately, compared to this relationship in the Control colony. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the callow fractions of the experimental colonies in experiment 3. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for all the callow fractions for the relationship between the ranked frequency at which callows perform brood-related tasks in the experimental colonies in session 1 compared to session 3, with *z* calculated for this relationship for the Control colony, shows that there is no significant difference between this relationship for the experimental colonies and the Control colony in experiment 3.

ii. The flexibility of individual performance of external activity

Experiment 3

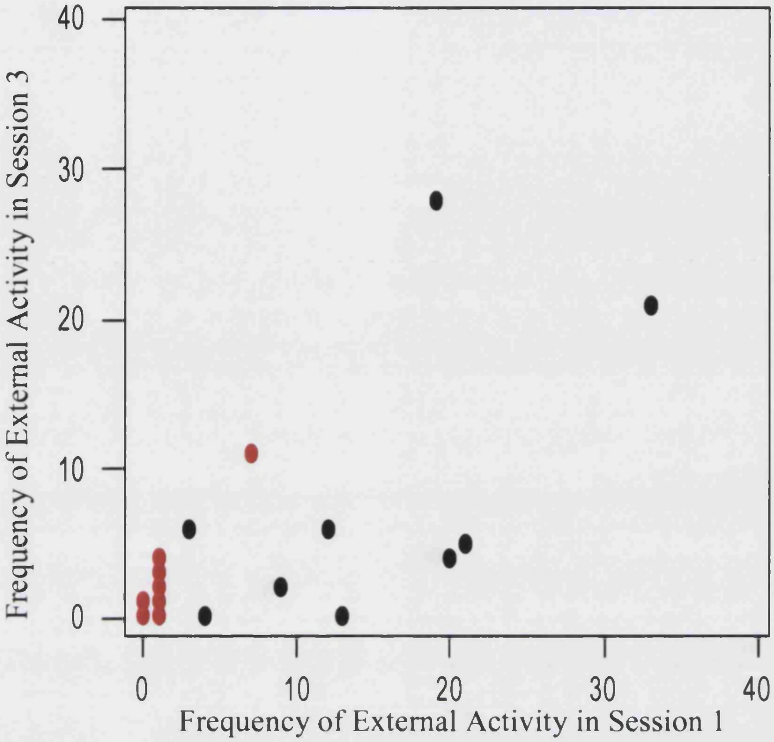
I carry out the same statistical analysis as in section 8.1ii.on the data from experiment 3. I compare the frequency at which individuals carry out external activity in session 1 for both the 'older' (original) and 'callow' (subsidiary) fractions, to the frequency at which these individuals perform external activity in the relevant fraction during session 2; and in the united colonies in session 3.

The relationship between the frequency at which individuals perform external activity during session 1 compared to the frequency at which they perform external activity in the other sessions, is shown in the graphs below for the Control Colony (Colony C_3). Individuals that were identified as callows when the colony was marked, prior to the beginning of session 1, are shown in red, as before.

Colony C_3 - Control

Session 1 and Session 3

(a)



(b)

Session 1 and Session 2

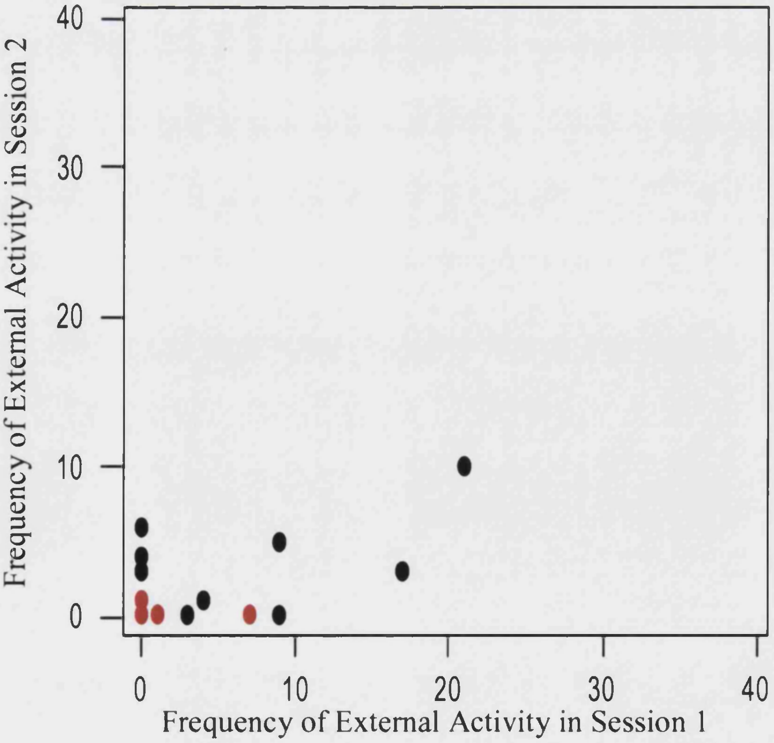
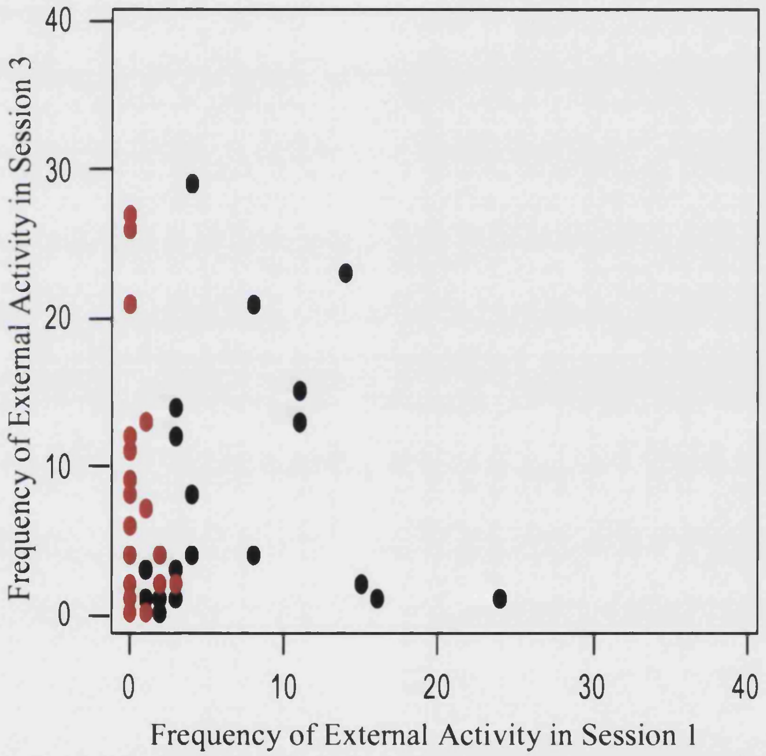


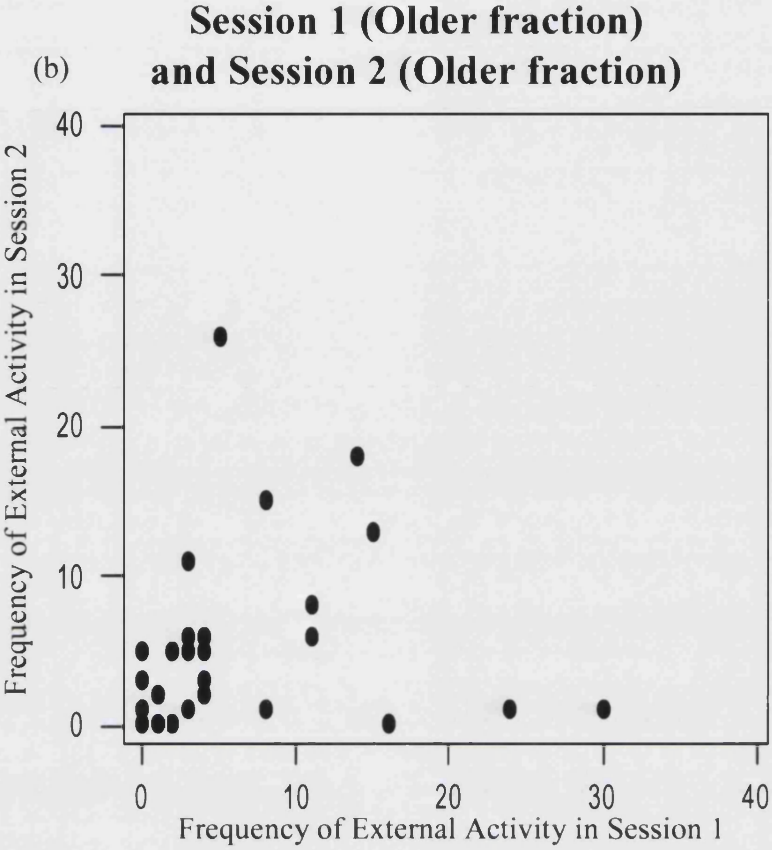
Figure 57 : The relationship between the frequency at which external activity is performed in (a) session 1 and session 3; and (b) session 1 and session 2, for Colony C_3, the Control, experiment 3. Callows are shown in red.

Colony 9 - An Example Experimental Colony

The graphs below show typical plots of the relationship between the frequency at which workers carry out external activity during session 1 compared to the frequency at which they carry out external activity in other sessions, for an example experimental colony (Colony 9). Individuals that were in the 'callow' (subsidiary) fraction of the colony during sessions 1 and 2 are shown in red.

Fig 58(a) **Session 1 (Callow and older fractions) and Session 3 (United fractions)**





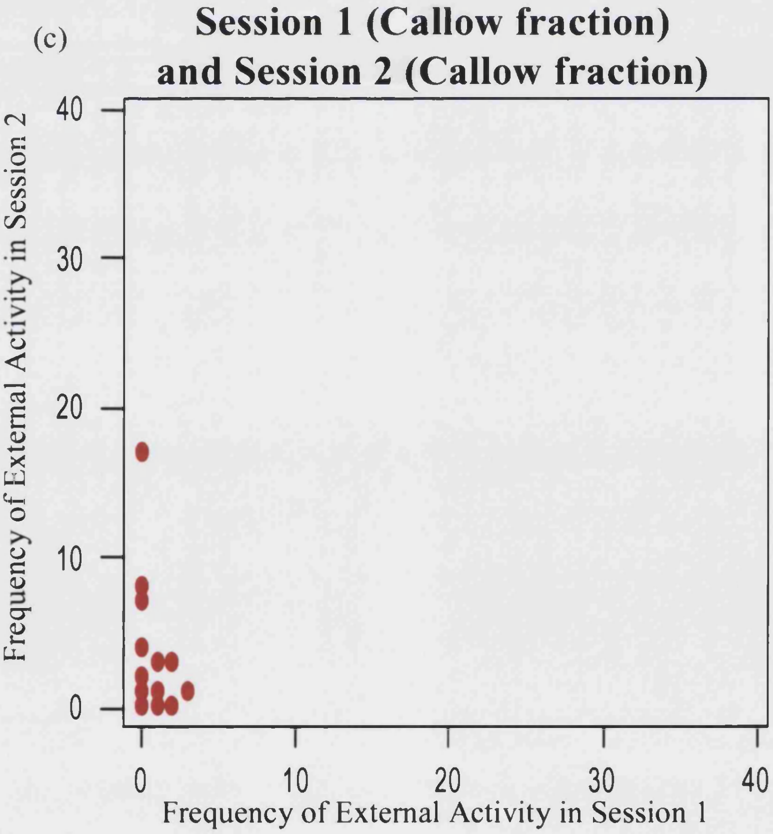


Figure 58 : The relationship between the frequency at which external activity is performed in (a) session 1 and session 3; (b) session 1 and session 2 (older workers); and (c) session 1 and session 2 (callow workers), for Colony 9, an example experimental colony. Callows are shown in red.

As before, I investigate the relationship between the frequency at which individuals carry out external activity in session 1 compared to the other sessions by calculating the Spearman's rank correlation coefficient of the relationship according to the procedures described at the beginning of this chapter. The results are shown in Table 62 (below). There is a significant, positive correlation between the relative frequency at which individuals carry out external activity in session 1 and the relative frequency at which they carry out external activity in the united colonies in session 3, for the Control, and for all four experimental colonies. The relative frequency at which individuals carry out external activity in session 1 is also positively correlated to the relative frequency at which they carry out external activity in session 2, for the Control, and also for the older (original) fraction of the experimental colonies. The relationship between the relative frequency at which the callows (in the subsidiary fraction) carry out external activity in session 1 and the relative frequency at which they carry out external activity in session 2 is not a statistically significant correlation, except for Colony 11, see Table 62 (below).

Fisher's z-transforms show that there is no significant difference between the correlation for the Control colony, and each the experimental colonies, in the comparison between the relative frequency at which external activity is performed in session 1 compared to session 3, see Table 61, below. These results are discussed in section 8.3iv.

Colonies Compared	r_{s1}	r_{s2}	n_1	n_2	z_1	z_2	t_s	Significance level $P<$
C_3 and 9	0.718	0.548	41	89	0.904	0.616	1.478	NS
C_3 and 10	0.718	0.664	41	67	0.904	0.800	0.506	NS
C_3 and 11	0.718	0.539	41	69	0.904	0.603	1.477	NS
C_3 and 12	0.718	0.563	41	60	0.904	0.637	1.272	NS

Table 61 : A comparison between the Spearman's rank correlation coefficients of the frequency at which individuals perform external activity in session 1 and the frequency at which they perform external activity in session 3, with that of Control colony C_3, calculated as a Fisher's z-transform.

Colony/Sessions Compared	r_s	n	Significance Level P<
Colony C_3			
Session 1 / Session 3	0.718	41	0.01
Session 1 / Session 2	0.766	42	0.01
Colony 9			
Session 1 / Session 3	0.548	89	0.01
Session 1 / Session 2 (Older Fraction)	0.593	42	0.01
Session 1 / Session 2 (Callow Fraction)	0.253	58	NS
Colony 10			
Session 1 / Session 3	0.664	67	0.01
Session 1 / Session 2 (Older Fraction)	0.589	33	0.01
Session 1 / Session 2 (Callow Fraction)	-0.073	21	NS
Colony 11			
Session 1 / Session 3	0.539	69	0.01
Session 1 / Session 2 (Older Fraction)	0.618	41	0.01
Session 1 / Session 2 (Callow Fraction)	0.680	29	0.01
Colony 12			
Session 1 / Session 3	0.563	60	0.01
Session 1 / Session 2 (Older Fraction)	0.563	34	0.01
Session 1 / Session 2 (Callow Fraction)	0.290	34	NS

Table 62: The relationship between individuals frequency of external activity compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

The results of a closer examination of the comparison between sessions 1 and 3 are given in Table 63, below. I calculate the Spearman's rank correlation coefficient separately for individuals that were in the original (older) and the subsidiary (callow) fractions of the colonies in sessions 1 and 2. The relationship between the relative frequency at which the older workers carry out external activity in session 1 compared to the relative frequency at which they carry out external activity in session 3, is a significant, positive correlation in the experimental colonies. There is also a significant, positive correlation between the relative frequency at which the callows perform external activity in session 1 compared to session 3 for all the experimental colonies except for Colony 11, which does not show a significant correlation between these variables.

Colony/Sessions Compared	r_s	n	Significance level $p<$
Control Colony			
Session 1 / Session 3 (Older Fraction)	0.786	17	0.01
Session 1 / Session 3 (Callow Fraction)	0.544	24	0.01
Colony 9			
Session 1 / Session 3 (Older Fraction)	0.715	34	0.01
Session 1 / Session 3 (Callow Fraction)	0.319	55	0.05
Colony 10			
Session 1 / Session 3 (Older Fraction)	0.642	33	0.01
Session 1 / Session 3 (Callow Fraction)	0.670	34	0.01
Colony 11			
Session 1 / Session 3 (Older Fraction)	0.649	41	0.01
Session 1 / Session 3 (Callow Fraction)	-0.189	28	NS
Colony 12			
Session 1 / Session 3 (Older Fraction)	0.607	29	0.01
Session 1 / Session 3 (Callow Fraction)	0.391	31	0.05

Table 63 : The relationship between individuals frequency of external activity compared between sessions, calculated as r_s, Spearman's rank correlation coefficient, and tested as an ordinary product-moment correlation coefficient as n > 10 (Sokal and Rohlf, 1981 p.607).

I carry out further analysis on the results of experiment 3 to determine if sociotomy affects the absolute frequency at which individuals perform external activity. I compare the distribution of the frequency at which external activity is performed during session 2 of the experiment with the distribution of the frequency of external activity carried out in the relevant fraction during session 1, using Mann-Whitney U tests (see Table 65, below). I also compare the distribution of the frequency of external activity performed during the original (older) fraction during session 1 and the distribution of the frequency at which the older workers in the united colonies in session 3 carry out external activity. I carry out this analysis for the subsidiary (callow) fractions during session 1 compared to the callow workers in the united colonies during session 3.

Colonies / Sessions Compared	**N_1**	**N_2**	**η_1**	**η_2**	**W**	**Significance Level p <**
Colony C_3						
1 and 2	52	42	1.0	0.0	2792.5	0.01
1 and 3	52	42	1.0	0.0	2750.5	0.05
Colony 9						
1 and 26 (older fraction)	54	41	3.0	1.0	2801.0	NS
1 and 2 (callow fraction)	60	58	0.0	0.0	3259.0	0.01*
1 and 3 (older workers)	54	37	3.0	1.0	2636.5	NS
1 and 3 (callow workers)	60	55	0.0	0.0	3046.5	0.01*
Colony 10						
1 and 2 (older fraction)	36	33	0.0	0.0	1335.0	NS
1 and 2 (callow fraction)	40	21	0.0	0.0	1130.0	0.05*
1 and 3 (older workers)	36	39	0.0	0.0	1301.0	NS
1 and 3 (callow workers)	40	31	0.0	0.0	1470.5	NS
Colony 11						
1 and 2 (older fraction)	44	41	0.5	0.0	1974.5	NS
1 and 2 (callow fraction)	31	30	0.0	0.0	1000.0	NS
1 and 3 (older workers)	44	41	0.5	1.0	1839.5	NS
1 and 3 (callow workers)	31	28	0.0	0.0	925.0	NS
Colony 12						
1 and 2 (older fraction)	43	34	5.0	1.0	2013.0	0.01
1 and 2 (callow fraction)	31	41	0.0	0.0	1247.5	NS
1 and 3 (older workers)	43	31	5.0	1.0	1873.5	0.01
1 and 3 (callow workers	31	38	0.0	0.0	1073.0	NS

Table 65 : A comparison of the distribution of the frequency of performance of external activity in session 1 and session 2 (experiment 3), calculated separately for each fraction, for each colony, using Mann-Whitney U Tests. Comparisons of the distribution of the frequency of performance of external activity by older workers, and by callow workers, in the relevant fraction of session 1 compared to session 3. η_1 *= median frequency of external activity during session 1 of the experiment.* η_2 *= median frequency of external activity performed during the session to which session 1 is being compared. * = one-tailed test (null hypothesis* η_1 *is not less than* η_2, H_1 *:* η_1 *is less than* η_2*).*

I also compare the absolute frequency of external activity performed during each session for the experimental colonies with the relevant session of the Control colony (see Table 66). The results are discussed in section 8.3iv.

Session/ Colonies Compared	$N_{CONTROL}$	N_{EXP}	$\eta_{CONTROL}$	η_{EXP}	W	Significance Level p<
Session 1 – Older Fraction						
C_3 vs Colony 9	52	54	1.0	3.0	2676.5	NS
C_3 vs Colony 10	52	36	1.0	0.0	2582.0	0.05
C_3 vs Colony 11	52	44	1.0	0.5	2721.5	NS
C_3 vs Colony 12	52	43	1.0	5.0	2176.5	0.05
Session 1 – Callow Fraction						
C_3 vs Colony 9	52	60	1.0	0.0	3665.5	0.01
C_3 vs Colony 10	52	40	1.0	0.0	2891.0	0.01
C_3 vs Colony 11	52	31	1.0	0.0	2548.0	0.01
C_3 vs Colony 12	52	31	1.0	0.0	2456.0	0.01
Session 2 – Older Fraction						
C_3 vs Colony 9	42	41	0.0	1.0	1521.0	0.05
C_3 vs Colony 10	42	33	0.0	0.0	1668.5	NS
C_3 vs Colony 11	42	41	0.0	0.0	1709.0	NS
C_3 vs Colony 12	42	34	0.0	1.0	1434.5	0.05
Session 2 – Callow Fraction						
C_3 vs Colony 9	42	58	0.0	0.0	2183.0	NS
C_3 vs Colony 10	42	21	0.0	0.0	1329.0	NS
C_3 vs Colony 11	42	30	0.0	0.0	1685.0	0.01*
C_3 vs Colony 12	42	41	0.0	0.0	1926.0	0.05*
Session 3 – United Fractions						
C_3 vs Colony 9	42	92	0.0	0.5	2576.5	NS
C_3 vs Colony 10	42	67	0.0	0.0	2510.0	NS
C_3 vs Colony 11	42	69	0.0	0.0	2370.0	NS
C_3 vs Colony 12	42	69	0.0	0.0	2226.5	NS

Table 66 : A comparison of the distribution of the frequency of performance of external activity in each session of the experimental colonies with the relevant session of the Control colony , using Mann-Whitney U Tests. $\eta_{CONTROL}$ *= median frequency of performance of external activity in the relevant session of the Control.* η_{EXP} *= median frequency of performance of external activity in the relevant session of the experimental colony being considered. * = one-tailed test (null hypothesis* $\eta_{CONTROL}$ *is not greater than* η_{EXP}, H_1 *:* $\eta_{CONTROL}$ *is greater than* η_{EXP}*).*

Multiple comparisons using pooled data

I determine that there a is significant relationship between the ranked frequency at which individuals perform external activity in session 1 compared to session 3 for colonies in experiment 3. To determine if sociotomy has a significant effect on the relationship between the ranked frequency at which individuals perform external activity in session 1 (callow and older fractions separately) compared to session 3 (after unification) I perform multiple comparison analysis for the callows and the older workers separately. Using the method outlined in Chapter 7, I determine whether the colonies can be considered to be samples from a population exhibiting a common correlation between the ranked frequency at which individuals perform external activity in session 1 compared to session 3. I then compare the pooled *z* from the experimental colonies to *z* calculated for the Control colony, for the callows and the older workers separately for this relationship. The results are shown in Table 66a, below

$\bar{z}_{exp}$ session 1 and 3	χ^2_{exp} session 1and 3	Signif. Level$_{exp}$ session1&3 P<	$z_{control}$	$n_{exp\ session\ 1\&3}$	$n_{control}$	t_s	Signif. level p<
Callows							
0.485	4.675	NS	0.610	37	24	0.448	NS
Older workers							
0.787	0.582	NS	1.061	31.3	17	0.838	NS

Table 66a: Testing the relationship between the ranked frequency at which individuals perform external activity in session 1 compared to session 3 for combined colonies in experiment 3, calculated for the callows and older workers separately, compared to this relationship in the Control colony for the two fractions separately. Calculated as Fisher's z transform using pooled correlation coefficients where colonies can be considered samples from a population exhibiting a common correlation among the variables.

There is insufficient evidence to reject the null hypothesis of homogeneity for the separate fractions of the experimental colonies in experiment 3. I can consider the colonies to be samples from a population exhibiting a common correlation.

A Fisher's z transform, comparing the pooled *z* for the relationship between the relative frequency at which callows perform external activity in the experimental colonies in session 1 compared to session 3, to the *z* calculated for the callows for this relationship for the Control colony, shows that there is no significant difference between this relationship for the experimental colonies and the Control colony for the callows in experiment 3. This is also the case when multiple comparisons are used to compare the correlational relationships for the older workers in experiment 1 to the Control colony.

8.3 Discussion

An investigation of the flexibility of individual performance of brood-related tasks and external activity within the colonies

In Chapter 4, I analysed the spatial organisation of brood-related tasks and external activity in colonies of *Leptothorax albipennis*. I showed how the patterns of organisation observed for these tasks tend to be preserved throughout the manipulations carried out in the experiments. In Chapter 7, I examined spatial organisation in these colonies further, by comparing the relative positions of individual workers throughout the experiments. I showed that the relative spatial positions of individuals are resilient to sociotomy. In sections 8.1 and 8.2, above, I examined how individual task performance, in terms of brood-related tasks and external activity, is affected by the manipulations carried out in the experiments. In the following section I will discuss these results in relation to individual flexibility of task performance. The implications of these results for task allocation are considered further in Chapter 9.

The results are divided into the following sections:

8.1 An investigation of the flexibility of individual performance of tasks in colonies with a manipulated task structure

i. The flexibility of individual performance of brood-related tasks
ii. The flexibility of individual performance of external activity

8.2 An investigation of the flexibility of individual performance of tasks in colonies with a manipulated age structure

i. The flexibility of individual performance of brood-related tasks
ii. The flexibility of individual performance of external activity

For a summary of the methodology of all three experiments the reader is referred to Figure 9, Chapter 3.

i. An investigation of the flexibility of individual performance of brood-related tasks in colonies with a manipulated task structure

To examine the flexibility of individuals in terms of brood-related task performance, I determined how the frequency at which individuals perform brood-related tasks is affected by the removal of individuals associated with external activity in experiments 1 and 2. I examined how the frequency at which these removed individuals perform brood-related tasks is affected in session 3, experiment 2 (these fractions did not have any brood in experiment 1). After re-unification of the original and subsidiary fractions of the colonies in session 4, I examined how the performance of brood-related tasks overall, and for the removed and original workers separately, is affected, for both experiments 1 and 2. I plotted graphs of the frequency at which brood-related tasks are performed by individuals for compared sessions of the experiments. To determine if the individual relative performance of brood-related tasks is resilient to the manipulations, I ranked the frequency at which individuals perform these tasks for the two sessions to be compared, and calculated the Spearman's rank correlation coefficient for this relationship (see beginning of Chapter 4 for details of this procedure).

I also analysed the results to determine if sociotomy changes individual absolute performance of brood-related tasks in the colony between compared sessions. To do this I used Mann-Whitney U tests to determine if there is any difference in the distribution of the frequency at which these tasks are performed during each experimental session compared to the un-manipulated colonies in session 1, for each colony. These tests were also used to compare the distribution of the frequency at which brood-related tasks are performed in the Control colony compared to the relevant session for each experimental colony.

Experiment 1

The relationship between the frequency at which individuals in the Control colony (Colony C_1) perform brood-related tasks in session 1 compared to session

4 (experiment 1), is shown in Figure 47 (a). There is a highly significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in these two sessions, see Table 38. Individuals in the Control colony perform brood-related tasks at the same frequency relative to other individuals, in session 4 as they do in session 1. This is also the case for the comparisons between the other sessions for the Control colony (see Table 38, and Figure 48 for graphs of the frequency at which individuals carry out brood-related tasks in session 1 compared to (a) session 2, and (b) session 3). Thus, specialisation by individuals for brood-related tasks is maintained throughout the course of the experiment, despite the temporary removal of one third of the workers. I conclude, therefore, that the physical effect of removing individuals does not affect individual specialisation for these tasks.

I used Mann-Whitney U tests to compare the absolute frequency at which brood-related tasks are performed in session 1 compared to the other experimental sessions for the Control colony (see Table 40). There is no significant difference between the frequency at which brood-related tasks are performed in session 1 compared to any of other sessions. I conclude from this that the distribution of the frequency at which brood-related tasks are performed in the colony does not change significantly over the course of the experiment, despite the temporary removal of one third of the workers. Any significant differences in the distribution of the frequency at which brood-related tasks are performed in the experimental colonies can therefore be attributed to sociotomy.

I carried out similar analyses for the experimental colonies. I showed in Chapter 4, that the spatial organisation of brood-related tasks is preserved during the removal of workers associated with external activity from the colony in session 2, and after their removal (session 3, original fractions). I hypothesised that it is these remaining individuals that are responsible for the majority of brood-related tasks during session 1 before the workers associated with external activity are removed. Figure 49 (a) is a plot of the relationship between the frequency at which individuals perform brood-related tasks during session 1 compared to the frequency at which they perform these tasks during session 2, during which external workers are being removed, for an example experimental colony (Colony 3). Individuals that are removed as externals during session 2 are represented in red in this figure. Table 38 shows the results of correlation analyses performed on these variables. There is a significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in session 1 and the relative frequency at which these individuals perform brood-related tasks in session 2, for two of the four experimental colonies (Colonies 2 and 4). There is no significant correlation between the relative frequency at which individuals perform brood-related tasks in sessions 1 and 2 for Colonies 1 and 3. Therefore, I conclude that the removal of individuals associated with external activity from these colonies has some effect of the task profiles of the workers, despite the maintenance of spatial patterns of brood-related tasks (see Chapter 4), and the relative spatial positions of the workers (see Chapter 7). My hypothesis is that, although there is a tendency for individuals to perform brood-related tasks with a relatively high frequency during session 2 if they performed these tasks at a relatively high frequency during session 1, this tendency may be disrupted. One possible cause of this disruption is the presence of workers that perform brood-related tasks during sessions 1 and 2, but are removed during session 2 because they also exhibit external activity. This can indeed be clearly seen in Figure 49 (a), in which red data points represent the latter individuals. This graph also shows that these individuals tend to perform brood-related tasks at a lower frequency than the individuals that are not removed. I conclude that there are brood-work 'specialists' in sessions 1 and 2, that perform brood-related tasks with a relatively high frequency, and do not perform any external activity (and are consequently not removed from the colony). Also present are individuals that perform brood-related tasks (albeit at a lower frequency than the 'specialists') and external activity, and are consequently removed by the sampling procedure used in session 2. These individuals are discussed in detail later.

I compared the absolute frequency at which brood-related tasks are performed by each of the colonies in session 2 compared to session 1, using Mann-Whitney U tests (see Table 40). The distribution of the frequency at which brood-related tasks are performed is significantly higher in session 2 than in session 1 for

Colony 2. The analyses show that there is no significant difference between the distribution of the frequency at which brood-related tasks are performed in session 1 compared to session 2 in the other experimental colonies. The distribution of the frequency at which brood-related tasks are performed in Colony 2 during session 2 is also significantly higher than the distribution of the frequency at which these tasks are performed in the Control colony (see Table 41). There is no significant difference between the frequency at which these tasks are performed in the other experimental colonies and the Control colony during session 2. In all cases, however, the median number of brood-related tasks performed by an individual in each of these colonies is higher than the median number of these acts in the Control. This is evidence that individuals that do not perform external activity during session 2 (and are consequently not removed) tend to perform brood-related tasks at a higher frequency than the median frequency at which these acts are performed in an un-manipulated Control. In Colony 2, which exhibits significantly higher absolute frequencies of brood-related task performance than the Control colony, individuals maintain, and increase, their behavioural specialisation for brood-related tasks compared to session 1.

There is evidence that brood-work specialists are sufficiently flexible to respond to changing task demand in colonies of *Leptothorax albipennis* from which workers associated with external activity have been removed. I examined the association between the frequency at which individuals perform brood-related tasks in session 1 compared to the original fractions of the colonies in session 3. This relationship is difficult to draw conclusions from because the number of individuals that have retained their marks in these fractions of the experimental colonies is low. Figure 49 (b) is a plot of the relationship between the frequency at which individuals perform brood-related tasks in session 1 compared to the frequency at which these individuals perform these tasks in session 3, original fraction, for an example experimental colony (Colony 3). This graph shows that there is a tendency for a number of these individuals to remain resilient in their performance of brood-related tasks, but also that some individuals that perform brood-related tasks in session 1 do not perform these tasks in session 3. The results of correlation analyses on these data are shown in Table 38. The relative frequency at which an individual performs brood-related tasks in session 1 is not significantly correlated to the relative frequency at which she performs brood-related tasks in session 3 (original fraction). My explanation for this is that: first, there are low numbers of individuals in this fraction that have retained their marks for two of the experimental colonies; second, some individuals that perform brood-related tasks during session 1 change their task profile during session 3 as a result of sociotomy. My hypothesis is that the removal of all the workers associated with external activity during session 2 results in reduced demand for brood-related tasks in the colony, such that the remaining individuals alter their task profiles accordingly.

I compared the absolute frequency at which brood-related tasks are performed in the colonies in session 3 (original fraction) compared to session 1 (see Table 40), using Mann-Whitney U tests. As expected, the frequency at which brood-related tasks are performed during session 3 reflects the change in task profiles of some of the workers that have ceased to perform brood-related tasks. Consequently, the absolute frequency at which brood-related tasks are performed in the original fractions by former brood-work specialists, during session 3, is not significantly different from the frequency at which brood-related tasks are performed in session 1, for the majority of experimental colonies. It might be expected that the demand for brood-related tasks would increase, rather than decrease, as the ratio of the number of workers to the number of items of brood has decreased, compared to session 1. My explanation for the reduction in demand for these tasks is the reduction in forage available to the colony resulting from the absence of workers that previously specialised in external activity. This is hypothesised to lead to a reduction in task performance because a substantial proportion of brood-related tasks are connected with feeding the larvae, and my pooled results do not discriminate between tasks connected with feeding, and other types of task involving the brood, such as grooming. I have shown, in Chapter 4, that the frequency at which external activity is performed is substantially less in the original fractions during session 3, compared to session 1. It is noted that, despite changes in task demand and consequent adjustment to performance of these tasks, there is no disruption to the overall spatial organisation of brood-related tasks in these fractions of the colonies (see Chapter 4).

I note that the frequency at which brood-related tasks are performed is significantly higher in session 3 than in session 1 for one experimental colony (Colony 1). The frequency at which brood-related tasks are performed in Colony 1 during session 3 is also significantly higher than the frequency at which these tasks are performed in the Control colony (see Table 41). There is no significant difference between the frequency at which these tasks are performed in the other experimental colonies and the Control colony during session 3. One explanation for this is that the ratio of the number of workers to the number of items of brood in Colony 1 is such that task demand is affected, and the individuals remaining in this fraction of the colony perform brood-related tasks at a higher frequency than in the Control. An alternative explanation is that specialists in brood-related tasks lack the flexibility to respond to the removal of workers associated with external activity by reducing the amount of brood-related tasks they carry out. The latter explanation is considered unlikely as in three out of the four experimental colonies the brood-related task specialists do not differ significantly in the frequency at which they perform brood-related tasks from the complete, un-manipulated Control colony, despite their previous specialisation for brood work. I conclude that brood work specialists are sufficiently flexible to respond to changing task demand, and either increase or decrease the frequency at which they perform brood-related tasks accordingly.

It is expected that individuals will exhibit fidelity to their previous task profiles with regard to the performance of brood-related tasks in the colonies when they are re-united in session 4. I have shown, above, that there is a significant correlation between the relative frequency at which an individual in the un-manipulated Control performs brood-related tasks in session 1 compared to session 4 (Table 38). I have also shown that there is no significant change in the absolute frequency at which workers perform brood-related tasks in any of the experimental sessions compared to session 1 for this colony (Table 40). Therefore, when the experimental colonies are re-united prior to the beginning of session 4, the requirements of the colony for brood-related tasks is expected to be the same as in session 1.

In fact, only one of the four colonies, Colony 2, showed a significant correlation between the relative frequency at which individuals perform brood-related tasks in session 1 compared to the relative frequency at which they perform these tasks in session 4 (see Table 38). Figure 47 (b) is a plot of the relationship between the frequency at which individuals perform brood-related tasks during session 1 compared to the frequency at which they perform brood-related tasks during session 4 for an example experimental colony, Colony 3. In this graph, individuals that were in the subsidiary fraction of the colony are represented in red. The graph shows that the majority of the individuals that perform some brood-related tasks in session 1, but are removed because they also perform external activity, perform brood-related tasks with a frequency of zero in the re-united colony in session 4. This reduction in the frequency at which brood-related tasks are performed by some individuals is reflected by the results from Mann-Whitney U tests which compare the absolute frequency at which brood-related tasks are performed in session 4 to performance in session 1 for the experimental colonies (see Table 40). Brood-related tasks are performed at a significantly lower frequency in three out of the four experimental colonies. The remaining colony, Colony 2, does show a lower median frequency of brood work, but there is no significant difference between the overall distribution of the frequency at which brood-related tasks are performed in session 4 compared to session 1. Compared to the Control colony, there is no significant difference between the frequency at which brood-related tasks are performed in three out of the four experimental colonies. Colony 3 shows significantly less brood work. I conclude that sociotomy does affect individual performance of brood-related tasks in the colony. This is discussed in detail below.

My hypothesis is that individuals that were in the subsidiary fraction during session 3 cease to perform brood-related tasks because they had no opportunity to carry out these tasks in the subsidiary fraction of the colony during session 3, as there was no brood present. The importance of learning (and conversely, forgetting) in task allocation in these colonies is therefore suggested. I hypothesise that these individuals 'forget' how to perform brood work because they have ceased to perform it for a period of time. This explains the disruption to the relationship between the relative frequency at which individuals perform brood-related tasks compared between the two sessions. I analysed this relationship further for the four experimental colonies by calculating the Spearman's rank correlation coefficients separately for those individuals that had been the original fraction, and for those individuals that had composed the subsidiary fraction, in session 3 (see Table 39). When considered separately, a positive correlation coefficient is calculated for the individuals that had remained in the original colony for the two colonies with a sufficient number of individuals that had retained their marks, although this relationship is not significant for either colony. I attribute the lack of significance of these results to relatively low numbers of individuals that had retained their marks. The individuals that had been in the subsidiary fractions of the experimental colonies, when considered separately, did not demonstrate a significant correlation. In fact there was a trend towards negative coefficients for the relationship, although these were not high enough to be significant. I conclude, therefore, that there is evidence to support my hypothesis, (see above), that individuals that are removed from the original colonies and cannot perform brood-related tasks as there is no brood present in these colonies, 'forget' how to perform brood-related tasks when the fractions of the colony are re-united.

It will be revealing to examine these conclusions in the light of the results from experiment 2, when individuals do have access to brood in the subsidiary fractions during session 3. I hypothesis that these individuals should be sufficiently flexible to perform brood-related tasks in the subsidiary fractions, and will therefore 'remember' how to perform brood-related tasks when the fractions of the colony are re-united. If this is not the case, the explanation may lie in the degree of specialisation of individuals for brood-work. The brood-work 'specialists', discussed earlier, may be less prone to 'forget' how to perform brood-related tasks. Any individuals that exhibit brood-work in the subsidiary fractions of the colonies during session 3, in experiment 2, are not strictly 'specialists', as they have also performed external activity and consequently have been removed from the original colony.

Intriguingly, Figure 47 (b) shows that there are some individuals that were in the subsidiary fraction of Colony 3, that do resume brood-related tasks in session 4, despite having had no opportunity to do this in the subsidiary fraction of the colony. I showed in Chapter 4 that most of the individuals that composed the subsidiary fraction in session 3 are located relatively far from the centre of the brood pile after the fractions are re-united prior to session 4, and carry out little or no brood-related tasks. I noted, however, that there are a number of individuals located closer to the centre of the brood pile, and that they exhibit higher frequencies of brood-work. I hypothesised in Chapter 4, that these individuals were also closer to the centre of the brood pile during session 1, and carried out brood-related tasks in this session, but were removed because they also carried out external activity. A closer examination of the data reveals that these individuals are found closest to where the centre of the brood pile should have been, if there had been any brood present (taken as the position it occupied in session 1), in the subsidiary fraction during session 3. The individuals that subsequently carry out no brood-related tasks in the re-united fractions, are found further away from this point in the subsidiary fractions, despite having performed brood-related tasks with a relatively high frequency in session 1. Therefore, I conclude that spatial position in the colony may play a role in the processes of 'learning' or 'forgetting'. The role of 'learning' and 'forgetting' in processes of task allocation in these colonies is discussed further in Chapter 9.

What determines the spatial position of individuals in the subsidiary fractions and re-united fractions of the colonies, and therefore influences whether these individuals resume, or do not resume, brood-related tasks when the fractions of the colonies are re-united? The former individuals continue to be found relatively close to the area of the nest where the brood pile was located during session 1, in the subsidiary fractions during session 3. Conversely, the latter individuals are found further out in the subsidiary fractions, and continue to be found further out when the fractions are re-united. I hypothesise that these latter individuals carry out external activity in the subsidiary fraction, and continue to do so the in re-united colony, whereas the individuals that remain doing brood-related tasks in the united colony do not carry out external activity in the subsidiary fraction. I showed in Chapter 4 that the frequency at which individuals perform external activity increases with increasing distance from the brood pile in the subsidiary fractions. It has already been hypothesised that there is an efficient sorting procedure that allocates these individuals to spatial positions further from the centre of the colony. It could, therefore, be concluded that the performance of external activity overrides the potential for brood-related tasks in some individuals, whereas other brood-workers will stay in the nest and not perform external activity, even when there is no brood present to tend. This could be a reflection of individuals differing response thresholds for these tasks, or a reflection of the reproductive hierarchy in the colony (see section 2.4ii), especially considering the absence of the queen in the subsidiary fractions, and subsequent changes in reproductive conflict. The latter is considered unlikely, and is discussed further in Chapter 9, with reference to the results from Chapters 5 and 6.

Experiment 2

As previously noted, the disruption caused to Colony C_2, the Control colony in experiment 2, by the death of the queen, means this colony is not included in the analysis (although the results are given for the sake of completeness). Colony C_2 controlled for the effects of dyed, foreign brood on the colony, and this factor has been shown, in Chapter 4, not to affect the results. Colony C_1 is an adequate control for the remaining variables in this experiment. Consequently graphs from experiment 2 are given only for Colony 7, an example experimental colony, and these results are compared to Colony C_1.

I hypothesised in the discussion above that the results from experiment 2 will show that individuals that were removed to the subsidiary fractions of the colonies perform brood-related tasks in these fractions, and maintain their specialisation for these tasks when the fractions are re-united in session 4. I, therefore, expect a significant correlation between the relative frequency at which these ‘removed’ individuals perform brood-related tasks in session 1 compared to the subsidiary fractions of session 3. The explanation for this is that a number of the individuals removed, because they carry out external activity in session 2, also perform brood-related tasks. Therefore, the specialisation of these individuals for these tasks will continue in the subsidiary fractions when they are provided with brood. I hypothesise that these individuals will increase their frequency of performing brood-related tasks compared to session 1, up to the levels seen in the united colonies in session 1, reflecting changing task demand due to the absence of brood-work specialists. I expect that there will be no significant difference between the overall frequency at which brood-related tasks are performed in the subsidiary fractions of session 3 and the overall frequency at which brood-related tasks are performed in session 1. This is because individuals in the subsidiary fractions are sufficiently flexible to respond to the need for brood-related tasks in the subsidiary fractions and to alter their individual absolute performance to maintain the overall frequency.

It is expected that individuals in the original fractions of session 3 will also adjust their task profiles to reflect the required demand for brood-related tasks in these fractions. This is unlikely to lead to a lack of significant correlations between the relative frequency at which individuals perform brood work in session 1 compared to the original fractions of session 3, as relative specialisation should be maintained.

I also expect that the individuals removed to the subsidiary fractions of the colonies will maintain any specialisation they have for brood-related tasks when the fractions of the colonies are re-united. Therefore, the relative frequency at which individuals perform brood-related tasks in session 1 and session 4 will be a significant correlation. I hypothesise that there will be no difference between the absolute frequency at which brood-related tasks are performed in session 1and session 4, because these individuals return to their original task profiles when the fractions are re-united in session 4. The following is a discussion of the results from experiment 2 in the light of these hypotheses.

The relationship between the frequency at which individuals perform brood-related tasks in session 1 compared to the frequency at which these individuals perform brood-work in the original factions of session 3 is shown in Figure 50 (b), for an example experimental colony (Colony 7). The relationship between the relative frequency at which these individuals perform brood-related tasks in session 1 and session 3 is a significant, positive correlation for Colonies 7 and 8 (see Table 42). Insufficient numbers of individuals have retained their marks in Colonies 5 and 6 for this relationship to be examined. Therefore, I accept the hypothesis that brood-work specialists maintain their relative specialisation for brood-related tasks in the original fractions of the colonies. On the basis of these results, I conclude that any non-significant result for the same comparison obtained in experiment 1 is due to insufficient numbers of individuals that have retained their marks in these sessions, rather than any true reflection of changes in task performance due to experimental manipulations. This is because these fractions are treated identically in both experiments up to this point. The specialisation of the individuals in the original fractions during session 3, for brood-related tasks is reflected by the results of statistical analyses comparing the absolute frequency at which brood-related tasks are performed to the frequency at which these tasks are performed, overall, in session 1 (see Table 45). The individuals in the original fractions in session 3 perform brood-related tasks at a significantly higher frequency in session 3, than the individuals in the whole colony in session 1. The individuals in the original fractions of Colony 8 during session 3 perform brood-related tasks at a significantly higher frequency than the individuals in the Control colony during session 3 (see Table 46). This reflects the flexibility of these individuals in changing the frequency at which they perform brood-related tasks in response to changing task demand.

I hypothesised, above, that the individuals that are removed from the colonies during session 2 and placed in subsidiary nests, where they are provided with brood, will maintain their specialisation for brood work. Moreover, that these individuals will be sufficiently flexible to adjust the frequency at which they perform brood-related tasks to the levels seen in the whole colony in session 1, in response to changing task demand. Indeed, there is a significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in the subsidiary fractions of the colonies in session 3 compared to the frequency at which these individuals perform brood-related tasks in the united colonies in session 1 (see Table 42), for colonies in which a sufficient number of individuals have retained their marks. Moreover, multiple comparisons show that there is no significant difference between the pooled correlational relationships between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4 in the experimental colonies, compared to this relationship in the Control colony (see Table 46a). Figure 50 (c) is a graphical representation of brood-related task performance by these individuals in session 1 compared to session 3. With the exception of one individual, the frequency at which individuals perform brood-related tasks tends to increase in session 3 compared to session 1. This result demonstrates the flexibility of workers in performing brood-related tasks as they increase their performance in response to changing task demand in this session, resulting from the absence of brood-work specialists. This is also reflected by the absence of a significant difference between the absolute frequency at which brood-related tasks are performed by these individuals in the subsidiary fractions in session 3 and the united colonies in session 1 (see Table 45), or in the Control colony in session 3 (see Table 46). This is not the case for Colony 6, in which these individuals perform brood-related tasks at a significantly higher absolute frequency than in the united colony in session 1. My explanation for this is the low number of individuals that have retained their marks during this session in Colony 6. The specialisation for brood-related tasks by individuals in the subsidiary fractions of the colonies occurs despite the absence of the queen, and despite the presence of adopted, foreign, brood.

The relationship between the frequency at which individuals in session 1 perform brood-related tasks, and the frequency at which these individuals perform brood-related tasks in the re-united colonies in session 4, is shown in Figure 50 (a) for Colony 7. I hypothesised above that all the individuals would maintain their specialisation for brood-related tasks when the colonies are re-united. This is the case. The relationship between the relative frequency at which individuals perform brood-related tasks in session 1 compared to session 4 is a positive, significant correlation for colonies in which sufficient numbers of individuals have retained their marks (see Table 42). There is no correlation between these variables for Colony 6, but only 7 of the individuals in this colony were identifiable in both session 1 and session 4. However, Fisher's z-transforms show that there is no significant difference between the correlation coefficient for the above relationship in the Control colony compared to experimental colonies 6, 7 and 8. This statistic cannot be calculated for Colony 5 as insufficient numbers of individuals have retained their marks in session 4 to calculate the correlation coefficient. There is no significant difference between the absolute frequency at which individuals perform brood-related tasks in session 1 and session 4, for any of the experimental colonies (see Table 45). There is also no significant difference between the absolute frequency of brood-work performed in the Control colony during session 4 compared to any of the experimental colonies. I conclude that individuals are sufficiently flexible to adjust the absolute frequency at which they perform brood-related tasks in session 3 to the new task demand in session 4.

A closer examination of the specialisation for brood-related tasks by individuals in session 1 compared to session 4, for the experimental colonies, is shown in Table 44. When individuals that were in the original fraction of the colony during session 3, are considered separately to those that were in the subsidiary fraction, only the former show a significant correlation between the relative frequency at which they perform brood-related tasks in the re-united colony compared to session 1. My explanation for these results is that there are relatively low numbers of individuals that have retained their marks when the relationship is considered separately for those individuals that were in the subsidiary fraction. Moreover, any individuals that perform no brood-related tasks in session 1 then perform brood-related tasks with a very low frequency in session 4, and, conversely, individuals that are not recorded as performing any brood-related tasks in session 4, but do so at a very low frequency in session 1, have a relatively large influence on the statistic.

CONCLUSIONS

The following conclusions can be drawn:

Individuals in the Control colony, in which one third of the workers have been temporarily removed to control for the physical effect of removal, perform brood-related tasks at the same relative frequency throughout the experiment. Thus, the physical effect of removing individuals does not affect relative task specialisation for brood-related tasks. There is no significant difference between the absolute frequency of brood-related task performance by individuals in the Control colony in sessions 2, 3 and 4 compared to session 1.

There is some tendency for the relative frequency at which individuals perform brood-related tasks to be conserved in the experimental colonies despite the sequential removal of the individuals associated with external activity during session 2 (significant Spearman's rank correlation coefficient for 2 out of 4 colonies).

> The removal of individuals associated with external activity has some effect on the behavioural specialisation of workers for brood-work. My explanation for this is the presence in session 2 of individuals that carry out brood-related tasks, that are removed later while performing external activity.

Task specialisation for brood-related tasks among the remaining workers, after individuals associated with external activity have been removed, is maintained in colonies in which there are sufficient numbers of individuals that have retained their marks.

> These remaining individuals are responsible for the majority of brood-related tasks in the un-manipulated colonies during session 1, and are classed as 'brood-work specialists'. Moreover, in the majority of colonies, these individuals are sufficiently flexible to reduce the absolute level at which they perform brood-related tasks so that their performance

is not significantly different from that of the united colonies during session 1. I conclude that this reflects the reduced task demand for brood-related tasks in the colony that results from the absence of workers associated with external activity, and consequent decrease in the availability of forage.

The individuals that are removed from the original nests because they are associated with external activity, have no opportunity to carry out brood-related tasks in the subsidiary fractions of the colonies in experiment 1, as there is no brood present. Of these workers, some of those that performed brood-related tasks in the un-manipulated colonies during session 1 are still found very close to the centre of the nest in the subsidiary fraction, despite the absence of a brood pile. However, the majority of workers that also perform brood-related tasks in session 1 are found to be located relatively further away from the centre of the nest in the subsidiary fraction. It is hypothesised that the latter individuals carry out external activity in the subsidiary fraction, whereas the former individuals do not. This is examined in section 8.3ii., below.

Individuals removed from the colonies because they are associated with external activity are placed in nests with brood in experiment 2. Relative specialisation for brood-related tasks is maintained among these individuals in the subsidiary fractions of the colonies during session 3, for colonies containing sufficient numbers of individuals that have retained their marks, for this to be examined. These individuals are sufficiently flexible to respond to the changes in task demand in the colony and adjust the absolute frequency at which they perform brood-related tasks so that it is not significantly different from the levels observed for the entire colonies in session 1. This result demonstrates the flexibility of workers in performing brood-related tasks: individuals increase their performance due to changing task demand in session 3, subsidiary fractions, resulting from the absence of a large number of brood-work specialists. This occurs despite the absence of the queen.

Brood-related task specialisation by individuals is not maintained when the fractions of the colonies are re-united compared to session 1, if the individuals that were removed are placed in a nest without brood. Only one of the four colonies in experiment 1 (Colony 2), shows a significant correlation between the relative frequency at which brood-related tasks are performed by individuals in the united colony in session 1, compared to the re-united colony in session 4.

The majority of workers that perform brood-related tasks in the un-manipulated colonies during session 1, but are removed because they also perform external activity in session 2, do not perform any brood-related tasks when the fractions of the colonies are re-united in session 4. My hypothesis is that some of these individuals performed external activity in the subsidiary fraction, and continue to do so the re-united colony, but that the specialisation of removed workers for brood-related tasks ceases due to their lack of opportunity to perform brood-related tasks in the subsidiary fractions. This could be a reflection of individuals different response thresholds for these tasks compared to 'brood-work specialists', or a reflection of the reproductive hierarchy in the colony, and is discussed further in Chapter 9.

Task specialisation by individuals for brood-related tasks, is conserved from session 1, when the fractions of the colonies are re-united in session 4 if the removed individuals were placed in a nest containing brood. Moreover, multiple comparisons show that there is no significant difference between the relative performance of brood-related tasks in session 1 compared to session 4, and this relationship for the Control colony.

The workers are sufficiently flexible to adjust the absolute frequency at which they perform brood-related tasks so that there is no significant difference between this, and the absolute frequency of brood-work performance in the colonies in session 1, or with the Control colony in session 4.

ii. An investigation of the flexibility of individual performance of external activity in colonies with a manipulated task structure

To examine the flexibility of individuals in terms of their performance of external activity, I determined how the frequency at which individuals perform external activity is affected by the removal of individuals previously associated with external activity in experiments 1 and 2. I examine how the frequency at which these removed individuals perform external activity is affected, when they were in fractions without brood (experiment 1), and with brood (experiment 2). After re-unification of these two fractions of the colonies in session 4, I examined how the performance of external activity overall, and for the 'removed' and 'original' workers separately, is affected, for the colonies from experiments 1 and 2. I plotted graphs of the frequency at which external activity is performed by individuals, comparing different sessions of the experiments. To determine if individual performance of external activity is resilient to the manipulations, I ranked the frequency at which individuals perform these tasks for the two sessions to be compared, and calculated the Spearman's rank correlation coefficient for this relationship (see beginning of Chapter 4 for details of this procedure).

It should be noted that while the performance of brood-related tasks is determined directly from the photographic record, the performance of external activity is determined by direct observations of the colonies immediately after each photograph is taken. It is possible, therefore, for individuals to be identified as performing external activity without ever having been identified from the photographic record, and thus not having established any spatial position in the nest. Obviously these individuals could not be included in the analyses of the results in Chapter 4, as these analyses involved spatial position. As I observed that these individuals often demonstrate high frequencies of external activity, I considered it important that they be included in the analyses in sections 8.1 and 8.2.

Experiment 1

The relationship between the frequency at which individuals in the Control colony (Colony C_1), perform external activity in session 1 compared to session 4 is shown in Figure 51 (a). There is a significant, positive correlation between the relative frequency at which individuals perform external activity in these two sessions (see Table 47). Individuals in the Control colony perform external activity at the same frequency relative to other individuals in session 4 as they do in session 1. This is also the case for the comparisons between other sessions for the Control colony (see Table 47, and Figure 52 for graphs of the frequency at which individuals perform external activity compared to (a) session 2, and (b) session 3). Thus, as is the case with brood-work, specialisation by individuals for external activity is maintained throughout the course of the experiment, despite the temporary removal of one third of the workers. I conclude that the physical effect of removing individuals does not affect individual specialisation for tasks associated with external activity.

Figure 51 (a) indicates that there is some variation in the absolute frequency at which tasks are performed in session 1 compared to session 4, and this is also the case for the other session comparisons, see Figures 52 (a) and (b). I used Mann-Whitney U tests to compare the absolute frequency at which external activity is performed in session 1, compared to the other experimental sessions (see Table 49). There is significantly less external activity in session 2 compared to session 1, and there is also significantly less external activity in session 4 compared to session 1. However, there is no significant difference between the absolute frequency at which external activity is performed in session 1 and session 3. My explanation is that the temporary removal of one third of the workers results in a decrease in the frequency of performance of external activity measured during session 2. This is because of the removal a number of individuals that are associated with external activity (they are removed because they are the first third of the population to emerge after the nest is opened, and hence are associated with external activity). The reduction in the absolute frequency of external activity in session 4 compared to session 1 is likely to be the result of changing task demand through the sociogenesis of the colony. We know from the results above that the frequency of brood-work in the Control does not change significantly compared between sessions 1 and 4. The data does not, however, allow me to determine whether the decrease in external activity is attributable to a reduction in the requirements of the colony for food for the brood, as the distinction between brood-related tasks, and brood-feeding is not made. Nevertheless, these results mean that any decrease in the absolute frequency at which external activity is performed in the experimental colonies in session 4 compared to session 1, cannot definitely be attributed to sociotomy. However, any change in the relative specialisation of individuals for external activity in these colonies can be attributed to sociotomy.

In section 8.3i., above, I determined that relative specialisation for brood-related tasks is maintained despite sociotomy, if individuals in the subsidiary fractions of the colonies are provided with brood. If they are not, then specialisation for brood-related tasks among these individuals breaks down. In the latter case, some of the individuals that performed brood-work prior to their removal from the colony during session 2, remain located close to the centre of the new subsidiary nest, whereas the majority of individuals are located nearer to the periphery of the nest. I hypothesise that these former individuals do not carry out external activity in the subsidiary fraction, and that the lack of opportunity for them to perform brood-related tasks in this session over-rides any previous specialisation they had for brood-work. Therefore, I expect that specialisation for external activity will be maintained by individuals removed to the subsidiary fractions of the colonies in session 3 compared to session 1, when these individuals are provided with brood (i.e experiment 2). I expect that specialisation of individuals for external activity will be disrupted in colonies of the removed individuals when they do not have access to brood (experiment 1). I also expect that when the colonies are re-united with the individuals that are not provided with brood in the subsidiary fractions, specialisation will continue to be disrupted.

It will be revealing to examine the performance of external activity by the individuals remaining in the original fractions of the colonies during session 3. There is a crucial difference in these experiments between brood-related tasks and external activity. When the colonies are divided into fractions based on their performance of external activity, some of the individuals that are removed also performed brood-related tasks. However, the remaining individuals did not perform external activity (else they would have been removed) and consequently any performance of external activity by these individuals would reflect 'precocious' external activity. Therefore, I hypothesise that when the fractions of the colonies are re-united in experiment 2, any disruption to specialisation for external activity can be attributed to such 'precocious' individuals, if the performance of external activity does, in some way, override specialisation for brood-work.

During, and after, the removal of workers associated with external activity from the experimental colonies (sessions 2 and 3 (original fraction), respectively) there is a drastic reduction in the frequency of external activity observed in the colonies during the experimental periods. This has been discussed in detail in section 4.3iv. No external activity is recorded for Colonies 1, 2 and 3, during session 2. All the workers associated with external activity had been removed in the bout of continuous removal at the beginning of session 2, prior to the beginning of the sampling procedure and the photographic record for that session). I recorded no external activity in the original fraction for colonies 1 and 2, during session 3.

The relationship between the frequency at which external activity is performed in the original fraction during session 3 compared to session 1, for one of the colonies that did show external activity in this fraction during session 3, is shown in Figure 53 (b). Firstly, as discussed in Chapter 4, the colony exhibits very low absolute frequencies of external activity during this session. Secondly, most of the individuals that do exhibit some external activity, also exhibit some external activity in the un-manipulated colony during session 1. In Chapter 4, I hypothesised that individuals that carry out external activity in the original fraction of session 3 could either be individuals that carry out external activity in session 1, but were not removed, or, alternatively, they are precocious foragers that have not previously carried out external activity. Only two individuals demonstrate external activity in session 4, but not in session 1, and can therefore be placed in the latter category (I recorded only one act of external activity for each of these individuals during session 4, and consequently they are seen as a single point in Figure 55 (b)). The individuals that carry out external activity in session 3 are therefore a combination of workers with previous experience of external activity in session 1, and these precocious foragers. In the colonies in which external activity is recorded during these sessions, there is no significant correlation between the relative frequency at which individuals carry out external activity in session 2, or in the original fraction of session 3, compared to session 1, see Table 37. However, the frequencies of external activity are very low and it is, therefore, difficult to establish a significant correlation.

I analysed the performance of external activity by individuals in the subsidiary fractions of the experimental colonies during session 3. There is a significant, positive correlation between the relative frequency at which individuals in the subsidiary fraction of Colony 1 perform external activity in session 1 compared to session 3. This is not the case for the other experimental colonies. The relationship between the absolute frequency at which individuals perform external activity in the subsidiary fraction during session 3 compared to their performance of external activity during session 1 is shown in Figure 53 (c), for an example experimental colony, Colony 3. The absolute frequency at which individuals perform external activity is higher in the subsidiary fraction of session 3, than for these individuals in session 1, for this colony. Mann-Whitney U tests show that this is reflected in the significantly higher frequency of external activity in this fraction of the colony than for the overall colony during session 1 (see Table 49). Although this does not apply to the other experimental colonies, the median frequency of external activity exhibited by these individuals is higher than the median frequency of performance of the workers in the Control colony, during session 3. The absolute frequency at which external activity is performed is significantly higher in these colonies than in the Control in two out of four cases, when compared using Mann-Whitney U tests, see Table 50. Figure 53 (c) shows that a number of the individuals in the subsidiary fractions of Colony 3, carry out no external activity in session 1, but demonstrate relatively high levels in session

3.

Individuals that perform brood-related tasks during session 1, but are removed because of their external activity, and subsequently do not perform brood-related tasks in session 4, change their task profiles in session 3. I hypothesised in section 8.3i., above, that this is a result of the absence of brood, and consequently, of opportunities to perform brood-related tasks, in session 3, subsidiary fractions. This is, in fact, the case in the subsidiary fractions during session 3. These individuals demonstrate a high absolute frequency of external activity in session 3, despite having performed little or no recorded external activity in session 1. I also hypothesised that of the individuals that perform brood-related tasks in session 1, but are removed in session 2, because they also demonstrate external activity, those that exhibit brood-related tasks subsequently to re-unification of the fractions of the colonies in session 4, show no external activity in the subsidiary fractions during session 3. These individuals are located closest to where the brood pile should have been in the nest in session 3, and do not perform any external activity, as hypothesised. Thus, the explanation for the disruption to the patterns of relative performance of external activity is due to the individuals that exhibit very low frequencies of external activity in the un-manipulated colonies during session 1, but then specialise as high performing externals in the subsidiary fractions during session 3. Is this phenomenon of changing specialisation caused by the absence of brood in the subsidiary fractions? This is discussed with reference to the subsidiary fractions of the experimental colonies in experiment 2, which do contain brood.

When the original and subsidiary fractions of the colonies are re-united in session 4, there is some tendency for the relative frequency at which individuals perform external activity to be significantly correlated to the relative frequency at which they perform external activity in the un-manipulated colonies during session 1 (for two of the four experimental colonies, see Table 47). I examined these correlations more closely by calculating separate correlation coefficients for the individuals in the re-united colonies that had been in the original fractions of the colonies during session 3, and for those that had been in the subsidiary fractions (see Table 48). The colonies that showed significant correlations between the overall relative frequencies of individual performance of external activity between sessions 1 and 4, also exhibit significant correlations between the relative frequency at which individuals that had been in the subsidiary fractions perform external activity in session 4 compared to session 1. I did not find significant correlations between the relative frequency at which individuals that had been in the original fractions during session 3 performed external activity in session 4 compared to session 1, for any of the experimental colonies. However, the numbers of the latter that retained their marks were relatively low, and the absolute frequency at which these workers performed external activity is also too low for a meaningful statistical analysis.

The relationship between the absolute frequency at which individuals perform external activity in session 1 compared to session 4 is shown in Figure 51 (b), for an example experimental colony (Colony 3). Individuals that had been in the subsidiary fraction of the colony during session 3 are shown in red, individuals that had been in the original fraction, in black. Again, individuals tend to exhibit higher frequencies of external activity in session 4 than in session 1. I analysed the absolute frequency of external activity in session 4 compared to session 1 for each of the experimental colonies (see Table 49). There is a significant increase in the frequency of external activity in session 4 compared to session 1 for Colony 3. However, this is not the case for the other experimental colonies – there is a significant decrease in the frequency of external activity in session 4 compared to session 1 for Colonies 2 and 4. As demonstrated above, external activity is performed at a significantly lower frequency during session 4 compared to session 1 for the Control colony. This natural decrease in the frequency of external activity, attributed to the normal sociogenesis of the colony, could have affected the results in the experimental colonies. I compared the distribution of the frequency of external activity in the experimental colonies during session 4 with the same distribution in the Control colony during session 4, using Mann-Whitney U tests. The results are shown in Table 50. There is no significant difference between the frequency of external activity exhibited by the Control colony during session 4 and during this session for the experimental colonies.

In summary, there is no difference in the absolute frequency of performance of external activity in the experimental colonies during session 4 compared to the Control colony. However, it has been seen that there is a tendency for the relative specialisation of individual workers for these tasks, to be disrupted. How can this be explained? My explanation is based on the observation that a small number of individuals that carry out external activity in the original fraction during session 3, continue to perform external activity in the re-united colony in session 4 (see Figure 51 (b). Several of these individuals actually increase the frequency at which they perform external activity in session 4 (some of these points on the graph are obscured). I hypothesised, above, that the individuals that were in the subsidiary fraction of session 3, and had carried out brood-related tasks in session 1, but stopped this in session 4, continue to carry out external activity in session 4. I showed that these individuals did perform external activity in session 3. A closer examination of the data shows that this is indeed the case. This may be the explanation why the correlation of the relative frequency at which individuals perform external activity in session 1 compared to session 4 is not always significant. Individuals that do not perform any external activity in the subsidiary fraction during session 3, but are located near to where the brood pile would have been, do not perform external activity in session 4 either. Consequently, the conclusions I reached concerning these individuals in section 8.3i., above, on specialisation for brood-related tasks, are confirmed. I discuss whether the disruption to the behaviour of these individuals in the subsidiary colony is caused by the absence of brood, in relation to the results from experiment 2, below. I also discuss whether their change from specialisation for brood-related tasks and external activity to solely external activity continues even when the fractions of the colony are re-united.

Experiment 2

The general trends observed in the experimental colonies in experiment 2 are consistent with the hypotheses suggested above. The pattern of external activity observed in the original fractions of the experimental colonies in session 3 is similar to that observed in the original fractions in experiment 1. Again, the frequency of external activity observed in these fractions is dramatically reduced, compared to the activity seen in the other sessions. In fact, I did not record any external activity for three of the four experimental colonies. There is a significant, positive correlation between the relative frequency at which individuals perform external activity in session 1 compared to the relative frequency at which these individuals perform external activity in the original fraction during session 3, for the colony in which I did observe some external activity (Colony 8, see Table 51). As observed for the experimental colonies in experiment 1, a large number of the individuals that do exhibit external activity in this session, only perform it at a low frequency. However, a number of the individuals in Colony 8 perform external activity at higher frequencies in the original fraction during session 3, and therefore it is possible to establish a significant correlation. The non-significance of the results obtained for this session in experiment 1 is therefore likely to be due to the low levels of external activity, as is suggested above. Moreover, I also examined the absolute frequency at which external activity is performed in the original fraction of Colony 8, during session 3, using Mann-Whitney U tests. There is no significant difference in the frequency at which external activity is performed in this session compared to session 1 (see Table 54), or to session 3 of the Control colony (see Table 55). Thus, these individuals are sufficiently flexible to respond to changing task demand within the colony resulting from the removal of individuals associated with external activity, and exhibit the same absolute frequency of external activity as an un-manipulated colony. The relationship between the frequency at which individuals perform external activity in the original fraction during session 3 compared to their performance of external activity during session 1, is shown in Figure 54 (c). The graph shows that, although there is some precocious external activity, there are a number of individuals that perform external activity during session 1 as well as session 3. These individuals do not perform external activity during session 2, or they would have been removed into the subsidiary fraction of the colony. However, all these individuals exhibit a relatively low frequency of external activity during session 1.

There is a significant, positive correlation between the relative frequency at which individuals perform external activity during session 1 compared to the frequency at which they perform these tasks in the subsidiary fraction of the colonies during session 3, for Colonies 7 and 8 (see Table 51). This is an important result. I hypothesised, above, that disruption to the behavioural specialisation of workers is caused by the absence of brood in the subsidiary fractions during session 3 for the experimental colonies in experiment 1, and that this results in continued disruption when the fractions of the colony are re-united in session 4. Figure 54 (c), again shows that individuals have a tendency to perform external activity at a higher frequency in session 3 than in session 1. Statistical analyses using Mann-Whitney U tests show that, in fact, there is a significantly higher frequency of external activity by the individuals in the subsidiary fraction during session 3 compared to the frequency of external activity in the intact, un-manipulated, colony during session 1 for three of the four experimental colonies (see Table 54). There is a significantly higher frequency of external activity by these individuals compared to the frequency of external activity in the Control colony during session 3 in the case of every colony (see Table 55). This is a reflection of changing task demand in the subsidiary fraction due to the changes in the ratio of the number of workers to the amount of brood present in these fractions. Individuals in Colonies 5 and 6 do not exhibit a significant correlation between the relative frequency at which they perform external activity in the subsidiary fraction during session 3 and session 1. Colony 5, again, shows a considerable amount of variation, as discussed above, and a large number of individuals still exhibit the general trend seen so clearly in Colonies 7 and 8. The results of Colony 6 are disrupted by a very small number of individuals that perform external activity with a much higher frequency in session 1 than they do in session 3. Thus, the result is not significant due to the relatively large influence of these individuals on the correlation coefficient, reinforced by the relatively low number of individuals that have retained their marks. I conclude that the disruption to the specialisation of workers in the subsidiary fractions of the colonies during session 3 in experiment 1, is caused by the absence of brood. Individuals change their behaviour from performing brood-related tasks and external activity, to solely performing external activity.

There is a significant, positive correlation between the relative frequency at which individuals perform external activity in the un-manipulated colonies during session 1 and the relative frequency at which they perform external activity in the re-united colonies during session 4, for Colonies 6, 7 and 8 (see Table 51). The relationship between individual performance of external activity during session 1 compared to session 4 is also significant when those individuals that were in the original fractions, and those that were in the subsidiary fractions, during session 3, are considered separately, where there is sufficient activity for correlations to be established (see Table 53). The correlation coefficient of the relationship between the relative frequency at which individuals in the Control colony perform external activity in sessions 1 and 4, is not significantly different from the correlation coefficients for this relationship for each of Colonies 6, 7 and 8, when compared using Fisher's z-transforms (see Table 52). Moreover, multiple comparisons show that there is no significant difference between the pooled correlational relationships between the relative frequency at which individuals perform external activity in session 1 compared to session 4 in the experimental colonies, compared to this relationship in the Control colony (see Table 55a). I conclude that behavioural specialisation is not affected by sociotomy in these colonies.

Colony 5 does not show a significant correlation between the relative frequency at which individuals perform external activity in sessions 1 and 4. A closer examination of the results for this colony reveals that there are a large number of individuals which do exhibit similar performance of external activity in sessions 1 and 4. However, the results are affected by the strong trend for a number of the workers in this colony to substantially increase their performance of external activity between sessions 1 and 4, and also by individual variation as discussed below. There are insufficient data regarding spatial organisation of brood-work available for this colony because a large number of individuals in this colony have not retained their marks. Therefore, I cannot draw any conclusions regarding the influence of the above factors on specialisation for external activity. The important point to note is that a large number of the individuals in this colony do exhibit the same trends as the other experimental colonies. The lack of significance of the correlation coefficient may well be due to individual variation, rather than any real effects experienced by the colony due to sociotomy. I discuss individual variation, and its possible causes, as well as the possible effects this may have on the general patterns observed for the colonies, below.

The relationship between the absolute frequency of performance of external activity by individuals during session 1 compared to their performance during session 4 is shown in Figure 54 (a) for an example experimental colony (Colony 7). As in experiment 1, there is an evident trend towards increased frequency of external activity for most individuals in the colony in session 4, compared to session 1. There is no significant difference between the absolute performance of external activity during each of the experimental colonies 6, 7 and 8 during session 4 compared to the absolute frequency of performance of external activity in this session in the Control colony, when the results are compared using Mann-Whitney U tests (see Table 55). There is a significant increase in the absolute performance of external activity by individuals in Colony 5 during session 4 compared to the Control, and Colony 5 is discussed above. No meaningful conclusions can be drawn by analysing the relationship between the absolute frequency of performance of external activity in each colony during session 4 compared to session 1, because of the change in the frequency of performance of external activity exhibited by the Control colony.

There is some individual variation within the experimental colonies. Examining Figure 54 (a) more closely reveals that there are two individuals that do not follow the trend of maintained specialisation, despite the overall significance of the relationship for this colony. These two individuals perform substantially more external activity in session 1, than in session 4. This is also the case for one individual in Colony 6, and this may have had a relatively greater effect on the correlation coefficient obtained for this relationship in Colony 6, due to the lower number of individuals in this colony that retained their marks. This may have been the cause of the resulting p value being greater than 0.01 when the correlation coefficient for this relationship is tested for significance (although the correlation is still significant). In Colony 6 this unusual individual actually performs a relatively high number of brood-related tasks in session 1, but is not recorded as performing any brood-related tasks in the subsidiary fraction of session 3, and only performs one brood-related task during session 4. The photographic record does not show that the two individuals concerned in Colony 7 perform any brood-related tasks. It is important to note, therefore, that deviation from the general trend is observed for a small number of individuals in some colonies. The individuals discussed here spend a relatively large amount of time outside the nest, and therefore I collected little or no information about their spatial positions during the different sessions of the experiment. It is likely that the general activity of these individuals decreases throughout the course of the experiment, and as they do not follow the general trends, it is unlikely that this reduction in activity is caused by the manipulations performed on the colonies. It is more likely that the decrease in activity observed in these individuals is a result of internal factors, for example, senescence.

I concluded, above, that the change in behavioural specialisation by workers that perform both external activity and brood-related tasks during session 1 to the performance solely of external activity in the subsidiary fractions during session 3 for the colonies in experiment 1, continues when the fractions of the colony are re-united. I have shown, above, that when removed workers are placed in a subsidiary nest which contains brood, specialisation for brood-related tasks, and external activity, is generally not disrupted, save for individual variation which has been discussed in detail above.

CONCLUSIONS

The following conclusions can be drawn:

Individuals in the Control colony perform external activity at the same relative frequency throughout the experiment. Thus, the physical effect of removing individuals does not affect task specialisation for external activity.
There is some variation in the absolute frequency at which external activity is performed in the Control colony during session 1 compared to the other sessions. There is a significant decrease in the absolute performance of external activity in session 2, and I attribute this the absence of one third of the workers for one week. There is also a reduction in the absolute performance of external activity in session 4. This is attributed to changes in task demand through the natural sociogenesis of the colony.

During, and after the removal of workers associated with external activity (sessions 2 and 3, original fraction, respectively) very little external activity is recorded for the remaining individuals.

> When there is a reasonable amount of external activity, relative task specialisation is conserved among individuals that exhibited external activity in the un-manipulated colony.
> Precocious external activity is recorded for some individuals.

There is generally little tendency for removed individuals to retain their behavioural specialisation for external activity in experiment 1. These individuals are removed to a subsidiary fraction in a nest that does not contain any brood (no significant correlation for three of the four experimental colonies).

> The absolute frequency at which these workers carry out external activity tends to be higher than the frequency at which they carry out external activity in the un-manipulated colony in session 1. Indeed, some precocious external activity occurs in these fractions during session 3. This is attributed to changes in task demand resulting from

the absence of brood, and a large proportion of the workforce, in these fractions.

The relative performance of external activity by removed individual workers when they are placed in nests containing brood, in experiment 2, is maintained in colonies where sufficient numbers of individuals retain their marks. Behavioural specialisation is retained by individuals when brood is present in these fractions. The absence of the queen, and a proportion of the workers, does not affect the relative specialisation of individuals for external activity.

This is the first demonstration of social resilience despite the absence of the queen and a large proportion of the workers, and is evidence of the considerable resilience of relatively small colonies of this 'simple', monogynous, ant to drastic changes in the composition of colony.

The absolute frequency of performance of external activity by these individuals is significantly higher than in session 3 of the Control colony. This is attributed to changes in the brood to worker ratio.

There is some tendency for task specialisation by individuals, in terms of external activity, to be conserved when the fractions of the colonies are re-united, compared to individual task specialisation in the colonies before workers associated with external activity are removed in experiment 1. This occurs despite the absence of brood, and subsequent disruption to specialisation, in these fractions during session 3. There is a significant, positive correlation between the relative performance of external activity in session 1 compared to session 4 in two of the four experimental colonies.

The disruption to task specialisation for external activity in the other two colonies is largely attributable to the performance of external activity in session 4 by individuals which did not show external activity in session 1. These consist first, of individuals that exhibit external activity in the original fraction during session 3, and continue to do so in session 4 (the precocious individuals, mentioned above); second, the workers that perform both external activity and brood-related tasks in session 1, and then cease brood-related tasks in the subsidiary fraction during session

3, and perform solely external activity. These individuals continue to perform external activity in the re-united colony, and do not perform brood-related tasks.

This confirms the hypothesis that the performance of external activity may override the potential for brood-related tasks, if brood-related tasks are unavailable, in some individuals. This could be a reflection of individuals different response thresholds for these tasks, or a reflection of the reproductive hierarchy in the colony.

Task specialisation by individuals, in terms of external activity, is conserved when the fractions of the colonies are re-united compared to individual performance of external activity in the colonies before workers associated with external activity are removed in experiment 2. The relationship between relative frequency at which individuals perform external activity in session 1 compared to session 4 is not significantly different from this relationship for the un-manipulated Control colony. In this experiment, removed individuals are placed in a nest with brood. It is, therefore, the absence of brood, and the subsequent disruption to worker task profiles in experiment 1, that results in the trend towards continued disruption of specialisation in the re-united colonies. Social resilience in terms of external activity occurs in the colonies in experiment 2, which have access to brood in session 3.

There is evidence that the absolute performance of external activity during session 4 is not significantly different from the absolute performance of external activity in the Control colony during session 4.

An investigation of the flexibility of individual performance of tasks in colonies with a manipulated age structure

Experiment 3

iii. An investigation of the flexibility of individual performance of brood-related tasks in colonies with a manipulated age structure

I compared the relative frequency at which individuals perform brood-related tasks in session 1 to session 3, for the Control colony in experiment 3 (Colony C_3). The relationship is a highly significant positive correlation (see Table 56). Thus, individual specialisation for brood-related tasks in this colony is preserved throughout the length of the experiment. I also analysed this relationship for the older workers and the callows in the Control colony separately (see Table 58). There is a significant, positive correlation between the ranked frequency at which callows perform brood-related tasks in session 1 compared to session 3. However, I cannot establish a significant correlation for this comparison for the older workers. My explanation for this is the relatively low numbers of older individuals that retained their marks in session 3 (n=10). Specialisation for brood-related tasks is also conserved in session 1 compared to session 2 (see Table 56). Colony C_3 was un-manipulated except for marking and subsequent emigration of the colony. Thus, any differences in task specialisation among workers of different ages between the Control and the experimental colonies, is due to the removal of the callow cohort from the experimental colonies prior to the beginning of session 1, and their subsequent re-unification prior to session 3. There is some variation in the absolute frequency at which individuals in the Control colony perform brood-related tasks in session 1 compared to session 2, and compared to session 3 (see Figures 55 (a) and (b) respectively). However, analyses using Mann-Whitney U tests show that there is no significant difference between the absolute frequency at which brood-related tasks are performed in session 1 and session 2, or session 1 and session 3, in the Control colony (see Table 59). I showed in Chapter 4 that brood-related tasks are spatially organised, and that both older and younger individuals perform these tasks. This is clearly seen in Figures 55 (a) and (b), where callows are shown in red, and older individuals are shown in black.

I examined whether specialisation for brood-related tasks in the experimental colonies is preserved between sessions 1 and 2. There is a significant, positive correlation between the relative frequency at which individuals perform brood-related-tasks in session 1 compared to the relative frequency at which they perform these tasks during session 2 (see Table 56). This is the case for both the 'callow' and the 'older' fractions of the colony. Thus, individual specialisation for brood-related tasks is preserved through sessions 1 and 2 of the experiment by both the callow workers, when they are in colony fractions with foreign brood, and the older workers in the colony fractions with the original brood.

The relationship between the absolute frequency at which individuals perform brood-related tasks in session 1 compared to the frequency at which they perform these tasks in session 2 is shown in Figures 56 (a) and (b), for the 'older' and 'callow' fractions of Colony 9 respectively. Analysis using Mann-Whitney U tests shows that there is no significant difference between the distribution of the frequency at which brood-related tasks are performed during session 1 and session 2, for the workers in either the 'older' or the 'callow' fractions of the colony for three of the four experimental colonies (see Table 59). Colony 10 is an exception to this. The absolute frequency at which individuals in the 'older' fraction of Colony 10 perform brood-related tasks during session 2, is significantly higher than the absolute frequency at which these individuals perform brood-related tasks during session 1. Conversely, the absolute frequency at which individuals perform brood-related tasks decreases significantly in session 2 compared to session 1 in the 'callow' fraction of this colony.

I also compared the absolute frequency at which brood-related tasks are performed in each fraction of the experimental colonies with the absolute frequency at which brood-related tasks are performed in the united Control colony, using Mann-Whitney U tests (see Table 60). There is evidence that individuals in the older fractions of the experimental colonies are sufficiently flexible to respond to the change in task demand resulting from the absence of callow workers. This leads to levels of absolute performance of brood-related tasks that are not significantly different from those which are observed in the united Control colony during the relevant session. This is evident for one colony in session 1 (Colony 10), and two colonies in session 2 (Colonies 9 and 12). Individuals in the older fraction of Colony 10 actually exhibit significantly higher frequencies of performance of brood-related tasks during session 2 than the united Control colony during this session. Thus, there is evidence that older individuals are sufficiently flexible to respond to the demand for brood-related tasks when all the younger individuals are absent. This response occurs in a larger number of colonies in session 2 compared to session 1, and this indicates that individuals respond increasingly over time, or increase their specialisation over time. I hypothesise that these older individuals will revert to their former behavioural profiles when the younger workers are re-united with them. I discuss whether the length of time the callows are absent affects the tendency for behavioural flexibility, below.

The callows from Colony 9 have never been in the presence of older workers in an undisturbed colony, and yet they exhibit task specialisation for brood-related tasks. Moreover, the pattern of specialisation is very similar to that seen in the un-manipulated Control colony. I determined above, that there tends to be no significant difference between the frequency at which brood-related tasks are performed by the callow fractions of the experimental colonies in sessions 1 and 2. Colony 10 exhibits a lower frequency of absolute performance of brood-related tasks during session 2 compared to session 1 (see Table 59). My explanation for this is that the callow individuals in this colony are responding flexibly over time to the changing task demand in the colony resulting from the absence of all the older workers. I compared the distribution of the frequency at which brood-related tasks are performed by the callow fractions during session 1, and during session 2, with the distribution of the frequency at which these tasks are performed by individuals in the united Control colony in the relevant session (see Table 60). There is evidence that individuals in the callow fractions of the experimental colonies are sufficiently flexible to respond to the change in task demand, resulting from the absence of all the older workers. This leads to distributions of the frequency at which brood-related tasks are performed that are not significantly different from those that are observed in the united Control colony during the relevant session. Again, there is evidence that the response of the callow individuals increases over time. The distribution of the frequency at which individuals perform brood-related tasks tends to become more similar to the Control colony as the experiment progresses. All four experimental fractions exhibit brood-related tasks at a frequency that does not differ significantly from the Control colony during session 2. Only two of the four experimental colonies exhibit this response during session 1 (see Table 60).

I compared the individual specialisation for brood-related tasks in session 1 with session 3, considering firstly, the older workers and the callow workers together, and secondly, separately. There is a highly significant, positive correlation between the relative frequency at which individuals perform brood-related tasks in session 3 compared to the relative frequency at which they perform brood-related tasks in session 1, when the older workers and callow workers are considered together, for every experimental colony (see Table 56). Thus, individual specialisation for brood-related tasks is maintained by the workers when the fractions are united. There is no significant difference between the correlation coefficient of this relationship for each experimental colony and the correlation coefficient for the same relationship in the Control colony, compared using Fisher's z-transforms (see Table 57).

I analysed the resilience of specialisation compared between sessions 1 and 3 for the older workers and the callows separately. The results are shown in Table 58. There is a highly significant, positive correlation between the relative frequency at which older workers perform brood-related tasks in session 1 and session 3, for all four experimental colonies. There is also a significant, positive correlation between the relative frequency at which callows perform brood-related tasks in the united colonies in session 3, compared to the frequency at which they perform brood-related tasks in the separate fractions in session 1, for three of the four experimental colonies. Colony 11 is an exception to the latter result, and the callows in this colony do not exhibit resilience in their specialisation for brood-related tasks between sessions 1 and 3. This is discussed with reference to the patterns of external activity observed in this colony, see below. In colonies where I established a significant correlation between the ranked frequency at which individuals perform brood-related tasks in session 1 compared to session 3, I performed multiple comparisons. I show that there is no significant difference between the pooled correlational relationships between the relative frequency at which callow individuals perform brood-related tasks in session 1 compared to session 3 in the experimental colonies, compared to this relationship for the callows in the Control colony (see Table 60a). I do not perform this analysis for the older workers, as I cannot establish a significant correlation between the ranked frequency at which the older individuals in the Control colony perform brood-related activity in session 1 compared to session 3, as explained above.

The absolute performance of brood-related tasks was also examined. I compared the frequency distribution of the performance of brood-related tasks by the older workers in session 1 with the frequency distribution of their performance in the united colonies in session 3 using Mann-Whitney U tests (see Table 59). There is no significant difference between the frequency at which brood-related tasks are performed by the older workers in session 1 compared to session 3 except in Colony 12, which exhibits a significantly lower frequency of brood-work. Thus, the older workers tend to adjust their levels of performance in response to the changing task demand, which results from their re-unification with the callow cohort. I also compared the absolute performance of brood-related tasks by the callow individuals in session 1 with their performance in session 3 (see Table 59). There is no significant difference between the absolute frequency at which brood-related tasks are performed by the callows compared between sessions 1 and 3 in two of the four experimental colonies. The callows in Colony 10 exhibit a significantly lower frequency of brood-work in session 3 than they did in session 1. Conversely, the callows in Colony 12 exhibit a significantly higher frequency of brood-work in session 3. I also compared the absolute performance of callows and older workers in session 3 with the absolute performance of the workers in the Control colony. There is no significant difference between the absolute frequency at which brood-related tasks are performed in the Control colony during session 3 and the frequency at which these tasks are performed in each of the united experimental colonies in this session (see Table 60). I conclude that the individuals are sufficiently flexible in their performance of brood-related tasks to respond to the changed task demand in the re-united colonies in session 3, so that there is no significant difference between the overall performance of these colonies and that of an un-manipulated colony. Despite this, relative specialisation, by both callow and older individuals is preserved after re-unification, and the two fractions intermingle both behaviourally, as can be clearly seen in Figure 56 (a), and spatially, as I determined in Chapter 7. I conclude that individual experience in performing these tasks, rather than age, leads to their continued relative specialisation, and that absolute performance of tasks is adjusted according to changing task demand. The mechanism suggested in Backen *et al.*, (2000) which can explain the results in experiments 1 and 2, is also consistent with these results and the conclusions I have drawn above. This is discussed further in Chapter 9.

CONCLUSIONS

The following conclusions can be drawn:

Artificially established single age cohort colonies of completely inexperienced callows establish individual specialisation for brood-related tasks.

This specialisation by individuals is consistent from the beginning of session 1 to the end of session 2 (10 weeks in total).

There is no significant difference between the frequency distributions of the absolute performance of brood-related tasks during session 1 and session 2, for the workers in the 'callow' fractions of the colonies, for three of the four experimental colonies.

> Individuals in the callow fractions of the experimental colonies are sufficiently flexible to respond to the change in task demand resulting from the absence of all the older workers. This leads to levels of absolute performance of brood-related tasks that are not significantly different from those which are observed in the united Control colony during the relevant session.
>
> Absolute performance of brood-related tasks by the 'callow' fractions tends to become more similar to the Control over time.

Colonies from which the entire callow cohort has been removed also establish individual specialisation for brood-related tasks.

> This specialisation by individuals is consistent from the beginning of session 1 to the end of session 2 (10 weeks in total).
>
> There is no significant difference between the absolute performance of brood-related tasks during session 1 and session 2 for the workers in the 'older' fractions of the colony, for three of the four experimental colonies.
>
> Individuals in the 'older' fractions of the experimental colonies are sufficiently flexible to respond to the change in task demand resulting from the absence of callow workers. This leads to levels of absolute

performance of brood-related tasks that are not significantly different from those which are observed in the united Control colony during the relevant session.

Absolute performance of brood-related tasks by the 'older' fractions tends to become more similar to the Control over time.

When the 'older' and 'callow' fractions of the colony are re-united, there is generally no disruption to the pattern of brood-related task performance among the workers compared to when they were in separate fractions. Thus, individual specialisation for brood-related tasks is maintained by the workers when the fractions are re-united.

There is no significant difference between the absolute performance of brood-related tasks in the Control colony during session 3 and the performance of these tasks in each of the united experimental colonies in this session.

Individuals are sufficiently flexible in their performance of brood-related tasks to respond to the changed task demand in the re-united colonies in session 3. There is no significant difference between the frequency at which brood-related tasks are performed in the united colonies (when the callows and older workers are considered together) compared to un-manipulated colonies. Despite the continued individual specialisation exhibited by individuals in the re-united colonies, the two fractions intermingle both behaviourally and spatially.

Multiple comparisons show that there is no significant difference between the pooled relationships for the callow individuals in the experimental colonies compared to the Control colony.

Individual experience in performing these tasks, rather than age, leads to their continued relative specialisation, and absolute performance of activity is adjusted according to changing task demand. This is discussed further in Chapter 9.

iv. An investigation of the flexibility of individual performance of external activity in colonies with a manipulated age structure

I compared the relative frequency at which individuals perform external activity in session 1 with the relative frequency at which they perform external activity in session 3, for the Control colony in experiment 3 (Colony C_3). The relationship is a highly significant, positive correlation (see Table 62). I conclude that individual specialisation for external activity in this colony is maintained throughout the length of the experiment. Individual specialisation for these tasks is also preserved in session 1 compared to session 2 (see Table 62). As colony C_3 was not manipulated (except for marking and subsequent emigration of the colony), any difference in the maintenance of specialisation in the experimental colonies, compared to the Control, is attributed to sociotomy.

The relationships between the absolute frequency at which individuals in the Control colony perform external activity in session 1 compared to session 3, and in session 1 compared to session 2, are shown in Figures 57 (a) and (b), respectively. In these graphs, callows are represented in red and older workers are represented in black. Analyses using Mann-Whitney U tests, show that there is a significant decrease in the absolute frequency at which individuals perform external activity in session 2 compared to session 1, and in session 3 compared to session 1 (see Table 65). My explanation for this change in the absolute performance of external activity is a seasonal decrease in demand for these tasks, resulting from natural changes in the relative proportions of the colony constituents through the season. Such a decrease is not observed in the Control colony used in experiments 1 and 2 (Colony C_1). This reflects the later time during the season at which experiment 3 is performed.

I showed in Chapter 4 that individuals that perform external activity are spatially organised in the nest. The frequency at which individuals perform external activity increases as their median distance from the centre of the colony increases. Younger individuals are located closer to the centre of the colony, and they perform external activity at a lower frequency than older individuals. This is clearly seen in Figures 57 (a) and (b).

I examined whether individual specialisation for external activity in the experimental colonies is preserved between sessions 1 and 2. There is a significant, positive correlation between the relative frequency at which individuals perform external activity in session 1 compared to the relative frequency at which they perform these tasks during session 2, for the older fractions of the colonies (see Table 62). I conclude that individual specialisation for external activity is preserved through sessions 1 and 2 of the experiment by the older workers when they are in a nest with the colony's original brood, but from which all the younger workers have been removed.

There is no significant correlation between the relative frequency at which the individuals in the callow fractions of three of the four experimental colonies perform external activity in session 1 compared to session 2 (see Table 62). There is a significant correlation between the relative frequency at which individuals in the callow fraction of Colony 11 perform external activity in session 1 compared to session 2. I conclude that in the majority of experimental colonies individual specialisation for external activity in the callow fraction is not maintained when session 2 is compared to session 1. I attribute this to the low levels of external activity exhibited by these individuals during session 1. The relationship between the absolute performance of external activity by the callow fraction of an example experimental colony (Colony 9) in session 1 compared to session 2 is shown in Figure 58 (c). This graph shows that during session 1 these individuals perform external activity at low frequencies. As specialisation for these tasks is less pronounced among these callow workers, the relative frequency at which an individual performs external activity can be considerably affected by small changes in absolute performance and this leads to a non-significant result. A closer examination of the callow fraction of Colony 11 (in which a significant correlation is established between the relative performance of external activity in sessions 1 and 2) reveals that only two individuals perform any external activity during session 1. These individuals continue to perform external activity in session 2 at the same relative frequencies (although higher absolute frequencies), resulting in a significant correlation. Therefore, comparing the relative specialisation for external activity of individuals in the callow fractions of the experimental colonies between sessions 1 and 2 is not meaningful due to the very low levels of external activity exhibited by these individuals in session 1. It is more meaningful to compare absolute performance of external activity between these two sessions. This also highlights the disadvantage of using Spearman's rank correlation analyses to compare relative specialisation. If absolute frequencies of performance of the relevant task are low, and the number of individuals involved in the analysis is also low, a significant correlation can be established where there is no biologically meaningful pattern.

The relationship between the absolute performance of external activity in session 1 compared to session 2 is shown in Figures 58 (a) and (b), for the 'older' and 'callow' fractions of Colony 9, respectively. I will discuss the results for the older fractions of the experimental colonies first.

I have already shown that specialisation for external activity is maintained when session 2 is compared to session 1 among the older workers. I compared the absolute performance of external activity by these individuals in session 2 and session 1, using Mann-Whitney U tests. There is no significant difference between the absolute frequency at which external activity is performed in session 1 compared to session 2, for three of the four experimental colonies (see Table 65). There is a significant decrease in the frequency at which external activity is performed by the older fraction of Colony 12 in session 2 compared to session 1. The Control colony also demonstrates a decrease in absolute frequency at which external activity is performed in session 2 compared to session 1. Therefore, from this analysis, it cannot be established whether or not the decrease in absolute performance observed in Colony 12 results from sociotomy.

I also compared the absolute performance of external activity in the older fractions of the experimental colonies with the absolute performance of external activity by the united Control colony, in the relevant session (see Table 66). There is no significant difference between the absolute performance of external activity in session 1 compared to the Control for two of the four experimental colonies. Of the remaining colonies, there is a significant increase in the absolute performance of external activity in session 1 of Colony 12 compared to the Control, whilst there is a significant decrease in the absolute performance of the workers in Colony 10 compared to the Control. I also compared the absolute performance of external activity by the older fractions of the experimental colonies in session 2, compared to the performance of the united Control colony during this session. Again, there is no significant difference in the absolute performance of external activity for this comparison, for two of the four experimental colonies (Colonies 10 and 11). There is a significant increase in absolute performance of external activity in the older fractions of experimental Colonies 9 and 12, compared to the Control (see Table 66). I conclude that the absolute performance of external activity is more affected by inter-colony variation, for example the ratio of the number of workers to the number of items of brood in the colony, or population size, than by the removal of all the young workers in the colony. This is not surprising, as it is the older workers that are primarily responsible for external activity in the colonies. However, it is important to note that the absence of the younger workers in the colony does not affect continued individual specialisation for external tasks, despite the fact that some workers perform brood-related tasks.

As I concluded above, whether relative specialisation for external activity is maintained among the callow workers when session 2 is compared to session 1 cannot be determined because of the low levels of external activity exhibited by these workers. I compared the absolute performance of external activity by the callow fractions in session 2 and session 1 to determine if the absence of older workers results in these inexperienced callows increasing their absolute performance of external activity. I compared the absolute performance of external activity by these workers in session 1 and session 2, for each of the experimental colonies, using Mann-Whitney U tests (see Table 65). The absolute performance of external activity is significantly higher in the experimental colonies in session 2 compared to session 1, for two of the four callow fractions (Colonies 9 and 10). There is no significant difference in the absolute performance of external activity in sessions 1 and 2 by the callow fractions of Colonies 11 or 12. Again, it is more meaningful to compare the performance of external activity in session 1, and in session 2, with the relevant session of the Control colony to eliminate any seasonal changes in performance.

I examined the absolute performance of external activity in the callow fractions of each experimental colony during session 1, and during session 2, with the performance of external activity in the relevant session of the united Control colony, using Mann-Whitney U tests (see Table 66). The absolute performance of external activity in the callow fractions of all four experimental colonies is significantly lower than in the united Control colony during session 1. The callows tend to perform external activity at a lower frequency than the absolute performance of a united colony. When I compared the absolute performance of brood-related tasks in the callow fractions during session 2 and the Control colony during this session, I found no significant differences. I conclude that callows will readily perform brood-related tasks, even when they have no previous experience of these tasks, but will not readily perform external activity in the 'callow' fractions. One explanation for this result is that callows have an innate tendency to perform brood-related tasks, as oppose to external activity. However, I hypothesise that callows are more likely to carry out brood-related tasks as they are likely to be encountered, physically, before the nest entrance is encountered. Moreover, there is evidence that the callows do adjust their absolute performance of external activity as the experiment proceeds. There is no significant difference between the absolute performance of external activity by the callow fractions of two of the experimental colonies (Colonies 9 and 10) during session 2 and the united Control colony (older and younger workers considered together). Thus, despite their lack of experience, and lack of any opportunity to learn from older workers in the colony, the callows may increase the frequency at which they perform external activity to the extent that there is no significant difference between their absolute performance and the performance of an un-manipulated colony. This occurs despite the absence of a queen, and the presence of adopted brood. It will be revealing to determine if this increase in absolute performance of external activity by the naïve callows is maintained when the callows are united with the older workers. I hypothesise that the individual specialisation by callows for external activity will not continue after unification. Specialisation of the older workers in the colonies for brood-related tasks, which is established by their flexible response to the absence of younger workers in sessions 1 and 2, continued in session 3. However, it is assumed that these older individuals had experience of performing brood-related tasks in their life (as younger individuals, as these tend to perform brood-related tasks at relatively high frequencies). The callows are completely naïve to external activity (as they have only just eclosed), and therefore this is not behavioural 'reversion', but a precocious behaviour. I hypothesise that this will not continue in session 3, and this is discussed below.

I compared the individual specialisation for external activity in session 1 with session 3. First I consider the older workers and the callow workers together. Second, I consider the two fractions separately. There is a significant, positive correlation between the relative frequency at which individuals perform external activity in session 3 compared to the relative frequency at which they perform external activity in session 1, when the older workers and the callows are considered together (see Table 62). Thus, individual specialisation of the workers, established when they existed in separate fractions, is maintained by the workers when the fractions are united. There is no significant difference between the correlation coefficient of this relationship for each experimental colony and the correlation coefficient for the same relationship in the Control colony, compared using Fisher's z-transforms (see Table 61).

I analysed the resilience of specialisation compared between sessions 1 and 3, for the older workers and the callows separately. The results are shown in Table 63. There is a significant, positive correlation between the relative frequency at which older workers perform external activity in session 1 and session 3 for all the experimental colonies. There is a significant, positive correlation between the relative frequency at which the callows perform external activity in the united colonies in session 3 compared to the frequency at which they perform these tasks in session 1 for three of the four experimental colonies. Thus any specialisation for external activity established by the callows in session 1 is preserved, or re-established, in session 3. Moreover, multiple comparisons for colonies exhibiting a significant relationship between ranked frequency at which individuals perform external activity in session 1 compared to session 3, show that there is no significant difference between the pooled correlational relationships in session 1 compared to session 4 in the experimental colonies, for either the callows or the older workers, considered separately, compared to this relationship in the Control colony (see Table 66a).

Colony 11 is an exception to the rule, and the callows do not exhibit resilience in their relative specialisation for external activity between sessions 1 and 3. I analysed the relationship between the relative frequency at which these individuals in Colony 11 perform brood-related tasks in session 1 and session 3. Again, Colony 11 was an exception to the general trend of significance in this relationship. The callows in Colony 11 do not exhibit resilience in their specialisation for brood-related tasks or for external activity. My explanation for these results stems from the observation that the callow fraction of Colony 11 consists of fewer individuals than the callow fractions of the other colonies. Behaviour may be disrupted in colonies when the population has been artificially reduced below a certain threshold. I suggested, in section 7.3i. that the removal of a relatively large proportion of Colony 4 into the subsidiary fraction disrupts subsequent spatial organisation among the remaining individuals in the original fraction during session 3. This may be connected with disruption to the mechanism by which specialisation is maintained after it has been established.

I also examined the absolute performance of external activity. I compared the absolute performance of external activity by the older workers in session 1 with their absolute performance of external activity in session 3, using Mann-Whitney U tests (see Table 65). There is no significant difference between the absolute performance of external activity by the older workers in session 1 compared to session 3, except in Colony 12, which exhibits significantly less external activity in session 3. This is a similar pattern to that established with regard to brood-related activity, and I conclude that, in the majority of colonies, older workers adjust their levels of performance of both brood-related tasks, and external activity, in response to changes in task demand resulting from their re-unification with the callow cohort. I also compared the absolute performance of external activity by the callows compared between the sessions. There is no significant difference between the absolute performance of external activity by the callows between sessions 1 and 3 for three out of four of the experimental colonies. The callows in Colony 9 exhibit significantly more external activity in session 3 than they did in session 1.

In order to eliminate any effect resulting from the seasonal decrease in the absolute performance of external activity established for the Control colony, I compared the performance of the callows, and the older workers, in session 3, for each experimental colony, with the absolute performance of the workers in the Control colony (see Table 66). There is no significant difference between the absolute performance of external activity by the workers in the Control colony during session 3 and the absolute performance of these tasks in this session in each of the experimental colonies. I conclude that individuals are sufficiently flexible in their performance of external activity to respond to the changed task demand in the united colonies in session 3. Thus, there is no significant difference between the overall performance of these colonies and that of the un-manipulated Control. Relative specialisation for external activity tends to be maintained, and it has already been noted that the two fractions intermingle behaviourally, and spatially. This confirms the conclusions reached in section 8.3iii., that individual experience in performing tasks leads to continued relative specialisation, and that absolute performance of external activity is adjusted according to changing task demand.

CONCLUSIONS

The following conclusions can be drawn:

Artificially created single age cohort colonies of completely inexperienced callows establish specialisation for external activity.

This specialisation occurs over a period of adjustment by the colony. As the absolute performance of external activity during session 1 is relatively low, it is not meaningful to compare relative specialisation among the callows for these tasks between sessions 1 and 2. The absolute performance of external activity is significantly higher in session 2 than in session 1 for two of the four experimental colonies. The absolute performance of external activity is significantly lower in the callow fractions of all four experimental colonies compared to session 1 of the united Control colony. However, there is evidence that the callow fractions are sufficiently flexible to accommodate changing task demand resulting from the absence of older workers after a sufficient period of time. The absolute performance of external activity by two of the four callow fractions in session 2 is not significantly different from the absolute performance of the Control colony during this session.

Colonies from which the entire callow cohort has been removed also establish specialisation for external activity.

Individual relative specialisation is consistent from the beginning of session 1 to the end of session 2 (10 weeks in total). There is no period of adjustment, and this is attributed to the fact that it is the older individuals that tend to be responsible for external activity in un-manipulated colonies (see Colony C_3).

There is no significant difference between the absolute performance of external activity in session 1 compared to session 2 by the older fractions of three in the four experimental colonies. The remaining colony exhibits significantly less external activity in session 2 compared to session 1, but this trend is also observed in the Control colony, and may be due to seasonal changes.

The absolute performance of external activity by the older fractions of the experimental colonies is influenced by inter-colony variation.

It is important to note that relative specialisation among older individuals for external activity is not affected by the absence of the younger age cohort, despite the fact that some of the older workers perform brood-related tasks.

When the 'older' and 'callow' fractions of the colony are united, there is no disruption to individual relative specialisation for external activity compared to the separate fractions in session 1, when the colony is considered as a whole.

There is no significant difference between the correlation coefficients for this relationship for each experimental colony, and the correlation coefficient for the same relationship in the Control colony.

Individual relative specialisation for external activity is maintained by the older individuals, when they are considered separately from the callows, for the comparison between sessions 1 and 3. There is no significant difference between the pooled correlation coefficients for these relationships with the relationship for the older workers in the Control colony.

Individual relative specialisation for external activity tends also to be maintained by the callows, when considered separately, although this pattern is disrupted in colonies where the callow fraction is relatively small. This may be a result of disruption to the mechanism that maintains specialisation. There is no significant difference between the pooled correlation coefficients for these relationships with the relationship for the callow workers in the Control colony.

There is no significant difference between the overall absolute performance of external activity by the workers in the united colonies in session 3 and the absolute performance of external activity in the Control colony.

Thus, relative specialisation is maintained by workers when the fractions of the colony are united, and is concluded to be dependent on experience. Absolute performance may change in response to changing task demand.

Chapter 9

Discussion

i. Introduction

This thesis has been concerned with certain of the proximate mechanisms that underpin the ultimate explanations of the undisputed success of eusocial insects. In Chapters 1 and 2, I reviewed the ecological and evolutionary measures of this success, and discussed its causes. I concluded that the presence of a reproductive division of labour, one of Wilson's (1971 p.4) three traditional defining traits of eusociality, and the consequent facilitation of divisions among other sorts of work, is likely to be the primary cause of the success of eusocial insects.

Division of labour has allowed colonies of eusocial insects to perform tasks more efficiently than a colony of generalists. However, the colony must retain sufficient flexibility to respond to changing environmental conditions, and changing colony requirements, or these efficiencies will be lost. This is especially important in species with small (*sensu* Franks, 1999) colonies, which have a relatively small buffer against changing conditions. Such 'simple' (*sensu* Bourke, 1999) colonies are characterised by monomorphic workers, and retention of 'totipotency' with regard to their ability to reproduce and perform other tasks. There is no colony leader directing activities. The mechanisms whereby workers may be allocated among particular tasks according to colony needs are reviewed in Chapter 2.

Eusocial insect colonies have often been compared to autonomous organisms, and have been considered to be 'superorganisms' (Wheeler, 1911). Recently, it has begun to be appreciated that mechanisms used to explain processes of organisation in autonomous organisms may also be used to explain processes of organisation in eusocial insect colonies. The phenomenon of self-organisation, originally conceived in the context of chemistry and physics, has been used to shed light on aspects of eusocial insect biology, and is potentially important in understanding processes of task allocation. Sendova-Franks and Franks (1999) and Cox and Blanchard (2000) have discussed mechanisms of task allocation with reference to processes involving assembly, utilising templates, or by self-assembly processes. Sendova-Franks and Franks (1999) conclude that the phenomenon of 'social resilience', a process which may prove fundamental to maintaining the efficiency of division of labour, may be explained by such processes.

ii. Main Aims of the Experiments

The experiments in this thesis utilise *sociotomy* techniques, pioneered by Lenoir (1979a, b) and Lachaud and Fresneau (1987), to allow unique insights into the organisation of work, and the extent of flexibility in colonies of the ant, *Leptothorax albipennis*. The experiments aim to determine whether age, or experience, is crucial in determining the behaviour of an individual. The limits under which social resilience maintains organisation of the colony are tested, to distinguish between the role of templates and that of self-assembly processes in this organisation (see also Backen *et al.*, 2000). Patterns of interaction among the workers, and the role of the queen, her response to sociotomy, and her possible influence on the organisation of the colony, are also examined. The absence of 'hard-wired' physical connections between the components of eusocial insect colonies means that colonies can be experimentally manipulated relatively easily. The technique of sociotomy involves taking the colony apart by splitting it into fractions on the basis of various factors (specifically: task, experiments 1 and 2; and age, experiment 3). Consequently, the organisation of the colony and how it functions may be determined by examining the effects on subsequent task allocation and individual flexibility.

These are the first sociotomy experiments to be carried out on small (*sensu* Franks, 1999), simple (*sensu* Bourke, 1999) colonies, where the effect of the loss of colony members is relatively greater. All the individuals in the colonies can be uniquely marked, and followed over relatively long-term experiments. Elements of spatial organisation can also be examined. The results of these experiments are particularly important as they examine task allocation in the 'broader sense' of 'roles', in terms of brood-related tasks or external activity. The majority of manipulative experiments aiming to examine processes of task allocation and determine the extent of flexibility of eusocial insect colonies, have concentrated only on task switching within roles (see Blanchard *et al.*, 2000; for example Gordon, 1986, 1987, 1989).

iii. Main Findings of the Experiments

I briefly discuss the findings of the experiments separately for the 'divided' colonies ('original' or 'subsidiary' fractions, session 3, experiments 1 and 2; 'older' or 'callow' fractions, sessions 1 and 2, experiment 3), or 're-united' (session 4, experiments 1 and 2; session 3, experiment 3). The relative spatial positions of colony members, and relative and absolute performance of tasks by individuals in the fractions, are compared to their relative positions, and the relative and absolute performance of tasks, in the undivided, un-manipulated colonies of the Control, or the experimental colonies prior to manipulation (session 1, experiments 1 and 2). Note the two fractions of the colonies involved in experiment 3 (Colonies 9-12) have never been united as adults prior to session 3; the young age cohort of workers are removed prior to their eclosion. For a summary of the methodology of all three experiments the reader is referred to Figure 9, Chapter 3. For a more detailed discussion of the results, see Chapters 4-8.

❶ The spatial organisation of brood-related tasks and external activity

I discussed spatial organisation in eusocial insect colonies in Chapter 2. Highly structured organisation could enhance efficiency in eusocial insect colonies. The extent of spatial organisation of particular tasks within the colony, and its possible importance in maintaining the efficiency of division of labour, is only beginning to be appreciated (for example Sendova-Franks and Franks 1994, 1995a, 1999). I determined that brood-related tasks, and external activity, are organised in a highly structured way in un-manipulated colonies of *Leptothorax albipennis* (Colony C_1, Colony C_3, and experimental colonies 1-8 during session 1).

In more detail, the frequency at which brood-related tasks were performed decreased with increasing distance from the colony centre. Workers with a median position in the nest above a certain threshold distance from the centre of the brood pile, performed brood-related tasks at a frequency of zero, or very close to zero.

Moreover, the relationship closely approximated an exponential decay. There was a significant, negative correlation between the relative frequency at which individuals performed brood-related tasks and their relative median distance from the colony centre in the un-manipulated colonies. Conversely, the pattern of external activity was such that the frequency at which individuals performed external activity increased as their median distance from the colony centre increased. These colonies exhibited a significant, positive correlation between the relative frequency at which individuals performed external activity and their relative median distance from the colony centre.

Original and Subsidiary Fractions - Experiments 1 and 2

I aimed to determine if the spatial patterns of tasks were still evident in colonies that had been divided into two fractions on the basis of previous performance of external activity. In the subsidiary fractions, individuals had previously exhibited external activity, and in the original fractions, they had not. I expected that the spatial patterns in the subsidiary fractions of the colony would re-establish themselves. This was because individuals in these 'simple' colonies need to maintain some level of 'totipotency' with regard to task performance, and also because spatial organisation of tasks leads to increased efficiency. Individuals in the subsidiary fractions were likely to be older, and have previously performed brood-related tasks. Moreover, some had performed both external activity and brood-related tasks during the session in which they were removed. These 'removed' individuals were, therefore, likely to be able to revert to performing brood-related tasks if required. The subsidiary fractions generated from the colonies in experiment 2 were provided with brood in order to determine whether patterns of brood-related tasks were re-established. These colonies were also used to determine if patterns of external activity were maintained. The subsidiary fractions generated from the colonies in experiment 1 were not provided with brood, and were only tested for patterns of external activity.

Indeed, the spatial patterns of brood-related activity in the subsidiary fractions of the colonies in experiment 2 were established (in colonies where sufficiently high numbers of workers retained their marks). Similar patterns of external activity were established for the subsidiary fractions of the colonies in both experiments 1 and 2, as expected.

The establishment of spatial patterns of tasks in the 'original' fractions of the colonies was likely in the case of brood-related tasks, as these individuals had previously had a relatively high probability of performing these tasks. The establishment of patterns of external activity among these individuals was less likely as they had not exhibited external activity (or they would have been removed). However, I expected that individuals would be sufficiently flexible to exhibit 'precocious' external activity, as the colony demands had changed.

The original fractions of the colonies did indeed exhibit similar spatial patterns of brood-related tasks (in colonies where sufficient numbers of individuals had retained their marks). Levels of external activity exhibited by the original fractions of the colonies, in both experiments 1 and 2, were too low for spatial patterns to be established. However, some external activity did take place, and this provides evidence of the flexibility of workers in responding to changing colony requirements resulting from the loss of a large proportion of the population. I hypothesise that, if these fractions were left for a longer period of time, the spatial patterns of external activity would eventually be established.

In Chapter 7, I investigated the resilience of colony spatial organisation in terms of the relative positions of individually recognisable workers. I compared the relative spatial position of individuals in the subsidiary, and in the original fractions of each colony, with their relative spatial position in the un-manipulated colony in session 1. I determined that individuals tended to remain in the same relative positions in either the original or subsidiary fraction of the colony, as they did in session 1. Again, this strongly suggests the resilience of spatial organisation despite sociotomy.

Re-United Fractions - Experiments 1 and 2

When the two fractions of each colony were re-united, I expected that individuals would be sufficiently flexible to resume their original tasks, and thus, that spatial patterns of brood-related tasks, and of external activity, would be re-established. The spatial patterns were, in fact, re-established. In Chapter 8, I analysed the individual relative performance of each task in the un-manipulated colonies, and compared this with their relative performance of these tasks in the re-united colonies. I aimed to determine if the spatial patterns were re-established because individuals resumed their original tasks, or whether individuals had changed their task in response to their experience in the subsidiary fractions of the colonies, and it was merely the overall spatial patterns that had been conserved. I discuss the results of this analysis below, but it is clear that spatial patterns of behaviour are very pronounced, and tend to be maintained despite sociotomy.

In Chapter 7, I compared the relative spatial position of individuals in the re-united colonies with their relative spatial position in the un-manipulated colonies. I determined that the relative position of individuals was conserved. This result is particularly striking as the colonies were not emigrated during, or subsequently to, re-unification. The workers were capable of re-establishing the same relative positions in session 4 as they had occupied in the un-manipulated colonies. It has been established that workers will re-adopt their relative positions (and resume their tasks) after an emigration (Sendova-Franks and Franks, 1994). It has not previously been shown that workers will resume their relative spatial positions after sociotomy, and without an emigration. Again, the resilience of spatial organisation is emphasised. This resilience strongly suggests that spatial organisation may be very important in maintaining efficiency.

Divided Fractions - Experiment 3

In experiment 3, colonies were divided on the basis of age, rather than task. The subsidiary, 'callow', fractions contained individuals that had been removed as callows, and had never, as adults, been part of the united colony. The original 'older' fractions contained the individuals that remained in the colony after the young cohort had been removed. I expected patterns of external activity and brood-related tasks in the 'older' fraction to be consistent with those established for the un-manipulated colonies. My reasoning for this was that these individuals, collectively, had previously performed all the jobs in the colony. The removal of the pupae should have had no effect on these individuals' continuing ability to perform these tasks if required. This, indeed, tended to be the case. The patterns of both brood-related tasks, and external activity, were very similar to those observed for the experimental colonies in experiments 1 and 2, during session 1, and for the Controls, Colonies C_1 and C_3.

In the subsidiary, 'callow', fractions of these colonies, I did not expect such pronounced spatial organisation of tasks. These individuals had not previously existed as eclosed adults within a colony, and had no experience of performing any tasks. In fact, very similar patterns were established for both brood-related task performance, and the performance of external activity. However, as for the subsidiary fractions of the colonies in experiments 1 and 2, levels of external activity tended to be relatively low. I hypothesised that this was related to the relatively higher probability of callows physically encountering brood items. There is evidence that callows are flexible regarding external activity. The absolute performance of external activity in the callow fractions tended to become more similar to that seen in the Control, as the experiment progressed. The striking results from these subsidiary fractions strongly suggest the resilience of spatial patterns of organisation, and moreover, that they become established spontaneously. They also emphasise the flexibility of individuals, even those with no previous experience of external work, or in fact, of any work, in performing external activity, albeit at a lower level than an un-manipulated colony.

United Fractions - Experiment 3

The final part of experiment 3 was to unite the two fractions of each colony and determine whether the spatial organisation of tasks was maintained. I expected the overall patterns to be preserved, but for the individuals from the 'all young' fraction to become situated further towards the centre of the colony, and perform predominantly brood-related tasks, leaving the older workers to perform the external tasks. This was emphatically not the case. The spatial patterns were maintained overall, but, crucially, when the two fractions were tested for the significance of spatial patterns separately in the united colony, both the 'callow' and the 'older' fractions tended to show significant patterns of spatial organisation for both types of task. This was investigated further by the analysis in Chapter 8 when I examined individual performance, and is discussed below, but this strongly suggested that the behaviour of workers is determined by their experience of tasks, rather than their age.

In Chapter 7, I analysed the results from experiment 3 to determine the resilience of individual spatial position. As for the previous experiments I determined that relative spatial position was conserved throughout the experiment. This result is even more striking however, because the colonies were not emigrated during, or subsequently to unification, and some of these individuals from the 'all young' fractions occupied positions relatively close to the colony centre, rather than the periphery. Impediments to the progression of these individuals to the centre of the colony when the fractions were united, such as other workers or brood, were not sufficient to overcome the resiliency of the spatial structure. Perhaps this, more strongly than any other finding, emphasises the role that spatial structure might play in task allocation. Individuals established the same relative positions in the colony as they did when they were in a fraction on their own, even when this distribution does not correspond to 'classical' patterns predicted by a correlation between age and task. It is experience that is important in determining these patterns.

❷ Individual behavioural flexibility

Having established that tasks are highly spatially organised in colonies of *Leptothorax albipennis*, and that this organisation is maintained despite sociotomy by the resilience of individuals relative spatial positions, I analysed in Chapter 8, the resilience of task performance by individuals. This can be viewed as the second component to social resilience, the first being the spatial element.

I compared the relative individual performance of brood-related tasks, and of external activity, in each of the experimental sessions, with relative individual performance in the un-manipulated colonies, for the colonies in experiments 1 and 2. I compared the relative individual performance of the tasks in the divided 'callow' and 'older' fractions in session 1 with the relative performance of individuals in the united colonies in session 3, for the colonies in experiment 3. I also analysed the absolute performance of each of the tasks in each experimental session compared with session 1, and also with the relevant session of the Control. This analysis was performed to determine whether the absolute performance of brood-related tasks, and of external activity, was resilient to sociotomy. Comparisons with the relevant session of the Control colony eliminated any fluctuations in the absolute performance of tasks due to naturally changing task demand across the season. The analysis performed in Chapters 4 and 7, summarised above, demonstrated the pronounced spatial patterns of brood-related tasks, and of external activity in these colonies, and moreover, the resilience of this spatial organisation to sociotomy. The analysis in Chapter 8 was particularly important, as it aims to determine whether spatial organisation is a cause or an effect of task allocation. If spatial organisation is resilient in situations where behavioural organisation is not, it can be concluded that spatial fidelity occurs first, and task specialisation proceeds subsequently. The main findings for each of the divided fractions, and the united fractions, are outlined below. For a fuller discussion see Chapter 8.

Original and Subsidiary Fractions - Experiments 1 and 2

In un-manipulated colonies, relative specialisation for brood-related tasks was consistent throughout the experiment. Moreover, the absolute performance of these tasks did not change significantly over this time. There was some disruption to individual relative specialisation for brood-related tasks in the experimental colonies when the original fractions of the colonies during session 3 were compared to relative individual specialisation in session 1. There was evidence that specialisation for these tasks was resilient to the removal of individuals associated with external activity, but patterns were not always consistent. In a number of colonies, the absolute performance of brood-related tasks did not differ significantly from the Control, or from performance during session 1 in the relevant experimental colony. However, when absolute performance was established to be significantly different from the Control, the trend was always for absolute performance of brood-related tasks in the original fractions of the colony to be higher than that observed during session 1, or in the Control during session 3. My explanation for this was the higher ratio of the number of workers to the number of items of brood in the original fractions of the colonies, compared to session 1, or in the Control colony during session 3, resulting from the removal of a proportion of the workers from the original colonies. In order to preserve the overall level of brood-related tasks, individuals must have increased their absolute performance of these tasks.

The resilience of individual performance of brood-related tasks in the subsidiary fractions of the experimental colonies could only be examined in experiment 2, as subsidiary fractions did not contain brood in experiment 1. However, the spatial organisation of the individuals in the subsidiary fractions in experiment 1, was resilient to sociotomy, despite the absence of the queen, the brood, and a large proportion of the workers, all of which may be the source of a template for re-organisation. This is the first line of evidence that strongly suggests that spatial organisation is established prior to task specialisation, and that this still occurs even when the task is completely absent.

In experiment 2, the subsidiary fractions were provided with brood. Specialisation for brood-related tasks was maintained by individuals in the subsidiary fractions compared to the individual's relative performance in the colonies prior to sociotomy (in colonies with sufficient numbers of individuals that had retained their marks). There was evidence that individuals were capable of adjusting their absolute performance of brood-related tasks to be indistinguishable from the absolute performance of these tasks in the Control colony, or in the relevant experimental colony prior to manipulation. This strongly suggests that individuals are sufficiently flexible to respond to changing task demand, and adjust the absolute frequency at which they perform brood-related tasks after being removed from a proportion of the colony.

Relative specialisation for, and absolute performance of, external activity, was also preserved throughout the experiment, in un-manipulated colonies. Crucially, removed individuals in the experimental colonies may also have exhibited brood-related tasks, but the individuals remaining in the original fractions of the experimental colonies were unlikely to have exhibited external activity. Levels of external activity exhibited by the original fractions of the experimental colonies were consequently very low.

When the subsidiary fractions were examined, relative specialisation for external activity tended to be resilient to sociotomy only if brood was present in these fractions (i.e experiment 2). I hypothesised that the disruption to task allocation in the subsidiary fractions of the colonies in experiment 1 was attributable to drastic changes in task demand resulting from the absence of brood. The requirements for external activity, such as foraging, may have been drastically reduced, despite the resilience of the spatial organisation of these individuals.

Re-United Fractions - Experiment 1 and 2

I compared the relative frequency at which individuals in the Control colony performed brood-related tasks in session 1 and session 4. Relative specialisation for brood-related tasks was preserved. Moreover, the absolute performance of brood-related tasks did not change significantly when these two sessions were compared. Relative specialisation for external activity was also maintained, but the absolute performance of external activity was significantly reduced in session 4 compared to session 1 in the Control. I hypothesised that this is a result of natural changes in task demand resulting from seasonal changes in the ratio of the number of workers to the number of items of brood. An example of a seasonal change that would alter task demand is the increasing stages of maturity reached by the brood (pupae do not require feeding). Moreover, as pupae eclose, the ratio of workers: brood would increase.

Relative specialisation for brood-related tasks tended to be disrupted in session 4 compared to session 1, in colonies in which the subsidiary fractions had no access to brood in session 3 (i.e. experiment 1). The absolute performance of brood-related tasks in these colonies tended to be significantly reduced. This occurred despite the maintenance of relative spatial positions, and is therefore strong evidence that first, spatial fidelity is established prior to behavioural specialisation, and second, that behavioural specialisation may be strongly dependent on experience. There was also disruption to both the relative specialisation for, and absolute performance of, external activity, in the colonies in experiment 1. In colonies where the subsidiary fractions were provided with brood, relative specialisation for brood-related tasks, and for external activity was retained. There was no significant change in absolute performance of these tasks compared to the Control colony. The disruption to relative specialisation for, and absolute performance of, brood-related tasks, and external activity, in the experimental colonies in experiment 1 can therefore be attributed to the absence of brood in their subsidiary fractions in session 3, and subsequent changes in behavioural specialisation. The disruption was not a result simply of changing task demand, as task demand should be the same in the united fractions as in the Control colony in session 4. The absence of brood did not affect the maintenance of spatial organisation, and this strongly suggests that the brood is not required as a template in spatial re-organisation. This finding does not preclude the involvement of templates in 'fine-tuning'; but demonstrates, for the first time, that templates are not a necessary condition for social resilience. I hypothesise, therefore, that it is the recent experience (or lack of experience) of tasks by individuals, which determines their subsequent behavioural specialisation.

Divided Fractions - Experiment 3

In the un-manipulated Control colony in experiment 3, relative specialisation for brood-related tasks, and for external activity, was retained throughout the experiment. The absolute performance of brood-related tasks did not change significantly over the course of the experiment, but there was a significant reduction in the absolute performance of external activity. I hypothesised that this results from a natural change in task demand resulting from seasonal changes in the ratio of the number of workers to the number of items of brood. Relative specialisation for brood-related tasks, and for external activity, tended to be maintained in the younger, and in the older, fractions of the experimental colonies. I concluded that individuals in the 'older' fractions were sufficiently flexible to respond to changing task demand so that the absolute performance of brood-related tasks, and of external activity, did not tend to differ significantly from the Control.

Relative specialisation for brood-related tasks was retained in the 'callow' fractions of the colonies between sessions 1 and 2. The absolute performance of external activity tended to be relatively low, and thus it was not meaningful to examine relative specialisation. The absolute performance of external activity in session 1 was significantly lower than in the Control. Absolute performance of these tasks in the 'callow' fractions tended to increase in session 2, to the level where it did not differ significantly from the Control for two of the four 'callow' fractions of the experimental colonies.

United Fractions - Experiment 3

Relative specialisation by individuals for both brood-related tasks, and external activity, was retained when the two fractions of each colony were united, compared to relative specialisation in session 1. I have already established that relative spatial positions were re-established by individuals in the united fraction compared to their positions in the fractions when they existed separately. Moreover, it can clearly be seen in Figure 46(a), Chapter 7, that the actual spatial positions of young and old individuals completely overlapped when the two fractions were united (compare to Figure 45(a) where younger individuals were clearly located closer to the centre of the nest).

The crucial difference between experiments 1 and 2, and experiment 3, should be noted. In experiments 1 and 2, the two fractions existed together in a united colony in session 1, and were later re-united in session 4. In experiment 3, the two fractions never existed as eclosed adults in a united colony, but were united in session 3 after existing as separate fractions. The findings of experiment 3 strongly suggest that performance of tasks is dependent on experience, rather that age. Sociotomy techniques had been used to change the experience of individuals and resulted in artificially created colonies in which individuals exhibited specialisation for particular tasks. This relative specialisation was retained when the fractions of the colonies were re-united, and can therefore be concluded to be dependent on experience, rather that any innate specialisation resulting from their age. This is because individuals did not establish the familiar patterns seen in un-manipulated colonies. As the evidence strongly suggests that spatial organisation is established prior to task allocation, the hypothesis can be qualified further as: spatial organisation is dependent on experience, rather than age, and task specialisation occurs subsequently, depending on task demand.

❸ The influence and behaviour of the queen

Although it has long been established that there is no 'leader' directing activities in the colony, there is evidence that the integration of activity in eusocial insect colonies may be more centralised in 'simple' (*sensu* Bourke, 1999) species (Robinson, 1992). The experimental removal of foragers in colonies of polistine wasps resulted in increased activity and aggressive interactions by the queen (Gamboa *et al.*, 1990) leading to increased foraging activity by the remaining workers. Sendova-Franks and Franks (1995b) suggested that workers use queen-workers interactions firstly, to update their information about the condition of the queen, as any change in the queen's fertility alters the interests of workers in terms of what, and how much, brood to produce. Secondly, Sendova-Franks and Franks (1995b) hypothesised that workers may use queen-worker interaction to adjust their spatial and social position in the colony, and subsequently contribute to the efficiency of the colony in the division of labour. Powell and Tschinkel (1999) suggested a hypothesis whereby these two factors become linked. They suggested reproduction-based dominance interactions control worker movement and location in *Odontomachus brunneus* and that this mechanistically governs task allocation and establishes a division of labour for non-reproductive tasks.

In Chapter 5, I investigated patterns of interaction between workers and the queen in experiments 1, 2 and 3. I established that there was a spatial pattern to such interactions. Interactions were more likely to occur near to the centre of the colony where the queen was usually situated (see also Sendova-Franks and Franks, 1995a: *Leptothorax unifasciatus*). I determined that this spatial pattern was not disturbed by sociotomy. Moreover, I established that there was a general tendency for the distribution of distances of the queen from the centre of the colony to increase over the course of the experiment in the un-manipulated Control. The queen's zone of movement was significantly increased in session 4 compared to session 1 in the experimental colonies. As this trend was observed in both the Control, and the experimental colonies, I hypothesised is that it does not result from sociotomy.

Most importantly, the absence of the queen in the subsidiary fractions of experiments 1 and 2, and the 'callow' fractions of the colonies in experiment 3, did not affect the resilience of worker spatial organisation. Therefore, my findings cast doubt on Sendova-Franks and Franks' (1995b) hypothesis that queen-worker interactions are utilised by workers to adjust their spatial and social position in the colony. Any influence the queen may have either directly via contact with workers or by chemical communication, or indirectly, via interactions resulting from her absence, are not necessary for the resilience of spatial organisation of workers in these colonies. Interactions among the workers, both in the presence and absence of the queen are discussed further below.

● Interactions among the workers

In Chapter 6, I investigated whether there were any spatial patterns in the interactions among the workers, and how these were affected by sociotomy. Although there was some tendency for the frequency at which workers interacted with each other to decrease with their increasing distance from the colony centre, the occurrence of this pattern was not related to any particular stage of sociotomy, and frequently did not occur at all. However, there was some consistency within colonies towards the occurrence of these trends. I hypothesised that such spatial patterns occurred in colonies where the absolute frequency of interaction was relatively high. There was no correlation between the size of the worker population and the absolute intensity of interactions. This can only be explained, therefore, by inter-colony variation. There was evidence that strongly suggested that the absolute frequency at which workers interacted with each other increased as a result of seasonal changes and sociogenesis. The absolute frequency at which workers interacted with each other was also affected by sociotomy. In the original fractions from which individuals associated with external activity had been removed, during experiment 1, there tended to be significantly less worker-worker interaction than in the Control. Conversely, in the subsidiary fractions of these colonies, there tended to be significantly more worker-worker interaction.

Gordon and Mehdiabadi (1999) hypothesised that cues based on interaction rate enable workers to respond to changes in worker number. The rate of worker-worker interactions may provide individuals with environmental cues as to requirements, via local, simple, events, and may explain how the colony responds to changing conditions without presupposing global or collective knowledge.

iv. Mechanisms of Task Allocation

The conclusions reached above lead one inevitably to hypothesise mechanisms by which spatial organisation may be established in situations of drastically changing colony conditions, or manipulations, such as those employed in experiment 1, 2 and 3 in this thesis. Mechanisms involving individuals responding to simple, local, factors, according to simple rules, without any explicit coding via an influence external to the workers themselves such as structure or direction, are very appealing. The appeal results from the mechanisms' independence from the accumulation and processing of huge amounts of complex information by individuals or any central influence by the queen (Bonabeau *et al.*, 1998). Such robust and generic principles of organisation have proved increasingly important to the understanding of biological organisation at many levels, and may prove fundamental to understanding organisation and efficiency in eusocial insect societies.

Although self-organisation theory has already provided the basis for explanations of varied phenomena in eusocial insect biology, there has previously been little evidence to support models of task allocation based on such simple principles. Moreover, many previous hypotheses that have attempted to explain task allocation with reference to processes of self-organisation, have relied on external influences, such as templates. This work provides evidence, for the first time, that strongly suggests that the mechanisms by which spatial organisation, and consequently task specialisation, are established, do not require templates. Spatial patterns still persist despite the absence of the queen, the brood and some of the workers, see also Backen *et al.* (2000, Appendix A). Moreover, individuals re-adopt the same relative spatial positions whether they are situated relatively far or relatively close to the centre of the brood pile. Consequently, the colony need not be emigrated during re-unification for individuals to show spatial fidelity. The 'sorting' procedure enabling individuals to re-adopt their positions relative to other individuals in the nest must be extremely efficient. I also conclude that mechanisms of task allocation are independent of age. The results of the experiments in this thesis strongly suggest that task allocation in *Leptothorax albipennis* is heavily dependent on experience. As experience is usually correlated age, this explains the correlations often observed between age and task.

Sendova-Franks and Franks (1999) and Backen *et al.* (2000) hypothesised that the mechanism for social resilience must be based on self-assembly, a process that is not affected by the absence of a potential sources of a template, such as the queen, or the brood. Moreover, such a process is also unaffected by the absence of a proportion of the work force, as has been shown to be the case in practice. Backen *et al.*, (2000) also hypothesised that the mechanism may be based on continuos variation in the level of mobility of the workers (see Appendix A for more details). Future work is concentrated on the 'variable mobility hypothesis' outlined above, and in Backen *et al.* (2000, Appendix A). Mathematical modelling is being used to determine whether patterns of task allocation can be explained by individuals sorting themselves on the basis of mobility rates. Such a mechanism could explain the role of processes of 'learning' and 'forgetting', in relation to these mobility rates. It must be stated that, as with all explanations of eusocial insect phenomena involving self-organisation, external influences on the colony are not precluded. Templates may provide a mechanism for the 'fine-tuning' of social resilience, and the role of response thresholds in explanations of the importance of experience in task specialisation, should not be over-looked.

I conclude that the resilience of spatial organisation in *Leptothorax albipennis* can be accounted for by a self-organised process of self-assembly. Templates are not a necessary condition for social resilience in this species. The results from these experiments strongly suggest that relative specialisation is dependent on experience, rather than directly from age. Moreover, the evidence strongly suggests that spatial organisation is established prior to task allocation, and I therefore conclude that spatial organisation is dependent on experience, rather than age, and task specialisation occurs subsequently, depending on task demand. Given the ecology of this species and potentially rapidly changing environmental conditions, task allocation based on such mechanisms could prove to be of fundamental importance. Such mechanisms could explain the resilience of colonies to fluctuations in both the internal colony situation, and in environmental conditions.

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Appendix A

<u>Backen *et al.*, 2000</u>

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ORIGINAL ARTICLE

Sarah J. Backen · Ana B. Sendova-Franks
Nigel R. Franks

Testing the limits of social resilience in ant colonies

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Abstract Social resilience is the ability of *Leptothorax* ant colonies to re-assemble after dissociation, as caused, for example, by an emigration to a new nest site. Through social resilience individual workers re-adopt their spatial positions relative to one another and resume their tasks without any time being wasted in worker re-specialisation. Social resilience can explain how an efficient division of labour can be maintained throughout the trials and tribulations of colony ontogeny including the, often substantial, period after the queen dies when the ability to conserve worker social relationships may be essential for efficiency to be maintained. The mechanism underlying social resilience is, therefore, expected to be robust even in the absence of many of the colony's components, such as the queen, the brood and even a large proportion of the workers. Such losses are likely, given the ecology of this genus. Using sociotomy experiments, we found that social resilience can occur in the absence of the queen. Furthermore, the spatial component of social resilience can occur even when the queen, the brood, as well as a large proportion of the workers, are all absent simultaneously and hence many of the tasks are missing. We conclude, therefore, that social resilience is indeed robust. This does not, however, preclude worker flexibility in response to changes in task supply and demand. We propose a possible sorting mechanism based on worker mobility levels which might explain the robustness underlying this phenomenon.

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S.J. Backen · A.B. Sendova-Franks (✉) · N.R. Franks
Centre for Mathematical Biology
and Department of Biology and Biochemistry,
University of Bath, Claverton Down, Bath, BA2 7AY, UK

Present address:
A.B. Sendova-Franks, Faculty of Computer Studies and Mathematics University of the West of England,
Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK
e-mail: Ana.Sendova-Franks@uwe.ac.uk
Tel.: +44-117-3443370, Fax: +44-117-9763860

Key words Division of labour · Spatial efficiency · Social resilience · Ant · *Leptothorax*

Introduction

The incorporation of animals into eusocial colonies is one of the major transitions in evolution (Seeley 1989, 1997; Maynard Smith and Szathmáry 1997). In common with all other transitions to a higher level of biological organisation, the transition to eusociality has been accompanied by the evolution of labour division among the components of the system; here among the individuals in the colony.

Selection pressure for spatial efficiency is one of the main driving forces in the evolution of the division of labour. Spatially ordered work minimises the cost of travelling between items of the same task and fosters task specialisation (Bourke and Franks 1995).

In many social-insect colonies tasks are spatially segregated (Wilson 1976, 1985; Seeley 1982, 1985). Workers specialising in particular tasks, therefore, tend to occur in the spatial zones associated with these tasks (Sendova-Franks and Franks 1995a; Stradling et al. 1998; Tschinkel 1999).

In *Leptothorax* ant colonies, workers have individually specific spatial fidelity zones (SFZs), ordered in a sequence from the colony centre to the colony periphery. The position of an individual's SFZ is associated with the task she performs (Sendova-Franks and Franks 1995a).

Furthermore, after a massive colony disruption, such as an emigration to a new nest site, the workers can re-establish their SFZs relative to one another and resume their different (spatially ordered) tasks. We termed this phenomenon social resilience (Sendova-Franks and Franks 1994).

Social resilience allows division of labour to be re-established without any time and resources being wasted in worker respecialisation. Social resilience can, therefore, explain how an efficient division of labour might be maintained throughout colony ontogeny. This poses the following questions: how robust is the phenomenon of social resilience? Can the mechanism that allows individual workers to return to their relative spatial positions operate even when a substantial proportion of brood or workers, or indeed the queen, have been lost during emigration? Such losses are likely given the ecology of this species (Partridge et al. 1997).

This paper explores the limits of social resilience by applying the experimental technique of sociotomy (Lenoir 1979a, 1979b; Lachaud and Fresneau 1987). We removed particular colony components in order to test whether social resilience will occur in their absence.

The colony components we removed experimentally were as follows.

1. The queen. The queen is a potential source of a pheromone gradient and resides in the colony centre. Workers might be able to return to their relative positions by learning their own positions along this olfactory gradient.
2. The brood. The brood is organised in concentric circles of different brood types with the smallest items in the centre and the largest on the periphery, a pattern that is reconstructed after colony dissociation (Franks and Sendova-Franks 1992). It is possible that, after brood sorting, workers could return to their relative positions by having learnt the particular odours of the different brood types (Jaisson and Fresneau 1978).
3. Workers. It is, at least in theory, possible that workers are able to learn the identity of their neighbours and might be able to return to their relative positions by placing themselves between these particular individuals.

It might be the case that ant colonies can use all of these components in re-establishing the spatial and social structure of the worker population, and hence the presence of each of these components might be sufficient for social resilience to occur. With these considerations in mind we carried out two sets of experiments in order to answer the following questions. Firstly, can social resilience occur in the absence of the queen? Secondly, can social resilience occur when the queen, the brood and a large proportion of the workers are all simultaneously absent?

Methods

The experiments were carried out on seven colonies of the ant *Leptothorax albipennis* (Curtis) collected from flat rock crevices in Dorset, England. The colonies were housed in nests made from a pair of microscope slides separated by a thin cardboard perimeter (the details of the method used to culture colonies under laboratory conditions followed Sendova-Franks and Franks 1995a). All workers in a colony were marked individually with tiny drops of coloured paint on their gasters (Sendova-Franks and Franks 1993). The positions and behavioural acts of individual workers for a particular experimental session were determined from between 50 and 60 colour photographs (see Sendova-Franks and Franks 1995a for details).

For simplicity in our analysis we have reduced the spatial order to one dimension. The spatial position of a worker over a session was established as the median of the distribution of distances from the worker to the centre of the colony (the centre of the egg pile). For this reason, the spatial structure of a colony constitutes an array of the ranked individual positions (medians of SFZs) of all workers along the radius from the colony centre to its periphery (Sendova-Franks and Franks 1994, 1995a). Where the brood was absent, the centre for the session before the manipulation was utilised.

Experiment 1 tested the null hypothesis that social resilience does not occur in the absence of the queen. Colonies 1 and 2 were photographed in three sessions of 2.5 consecutive days each. In each session photographs were taken every half-hour for 9.5 h per day, from between 0800 and 0930 hours until 1730–1900 hours. The queen was removed between the second and third sessions during an emigration, provoked simply by removing the roof of the current nest site in the presence of another complete nest site nearby which the ants could colonise (see Sendova-Franks and Franks 1995b). Therefore, in the first two sessions the queen was present.

We refer to these henceforth as session "queenright 1" and session "queenright 2". The comparison between these two sessions was the control comparison. In the third session the queen was absent. We refer to this henceforth as a "queenless" session. The comparison between the queenright 2 and the queenless sessions was the experimental comparison.

Experiment 2 tested the null hypothesis that social resilience does not occur when the queen, the brood and a large proportion of the workers are all simultaneously absent. Colonies 3, 4, 5, 6 and the control colony C were studied in three sessions of 3 consecutive weeks each. The colonies were photographed during sessions 1 and 3 and experimentally manipulated during the intervening session 2. In each of sessions 1 and 3, photographs were taken four times a day between 0900 and 1700 hours at the beginning of a randomly selected 1-h period. In the experimental colonies, during session 2, any workers that ventured outside the nest entrance were removed and placed in a new arena with a new nest.

The removal procedure was carried out continuously for the first 3 h and during sampling intervals for the remainder of session 2. The removed workers formed "derived" colonies 3 to 6, respectively. These "derived" colonies contained no queen, no brood and only a proportion of the original worker population (in colonies 3 to 6 this proportion was 0.57, 0.61, 0.55 and 0.89, respectively), representing a sample of individuals with positions mainly but not exclusively in the peripheral half of the original colony. In the control colony, during session 2, the first third of the worker population was removed as they exited the nest during an emigration. They were returned to the parent nest after a week. This procedure controlled for any possible effect of the physical action of removing the workers and putting them into a new nest.

The effect of the absence of the queen, brood or nestmates on social resilience was measured in terms of changes in: (1) the relative spatial positions of workers, and (2) the relative task profiles of workers. Such changes were determined by, firstly, ranking the workers for each of the compared sessions according to their spatial positions, brood-care frequencies or their outside-the-nest frequencies, secondly, testing whether such rankings were significantly correlated, and thirdly, testing for significance the differences between the correlation coefficients for the control and experimental comparisons.

In every case, we tested for significance the difference between the correlation coefficients for the control and experimental comparisons as follows.

1. To make the comparison possible, we transformed the two correlation coefficients with Fisher's z-transform (Sokal and Rohlf 1995, equation 15.11).
2. We used a further approximation, called Hotelling's adjustment (z^*, Sokal and Rohlf 1995, equation 15.13), since in every case $10 \leq n < 50$.
3. We calculated the exact P value for the difference by employing the following procedure written in Minitab 9.1 (Minitab, State College, Pa.):

```
cdf difference k1;
norm 0 $\sigma_{z_1^*-z_2^*}$
let k1=2*(1–k1)
print k1
```

where cdf is the cumulative density function, *difference* is the difference between the transformed correlation coefficients z_1^* and z_2^*, norm 0 $\sigma_{z_1^*-z_2^*}$ is a normal distribution with a mean of 0 and standart error $\sigma_{z_1^*-z_2^*}$, and $k1$ is the probability value to be calculated.

This procedure calculates the following:

1. The probability that a random variable from a normal distribution with a mean, $\mu_{z_1^*-z_2^*}=0$ (according to the H_0 of no difference) and the observed standard error, $\sigma_{z_1^*-z_2^*} = \sqrt{\frac{1}{n_1-1} - \frac{1}{n_2-1}}$ (Sokal and Rohlf 1995, equation 15.14 and equation on p. 582), has a value less or equal to the observed *difference* between z_1^* and z_2^*;
2. The probability for a two-tailed test that the random variable is greater than the observed *difference*.

Spearman's rank correlation coefficient (r_s) was computed and tested as an ordinary product-moment correlation coefficient, since in every case $n>10$ (Sokal and Rohlf 1995). We calculated the exact P value for each correlation coefficient employing the above Minitab procedure for a mean, $\mu=r$-0=0 (according to the H_0 of no correlation) and the observed standard error, $s_r = \sqrt{\frac{1-r^2}{(n-2)}}$ (Sokal and Rohlf 1995).

Results

Experiment 1

In the absence of the queen, the workers in both colonies 1 and 2 re-established their spatial and task positions relative to one another with high fidelity after the emigration. In the experimental comparisons, between the queenright 2 session and the queenless session, the correlations between the rankings of workers in both colonies were significant for (1) spatial positions, (2) brood-care frequencies, and (3) outside-the-nest frequencies (Fig. 1, Table 1).

Furthermore, none of these correlation coefficients were significantly different to those for the respective control comparisons, between the queenright 1 session and the queenright 2 session (Table 1). These findings demonstrate that the presence of the queen is not a necessary condition for social resilience to occur.

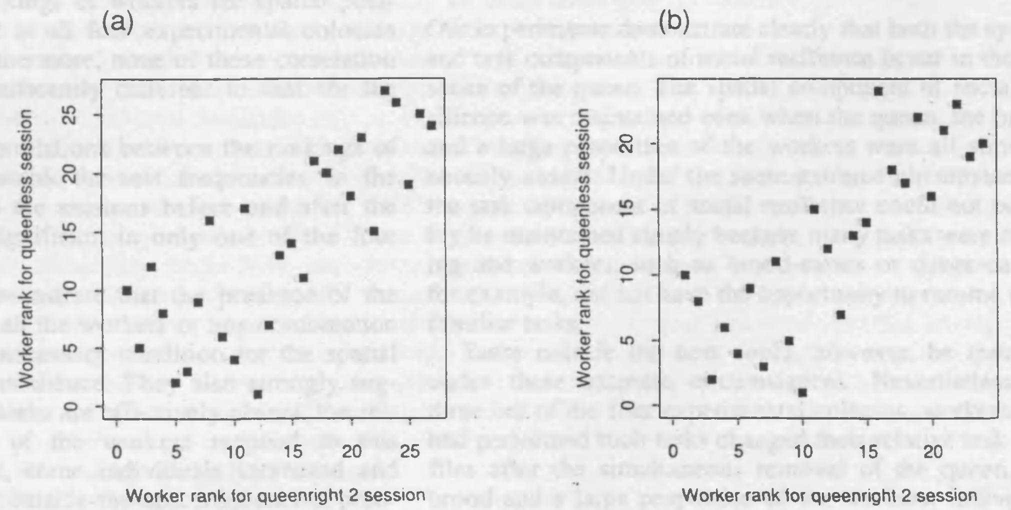

Fig. 1a,b Results of experiment 1 (an illustration): can social resilience occur in the absence of the queen? Correlations of the ranked spatial positions of all workers for the experimental comparison between the queenright 2 session and the queenless session. **a** Colony 1 (r_s=0.789, P<0.001, n=27); **b** colony 2 (r_s=0.812, P<0.001, n=24); calculation of P values as in Table 1

Table 1 Results of experiment 1 testing whether social resilience can occur in the absence of the queen. The difference between the correlation coefficients for control (subscript c) and experiment (subscript e) was tested for significance with Fisher's z-transform (Sokal and Rohlf 1995). The control comparison was between the queenright 1 and the queenright 2 sessions. The experimental comparison was between the queenright 2 and the queenless sessions. z^* Hotelling's adjustment of the z-transform for small sample sizes, $10 \leq n < 50$; exact values for P were calculated according to Sokal and Rohlf (1995), see Methods; where the value for P, with precision to the third decimal place, was 0.000, we used $P<0.001$

Workers ranked according to	Colony	Control			Experiment			z-transform	
		r_{s_c}	n_c	P	r_{s_e}	n_e	P	$\lvert z_c^* - z_e^* \rvert$	P
Spatial position	1	0.775	27	<0.001	0.789	27	<0.001	0.035	0.900
	2	0.926	24	<0.001	0.812	24	<0.001	0.480	0.106
Brood-care frequency	1	0.535	27	0.002	0.408	27	0.025	0.158	0.571
	2	0.914	24	<0.001	0.864	24	<0.001	0.233	0.433
Outside-the-nest frequency	1	0.863	27	<0.001	0.805	27	<0.001	0.186	0.505
	2	0.858	24	<0.001	0.853	24	<0.001	0.019	0.949

Table 2 Results of experiment 2 testing whether social resilience can occur when the queen, the brood and a large proportion of the workers are all absent simultaneously. The control comparison was between the sessions before and after the manipulation for control colony C. The experimental comparison was between the sessions before and after the manipulation for each of colonies 3 to 6. Test method, calculation of P values and abbreviations as in Table 1

Workers ranked according to	Colony	Control			Experiment			z-transform	
		r_{s_c}	n_c	P	r_{s_e}	n_e	P	$\lvert z_c^* - z_e^* \rvert$	P
Spatial position	C vs 3	0.764	26	<0.001	0.697	16	<0.001	0.162	0.620
	C vs 4	0.764	26	<0.001	0.511	13	0.049	0.450	0.200
	C vs 5	0.764	26	<0.001	0.617	27	<0.001	0.275	0.328
	C vs 6	0.764	26	<0.001	0.782	15	<0.001	0.013	0.969
Brood-care frequency	Not applicable								
Outside-the-nest frequency	C vs 3	0.723	26	<0.001	0.347	16	0.167	0.541	0.098
	C vs 4	0.723	26	<0.001	0.122	13	0.684	0.766	0.029
	C vs 5	0.723	26	<0.001	0.399	27	0.030	0.472	0.093
	C vs 6	0.723	26	<0.001	0.226	15	0.403	0.665	0.046

Experiment 2

Workers in the "derived" colonies 3 to 6 re-established their relative spatial positions with high fidelity even when the queen, the brood and a large proportion of their nestmates were absent. In the comparisons between the sessions before and after the manipulation the correlations between the rankings of workers for spatial positions were significant in all four experimental colonies (Fig. 2, Table 2). Furthermore, none of these correlation coefficients were significantly different to that for the control colony.

By contrast, the correlations between the rankings of workers based on outside-the-nest frequencies in the comparisons between the sessions before and after the manipulation, were significant in only one of the four colonies (Table 2).

These findings demonstrate that the presence of the queen, the brood and all the workers or any combination of the three is not a necessary condition for the spatial component in social resilience. They also strongly suggest that when many tasks are effectively absent, the relative task positions of the workers respond to this change. For example, some individuals increased and others decreased their outside-the-nest frequencies, probably because there was no brood to tend and, also, foraging requirements had been altered.

Discussion

The robustness of social resilience

Our experiments demonstrate clearly that both the spatial and task components of social resilience occur in the absence of the queen. The spatial component of social resilience was maintained even when the queen, the brood and a large proportion of the workers were all simultaneously absent. Under the same extreme circumstances, the task component of social resilience could not possibly be maintained simply because many tasks were missing and workers, such as brood-carers or queen-carers, for example, did not have the opportunity to resume their familiar tasks.

Tasks outside the nest could, however, be resumed under these extreme circumstances. Nevertheless, in three out of the four experimental colonies, workers that had performed such tasks changed their relative task profiles after the simultaneous removal of the queen, the brood and a large proportion of the workers. Individual

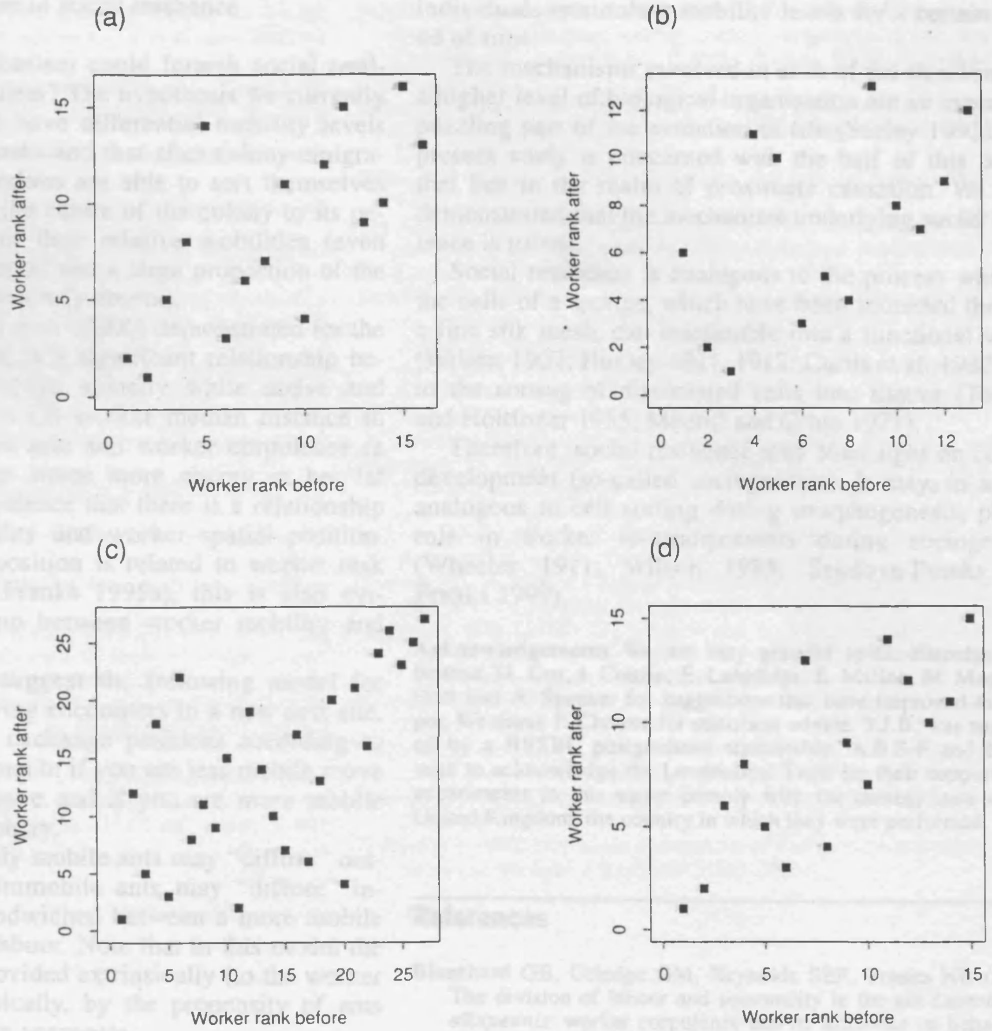

Fig. 2a-d Results of experiment 2 (an illustration): can social resilience occur when the queen, the brood and a large proportion of the workers are all simultaneously absent? Correlations of the ranked spatial positions of all workers for the experimental comparison between the sessions before and after the manipulation. **a** Colony 3 (r_s=0.697, P<0.001, n=16); **b** colony 4 (r_s=0.511, P=0.049, n=13); **c** colony 5 (r_s=0.617, P<0.001 n=27); **d** colony 6 (r_s=0.782, P<0.001, n=15); calculation of P values as in Table 1

workers either increased or decreased their outside-the-nest frequencies.

In sum, of the two constituent parts of social resilience, the spatial component is conserved while the task component could change. We conclude, therefore, that social resilience is a robust phenomenon. Its underlying mechanism clearly allows individual workers to return to their relative spatial positions under the most extreme circumstances. This is sufficient for social resilience to maintain an efficient division of labour. By returning individual workers to their relative spatial positions, social resilience ensures that all workers are restored either to their familiar tasks or to tasks in the neighbourhood of their familiar tasks, some of which they might have encountered previously. At the same time, by allowing for flexibility in the relative task profiles of workers, social resilience facilitates a colony-level adaptive response to any changes in task supply and demand.

Such robust social resilience should maintain an efficient division of labour in the likely event that components of the colony become lost during emigration. (Emigration occurs frequently in this species, Partridge et al. 1997). Furthermore, with regard to life-history strategies, the ability to conserve worker spatial relationships may be essential for efficiency to be maintained during the period of colony life after the queen dies (Franks et al. 1990). In small monogynous colonies, such as those of *Leptothorax*, workers can live for several years and hence colonies can have, after the death of the queen, an extensive orphanage period.

Franks et al. (1990) have shown that production of sexuals, including the production of sons by a small proportion of the workers, during the orphanage period can be of major importance for the fitness and inclusive fitness of all colony members. Therefore, a robust phenomenon, such as social resilience, that enables a colony to operate effectively after the loss of colony components, should be favoured by natural selection. Selection among colonies for greater efficiency is likely to have driven the evolution of task specialisation (Oster and Wilson 1978; Bourke and Franks 1995) and, in association with it, the maintenance of an efficient division of labour.

The possible mechanism of social resilience

What underlying mechanism could furnish social resilience with such robustness? The hypothesis we currently favour is that workers have differential mobility levels associated with their tasks and that after colony emigration to a new nest, workers are able to sort themselves along the radius from the centre of the colony to its periphery on the basis of their relative mobilities (even when the queen, the brood and a large proportion of the workers are all simultaneously absent).

Recently, Blanchard et al. (2000) demonstrated for the same species that there is a significant relationship between: (1) worker average velocity while active and worker corpulence, and (2) worker median distance to the centre of the brood pile and worker corpulence (a more corpulent worker stores more energy in her fat body). This is clear evidence that there is a relationship between worker mobility and worker spatial position. Since worker spatial position is related to worker task (Sendova-Franks and Franks 1995a), this is also evidence for a relationship between worker mobility and worker task.

On this basis, we suggest the following model for worker sorting. Following encounters in a new nest site, neighbouring workers exchange positions according to the following rule of thumb: if you are less mobile move towards the colony centre and if you are more mobile move towards the periphery.

In this way, relatively mobile ants may "diffuse" outwards and relatively immobile ants may "diffuse" inwards until each is sandwiched between a more mobile and a less mobile neighbour. Note that in this model the colony centre is not provided extrinsically (to the worker population) but intrinsically, by the propensity of ants from the same colony to aggregate.

Such a sorting procedure based on a general rule of thumb for worker-worker interactions in a new nest site, would provide not only a very simple mechanism but also one that is failure-secure and robust to the loss of colony components during an emigration (Sendova-Franks and Franks 1999). Furthermore, such a sorting procedure would facilitate an orderly and efficient re-allocation of tasks in the face of changing task supply and demand due to the loss of colony components. This procedure would enable workers in a new nest to take up new tasks closely related to their earlier tasks in agreement with the foraging-for-work model (Tofts and Franks 1992).

We suggest that individual workers acquire their relative mobility level in association with their task specialisation through a positive feed-back mechanism such as, for example, the ability of workers to learn (Deneubourg et al. 1987; Spencer et al. 1998; Theraulaz et al. 1998).

Thus, the more a worker performs a task, the more likely she is to perform it again and the more likely she is to move at a rate characteristic for the performance of this task. Memory, which is implicit in the process of learning, may in turn underlie the mechanisms by which individuals retain their mobility levels for a certain period of time.

The mechanisms involved in each of the transitions to a higher level of biological organisation are an especially puzzling part of the evolution of life (Seeley 1995). The present study is concerned with the half of this puzzle that lies in the realm of proximate causation. We have demonstrated that the mechanism underlying social resilience is robust.

Social resilience is analogous to the process whereby the cells of a sponge, which have been squeezed through a fine silk mesh, can reassemble into a functional whole (Wilson 1907; Huxley 1911, 1912; Curtis et al. 1982) and to the sorting of dissociated cells into tissues (Townes and Holtfreter 1955; Medoff and Gross 1971).

Therefore, social resilience may shed light on colony development (so-called sociogenesis). It may, in a way analogous to cell sorting during morphogenesis, play a role in worker re-arrangements during sociogenesis (Wheeler 1911; Wilson 1985; Sendova-Franks and Franks 1999).

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Appendix B

Population Counts

Colony	**Number of Workers**
Experiment 1	
C_1 (Control)	83
1	88
2	109
3	97
4	89
Experiment 2	
C_2	77
5	65
6	70
7	88
8	140
Experiment 3	
C_3 (Control)	62
9	141
10	82
11	83
12	91

***Table 1:** Table showing the population count of colonies used in experiments 1, 2 and 3.*