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Spatial and social organisation within nests of the ant Leptothorax unifasciatus (Latr.)

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SPATIAL AND SOCIAL ORGANISATION WITHIN NESTS OF THE ANT *LEPTOTHORAX UNIFASCIATUS* (LATR.)

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Abstract

The spatial positions of individually recognisable ants have been used in conjunction with their behaviour in order to achieve a simple and objective analysis of colony structure and organisation in the ant *Leptothorax unifasciatus* (Latr.). For this purpose extensive use has been made of computer based image analysis and the novel statistical approach of randomisation tests.

The concept of an ant's **Spatial Fidelity Zone** is introduced, its variation across colonies is examined, and the existence of colony spatial structure is demonstrated. The notion of **caste** is considered in this new light. Change of task over long periods of time is investigated. It is concluded that ants change their positions asynchronously along the colony spatial structure and hence age cohorts intermix, invalidating the idea of a hard-wired age polyethism. The effects of provoked emigration on colony spatial structure have been studied, both as another opportunity for task allocation and as a disturbance of colony everyday organisation. The results establish that task allocation is probabilistic and that colony spatial configuration and behavioural profile are stable. Finally, the spatial position of the queen and her interactions with workers are examined.

The novel approach of this study to problems of colony structure and organisation are of interest from the points of view of the evolution of insect societies, and sociality in general. Moreover, the results have general significance for organisational theories of both, biological and non-biological systems.

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Contents

Chapter 1

General Introduction

1.1 The Problem

A colony of ants is a curious combination of sociality and individuality. It is a coherent entity and yet consists of physically distinct and mobile ants. How then, does a colony of ants operate? Does it have a structure? Is it spatially ordered?

Just as anatomy and morphology are essential to our understanding of physiology, colony structure should be essential to our understanding of colony function. At present our concept of colony structure is limited to the division of labour that has been established in the societies of the eusocial Hymenopterans. The identification of distinct labour groups underlying the division of labour is based on perceived adaptiveness of function, however, and not on spatial order.

Despite the revival of the idea of the social insect colony as a superoganism in analogy with a multicellular organism (Wheeler 1928, Seeley 1989, Wilson and Sober 1989, Hölldobler and Wilson 1990), the colony is viewed as a loose assemblage of individuals that appears to lack any spatially ordered means of information acquisition or information processing in contrast to a multicellular organism which has a 3-dimensionally differentiated nervous system (Camazine 1993).

The idea that ant colonies are organised in dense heterarchies does not provide for any spatial order either (Wilson and Hölldobler 1988). This idea views the ant colony as a hierarchy-like organisation which comprises a nested system of labour categories from narrowly to more broadly defined, where the highest level is the whole membership. The heterarchy is also highly connected or "dense", in the sense that each individual member is likely to communicate with any other. The way this is realised, however, is not specified.

The study of colony organisation in social insects is at present characterised by two radically different and so far unreconciled approaches, defined by the theories of **optimisation** and **self-organisation**. On one hand, there is the view of the ant colony as an economic system, a factory within a fortress (Oster and Wilson 1978) that works towards maximising production of reproductives who in turn will found new colonies. From this viewpoint colony structure and function are subject to optimisation by natural selection at the colony level (Oster and Wilson 1978, p. 24) and although mechanisms of day-to-day organisation are not specified deterministic underlying processes are implied. On the other hand, there is the view of colony patterns of organisation as emergent properties of interactions between individuals or between individuals and the environment, where randomness, redundancy, simple rules and positive feed-back are of crucial importance (Pasteels et al. 1987). Optimisation models are useful for surmising **"strategies"** with an almost metaphysical purposefulness (Oster and Wilson 1978, p. 313 and p. 315). They consist of single complex algorithms that attempt to cover all possible eventualities but are intolerant to faults. Self-organisation models, on the other hand, emphasise mechanisms and **"tactics"** employing algorithms that are parsimonious and fault-tolerant. The top-down approach of optimisation theory is inspired by **human economics** (Oster and Wilson 1978) where optimality is an *a priori* consideration and randomness is unacceptable. The bottom-up approach of self-organisation theory is inspired by the self-organisation processes that were first observed in **physico-chemical** systems (Nicolis and Prigogine 1977) where efficiency is estimated only *a posteriori.*

The present work investigates ant colony spatial configuration, and aspires to contribute to our understanding of whether these two approaches to the organisation of social insect colonies are antagonistic or complementary (Pasteels et al. 1987).

1.2 The Approach

1.2.1 The Approach of the Present Work

An important characteristic of the approach taken in the present work is that it simplifies the problem of spatial organisation to 2 dimensions by using the ant species *Leptothorax unifasciatus* which lives in flat nests and forms small colonies, with the supposition that the results could be extrapolated to 3 dimensions. Data collection is based on making individuals recognisable by colour marks and sampling the range of their positions by taking colour photographs in sessions of regular time intervals. The positions of individuals are digitised as x- and y-coordinates within the nest with the help of image analysis equipment. Behavioural acts of individuals are recorded separately from the photographs.

In a dynamic system like the ant colony, spatial structure and function are hard to separate. The relative spatial positions of ants in the colony are a reflection of their movement and movement is itself a behaviour, a function. Even so, describing behaviour in terms of movement rather than behavioural patterns has the advantage of objectivity over subjectivity at the cost of reduced detail. Describing behaviour in terms of movement alleviates the problems of: (a) selecting only behavioural patterns that are deemed adaptive, (b) the subjectivity of establishing the beginning and end of a behavioural pattern. These are problems that lead to discrepancies when observations of the same species made by different authors and even by the same author at different times are compared (Jaisson et al 1988).

Randomisation tests, the application of which is relatively novel to biology, are widely used in the analysis. The general approach in the analysis is to avoid statistics that are based on models, because the models for the processes concerned could be very complicated. The price to be paid for this approach is that the tests employed are not as **powerful** as the usual **parametric** methods would be. In other words, the objective is to strike a balance between **robustness** and **power**. As it will become apparent, the statistics I use are very **robust** and since they reject the null hypotheses of no spatial structure, they clearly have sufficient **power** for my purposes. Therefore, hopefully they are well chosen.

1.2.2 Other Spatial Approaches

The spatial approach to animal behaviour is widely used in analyses of wildlife radio-tracking data (Doncaster 1990, White and Garrott 1990). This analysis has to deal with problems that are similar to the problems encountered in the present study; for example, what constitutes the home range of an individual and how to establish any static or dynamic interactions that may have occurred between individuals. However, there are crucial differences between the spatial analysis of wildlife radio-tracking data and the spatial analysis in the present study. These differences arise from the sociality of ants. An ant colony has a biologically meaningful centre - the egg pile, which can serve as a reference point for the positions of all ants and thus can simplify the analysis to 1 dimension. In addition, due to their sociality, ants of the same colony are easy to follow simultaneously while this is a problem with wildlife tracking. The present spatial approach is also different in that it does not make any *a priori* assumptions about the spatial distribution of the animal and makes extensive use of randomisation tests.

1.3. The Species

1.3.1 Systematics

The ant species *Leptothorax unifasciatus* (Latreille, 1798) (Kutter 1977, cited in Douwes and Stille 1991) belongs to the subfamily Myrmicinae of the family Formicidae. *Leptothorax* is a holarctic genus with 88 species from Europe (Agosti 1989, cited in Douwes and Stille 1991). The systematics of this genus is at present in a state of confusion (Douwes and Stille 1991).

1.3.2 Habitat

L. unifasciatus colonies are found in dry and sunny to semi-shaded areas (Martin 1988, 1990) where they usually nest in nearly flat crevices in rocks.

1.3.3 Colony Size

Up to 500 workers when the colony is mature (Martin 1988, 1990).

1.3.4 Size and Longevity of Queen and Workers

The longevity of the queen is about 10 years (Hölldobler and Wilson 1990, p. 169). The longevity of the workers is not less than 3 years in the laboratory (Hölldobler and Wilson 1990, p. 169; author, unpublished observations). The queen is 4.5 - 5mm long, workers are 2.5 - 3mm long on average.

1.3.5 Gyny, Morphism

L. unifasciatus is a monomorphic, monogynous species. No information is available about whether it is mono- or polyandrous.

1.3.6 Annual Cycle (based on the description of the annual cycle of *L. nylanderi* (Plateaux 1970, cited in Lane 1977a; and Martin 1988, 1990))

Colonies hibernate from October - November to March - April, when brood consists of larvae of diverse sizes. First eggs are laid at the end of April and the beginning of May. First prepupae and pupae appear in early June, and the first imagoes - in early July. Some larvae pass through two winters before eclosion. The rate of brood development decreases from mid-August onwards and is even arrested in the largest larvae at that time. The eclosion of adults terminates by the beginning of September. From mid-September onwards, the society is gradually preparing for hibernation evidenced by: a decrease in the number of eggs, reduced alimentary rates, grouping of the society, progressive closing of the entrance and general decrease of activity. The nuptial flight takes place in August - September always at a fixed hour (in the case of *L. nylanderi* - in the evening).

1.3.7 Food

No data is available on what *L. unifasciatus* feeds on in nature. In general *Leptothorax* ants do not interact directly with aphids, but lick the honeydew left by aphids on leaves and stems of plants (Wheeler 1910, cited in Lane 1977a). Ants of the species *Leptothorax acervorum* feed on Dipteran larvae and small Apterigotans, but do not attack insects longer than 0.5 mm (Dobrzanski 1966,cited in Lane 1977a).

1.3.8 Methods of Recruitment and Orientation

L. unifasciatus uses both, tandem-running and pheromone trails for recruitment to food sources, new nests and virgin areas (Lane 1977a). *L. unifasciatus* workers use both chemical and visual cues when foraging, but visual orientation, based on laboratory surroundings, dominates over chemical orientation. After the discovery of food workers lay down a trail which helps them to orient themselves when leading a tandem later, but does not recruit other nestmates directly. Individuals are able to distinguish their own trails among the trails of their nestmates. On the other hand, if their own trail is missing, they are able to follow the trails of their nestmates too (Aron et al. 1988). *L. unifasciatus* workers can distinguish new from old areas. Perhaps areas are chemically modified by the activity of the ants. This suggests that *L. unifasciatus* colonies mark their area and are territorial (Aron et al. 1986). The foraging and exploratory activities of *L. unifasciatus* vary considerably according to the state of the colony, the size of the colony, and the type of foraging area. Long-settled colonies show lower activity than recently-settled colonies. Smaller colonies have lower activity then bigger colonies. However, colony activity is influenced more by the length of time during which the nest has been inhabited by the colony than by absolute colony size.

In addition, recently-settled colonies show a higher level of foraging activity on a virgin area than on a familiar area, while long-settled 3 show no such difference (Aron et al. 1986).

1.3.9 Behaviour

The behavioural repertory of *L. unifasciatus* workers consists of approximately 21 behavioural acts (Chapman 1989; author, unpublished data). *L. unifasciatus* colonies sort their brood in concentric circles so that the type that needs least care (the eggs) is in the middle and the type that needs most care (the biggest larvae) is on the periphery. Ants are able to recreate quickly the same pattern after emigration to a new nest (Franks and Sendova-Franks 1992). *L. unifasciatus* ants are able to build a perimeter wall around their colony out of grit. This allows them to make their nest according to colony size (author, unpublished observations from nature and from the laboratory). Almost certainly *L. unifasciatus* employs the same simple rules that could account for building in *L. "tubero-interruptus"* - each builder, by pushing its building block into others, adds its work to existing structures (Franks et al. 1992).

1.3.10 Colony Activity Cycles

Colony activity in nests of *L. unifasciatus* occurs in bouts, approximately every 3 to 10 minutes. The cycle appears to be much longer during the night with activity increasing towards the daylight hours (Chapman 1989, p. 1). Colony activity in nests of *L. acervorum* and *L. "tubero-interruptus"* tends to cycle, although cycles are not truly periodic and are not of regular amplitude. Cycles in *L. acervorum* are approximately 20 minutes in duration, those in *L. "tubero-interruptus"* - approximately 35 minutes. Cycle length appears to be independent of colony size and brood to worker ratio (Hatcher 1992, p. 234; Tofts et al 1992).

1.3.11 Collection

Colonies of *L. unifasciatus* were collected in the spring of 1990, 1991, and 1992 from steep wooded hillsides above the village of Thompson in the Balkan mountains, Bulgaria. All colonies were collected from flat crevices in rocks. As a rule nests consisted of a single chamber where all the ants in the colony lived in close proximity. The colonies were aspirated in glass tubes where they stayed for about 3 - 5 days before being transported to the Ant Laboratory at Bath University.

1.3.12 Culturing in the Laboratory

The colonies were cultured in nests made from a pair of large microscope slides separated by a perimeter of cardboard to make a cavity of 35 mm x 21 mm x 1 mm (thin nest - only colony BG90UO was housed in this type of nest) or a cavity of size 35 mm x 23 mm x 1 mm (normal nest). A single nest exit passage of 4 mm x 2 mm x 1 mm was provided in one of the short walls of the nest. Each nest was placed in a petri dish, 100 mm x 100 mm x 17 mm, which served as an arena where the water and two food sources were situated (Fig. 1.1). In order to prevent ants going beyond the arena the petri dish walls were covered with FLUON.

Colonies were fed weekly on an *ad libitum* supply of honey solution (1 part in 10 parts of water) and *Drosophila* larvae.

1.3.13 Colony Annual Cycle as Observed in the Laboratory

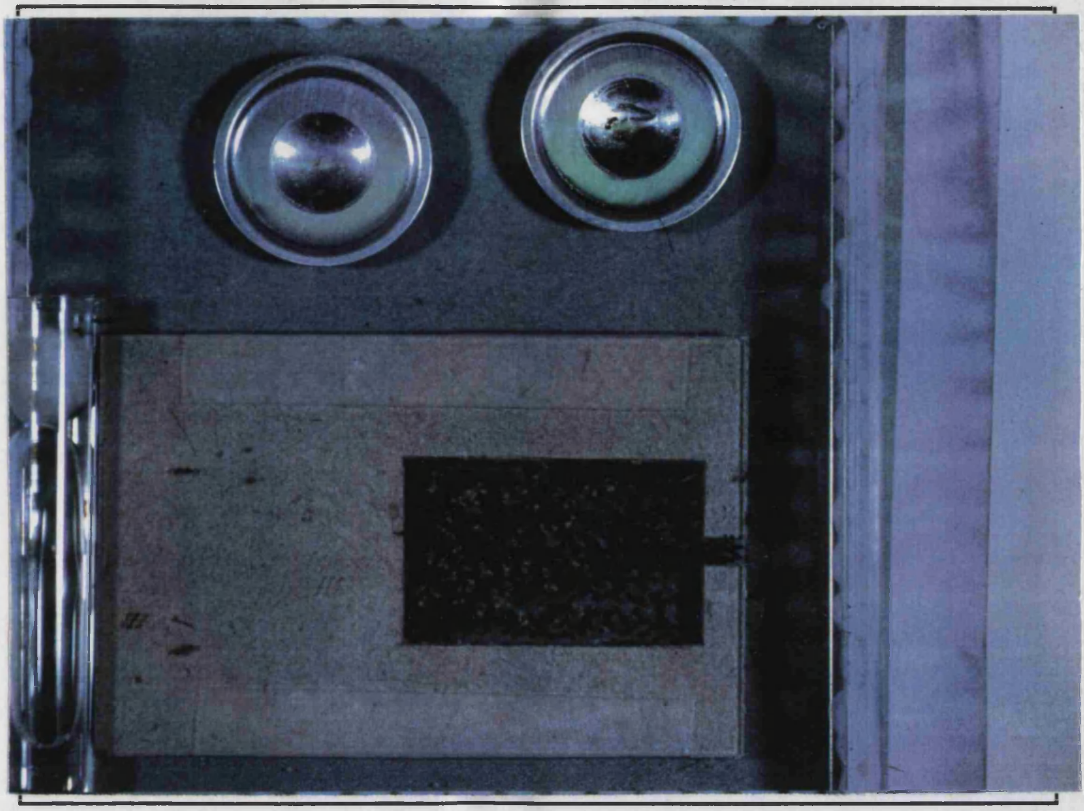


Figure 1.1: Laboratory nest and arena

L. unifasciatus colonies were put to hibernate from 5th October to 5th March each year by being placed in a cardboard box and left in the garage of a house where air temperature varied between 0 and 10^{0}C. In order to acclimatise, colonies were kept at an intermediate temperature for a week before and after the 1 period on the window sill of an unheated room. Throughout the rest of the year colonies were kept at an almost constant temperature of 20 - 23^{0}C maintained by the air conditioner in the Ant Laboratory at Bath University. The first eggs were laid immediately after the colonies were brought back into the laboratory. Prepupae and pupae started to appear in May and new imagoes emerged continuously but with diminishing intensity from June to mid-August.

1.3.14 Reasons for Choosing this Particular Species

The choice of ant species reflects the requirements of the approach (see section 1.2). *L. unifasciatus* forms small colonies that are easy to collect quickly in their entirety. The geometry of their nests in flat crevices in rocks is closely approximated by simple artificial nests made from two microscope slides separated by a perimeter of cardboard between them that allow the observation of all the ants all the time.

1.4 The Method

1.4.1 Data Collection

1.4.1.1 The Marking Technique

Ants were individually marked using the following technique. A slit (about 3 mm long) is made in the top surface of a block of foam plastic that is placed in a crystallisation dish (diameter 90 mm, hight 48 mm). The ant to be marked is placed into the slit so that only its gaster is visible. Then the ant is anaesthetised by piping at a low rate carbon dioxide into the bottom of the dish. The foam and anaesthetic make the ant easy to manipulate without causing it any injury. Tiny droplets of paint (PACTRA R/C polycarbonate, ketone-soluble model paint) are applied to the ant's gaster with a very thin entomological pin set in a match stick. Up to 4 droplets of paint can easily be applied to the gaster of an ant of this size so each ant can have its own individual mark. Then the ant is taken out of the restraining block and left in a new petri dish with a new nest. The paint dries in a minute or two, while the ant recovers in at most 10 min. The complete workforce of a colony is marked using the following procedure. The colony's nest is placed in a larger petri dish. Then the upper microscope slide is removed and placed about a centimetre away from the nest. This causes alarm and ants mix

Figure 1.2: A colony with individually marked workers

up. Each worker is marked in turn after being picked up at random. The queen and part of the brood together with the upper microscope slide on which brood is usually stuck are placed in the new arena sometime during the procedure. After the last worker is marked the rest of the brood is transferred to the new arena by either carefully using a brush (if the brood is scattered in the old arena) or by moving the lower slide of the old nest (if the brood is stuck to the remaining microscope slide). Note that the marking procedure includes an emigration of the colony to a new nest. Although marked ants seem to groom one another more often than usual for the first few days after they have been marked, such paint marks often persist for many months.

After the marking session each ant was made identifiable by drawing all the marks and assigning a number to each of them (Fig. 1.2).

Experimental work on *Myrmica* (Weir 1957) and *Leptothorax nylanderi* (Plateaux 1971) has shown that anaesthesia by carbon dioxide could cause damages to ant colonies, like colourless pupae (Plateaux 1971) and workers laying fewer eggs (Weir 1957). It could also be argued that marked ants behave differently from unmarked ants. However, no conspicuous signs of abnormality were observed in the marked colonies when compared to the rest of the colonies in the laboratory.

1.4.1.2 The Photographic Technique

Photographs were made on a fine-grain colour slide film (Kodak, Kodachrome ISO 64, 36 exposures), using a pair of synchronised flashes with 35 mm single lens reflex camera mounted on a Zeiss stereo dissection microscope. All photographs were taken against a black background. Photographs were taken in two types of sessions (see section 1.4.1.4). When separate photographs were made to record the brood pattern (sessions of type one) this involved turning the nest over. Doing this slowly and gently caused no noticeable disturbance of the ants. As a precaution, however, brood pictures were taken at the end of the day sequence so that the colony had till the next morning to recover if any disturbance was caused.

The scope of the photographs included only the nest cavity. Therefore, immediately after each photograph was taken the identities of the ants, both in the exit passage and outside the nest, were recorded.

The present method of using still photography for analysing ant social behaviour is similar in principle to that of Corbara et al. (1986, 1990).

1.4.1.3 Colonies and Sessions

The present study is based on observations and experiments with 10 ant colonies, 9 of which were recorded in photographic sessions. The sessions were of two types. One type of session covered 5 consecutive days and photographs were taken every half an hour for 9 1/2 hours per day, yielding 100 colour slide photographs. Sessions of type one started between 8:00 and 9:30 in the morning and continued until between 5:30 and 7:00 in the evening. The other type of session covered a month and was artificially defined because the colonies concerned were observed continuously for 6 months. During sessions of type two photographs were taken on as many days of the month as was possible, at the rate of at most 3 per day, yielding at least 50 colour slides per session. The first photograph for the day was taken some time in the morning, the second - around lunch time, and the third - in the late afternoon, avoiding fixed hours. During sessions of both types photographs were taken at temperature between 20 and 23^{0}C.

A total of 33 photographic sessions of observation were made (see Table 1.3), yielding approximately 2,500 slides and approximately 125,000 data points.

For more detailed information about the colonies and how they were observed, please look at Tables 1.1-1.5.

1.4.1.4 Justification of Sampling in Sessions of Type One

The method for establishing the position range within the nest for any individual ant is constrained by first, a big enough sample size and second, a time interval which is meaningful in terms of the speed of ant movement. One hundred is a statistically reasonable sample size, while half an hour is an interval potentially long enough for an ant to cover several times all possible positions in a nest of the used size.

Colony	Collected on	Marked on	Demography recorded on
BG90UO	11.04.1990	17.10.1990 08.06.1990	17.10.1990 10.06.1991
BG91UI	03.04.1991	23.04.1991	20.05.1991
BG91UJ	03.04.1991	28.06.1991	17.07.1991
BG91UQ	03.04.1991	02.07.1991	12.07.1991
BG91UL	03.04.1991	24.04.1991	30.04.1991
BG91UM	03.04.1991	26.04.1991	30.04.1991
BG91UN	03.04.1991	25.04.1991	30.04.1991
BG91US	03.04.1991	11.05.1992	13.05.1992
BG92U1	08.04.1992	08.05.1992	10.05.1992
BG92U5	08.04.1992	12.05.1992	15.05.1992

Table 1.1: List of the studied colonies

Colony	Workers (+ M/FM)	Total brood	Eggs	Larvae S M L	Pre-pupae	Pupae (+ M/FM)
BG90UO	60	75	0	41 13 21	0	0
BG91UI	84	128	9	47 14 29	23	6
BG91UJ	199 +8M	168	45	40 30 32	2	19
BG91UQ	189 +2M	88	45	19 5 5	0	14
BG91UL	42	95	12	46 21 15	1	0
BG91UM	82	177	12	110 46 9	0	0
BG91UN	52	110	13	57 28 12	0	0
BG91US	65	104	9	10 17 21	19	28
BG92U1	78	106	22	22 34 28	0	0
BG92U5	158	161	27	36 40 46	10	2

Table 1.2: Demography of each studied colony on the date shown in Table 1.1. Each colony had one queen.

Colony	**Number of sessions**	**Type of session**	**Purpose of observation**
BG90UO	5	type 1	change in colony configuration over time with two distinguishable age cohorts
BG91UI	2	type 1	effect of emigration
BG91UJ	2	type 1	effect of emigration
BG91UQ	2	type 1	effect of emigration
BG91UL	6	type 2	change in colony configuration over time
BG91UM	6	type 2	change in colony configuration over time
BG91UN	6	type 2	change in colony configuration over time
BG91US	2	1/2 type 1	effect of brood removal
BG92U1	2	1/2 type 1	effect of the removal of 1/2 of the ants
BG92U5	0	not filmed	worker specialisation in 5 emigrations

Table 1.3: Sessions of observation

In order to justify this statement, I will give an idea of how far on average an ant would go if an ant made a random walk in 2-D. Let us suppose that a *L. unifasciatus* ant moves outside the nest at a speed of 5mm/sec on average (an assumption based on observation). If this ant is making a random walk in a space without boundaries during a 30min (1800sec) interval, then the distance d between the end points of her walk would be:

$$d = s \times \sqrt{n},$$

where s is the step length and n is the number of steps (Franks and Fletcher 1983). For s = 5mm and n = 1800

$$d = 5 \times \sqrt{1800}$$

$$d = 5 \times 42.43$$

$$d = 212.15mm$$

Session	Time interval	brood av. no.	workers av. no.	Brood/ workers
BG90UO1	11.03.1991-15.03.1991	80	31	2.58
BG90UO2	15.04.1991-19.04.1991	90	28	3.21
BG90UO3	03.06.1991-07.06.1991	110	71	1.55
	same year age cohort marked			
	emigration on 08-09.06.1991			
BG90UO4	10.06.1991-14.06.1991	59	84	0.70
BG90UO5	02.09.1991-06.09.1991	53	84	0.63
BG91UI1	20.05.1991-24.05.1991	132	80	1.65
	emigration on 25.05.1991			
BG91UI2	26.05.1991-30.05.1991	120	66	1.82
BG91UJ1	29.07.1991-02.08.1991	185	165	1.12
	emigration on 03.08.1991			
BG91UJ2	05.08.1991-09.08.1991	176	150	1.17
BG91UQ1	15.07.1991-19.07.1991	83	165	0.50
	emigration on 20.07.1991			
BG91UQ2	22.07.1991-26.07.1991	74	155	0.48
BG91UL1	May 1991	80	42	1.91
BG91UL2	June 1991	47	43	1.09
BG91UL3	July 1991	36	49	0.74
BG91UL4	August 1991	22	48	0.46
BG91UL5	September 1991	20	47	0.43
BG91UL6	October 1991	16	46	0.35
BG91UM1	May 1991	189	82	2.31
BG91UM2	June 1991	198	92	2.15
BG91UM3	July 1991	173	138	1.25
BG91UM4	August 1991	123	160	0.77
BG91UM5	September 1991	106	156	0.68
BG91UM6	October 1991	102	153	0.67
BG91UN1	May 1991	94	51	1.84
BG91UN2	June 1991	69	52	1.33
BG91UN3	July 1991	54	72	0.75
BG91UN4	August 1991	34	83	0.41
BG91UN5	September 1991	32	81	0.40
BG91UN6	October 1991	28	77	0.36
BG91US1	18.05.1992-20.05.1992 (half day)	108	59	1.83
	removal of brood			
	emigration on 20.05.1992			
BG91US2	21.05.1992-23.05.1992 (half day)	17	55	0.31
BG92U11	11.05.1992-13.05.1992 (half day)	110	73	1.51
	removal of 1/2 of workers			
	emigration on 13.05.1992			
BG92U12	14.05.1992-16.05.1992 (half day)	102	42	2.43

Table 1.4: Time and duration of sessions

Session	Total no. averaged	marked	analysed	present in all sessions
BG90UO1	31	30	27	12
BG90UO2	28	27	24	12
BG90UO3	71	20	19	12
BG90UO4	84	72	69	58 (12o+46y)
BG90UO5	84	67	59	58 (12o+46y)
BG91UI1	80	77	73	57
BG91UI2	66	66	57	57
BG91UJ1	165	118	93	71
BG91UJ2	150	96	73	71
BG91UQ1	165	156	106	93
BG91UQ2	155	118	102	93
BG91UL1	42	43	39	30
BG91UL2	43	40	38	30
BG91UL3	49	39	38	30
BG91UL4	48	37	34	30
BG91UL5	47	34	32	30
BG91UL6	46	33	31	30
BG91UM1	82	81	78	63
BG91UM2	92	77	75	63
BG91UM3	138	73	71	63
BG91UM4	160	72	66	63
BG91UM5	156	66	63	63
BG91UM6	153	65	63	63
BG91UN1	51	51	50	36
BG91UN2	52	49	46	36
BG91UN3	72	46	46	36
BG91UN4	83	45	43	36
BG91UN5	81	41	39	36
BG91UN6	77	39	36	36
BG91US1	59	61	53	45
BG91US2	55	50	46	45
BG92U11	73	77	65	30
BG92U12	42	42	34	30

Table 1.5: Number of workers for each session

For comparison the nest size is 35 mm x 21 mm or 35 mm x 23 mm.

The second constraint gives rise to another potential problem. We do not know whether ants change their region of movement and if they do in what manner and with what speed. Hence it may not be possible to use together data collected in sessions of different duration. On the other hand, if the explored relationships are the same for sessions of both types, then they could be considered together.

1.4.1.5 Justification for Sampling in Sessions of Type Two

The definition of this type of session was artificial but not arbitrary. Sessions of the first type showed that 50 points were enough to give an ant the same spatial description as did 100.

1.4.1.6 Number and Age of the Ants Considered in Each Session

Observations during all sessions took into consideration all marked ants in the colony. A minimal number of marked ants lost their marks or died during a session (see Table 1.5, column 2). In addition, it was not always possible to identify all marked ants on a slide, because some of them were inverted on the upper slide of the nest and hence their marks were invisible to the camera. However, this shortcoming of the method should not have led to any bias in the established ant spatial distributions since there is no reason to believe that some ants were more likely to walk on the upper slide of the nest than others or that the same ant was more likely to walk on the ceiling in one part of the nest and on the ground - in another.

The score of an ant was determined as the sum total of: first, the number of recorded positions within the nest and second, the number of times she was either in the exit or outside the nest. Only ants which had scored at least 50% of the maximally possible score and at the same time had at least 5 recorded positions inside the nest were analysed. This criterion was a compromise between a reasonable sample size for positions within the nest and discarding all "foragers" from the analysis.

During some of the sessions all ants in the colony were marked. During the rest of the sessions the unmarked individuals (with the exception of the very small contingent of ants that have lost marks) consisted of the new generation for the year, that had eclosed after the marking session (Table 1.5).

1.4.1.7 The Digitising Technique

The positions of all marked ants on each photographic slide were digitised with the help of image analysis equipment and by using the following procedure. The slide is placed under a video camera in a consistent orientation so that the obverse side faces up and the nest entrance points to the right. The camera image is then "grabbed", i.e. stored as different grey values for 768 pixels x 576 pixels in the frame-storer of the connected PC computer. The "grabbed" image is projected on a black and white video monitor. A coordinate system on the image is produced by drawing a window of 652 pixels x 422 (or 392 for the narrow nest) pixels around the nest cavity, where the origin is in the upper left corner. Then the slide itself, which is colour, is projected on a slide projector. Ants on the black and white image are identified with the help of the identification list by finding them on the colour image. Then the coordinates of each identified ant are automatically recorded into a file (see section 1.4.3.1 for format of the data) by selecting the ant's petiole with a mouse controlled cursor and entering the ant's identification number. The resulting computer file with the identity of each recognisable ant and her coordinates is transferred to a workstation computer for analysis.

The digitising procedure was implemented by computer programmes written in BASIC by Tim Stickland and using functions written in C from the image analysis softwear. The finally chosen scale of the projected image was arrived at through trial and error. The window size was determined by the size of the nest cavity. One millimetre corresponded to approximately 18 pixels of the digitised image. When the position of an ant was digitised her identification number appeared on the image and this precluded the possibility of identifying the same individual twice.

Brood was digitised in a similar way as ants except for two differences. First, the position of each brood item was considered to be its centre. Second, brood items were identified according to type. The brood was classified into five categories: pupae (white and immobile but having the sculptured morphology of adults), prepupae (white and opaque but without adult sculpturing), large larvae (as with the other larvae light yellow and translucent), medium larvae, and lastly microlarvae and eggs. It is hard to distinguish between eggs and newly hatched microlarvae so these were grouped into the same category. Brood categories were coded numerically as follows: pupae (10), prepupae (9), large larvae (8), medium larvae (6), microlarvae and eggs (4).

The positions of the brood were digitised from one photograph per day in sessions of type one and from 4-5 photographs per month in sessions of type two. In sessions of type one the brood photographs were specially made by turning the nest upside-down. This was inappropriate to do for sessions of type two.

1.4.1.8 Behavioural Records

The behaviour of ants was recorded from the photographic slides after all of them were digitised. Behavioural acts were defined on the basis of observed contacts between the mouthparts of an ant and a nestmate or a brood item. The experience of the author with direct observations of behaviour helped to eliminate "neutral" contacts, e.g. when the mouthparts of an ant were touching passively an object while the ant herself was inactive. Within the data matrix one of the following 10 behavioural acts was possible to assign to the identity of the ant: contact with each of the 5 types of brood (see section 1.4.1.7), contact with a young or an old ant, contact with the queen, trophallaxis with larva, trophallaxis with an ant (see Appendix). For the final analysis the categories of contact with a young ant, contact with an old ant and trophallaxis with an ant were pooled together. In addition, the categories of trophallaxis with a larva was pooled with all other brood categories into the much broader category of contacts with brood, thus finally yielding only 3 broad categories of behavioural acts. This arrangement had the advantage of offering much greater sample sizes.

1.4.2 Experimental Procedures

1.4.2.1 Provoked Emigration

Three types of emigration were employed in the present work. First, the emigration, incorporated in the marking procedure, described in section 1.4.1.1. Second, emigration with the objective of following the division of labour that occurs during the process itself - described in section 4.2.2. Third, emigration as a perturbation of the colony - comprises the first steps described in section 4.2.2. In emigrations of the first type colonies were supplied with food immediately after they had settled down, after 12-24 hours. In emigrations of the second type, the colony was supplied with fresh food each day, approximately 30 min before the beginning of the experiment. In emigrations of the third type, colonies were provided with fresh food just before the commence of the photographic session.

1.4.2.2 Removal of 1/3 of the Workers

Within a setup for provoked emigration (see section 1.4.2.1) the upper microscope slide of the nest that housed colony BG92U1 was removed and 31 workers were taken away. The removed workers were selected on the basis that they had been mostly to be found on the brood pile during the preceding photographic session. After that the rest of the colony was left to emigrate to a new nest. The colony was successfully reunited after the experiment.

1.4.2.3 Removal of All the Brood Except the Eggs and Microlarvae

Within a setup for provoked emigration (see section 1.4.2.1) the upper microscope slide of the nest that housed colony BG91US was removed and 73 brood items were taken away, leaving only 3 microlarvae and 13 eggs. Then the rest of the colony was left to emigrate to a new nest. The colony was successfully reunited after the experiment.

1.4.3 Data Handling

1.4.3.1 The Format of the Data

Each colour slide photograph was digitised in terms of the ants' identity numbers and their x- and y-co-ordinates within the nest. In this way the primary type of raw data files was generated.

```
652 422 −> the size of the nest

0 28 43

18 305 208

31 412 144

.

.

. 0 - the queen
```

Each of the primary raw data files was checked for repeated identifications of ants as the same ant. Since it would have been very time consuming to determine which identification was the correct one, all such identifications were deleted from the respective data file. None of the ants were systematically misidentified.

When the complete number (between 50 and 100) of primary type raw data files was generated for a given session, new individual files were compiled - the secondary type of raw data files. These files consisted of the positional coordinates of particular individuals for the whole session.

```
0101 1 124 318
```

```
0102 1 414 319

0103 1 318 43

.

.

.

0120 1 114 281

0201 1 105 201

.

.

.
```

The first column in the above example contains the number of the slide - a time reference, the second column contains the ant's ID number, and the last two columns include the ant's positional coordinates.

Brood files were analogous to the primary type of raw data files for the ants, where the ant's ID number was substituted by the type of the brood.

On the basis of the length of each individual file and the available data for the identity of the ants that were in the exit or outside the nest, a list was compiled of all the ants observed during a session. Against the identity of each ant this list contained the number of slides the ant was identified on, the number of times she was recorded in the exit, and the number of times she was found outside the nest. With the help of this list individuals were included in the subsequent analysis on the basis of the criterion that an ant's total score should not be less than 50% of that possible for the session and she should have at least 5 recorded positions inside the nest (see section 1.4.1.6).

The centre of the colony was calculated as the centroid of all digitised brood of type 4 (microlarvae and eggs) for the respective session.

On the basis of the individual files (secondary type of raw data files) and the calculated centre of a colony for a particular session, a file containing the distances between the centre and each one of her positions was compiled for every ant.

1.4.3.2 Calculating the Median, the First and Third Quartile, and the Spread of a Distribution

The following algorithm was used to calculate quartiles:

```
if ( ((double)filelength/4 - filelength/4) < 0.5)

{

temp = filelength/4;

lowfourth = (file[temp - 1] + file[temp]) / 2;
```

```
templ = filelength - temp;
upfourth = (file[templ - 1] + file[templ]) / 2;
}
else
{
temp = (filelength/4 + 1);
lowfourth = file[temp - 1];
templ = filelength - temp;
upfourth = file[templ];
}
```

This algorithm does not work for sample sizes of less than 4. However, ants that had less than 5 positions in the nest were excluded from the analysis in the first place (see section 1.4.1.6).

The following algorithm was used to calculate the median:

```
if (filelength % 2 == 0)
```

```
{
temp = filelength/2;
median = (file[temp - 1] + file[temp]) / 2;
}
else
{
temp = filelength/2;
median = file[temp];
}
```

1.4.4 Statistical Methods

1.4.4.1 Randomisation test

Randomisation tests are tests for alternatives to null hypotheses of randomness. In comparison with more standard statistical methods, randomisation tests have two main advantages: (1) they are valid even without random samples; (2) it is easy to take into account the peculiarities of the situation of interest and use non-standard test statistics. The major disadvantage is that it is not necessarily possible to generalise the conclusions from a randomisation test to a population of interest. Randomisation tests can only be used to test hypotheses that involve comparisons between two or more groups (where randomisation involves swapping observations between groups), or hypotheses that say that the observations in one group are in random order (where randomisation involves generating alternative random orders) (Manly 1991).

The structural simplicity and power of randomisation tests have enormous appeal. The only reason they have not been used massively until now is that they require big computational power.

A randomisation test consists of the following steps: (1) Decide on the statistic. (2) Compute the statistic for the observed values of the used variable(s). (3) Consider the observed arrangement of values of the used variable(s) as a random sample of many possible but equally likely arrangements that could have arisen by chance. (4) Enumerate the possible arrangements of values of the used variable(s) and for each arrangement compute the statistic. (5) Compare the observed value of the statistic with the distribution of all its possible and equally likely values. (6) Decide whether the observed value of the statistic is deviant (= improbable) enough to reject the null hypothesis, i.e. that the observed arrangement of values of the used variable(s) is a random sample from all possible arrangements (based on Sokal and Rohlf 1981).

In some cases it is possible to enumerate all arrangements (permutations) of the observed values - a **permutation test**. However, very often this is practically impossible. Then, the way to proceed is to make a random sample of all possible permutations - **a sampled randomisation test.**

After a permutation test it is easy to determine whether the observed value of the statistic is in the upper or lower 5% rejection region of the simulated distribution. Similarly, when the simulated distribution is only a sample (sampled randomisation test) one should determine the percentage of simulated values which are equal or greater than the observed (if the observed value of the statistic is nearer to the upper end of the distribution) or are equal or smaller than the observed value (when it is nearer the lower end of the distribution). The confidence limits of the resulting percentage for the sample size can be determined on the basis of the binomial distribution (Sokal and Rohlf 1981, p. 794), thus establishing the probability of the observed value of the statistic for the entire number of possible permutations.

1.4.4.2 Spearman's Rank Correlation Coefficient

Spearman's rank correlation coefficient was tested as an ordinary product-moment correlation coefficient, because the sample sizes were always greater than 10 (Sokal and Rohlf 1981, p. 607).

Calculation was carried out within the S-plus statistical package (Becker et al 1988).

1.4.4.3 Runs Up and Down Test

Runs tests are used for testing whether events occur in a random sequence or whether the probability of a given event is a function of the outcome of a previous event. A **run** is defined as a succession of identical symbols which are followed and preceded by different symbols or by no symbols at all. The runs up and down version of the test can be carried out when **n** items are ordered in their natural sequence and the sign of the difference from the previous value is recorded for each item but the first. These signs would all be alike if the data were monotonically increasing or decreasing while cyclical data would show more than the number of runs expected in a random sequence of values. The test statistic (expected value of runs) is based on a normal approximation which is adequate for samples of $n >= 10$ and its value t_s is calculated as:

$$t_s = r - \frac{m_r}{s_r},$$

where **r** is the number of runs, m_r is the expected number of runs and s_r is its standard deviation. Consequently, when $t_s >= 1.96$ (i.e. at 95% significance level) it could be concluded that there was a regular alteration of differences, as might be caused by a cyclical phenomenon. On the other hand, when $t_s <= -1.96$ it could be concluded that one or more systematic trends were exhibited by the data.

It should be emphasised that runs tests apply to attributes only and take neither absolute magnitude nor order of magnitude into consideration (Sokal and Rohlf 1969, p. 624; Siegel and Castellan 1988, p.58).

The test was implemented by a programme written in C that was based on Sokal and Rohlf (1969, p. 628, box 17.3).

Trends and cyclicity in the data could also be established with the help of a randomisation test where the test statistic is the number of runs, and the number of runs is compared to the distribution of its values after hundreds of randomly chosen permutations of the order of the original data set. However, this approach would have been more time consuming and hence advantage was taken of the already existing test.

1.4.4.4 Random Number Generation

Random numbers were generated by a pseudo-random number generator based on the algorithm of Wichmann and Hill, 1982 written in C.

The authors give the cycle length of the generator as exceeding 2.78×10^{13} so that even if 1000 random numbers per second were used continuously, the sequence would not repeat for over 880 years. Later the authors (Wichmann and Hill 1984) corrected the period to 6.95×10^{12} which is only a quarter of the original one, but still large enough for any practical purposes.

1.4.4.5 Selection of Random Permutations

To choose random permutations of the integers from 1 to n the following method was used: (1) Generate n random numbers from a uniform distribution. (2) Put the n random numbers in an array where they will have indices from 1 to n. (3) Sort the random numbers in an ascending order. (4) Make an array of their indices. This array will be a random permutation of the integers from 1 to n.

Let $n = 5$

random numbers 0.51 0.01 0.72 0.12 0.68

indices 1 2 3 4 5

sort

sorted random

numbers 0.01 0.12 0.51 0.68 0.72

make an array of original indices

a random

permutation

of the integers

from 1 to 5: 2 4 1 5 3

1.4.5 Computer Programmes

Computer programmes were used as an aid to data handling and organisation, making calculations, and carrying out tests. The programmes were written in C, UNIX, and the language of the S-plus statistical and graphical package (Becker et al 1988). Since the employed algorithms are specified either directly or through references, the programmes themselves are not included in the Appendix but are available on floppy disks if examination is required.

Chapter 2

Division of Labour: Spatial Fidelity Zones, Behavioural Acts, and Tasks of Individual Ants

2.1 Introduction

In human societies division of labour has long been known to improve the productivity of work through the increase of dexterity in every particular worker, the saving of time otherwise lost in passing from one job to another, and the invention of machines which facilitate and abridge labour, thus enabling one worker to do the work of many (Smith, 1776 (1986), pp. 109 and 112). In human societies division of labour is limited by the size of the market (Smith 1776 (1986)). Only people living in big communities or working in large factories can afford to specialise in a particular job. In any particular manufacture the number and type of jobs, and even the way they are allocated among workers, is predetermined by economic forces. At the same time a ruling body exists in every factory to execute these economic laws in a way that is relevant to the particular situation of this factory.

Biological systems should also obey generic economic principles because efficiency is adaptive. In accordance with this division of labour is widespread in ant colonies. Empirical work has confirmed that instead of doing a little bit of everything individual workers specialise in a particular **task/role**, where **task** is used to denote a particular sequence of acts that accomplishes a specific purpose for the colony, such as foraging or nest repair, and has the effect of dividing labour (Hölldobler and Wilson 1990, p. 301, p.642). Division of labour has been reported in species across all subfamilies of the family Formicidae (Lenoir 1987). Only species from the less advanced (in evolutionary terms) subfamily Ponerinae have been found not to display any division of labour (Traniello 1978). In addition, observations of ontogenetic colony development reveal that increase in the size of the workforce is related to greater worker specialisation (Fresneau and Lachaud 1984, Gräf and Hölldobler 1991 and personal communication).

However, in contrast to human societies no ant society possesses either an autocratic or a democratic ruling body that can issue commands or make decisions. How then is labour divided among the workers at any particular time in any particular situation? The theoretical expectation that economic efficiency is adaptive and its confirmation in the omnipresence of division of labour in ant colonies has been interpreted to mean that ant colonies have been shaped by the forces of natural selection into a **strategic design** and similar to Swiss clocks that consist of levers and wheels ant colonies consist of individuals that have been turned into specialised working machines (Hölldobler and Wilson 1990, p. 298). The inference from this interpretation is that the process of worker differentiation should be deterministic.

Empirical results have so far failed to either support or falsify all the predictions of the hypothesis that ant colonies operate on the basis of deterministic behavioural differentiation of workers (Schmid-Hempel 1992). At the same time most empirical studies of division of labour are characterised by an approach that is implicitly biased in favour of the hard-wired mechanism of colony organisation. The method used in such studies is the compilation of ethograms. An ethogram incorporates the behavioural repertory together with the transition probabilities for each pair of behavioural acts and the distributions of the time spent in each behavioural act (Hölldobler and Wilson 1990, p. 300). A set of behavioural acts that are linked by high transition probabilities are defined as the **role** played by the individual(s) in the function of the colony. A **task** could be identical to a role, be only a part of it, or consist of behavioural acts that are distributed across two or more roles. Ethograms can be compiled for individuals, groups of individuals or the whole colony. For this purpose two recording methods are used depending on whether the ants are or are not individually recognisable. The **sporadic sampling** method involves following of randomly chosen individuals for short (1/2 - 1h) periods of time. The **systematic scanning** method involves following of all the ants at regular intervals or all the time (Jaisson et al. 1988).

Ethograms compiled for individual workers are biased because they include only behavioural acts that can be described in terms of function. This bias is even bigger when an ethogram is compiled for a whole morphological or age group since this is usually done by averaging the results for all group members and ignoring variation. Variation is likely to be treated as noise particularly when the sporadic sampling method has been employed and interindividual and intraindividual variation are impossible to separate.

The aim of this chapter is to introduce and immediately put into practice a new, objective and much simpler spatial method for describing ant colonies. This new method has been developed as a logical consequence of the difficulties the author encountered when using behavioural acts as the basis of description, and was inspired by the results of other studies that also use spatial methods (Franks and Sendova-Franks 1992, Sendova-Franks and Franks 1993). The ultimate purpose of this description is to set the scene for a further study into the mechanisms of ant social organisation.

This chapter deals with the problem of how to describe an ant in terms of her sampled spatial positions by using only one or two parameters, so that comparisons between colonies or between one and the same colony at different periods of time can be made in an objective way.

It introduces the concept of **Spatial Fidelity Zone (SFZ)** - the zone in the nest within which an individual ant moves and to which she is faithful. Using the ant species *L. unifasciatus* it demonstrates the existence of SFZs across colonies at different times of year. The relationship between the SFZ of an ant and the ant's behavioural acts is explored and on the basis of the accepted definition of task as a sequence of behavioural acts that accomplishes a specific purpose for the colony the following three questions are addressed: Is there any division of labour among workers? What constitutes a caste? How many castes are there? Lastly, the chapter discusses possible mechanisms for maintaining a SFZ, the possible adaptive significance of this behaviour, and the implications of the results for the way ant behaviour differentiates.

2.2 Materials and Methods

2.2.1 Colonies and Sessions

Colonies of *L. unifasciatus* were kept and studied as described in sections 1.3.12, 1.4.1, and 1.4.3. This chapter is based on all 33 photographic sessions of observation of colonies BG90UO, BG91UI, BG91UJ, BG91UQ, BG91UL, BG91UM, BG91UN, BG91US and BG92U1 (see Tables 1.1-1.5).

2.2.2 The Problem of Describing an Ant's Spatial Distribution in the Nest in Terms of Only One or Two Parameters at a Time

The aim was to describe an ant spatially in terms of only one or two parameters at a time, so that simple 2-D scatter plots could be used to depict patterns. Moreover, the use of only one or two variables would make it much easier to compare ants across colonies.

One possibility was to use the mean x-coordinate and the mean y-coordinate of all the ant's positions. However, there was a problem with how relevant these measurements would be to the situation and shape of the brood pile within the artificial nest (Fig. 2.1, a and b).

It is very important that any measurement of ant position refers to the position of the brood pile, because the brood pile is where most of the work is situated. Therefore, a good choice was the median distance of an ant from the centre of the brood pile (Fig. 2.1, c). A problem consequent on this is that the position of the centre of the brood pile must be determined. The mean x- and y-coordinates of all the brood should coincide with the biological centre, i.e. the egg pile, if the brood is arranged in concentric circles (Franks and Sendova-Franks 1992). More often than not however, this "ideal" shape is distorted into semicircles or even straight lines due to the geometry of the artificial nest (Fig. 2.1, b). To ameliorate this problem I defined the centre of the brood pile/the centre of the

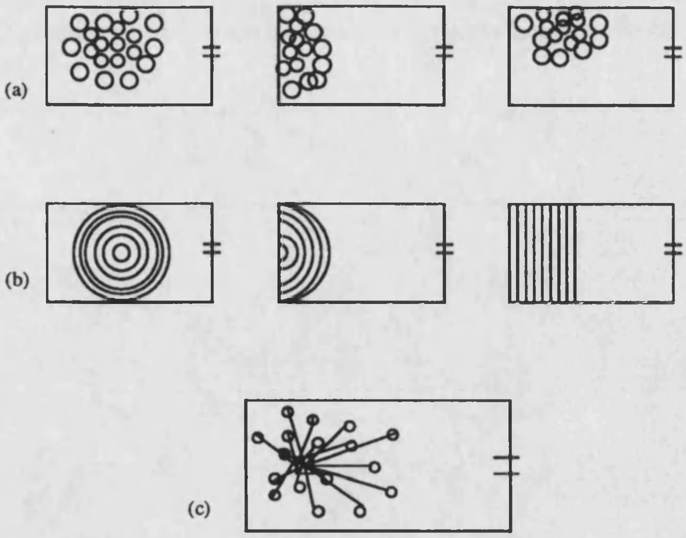


Figure 2.1: Problems of describing an ant's spatial distribution in the nest: (a) different positions of the brood pile; (b) different shapes of the brood pile. (c) Distances of an ant from the centre of the egg pile

colony as the point of the mean coordinates of all the eggs and microlarvae (eggs and microlarvae are difficult to separate). The eggs and microlarvae usually form a small and homogeneous group in the middle of the brood pile and hence their centroid is more representative of the biological centre of the colony.

2.2.3 Illustration of the Finally Chosen Measurements

On Figure 2.2 the finally chosen measurements are illustrated with a box plot. Box plots are often used to explore distributions of unknown type.

In this particular example the box plot represents the distribution of distances from the centre of the colony for one ant during one session. The median is the value in the distribution above and below which lie half of the data. The upper and lower quartiles delineate the first and the third quarters of the data respectively. The whiskers extend to the upper and lower extreme values. The

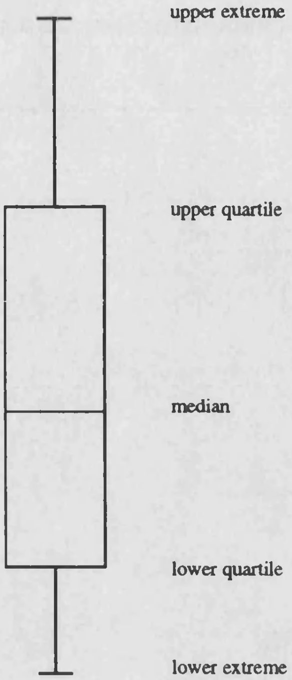


Figure 2.2: Box plot

median is a good measure for location, while the distance between the upper and the lower quartiles is a good measure for spread. My final choice was to use the median of an ant's spatial distribution as a measure for her position, the upper and lower quartiles - as measures for the boundaries of an ant‘s zone of movement, and the spread - as a measure for the size of this zone. The spread is a controversial choice of measure for the size of an ant's zone of movement, since it contains only the middle half of the distribution, i.e. the spread is a very robust measure, because it excludes the quartiles in which most of the variation occurs. Nevertheless, as I will demonstrate, this measure suffices the objectives of this study.

2.2.4 Randomisation Test

Ho is: The observed arrangement of ant spatial distributions and the observed median of their spreads can be arrived at when every individual ant is able to occupy with equal likelihood the position of any ant in the nest for a particular photograph (frame) and when this rule is true for all the photographs of the session, i.e. ants do not have SFZs. **Ha** is: The observed arrangement of ant spatial distributions and the observed median of their spreads can not be arrived at when every individual ant is able to occupy with equal likelihood the position of any ant in the nest for a particular photograph (frame) and when this rule is true for all the photographs of the session, i.e. ants do have SFZs. **Implementation:** Permutate the positions of the ants' ID numbers (except the queen's) according to a randomly chosen permutation in each raw data file of the primary type (see section 1.4.3.1) for a particular session.

raw data file -permutation-> permutated file

Ant ID X Y ————> Ant ID X Y

6 218 341 ————> 4 218 341

8 29 105 ————> 2 29 105

2 415 198 ————> 6 415 198

4 357 112 ————> 8 357 112

After that follow the usual steps of creating files (of the secondary raw data type) for each individual, calculating the spread of every ant's distribution of distances to the colony centre, and calculating the median of the spreads of all ants. Repeat the above procedure 100 times.

The test was carried out by using programmes written in C and UNIX shell (csh) script on a SUN workstation (see section 1.4.5).

2.2.5 Spearman's Rank Correlation Coefficient

(see section 1.4.4.2)

2.2.6 Runs Up and Down Test

(see section 1.4.4.3)

2.3 Results

2.3.1 Spatial Fidelity Zones

In order to investigate the relative positions of the spatial distributions of all ants in the nest, what are measures for the boundaries of an ant's zone of movement - the lower and the upper quartiles, are plotted against one another. The result is a configuration that approximates a straight line (Figures 2.3 - 2.9).

This means that the greater is the value of the lower quartile (boundary), the greater the value of the upper quartile (boundary) and that this relationship is approximately the same for all ants. The only exception is made by ants that have the lower boundaries of their zones above 400 - 500 pixels. Due to the limitation of the inflexible walls of the artificial nest, the upper boundaries of the zones of such ants do not have anywhere to expand (see sessions BG91UI1-2, BG91UJ1-2, BG91UQ1-2, BG90UO5, BG92U12, BG91US1-2, BG91UL1, BG91UM1-3). The threshold value for the lower boundary depends on the spread of the spatial

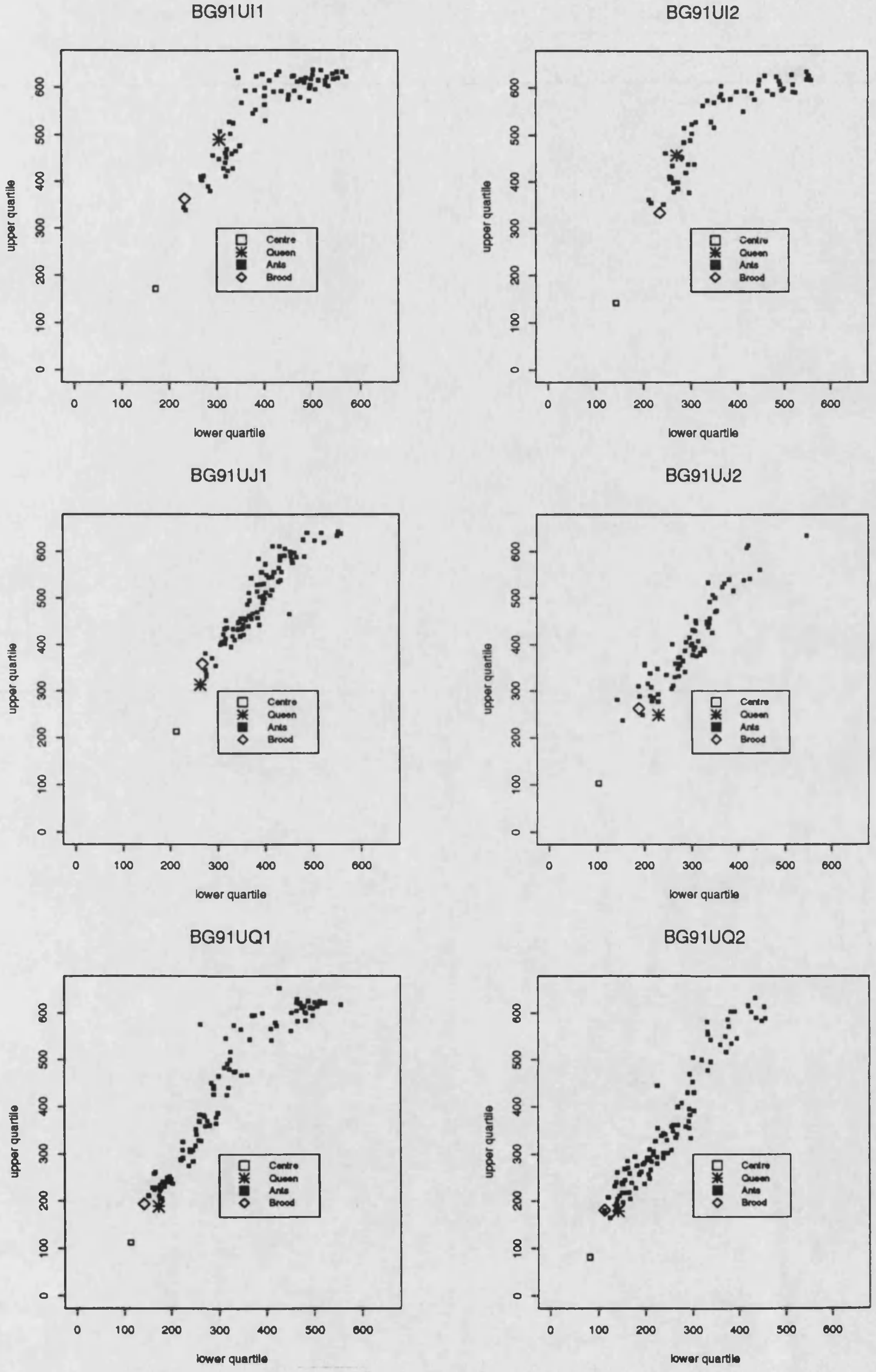


Figure 2.3: Distances of ants from the centre of the colony. Sessions BG91UI1-2, BG91UJ1-2, BG91UQ1-2

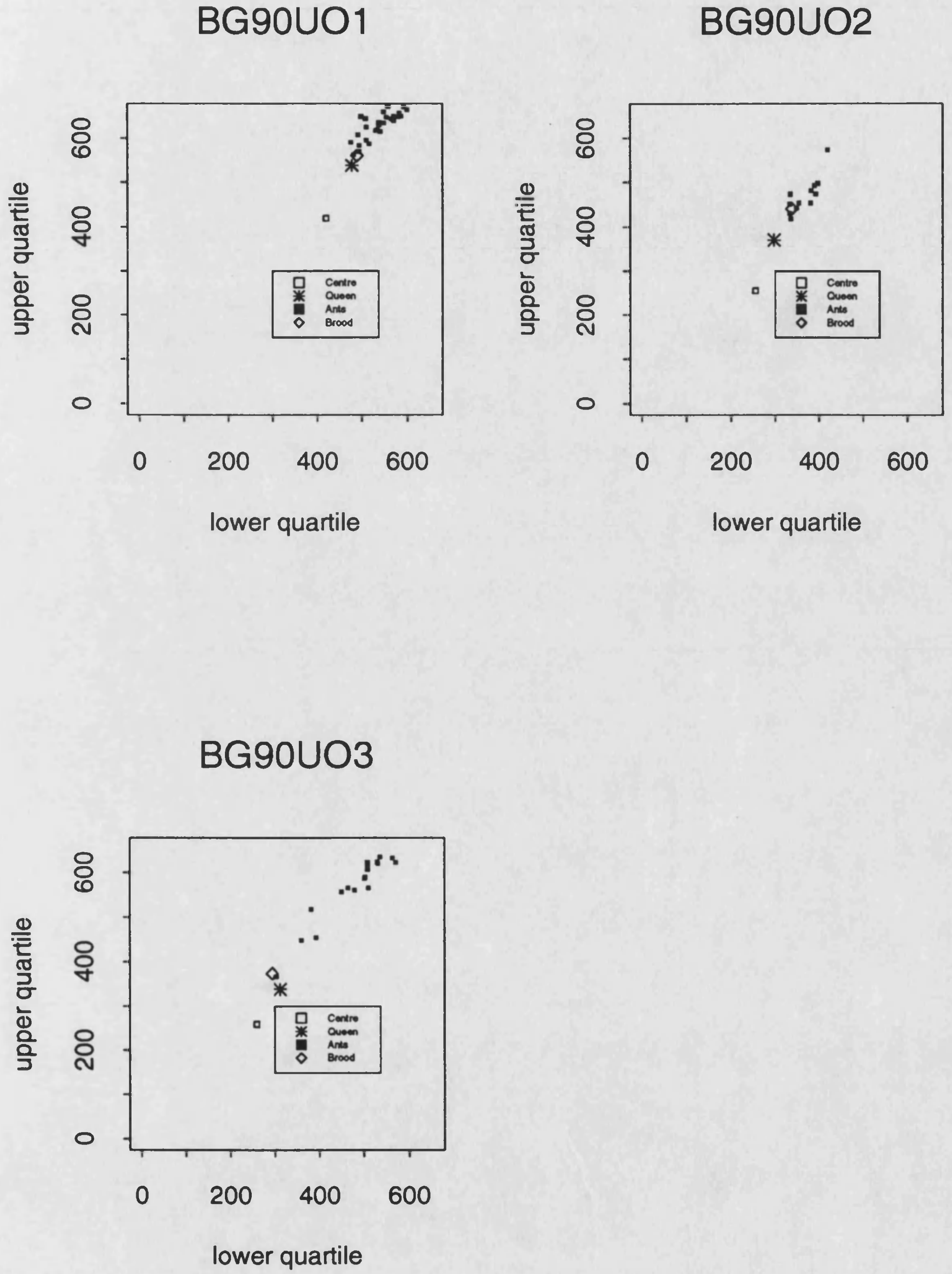


Figure 2.4: Distances of ants from the centre of the colony. Sessions BG90UO1-3

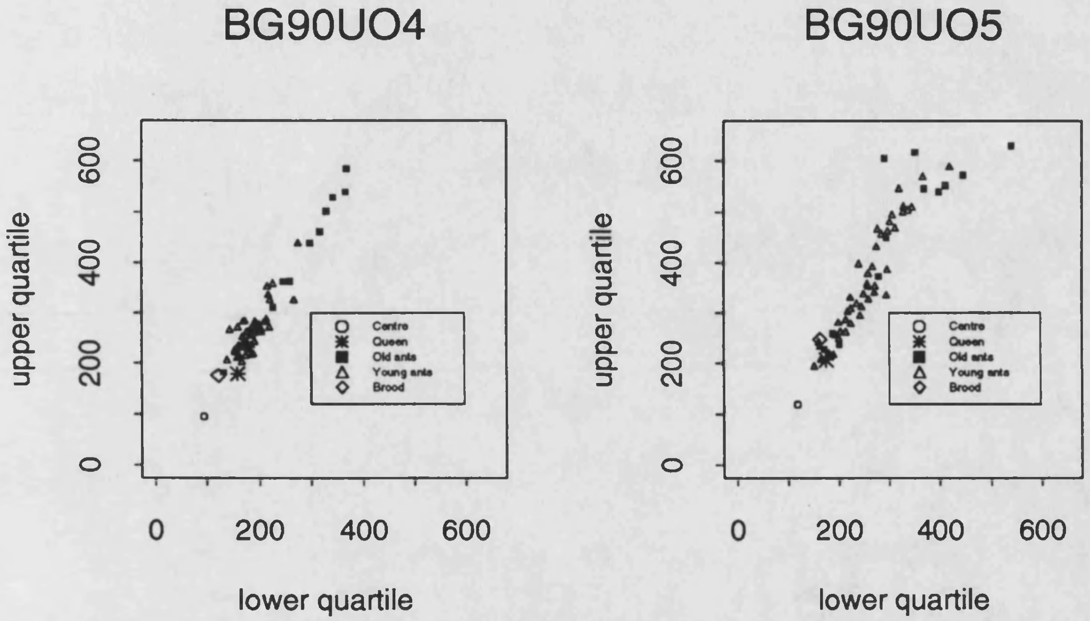


Figure 2.5: Distances of ants from the centre of the colony. Sessions BG90UO4-5

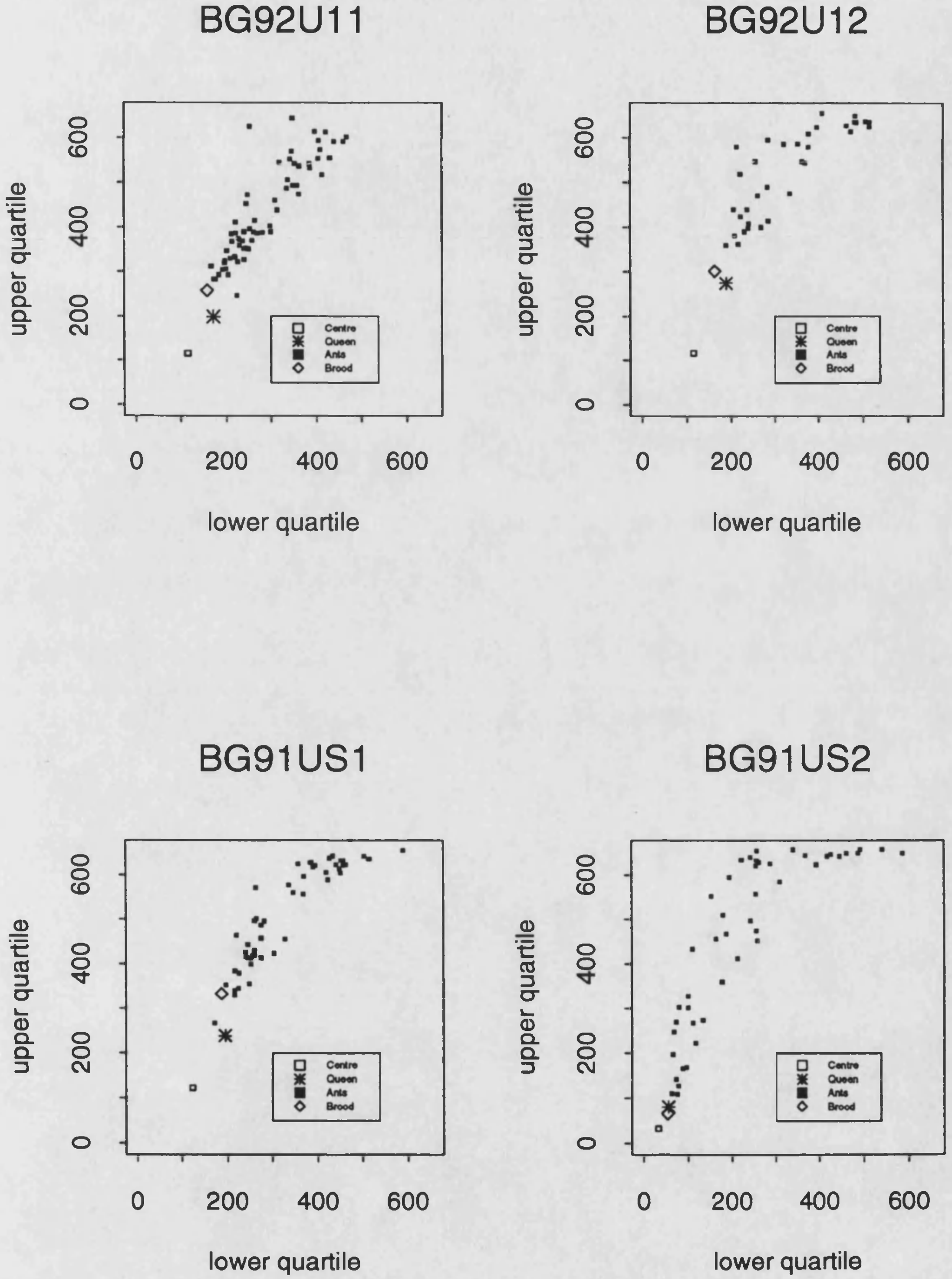


Figure 2.6: Distances of ants from the centre of the colony. Sessions BG92U11-2, BG91US1-2

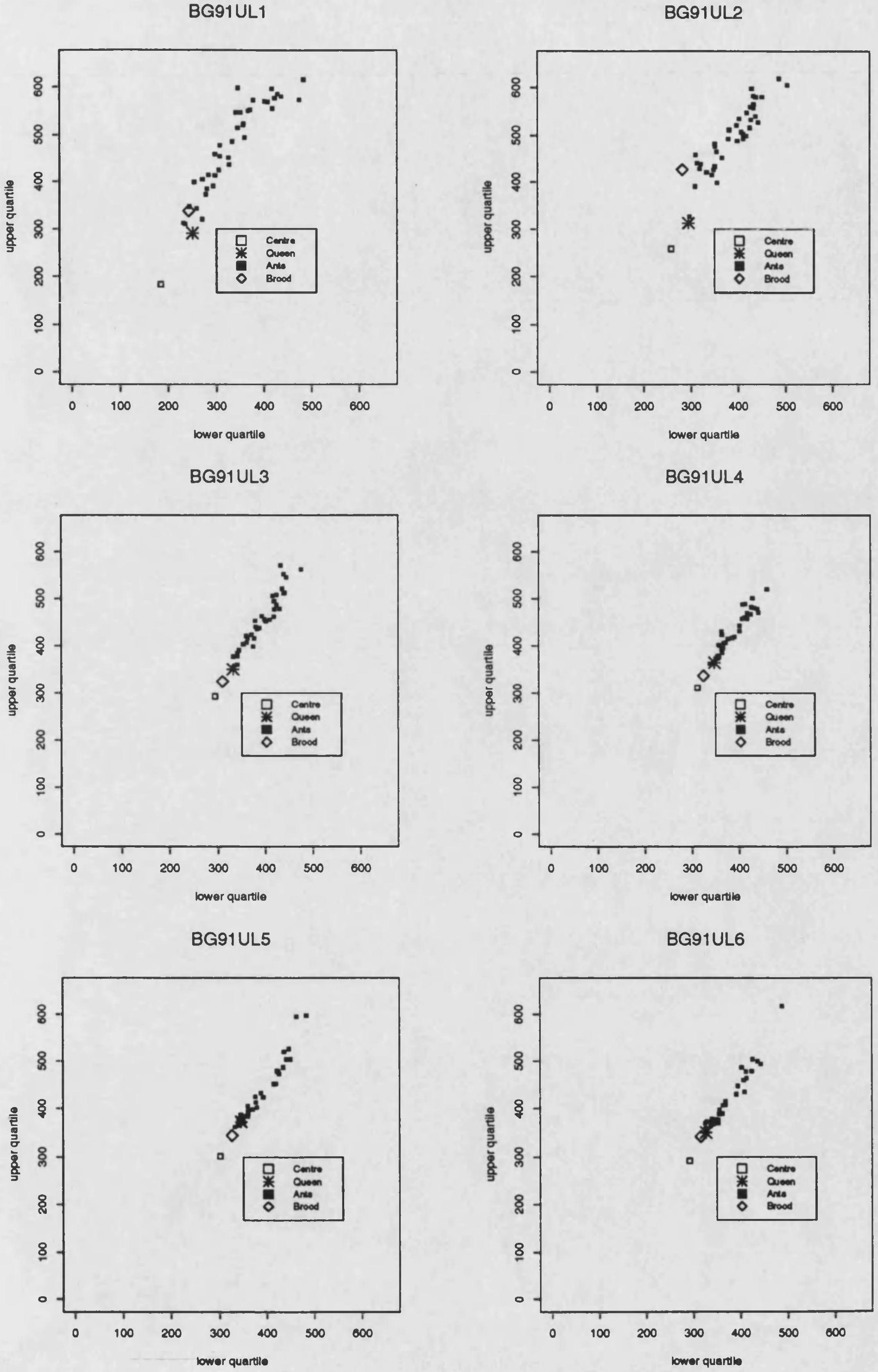


Figure 2.7: Distances of ants from the centre of the colony. Sessions BG91UL1-6

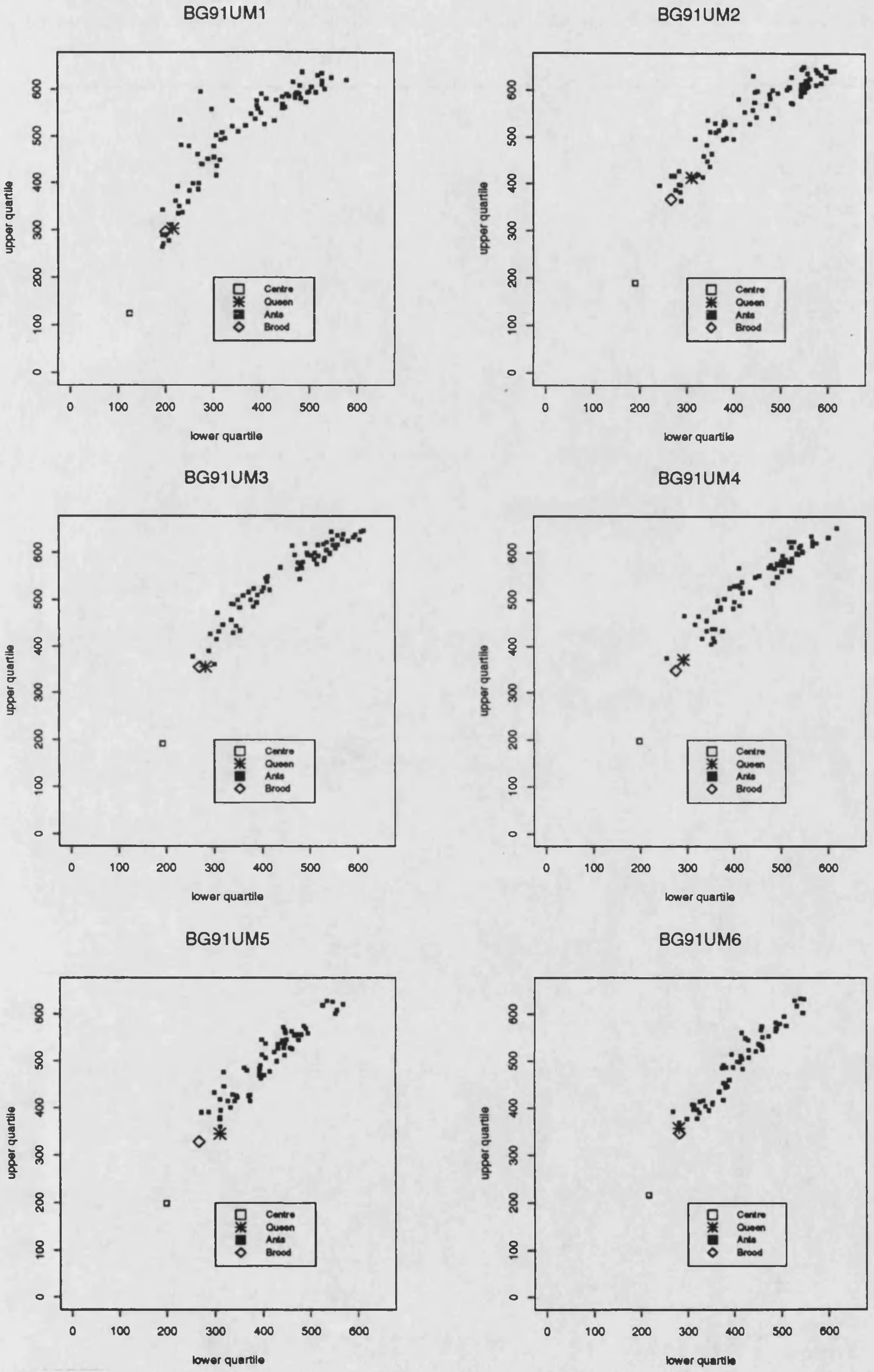


Figure 2.8: Distances of ants from the centre of the colony. Sessions BG91UM1-6

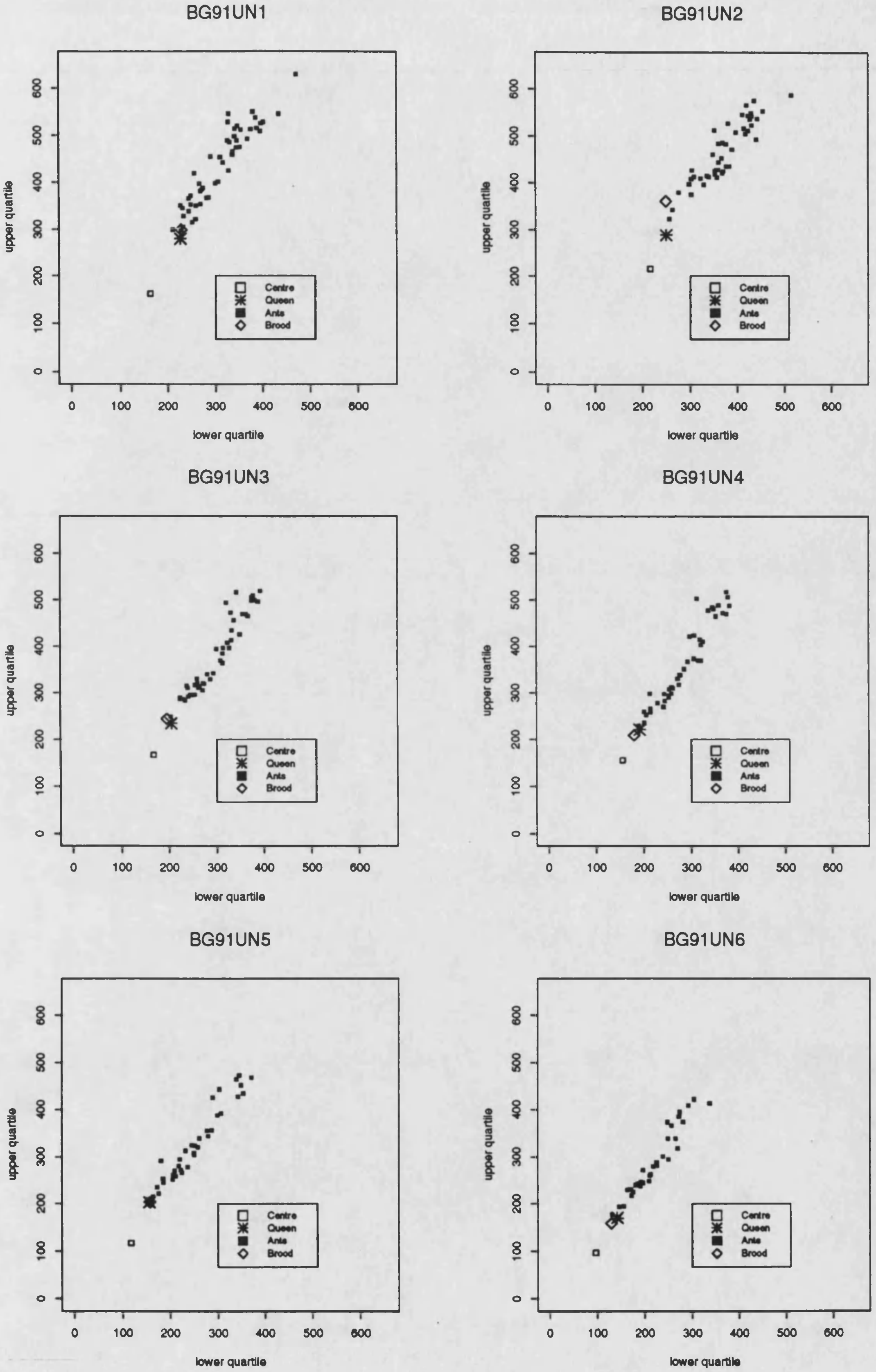


Figure 2.9: Distances of ants from the centre of the colony. Sessions BG91UN1-6

distributions of ants. The greater is the spread, the steeper the slope of the line and the smaller the threshold value for the lower boundary for which the upper boundary does not increase any more (see sessions BG91UM1-6).

The finding that the spatial distributions of the ants in a colony are arranged in a sequence suggests that individuals do not move equally throughout the nest but have restricted Spatial Fidelity Zones (SFZs). This is well illustrated by a comparison between a lower quartile - upper quartile plot for the real data and the same plot after a single randomisation (Fig. 2.10, a and b).

After a single randomisation the line structure disappears and the intervals for both the upper and the lower quartiles shrink to less than half of their previous size and are in the middle of the original intervals. This means that if all individuals were to be found anywhere in the nest with equal likelihood, their spatial distributions would have been different from those actually observed. In the random model, the spatial zones of all ants would have been clustered in the middle of the possible spectrum of distances from the centre of the egg pile. At the same time the median size of the distribution spreads would have been much greater, something that is very convincingly demonstrated with a randomisation test (Fig. 2.10, c and Table 2.1).

Here the chosen statistic is the median spread of the spatial distributions of all workers in the nest. It is evident that the observed value of the median spread is far away from the simulated sampled distribution - approximately two times as far as is the width of the distribution. None of the values in the distribution is equal to or smaller than the observed value. For a sample of 100 the 95% confidence limits for this percentage are 0.00% - 3.62% and the 99% confidence limits are 0.00% - 5.16% (Rohlf and Sokal 1981, p. 208). This means that the

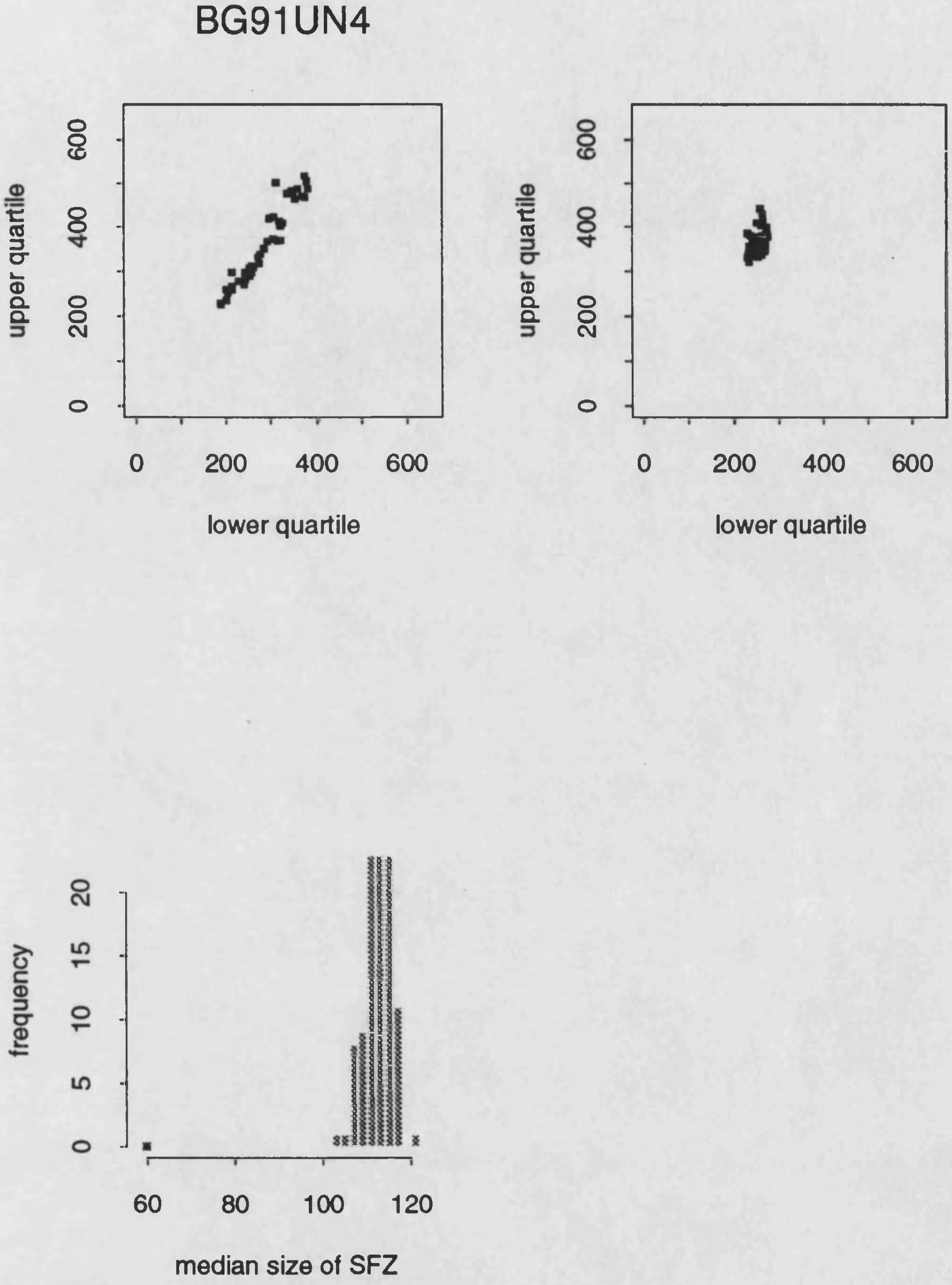


Figure 2.10: Randomisation test for demonstrating the existence of SFZs, session BG91UN4: (a) real data; (b) after a single randomisation; (c) result from the randomisation test, where * stands for the real value

Session	Median of distribution spreads for all ants	Minumum of the 100 simulated values for the same
BG90UO1	83.60	98.17
BG90UO2	102.26	145.78
BG90UO3	88.30	152.87
BG90UO4	84.27	108.06
BG90UO5	90.80	151.89
BG91UI1	132.25	217.83
BG91UI2	149.44	233.83
BG91UJ1	103.88	132.17
BG91UJ2	101.14	127.17
BG91UQ1	101.55	200.64
BG91UQ2	90.47	147.70
BG91UL1	145.35	178.97
BG91UL2	111.02	125.98
BG91UL3	53.18	81.02
BG91UL4	40.33	66.95
BG91UL5	37.11	63.96
BG91UL6	39.72	57.92
BG91UM1	129.12	235.29
BG91UM2	97.68	189.90
BG91UM3	97.72	165.03
BG91UM4	82.42	144.11
BG91UM5	88.26	128.42
BG91UM6	84.83	125.54
BG91UN1	123.82	152.50
BG91UN2	90.28	111.45
BG91UN3	70.85	99.67
BG91UN4	59.84	105.02
BG91UN5	69.41	108.36
BG91UN6	57.91	83.88
BG91US1	170.00	227.81
BG91US2	220.30	364.12
BG92U11	142.70	164.00
BG92U12	173.21	242.47

Table 2.1: Results from randomisation tests for the existence of ant SFZs

probability of obtaining this value for the median size of the spatial distribution on the basis of individuals moving randomly in the nest is less than 0.05, leading to the rejection of the null hypothesis and acceptance of the hypothesis that individuals do have SFZs.

Moreover, the SFZs are not clustered, but rather are arranged in a continuum. Only individual foragers appear to be "separated" from their nestmates and mainly in colonies that are too small for their nests (see sessions BG91UJ2 and BG90UO5, for example). This is very possibly an artifact. When colonies are given the opportunity to change the position of the nest boundaries (when the walls are made out of grit) such separation is unlikely to occur, at least not in such an extreme form (Franks et al. 1992).

NOTE: For an illustration of the spatial distributions of ants within the nest, please see the paper by Sendova-Franks and Franks 1993, a copy of which is bound with this thesis. The concept of SFZ in 1-D presented above is a deliberate abstraction which simplifies and facilitates analysis. It is beyond the purpose of this study to analyse the shapes of the SFZs in 2-D. My impression, however, is that they depend both on the shape of the nest and the shape of the brood distribution (see Fig. 2.1, b).

The following concepts, defined in this chapter, will be used repeatedly throughout this work: **Ant Spatial Position (Location)** - the median of the distribution of distances from the ant to the centre of the colony; **SFZ (Spatial Fidelity Zone)** - the interquartile range (spread) of the same distribution; **Colony Centre** - the centroid of eggs and microlarvae

2.3.2 Variation in the Size of the SFZ within a

Session

Figures 2.11 - 2.16 depict the variation in the size of SFZ within a colony for a particular session. This variation is structured, so that ants with positions further away from the centre have a greater size of SFZ. However, again there exists a threshold position, at 400 - 500 pixels, such that ants with a position further than this have a proportionately smaller size of SFZ. The result is a configuration approximating a curve, that is more or less pronounced depending on the greater or smaller median size of the SFZ for the session (see sessions BG91UI1-2, BG91UQ1-2, BG90UO5, BG91UL1, BG91UM1, for example). The curve-shaped relationship between position and size of SFZ is very probably an artifact, caused by the limitation of the inflexible walls of the artificial nest (restricted geometry).

If we accept that an artifact accounts for the curve, we can assume that the relationship between position and size of SFZ is in fact linear, which implies that the further away an ant is from the centre of the colony, the greater the size of her SFZ, i.e. the greater her mobility.

2.3.3 Variation in the Median Size of the SFZ Across Sessions

Figure 2.17, a examines the change in the median size of the SFZ over the active months of the year. There is a definite peak in May (even when the session with the maximal value, BG91US2, is excluded form the analysis) - the month when the greatest number of non-egg brood is present in the nest and when the size of the brood domains of care (Franks and Sendova-Franks 1992) is greatest. This finding could mean both, that the number and the state of the brood has an influence on the size of the worker SFZ, and that in May the workers themselves

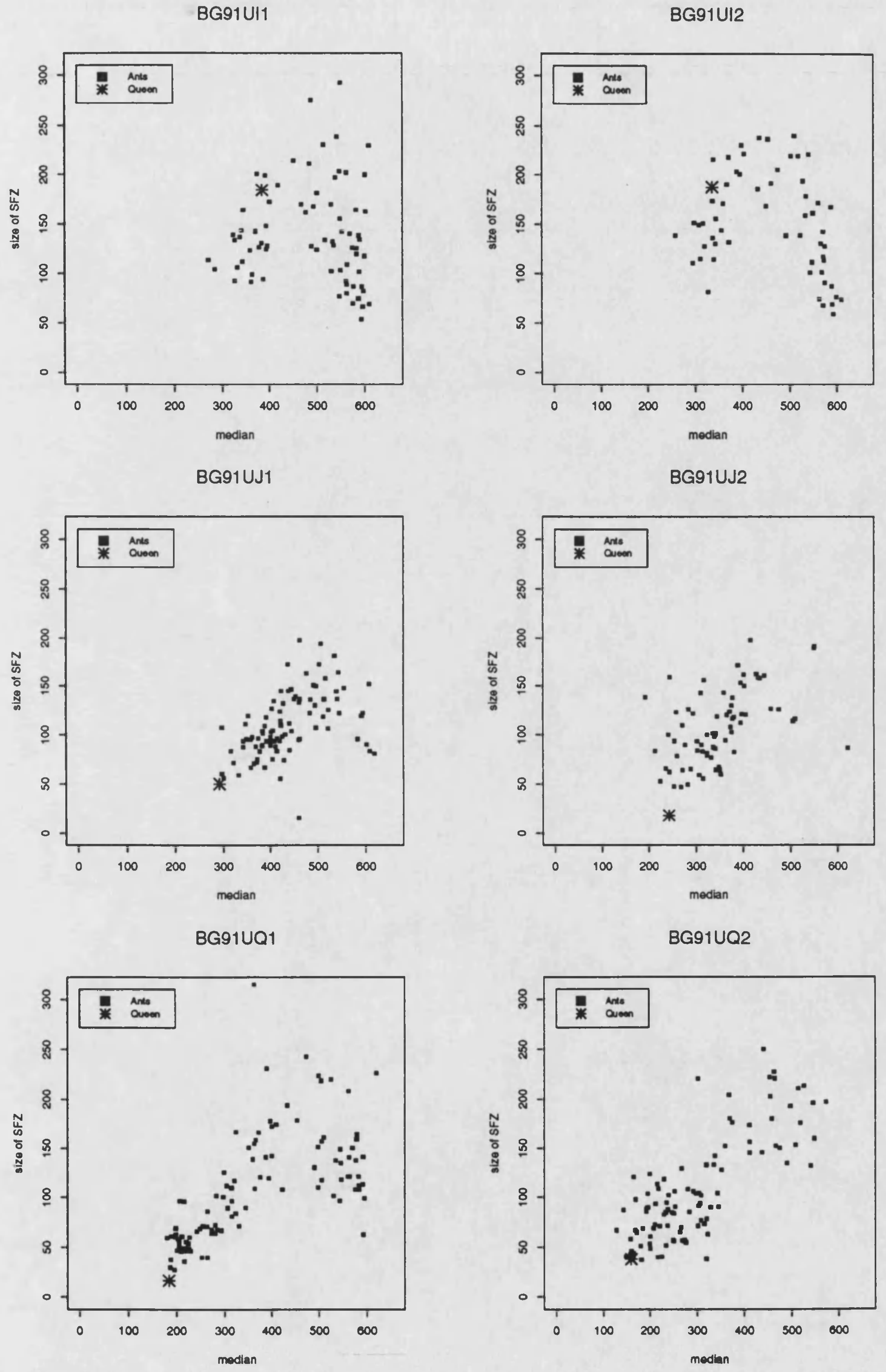


Figure 2.11: Relationship between the position of an ant and the size of her SFZ. Sessions UI1-2, UJ1-2, UQ1-2

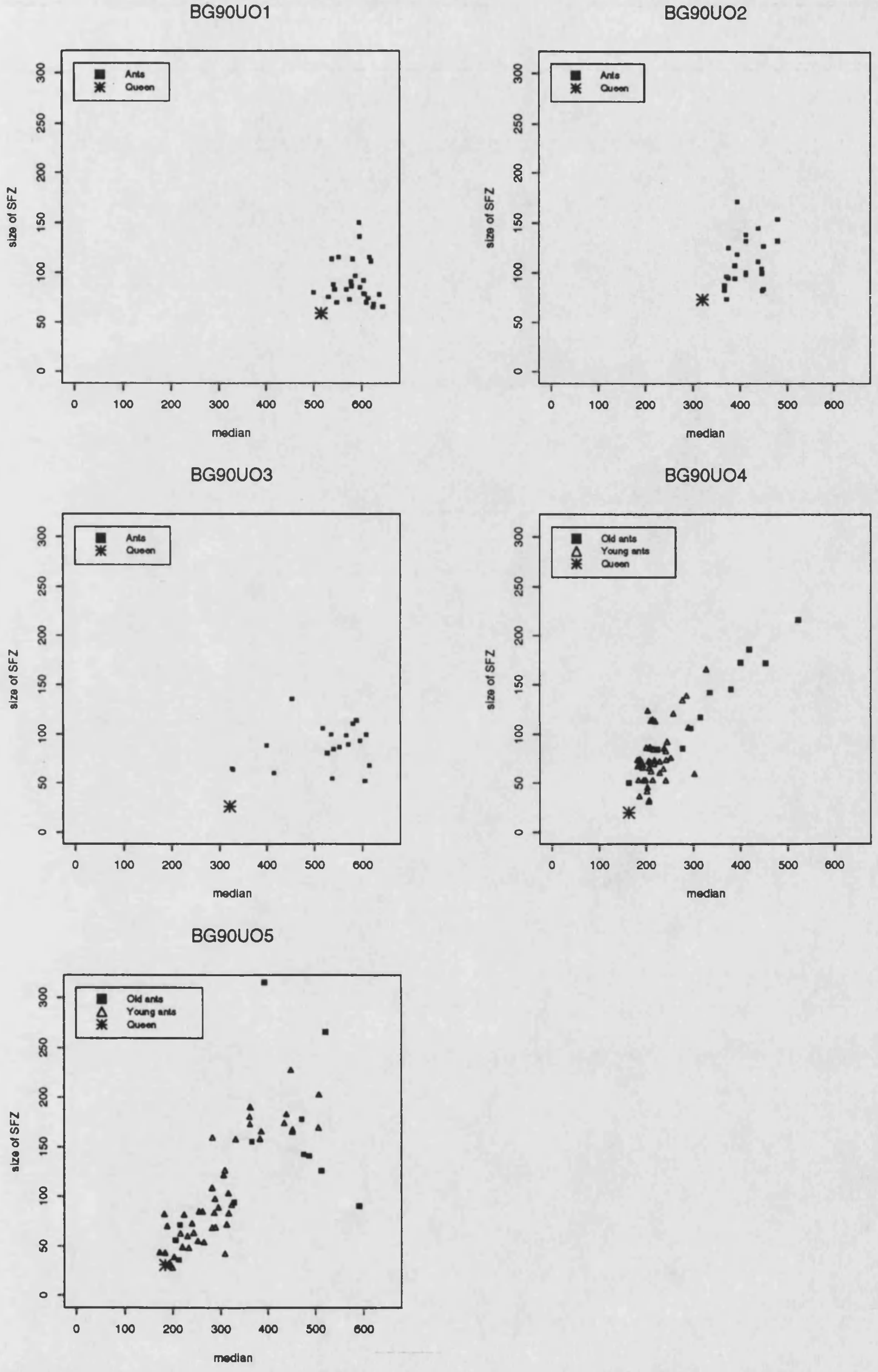


Figure 2.12: Relationship between the position of an ant and the size of her SFZ. Sessions BG90UO1-5

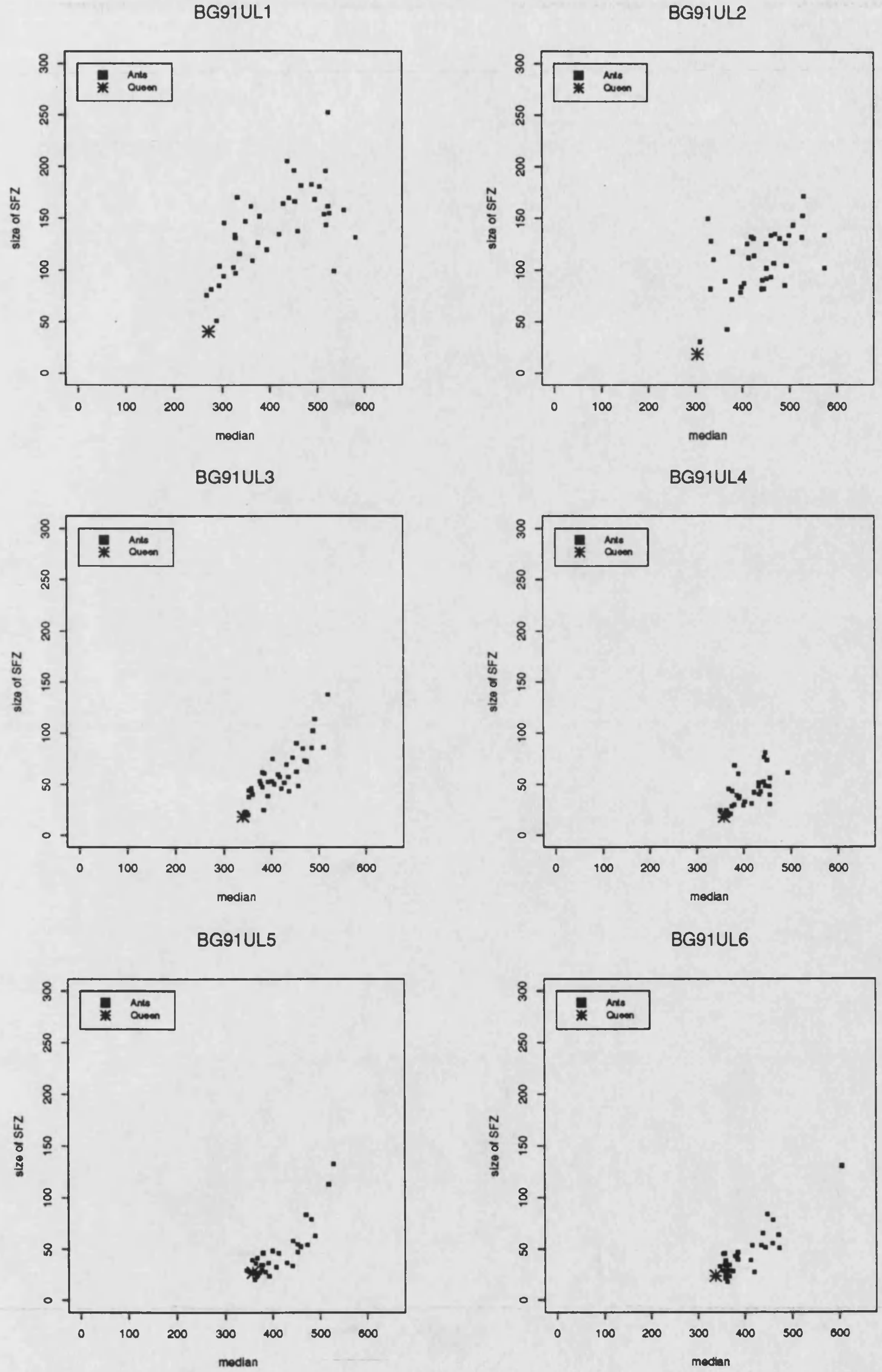


Figure 2.13: Relationship between the position of an ant and the size of her SFZ. Sessions BG91UL1-6

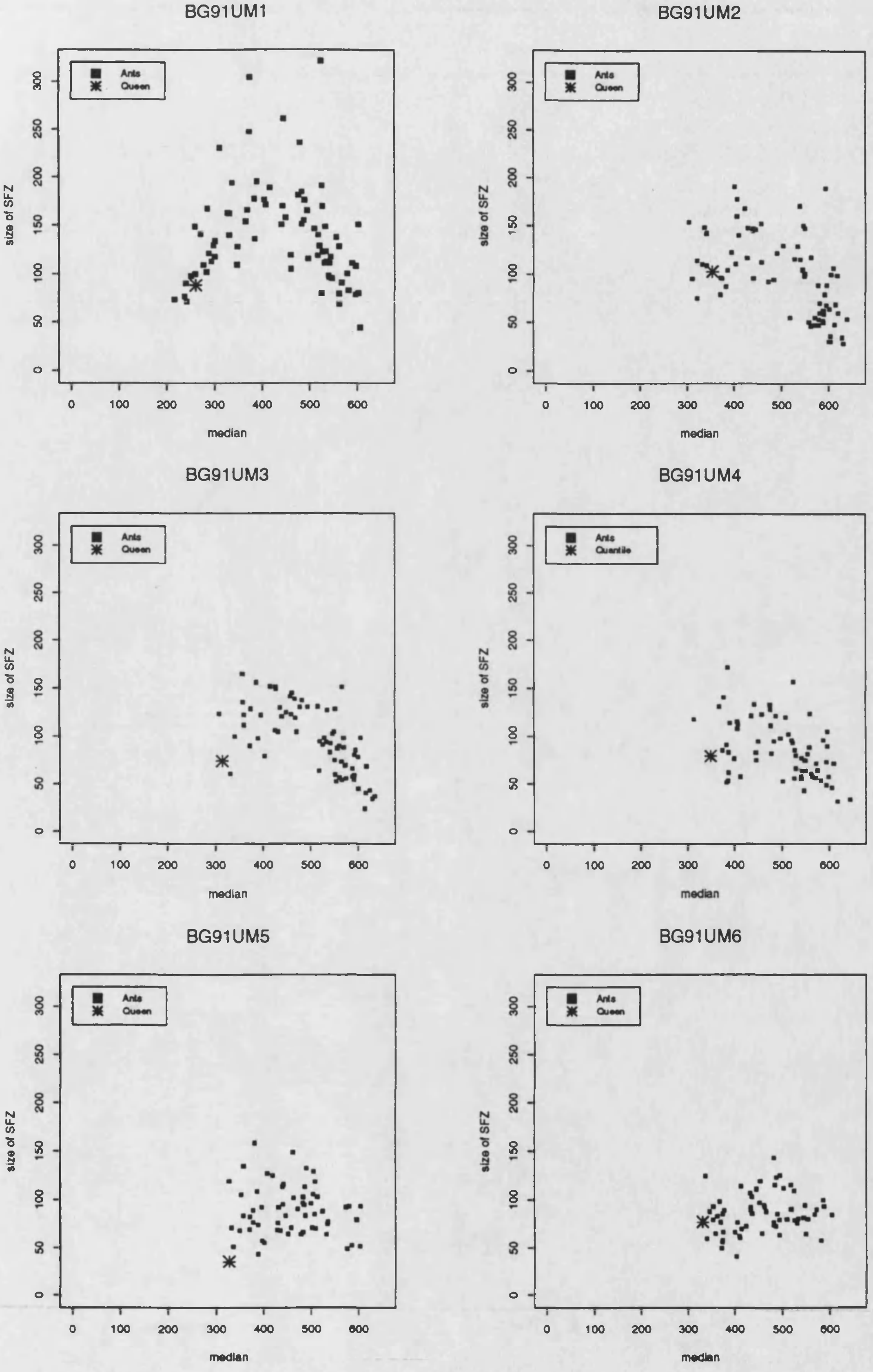


Figure 2.14: Relationship between the position of an ant and the size of her SFZ. Sessions BG91UM1-6

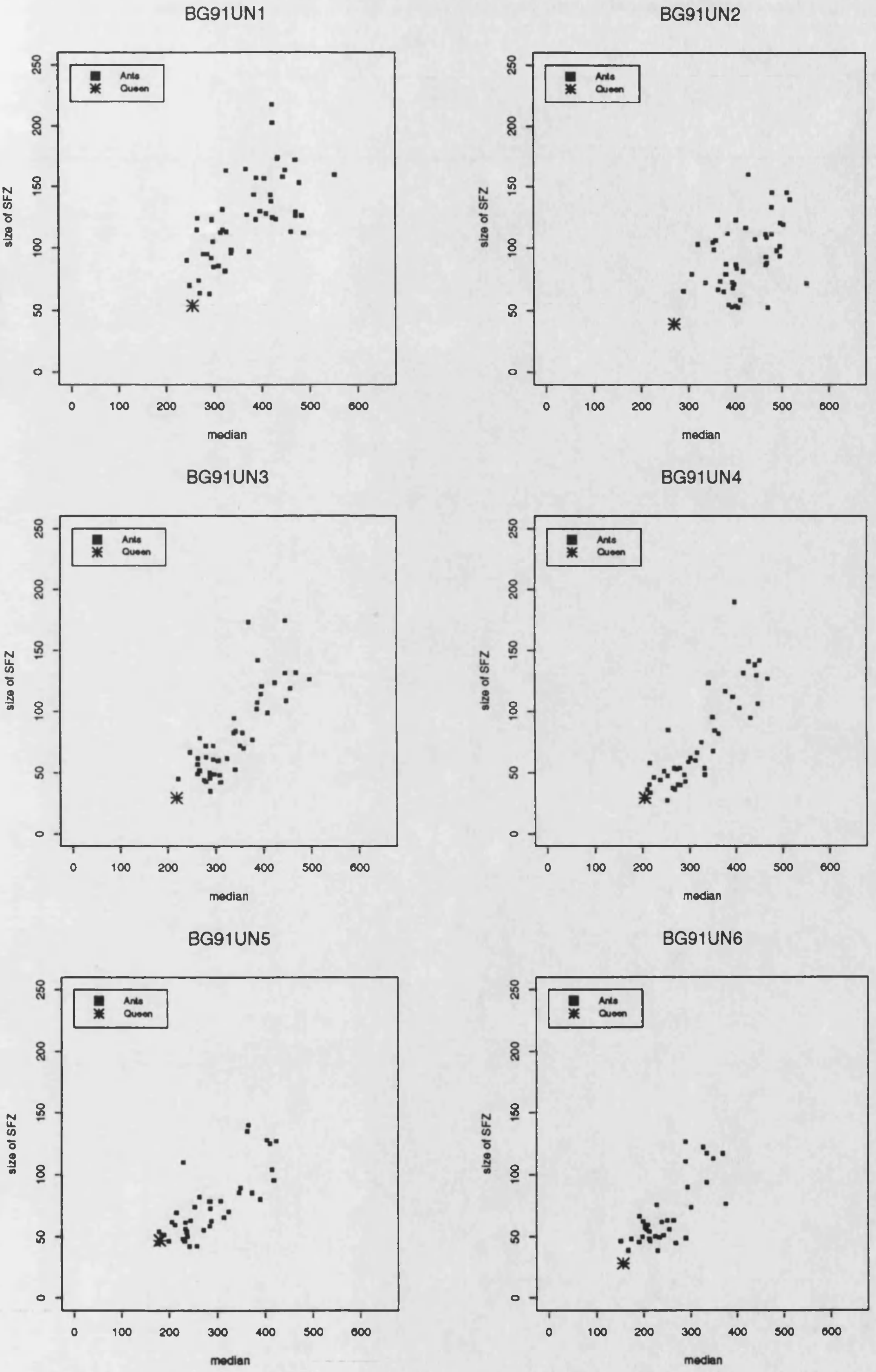


Figure 2.15: Relationship between the position of an ant and the size of her SFZ. Sessions BG91UN1-6

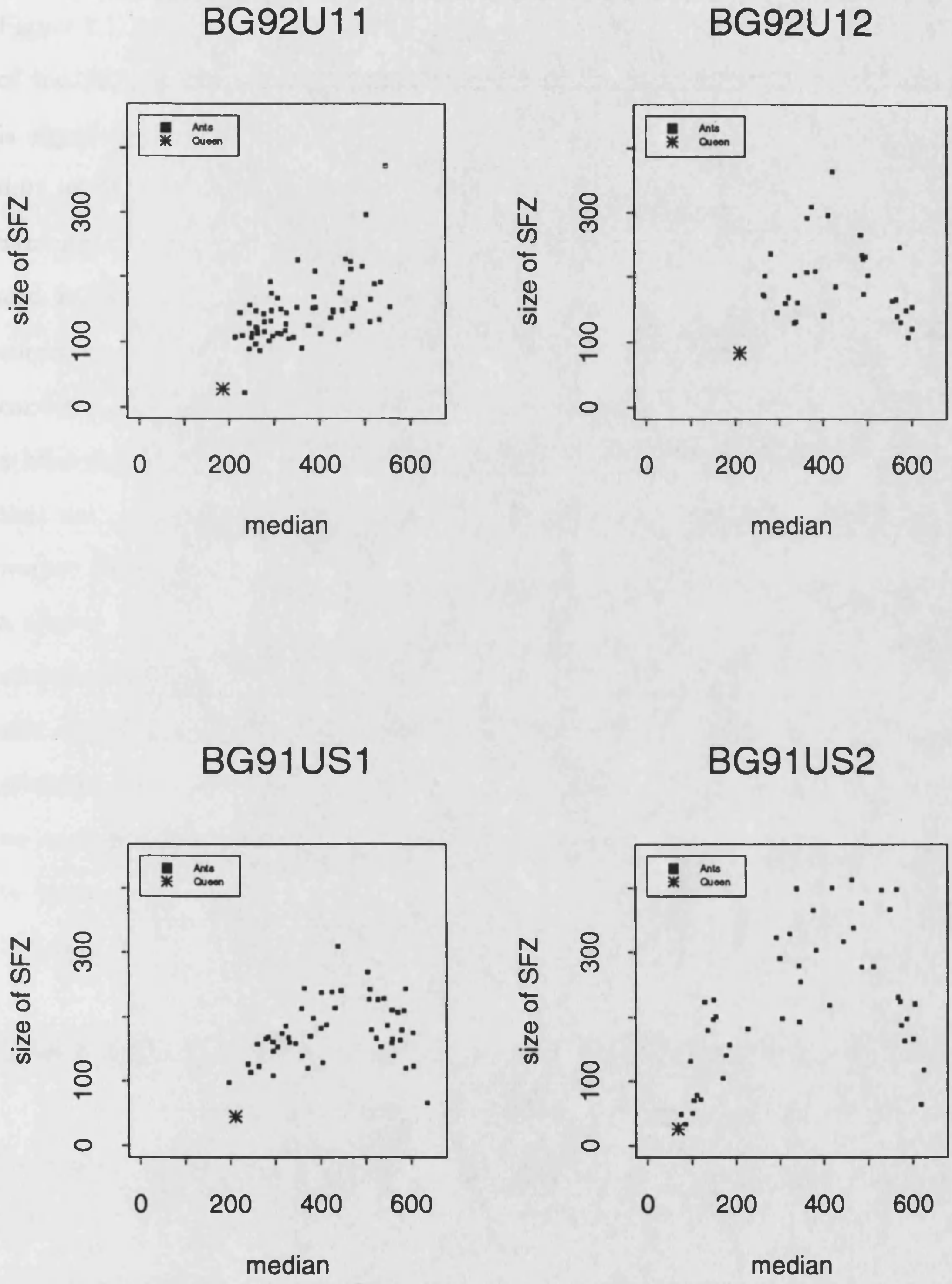


Figure 2.16: Relationship between the position of an ant and the size of her SFZ. Sessions BG92U11-2, BG91US1-2

are independently very active.

Figure 2.17, b examines the relationship between the variation in the median size of the SFZ and the brood to ant ratio during all 33 sessions. The relationship is significantly positive (Spearman's rank correlation coefficient = 0.58, $P < 0.01$ at 31 d.f.). This means that the more brood items the ants care for on average, the bigger the median size of the SFZ. However, for sessions BG90UO1 and BG90UO2 the values of brood to ant ratios are relatively great, but the corresponding median sizes of SFZ are relatively small. These two sessions were carried out in March and April respectively, when the brood was still partially in a hibernation state and presumably had a slow metabolic rate - a confirmation that not only the number but the state of the brood influences the size of the worker SFZ. Another exception is the point that represents session BG91US2, a session after the removal of all brood in the colony except for the eggs and microlarvae. The brood to ant ratio for this session is one of the lowest, but the size of SFZ is the highest. In other words, the brood is not the sole factor that governs the size of SFZ. It is quite possible that the ants themselves go through an annual change of metabolic rate (and consequently activity) that is similar to those of the brood, an idea supported by the significant positive correlation between the median size of SFZ and its variance (Fig. 2.17, c).

2.3.4 How Ants Move within their SFZs

In order to investigate whether ants move randomly or with some trend or regularity within their SFZ, a runs up and down test (Sokal and Rohlf 1969) was carried out for every ant in each of the type one sessions (including the half ones). The results from this test are plotted against the position of the ants on Figures 2.18 - 2.20. Lines are drawn across at the significant test values, -1.96 (values

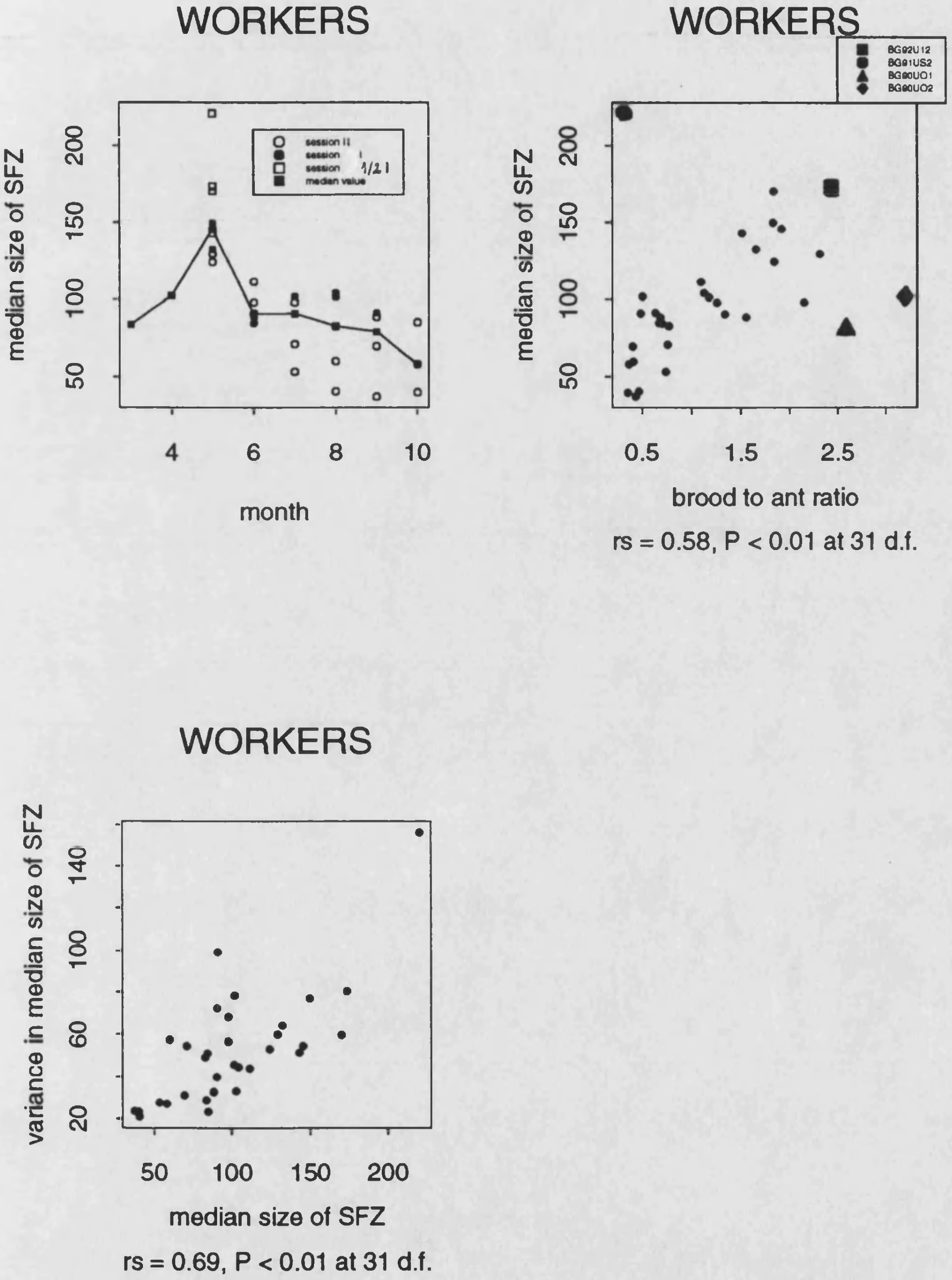


Figure 2.17: Relationship between colony median size of SFZ for a session and: (a) month of the year; (b) brood to ant ratio; (c) its variance

below this mean that there is a trend or trends in the pattern) and 1.96 (values above this mean that there is a regularity (cyclicity) in the pattern of movement).

Since, as suggested by a previous section, an ant's activity increases linearly with the increase in the distance between her position and the centre, the significant positive correlation between the position of an ant in the colony and the value of the test statistic for nearly half of the sessions (7 out of 15) appears to suggest that ants move nearer to and further from the centre in a cyclical fashion and that the values of the statistic are due to the relation between their activity and the sampling frequency. Note that half of the 8 sessions for which the correlation is not significant are in fact half sessions of type one (see section 1.4.1.3) and perhaps they were not long enough to allow a correlation to be established.

2.3.5 Relevance of the SFZ to What Ants Do

Figures 2.21 - 2.26 show the relationship between the location of an ant and the number of times she was recorded to do brood care.

These measures appear to be connected in a negative correlation - the further away an ant is from the centre of the colony, the less brood care she does. The slope of the correlation is steeper when the brood pile is smaller relative to the colony size (see BG91UQ1-2, for example). In addition, the further away the brood pile is from the exit, the greater the number of individuals that do not do any brood work (see BG90UO4, for example).

Ants spread the workload evenly over the work-surface by sorting the brood according to its domain of care. The higher the metabolic rate of a brood item, the greater the size of its domain of care (Franks and Sendova-Franks 1992).

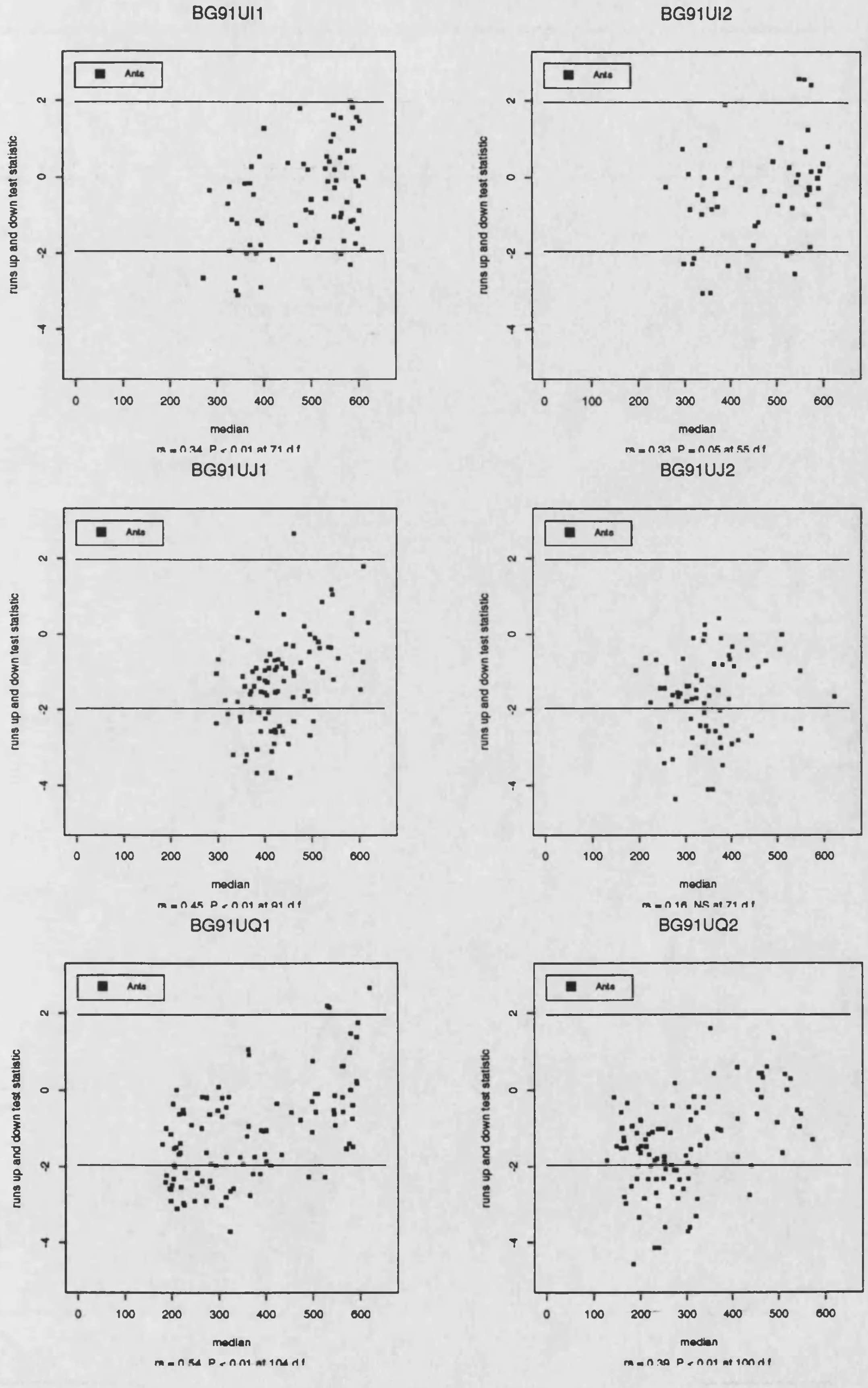


Figure 2.18: Relationship between ant position and her runs up and down test statistic. Sessions UI1-2, UJ1-2, UQ1-2.

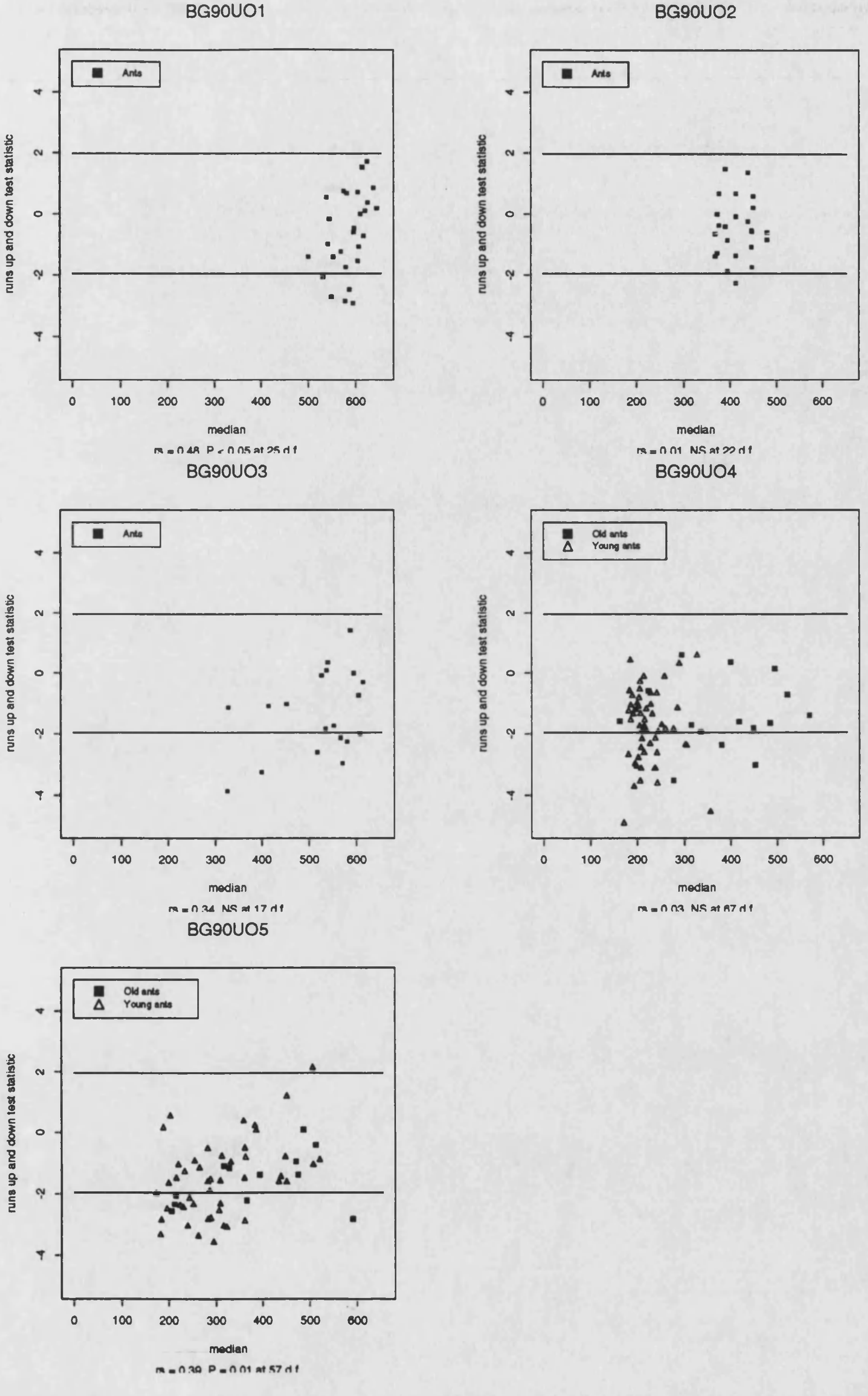


Figure 2.19: Relationship between ant position and her runs up and down test statistic. Sessions UO1-5

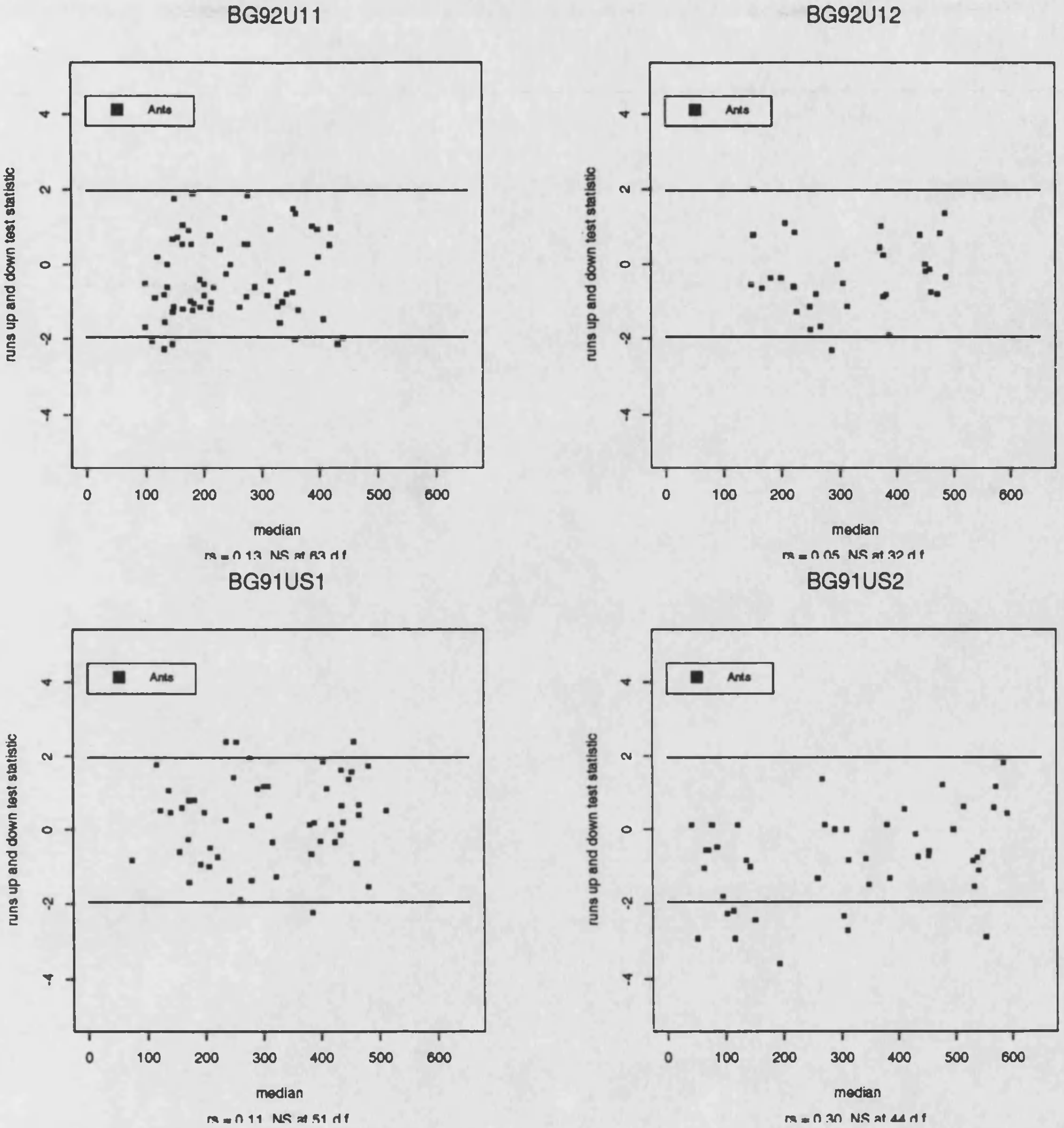


Figure 2.20: Relationship between ant position and her runs up and down test statistic. Sessions U11-2, US1-2

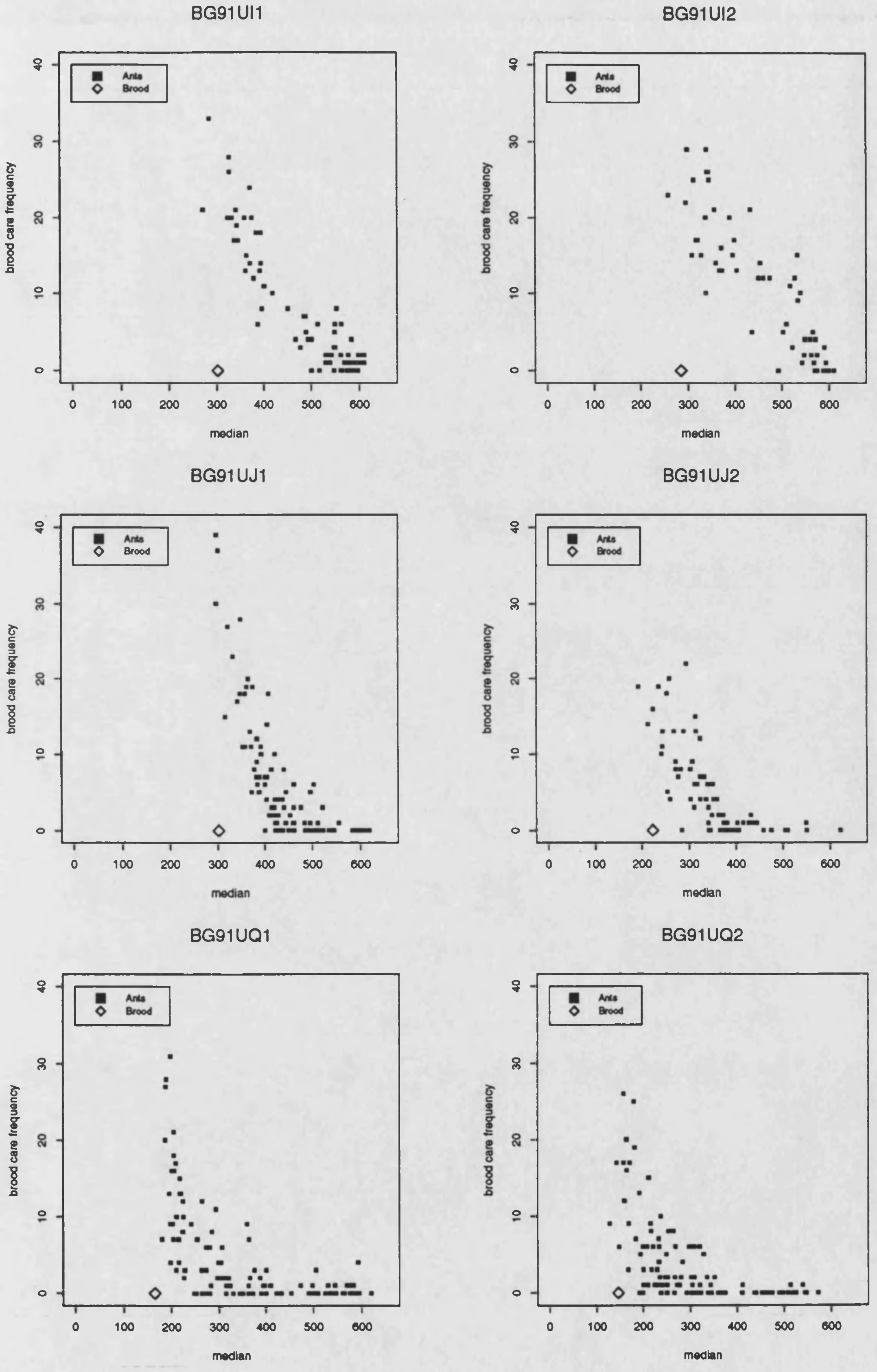


Figure 2.21: Relationship between ant position and brood care frequency. Sessions UI1-2, UJ1-2, UQ1-2

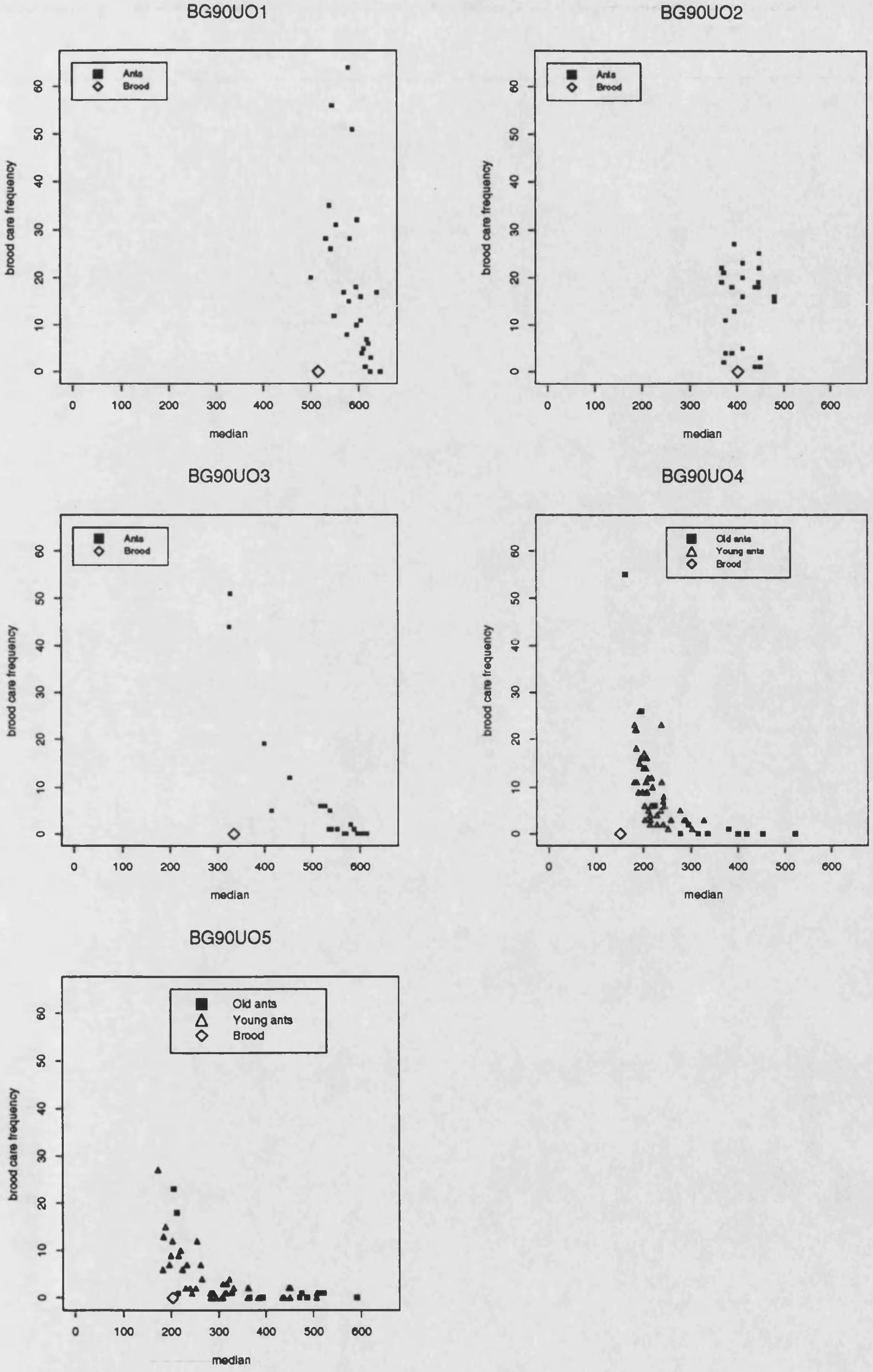


Figure 2.22: Relationship between ant position and brood care frequency. Sessions BG90UO1-5

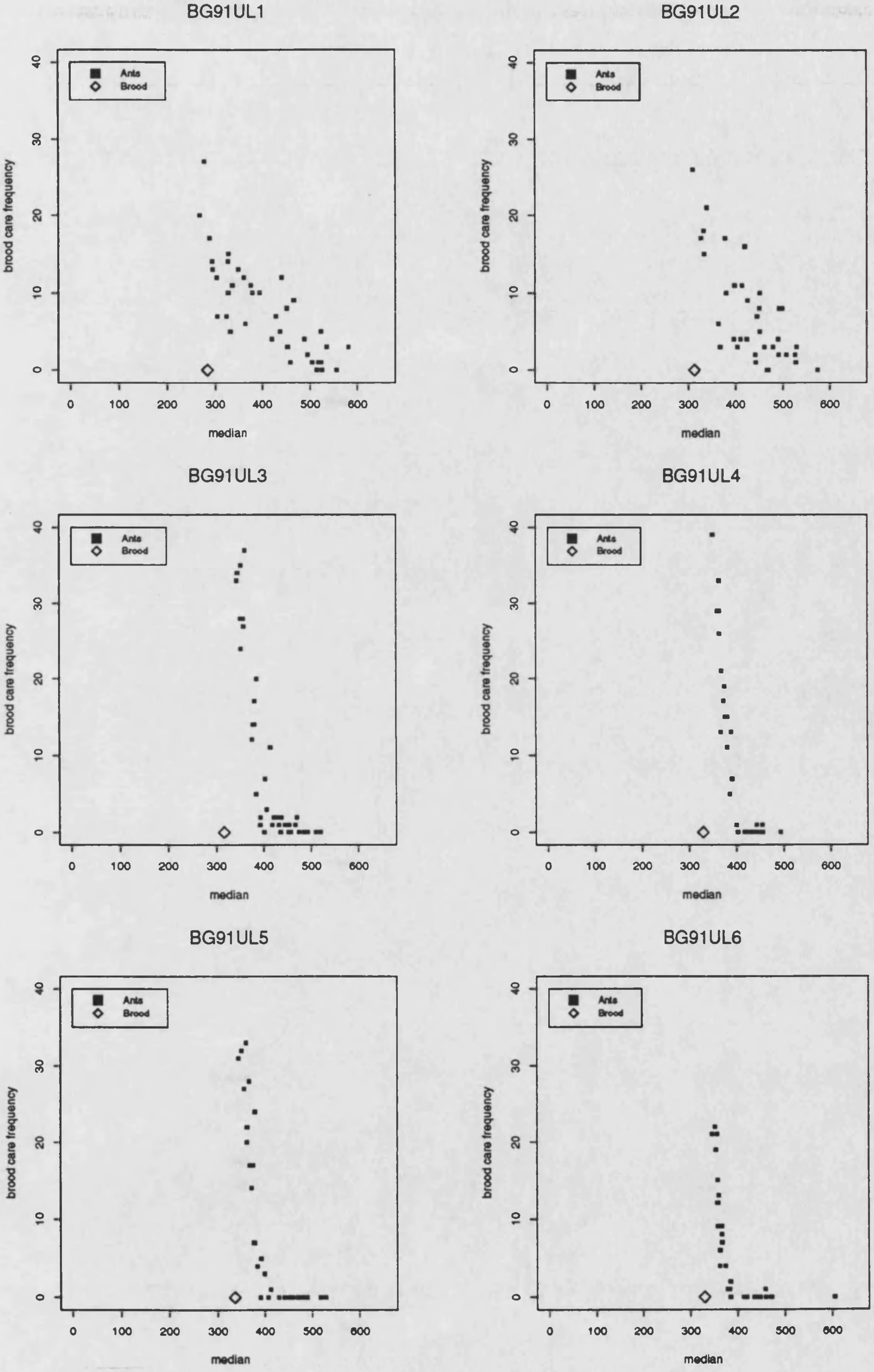


Figure 2.23: Relationship between ant position and brood care frequency. Sessions BG91UL1-6

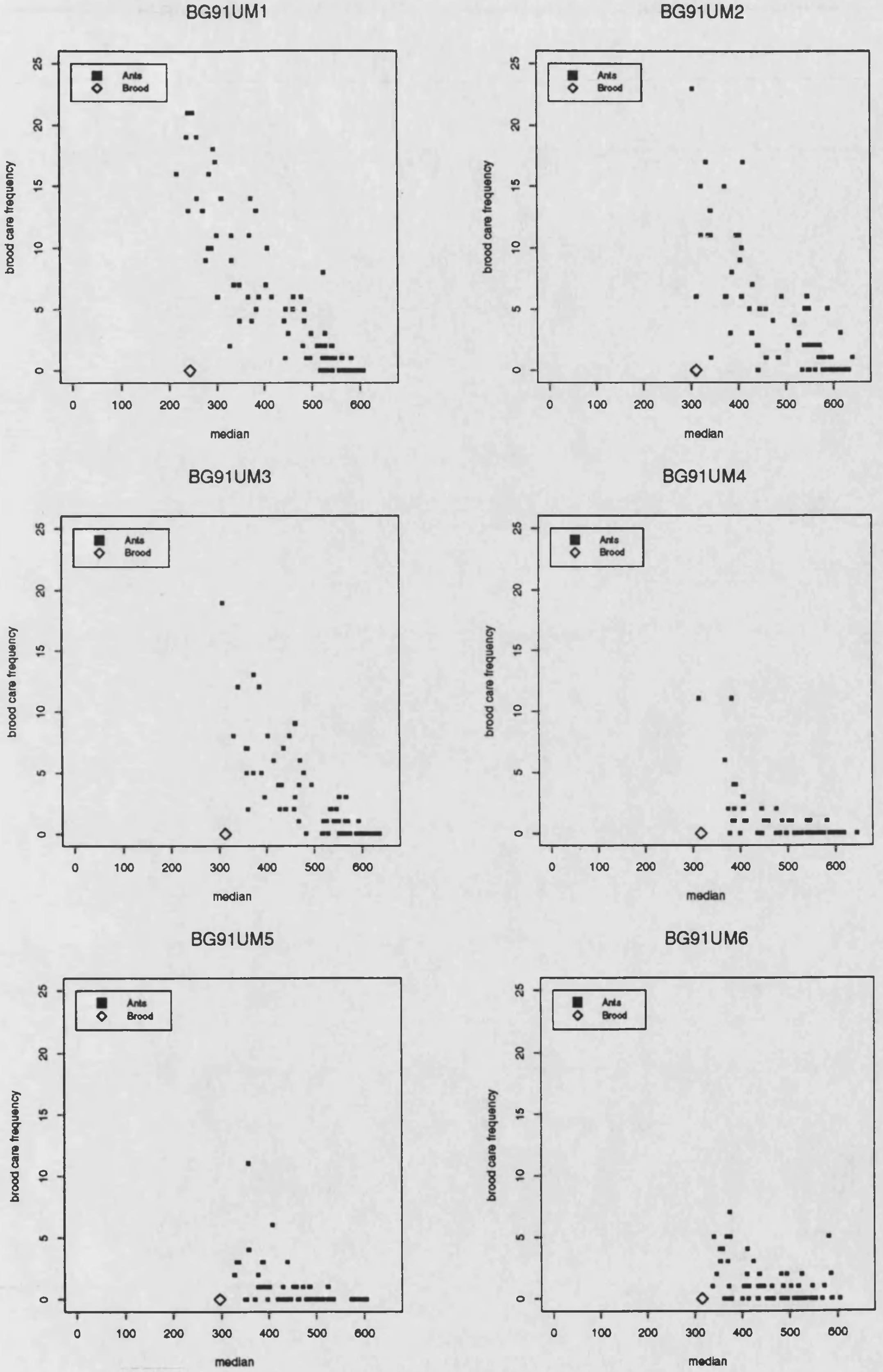


Figure 2.24: Relationship between ant position and brood care frequency. Sessions BG91UM1-6

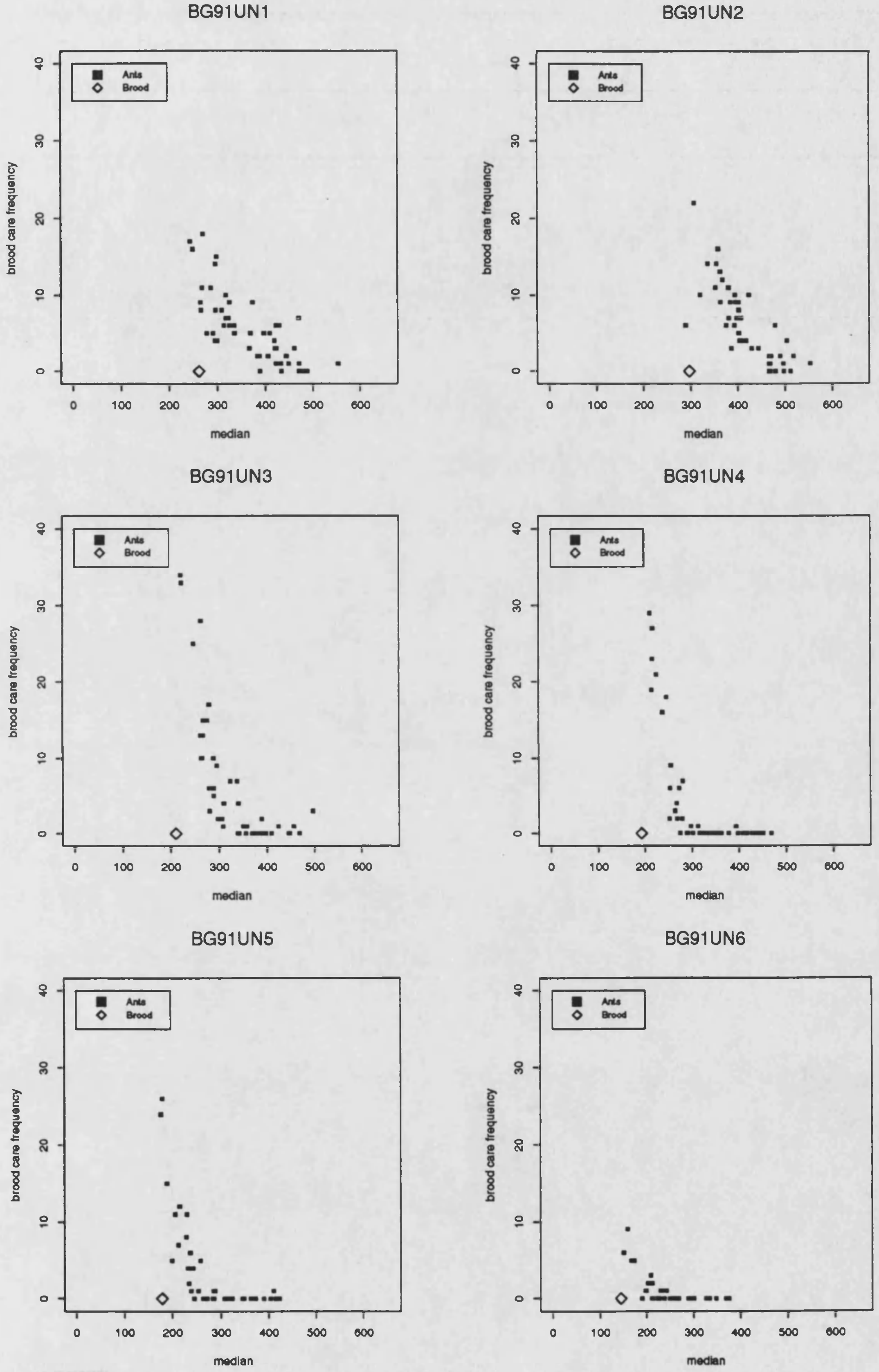


Figure 2.25: Relationship between ant position and brood care frequency. Sessions BG91UN1-6

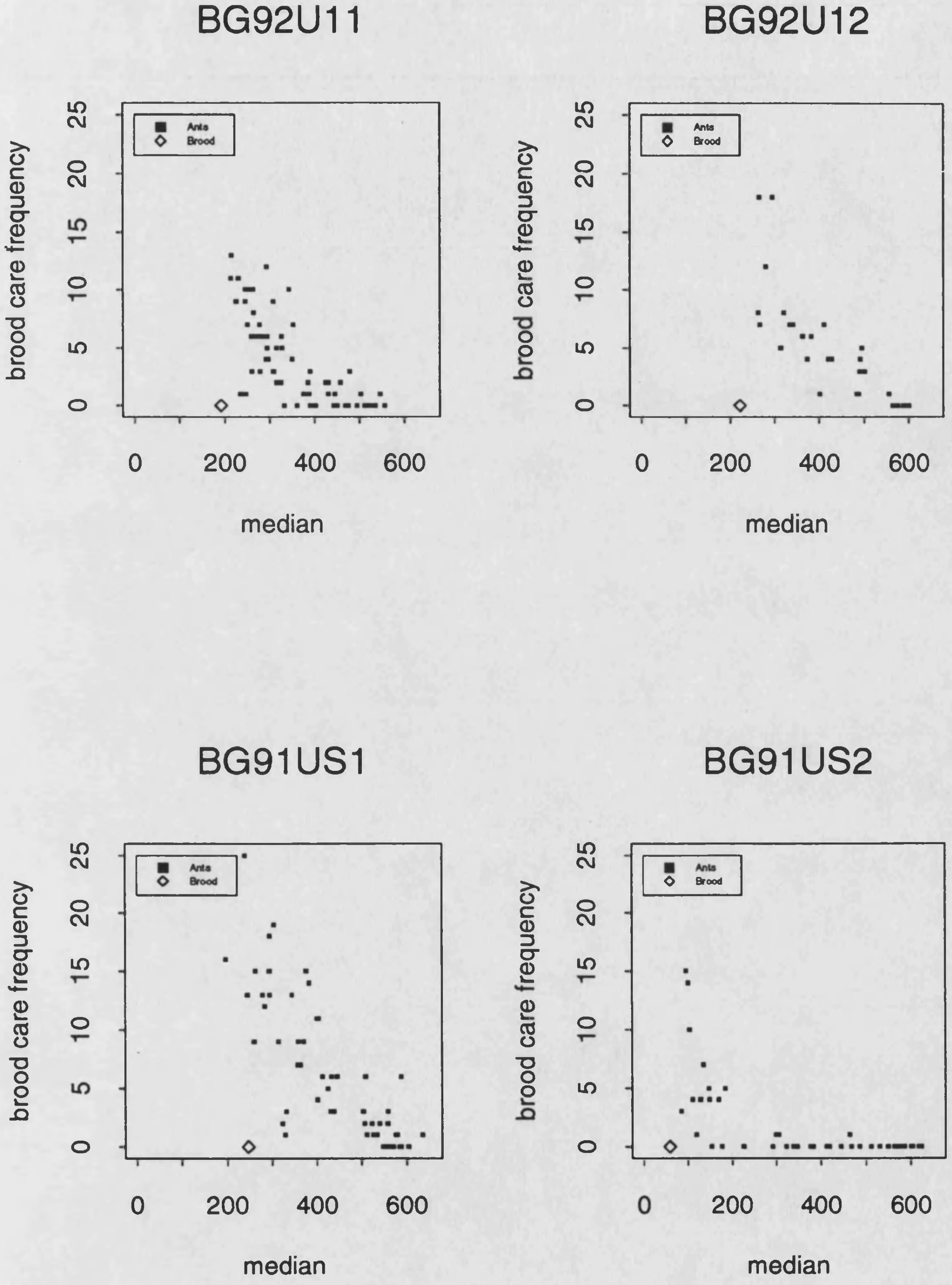


Figure 2.26: Relationship between ant position and brood care frequency. Sessions BG92U11-2, BG91US1-2

If the workload is evenly spread over the work-surface, why is it then that the further away ants are from the centre the less brood work they do?

The simplest explanation of this finding is that the further away the position of an ant is from the centre, the smaller the overlap between her SFZ and the distribution of the brood. This idea is supported by the relative position of the middle half of the brood distribution (see Figs. 2.3 - 2.9).

Not all tasks are performed within the nest. Another important task is that of foraging. In order to examine the relationship between the position of an ant in the nest and the number of times she has been out of the nest, the location of the ant is plotted against the sum total of the recorded times she has been either in the exit or outside the nest (Figs. 2.27 - 2.32).

These plots demonstrate that there is a threshold value for the ant position above which ants are very likely to go out of the nest. This threshold value depends on the size of the colony relative to the size of the nest and the distance of the colony from the exit. The smaller the colony and the further away from the exit is its brood pile, the smaller the threshold value for the position above which ants are very likely to get outside the nest.

Space does not appear to have much relevance to ant - ant contacts. It seems that ants are equally likely to interact with their nestmates. Only individuals that are in the centre or on the periphery of the colony appear to be less likely to communicate with their nestmates, which could be accounted for by their positions (see Fig. 2.33 for illustration). In contrast to this, queen - ant contacts are most likely to occur between the queen and workers that have positions closest to the centre of the colony, to which the position of the queen is usually nearest

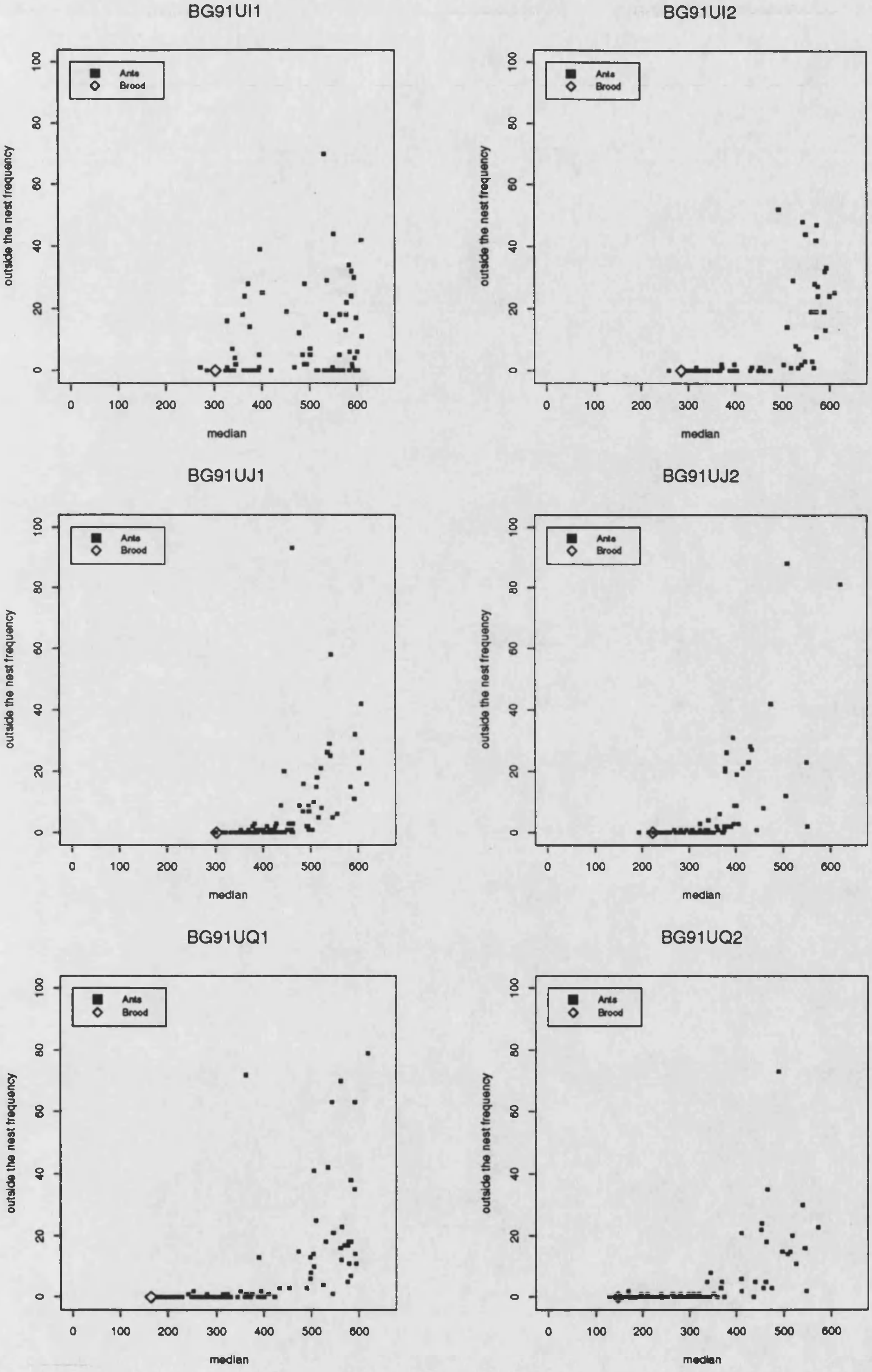


Figure 2.27: Relationship between ant position and outside the nest frequency. Sessions UI1-2, UJ1-2, UQ1-2

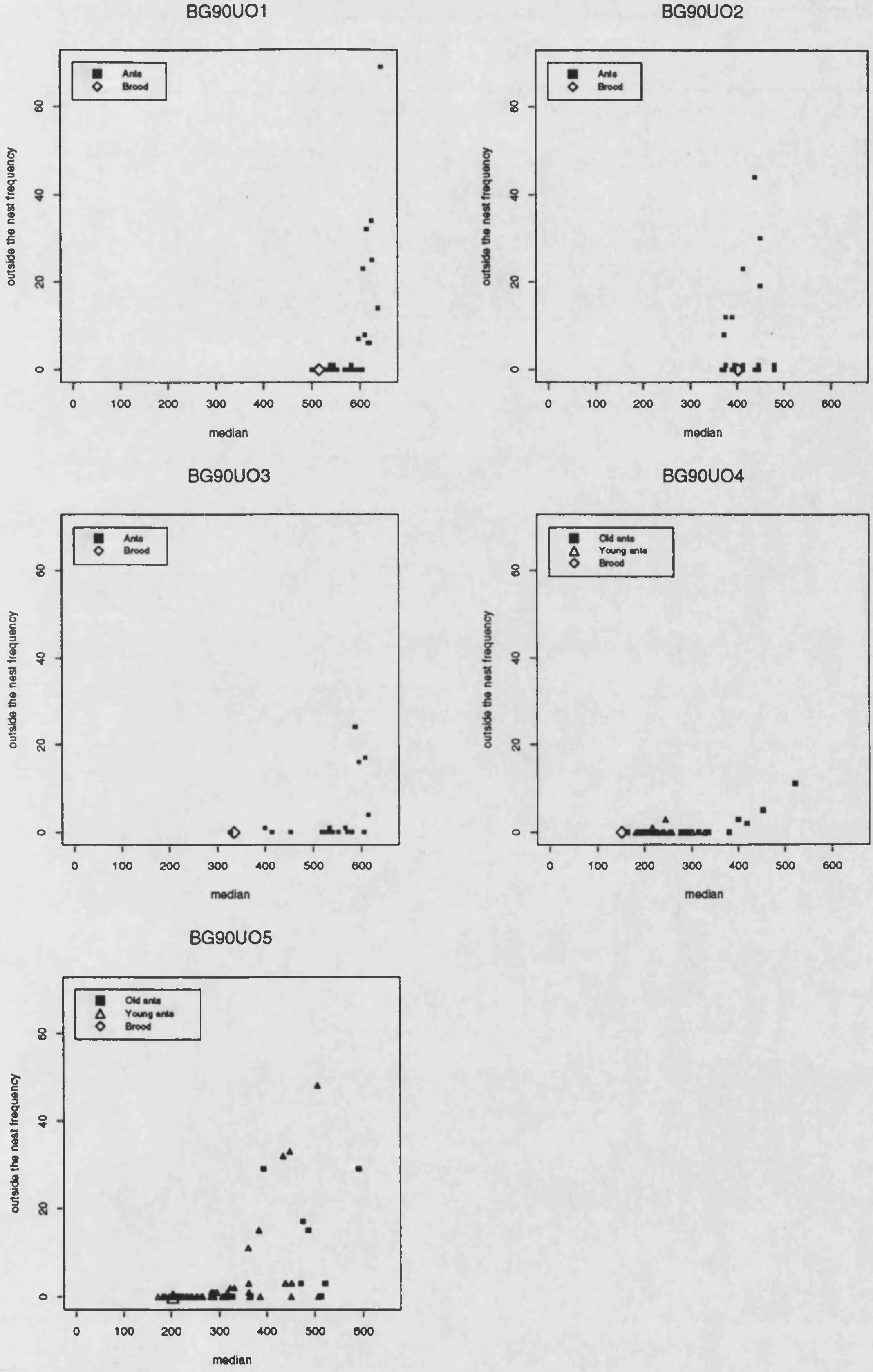


Figure 2.28: Relationship between ant position and outside the nest frequency. Sessions BG90UO1-5

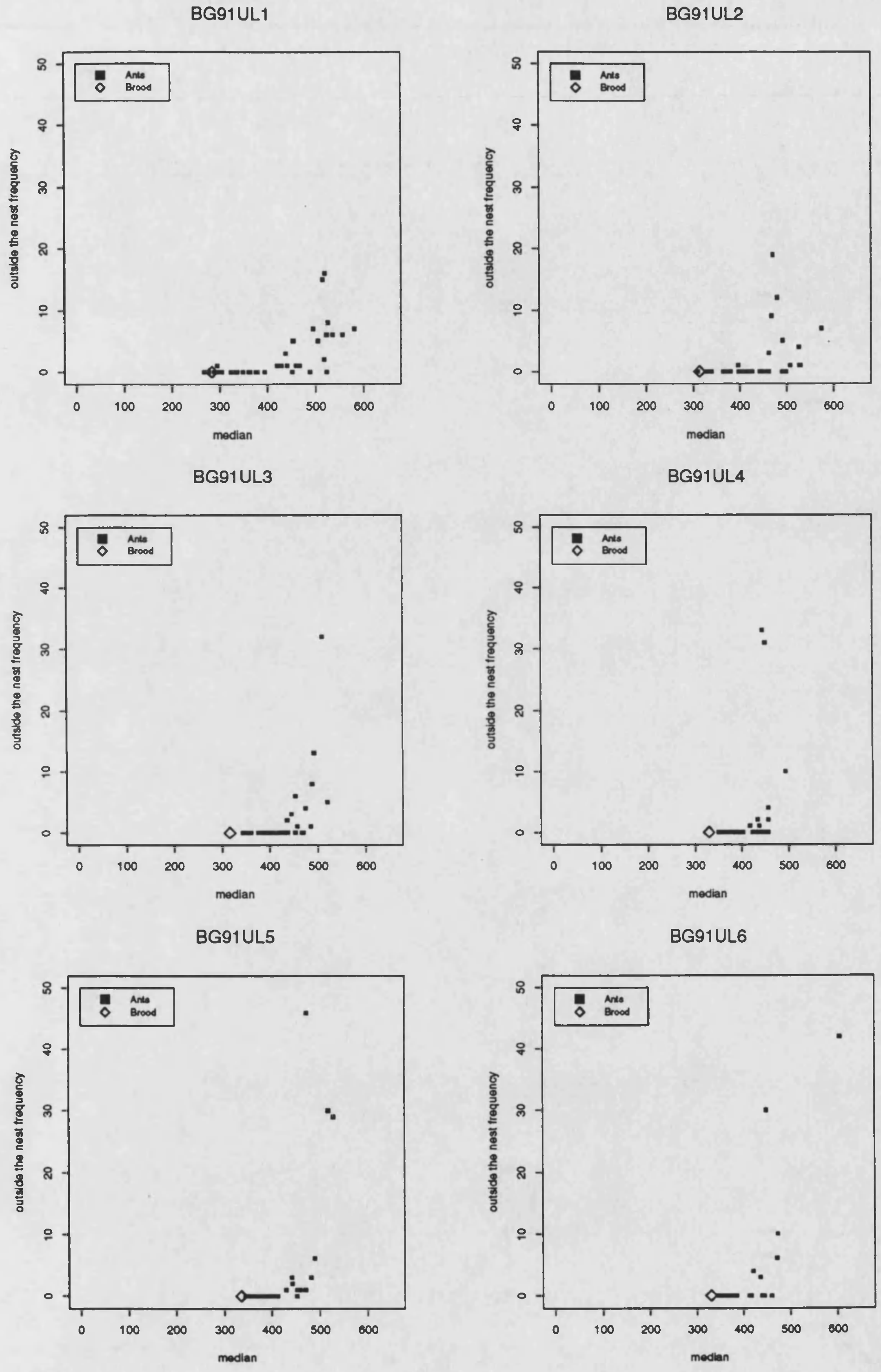


Figure 2.29: Relationship between ant position and outside the nest frequency. Sessions BG91UL1-6

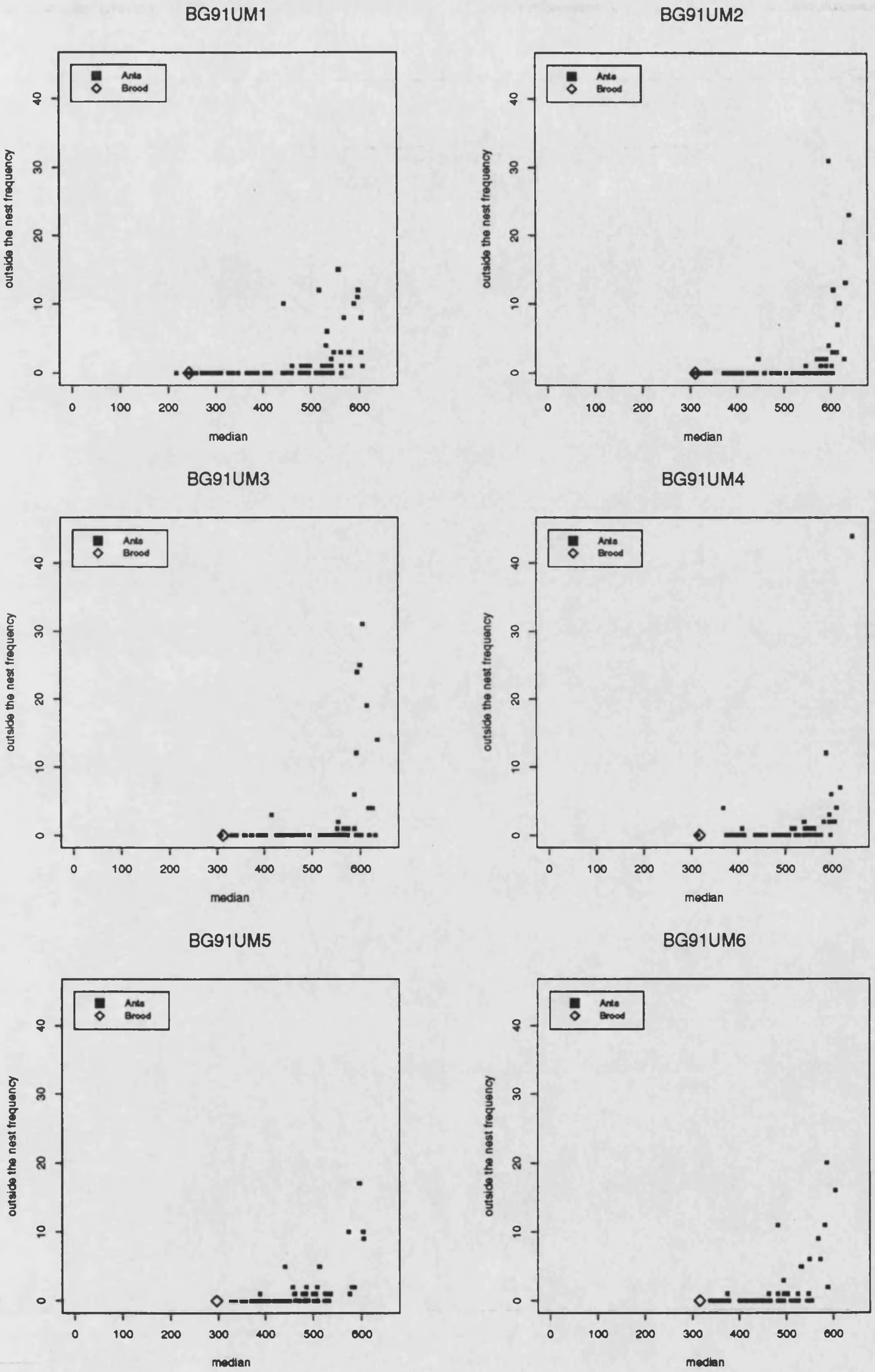


Figure 2.30: Relationship between ant position and outside the nest frequency. Sessions BG91UM1-6

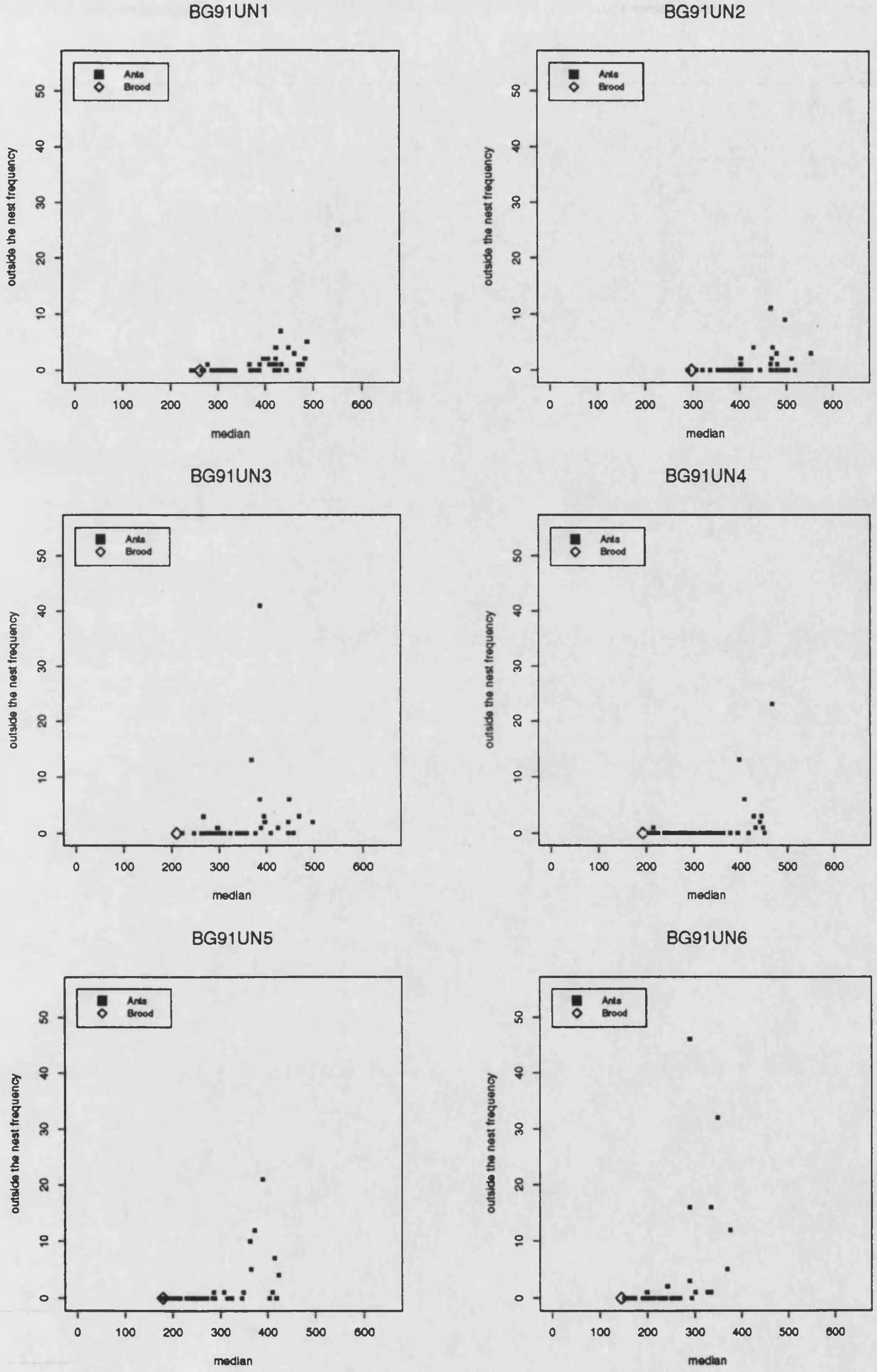


Figure 2.31: Relationship between ant position and outside the nest frequency. Sessions BG91UN1-6

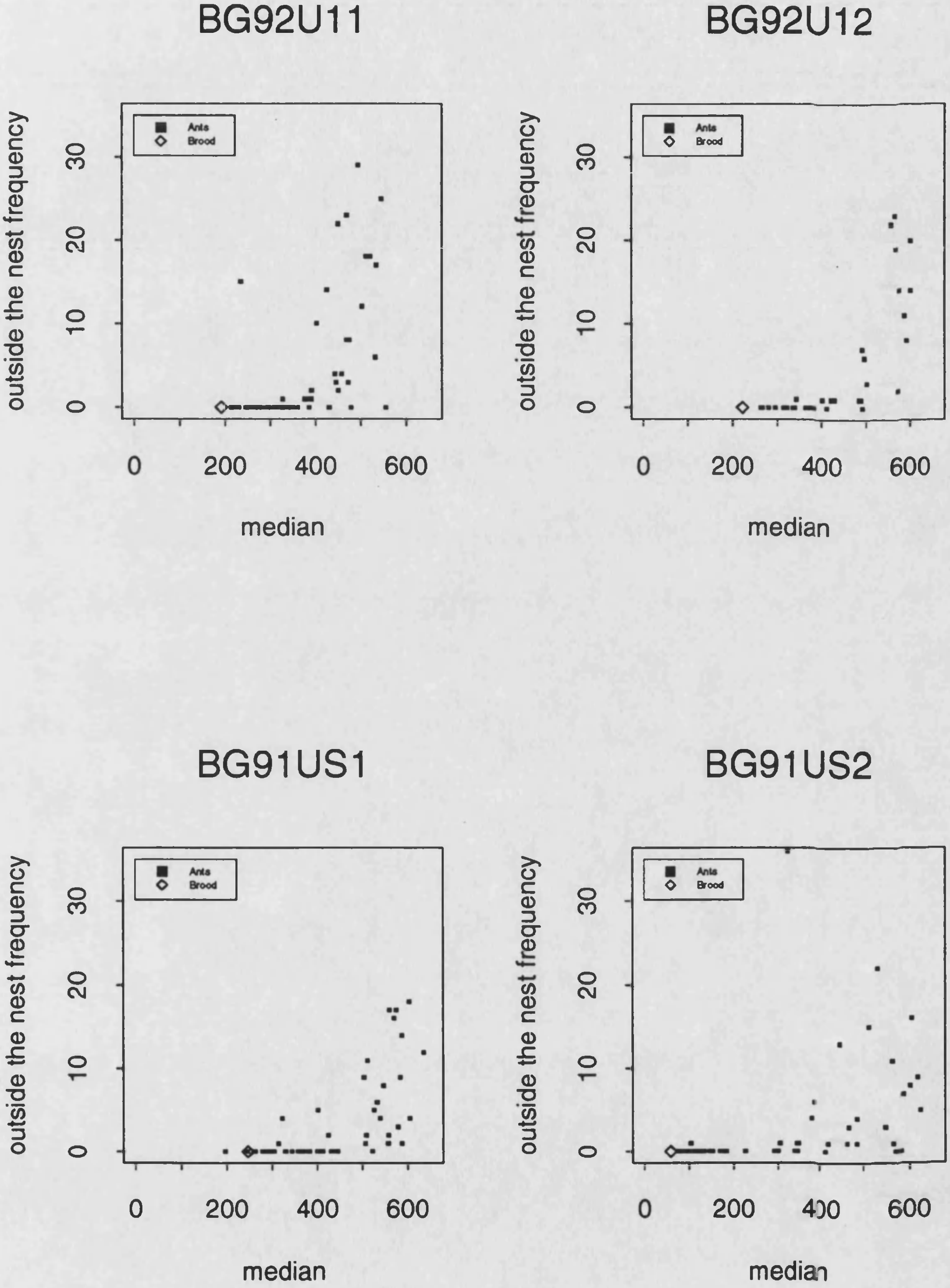


Figure 2.32: Relationship between ant position and outside the nest frequency. Sessions BG92U11-2, BG91US1-2

(see Fig. 2.34 for illustration).

2.3.6 SFZ and Age

The longevity of a *L. unifasciatus* worker is not less than 3 years, the colony produces only one generation per year and callows eclose almost continuously from June to August inclusive (see sections 1.3.4 and 1.3.6). According to the idea of age-based polyethism the age cohort of at least one year old workers should be spatially separated from the cohort of same year workers, since the tasks of looking after different types of brood and foraging are themselves separated in space. Two symbols are used on Figure 2.5 in order to distinguish these two age cohorts.

It is immediately obvious that the two age groups are not segregated into two spatial groups with the young in the middle and the old on the periphery, as would be expected on the basis of an age based division of labour.

On the other hand, in July (BG90UO4) the younger generation tends to be in the middle, while the older generation is spread out more or less evenly. By September (BG90UO5) the younger generation has spread approximately evenly while the older generation seems to have its greater weight on the periphery, though some old ants are still in the very centre. Three of these old ants remained in the same region in May 1993 when they were three years old (data not shown).

Chapter 3 will shed more light on the association between age and task.

2.4 Discussion

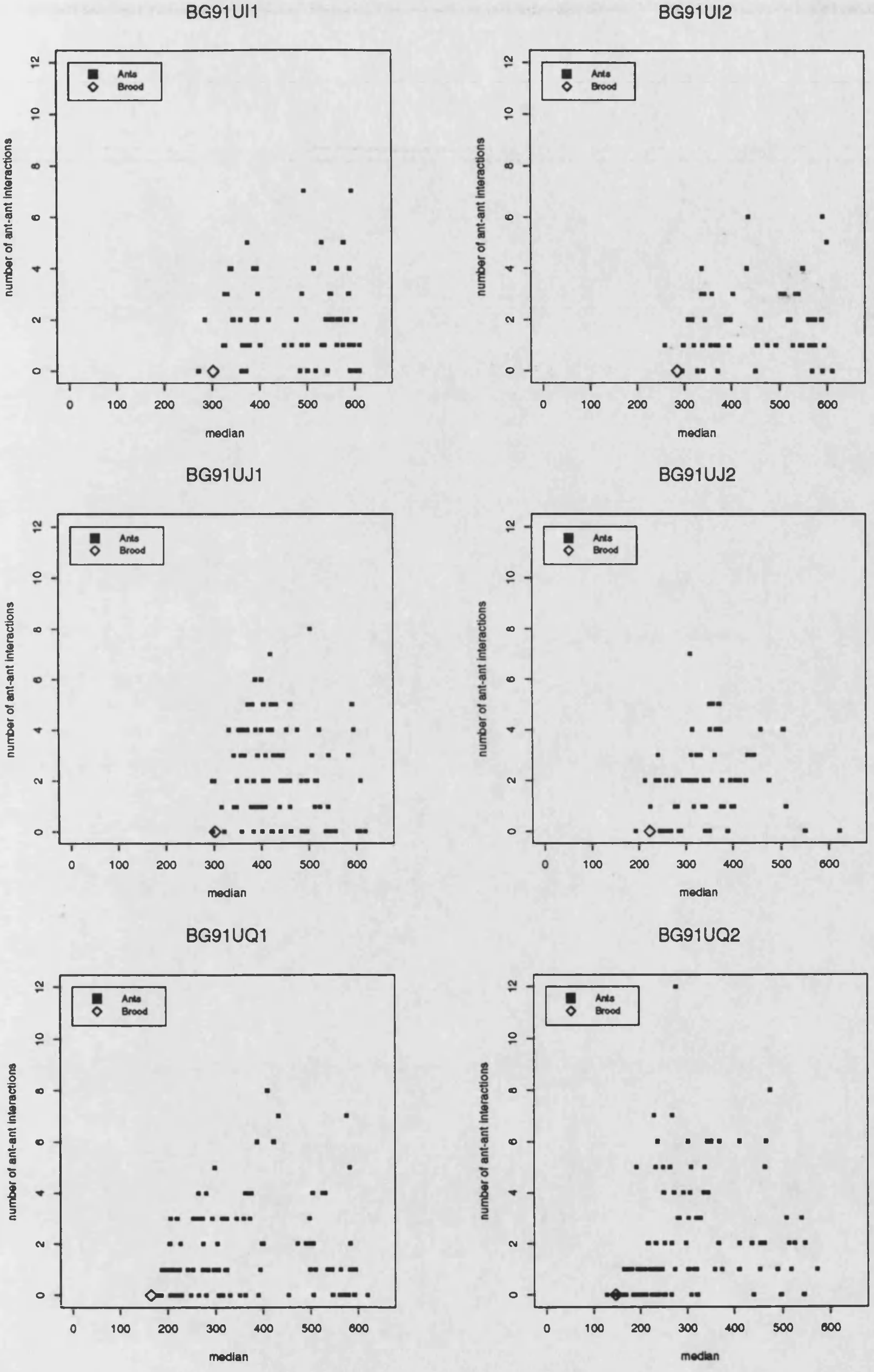


Figure 2.33: Relationship between ant position and number of ant-ant interactions. Sessions UI1-2, UJ1-2, UQ1-2

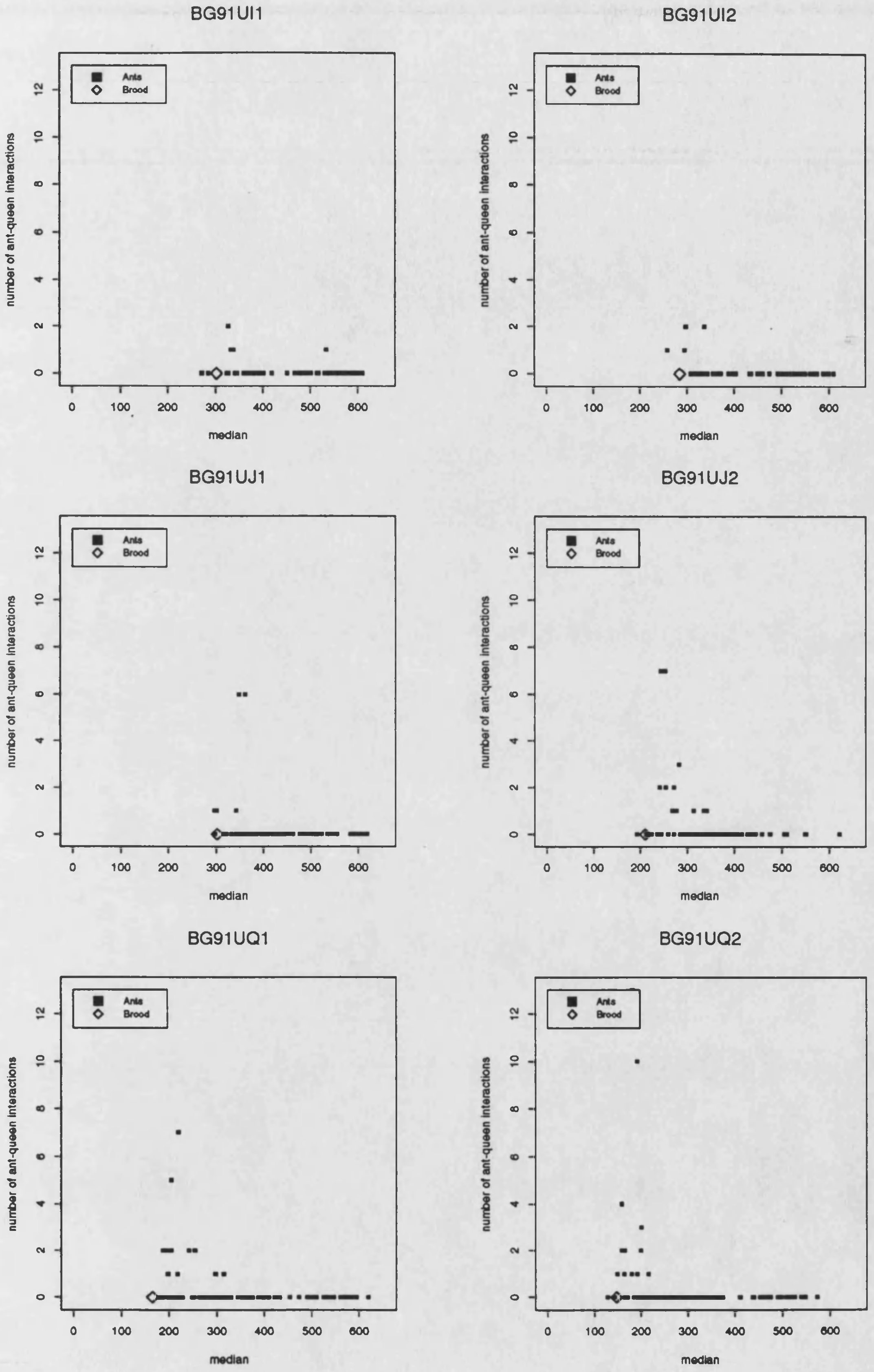


Figure 2.34: Relationship between ant position and number of ant-queen interactions. Sessions UI1-2, UJ1-2, UQ1-2

In this chapter I have used a novel method, based solely on spatial data, to describe colonies of the ant *L. unifasciatus*. The study reveals that an individual ant does not move randomly everywhere possible within the nest, but is to be found within a restricted area, which I have termed its Spatial Fidelity Zone (SFZ). This method reveals a spatial structure within the nest that has not been noticed before. Not only is the brood sorted in concentric circles so that the smallest items are in the middle and the biggest items are on the periphery (Franks and Sendova-Franks 1992), but the ants themselves form a continuum of partially overlapping SFZs. The type of analysis used in the present study simplifies these zones to one dimension. However, judging by the circular shapes of the brood arrangement and the nest wall that ants build themselves in nature or in the laboratory when presented with debris (Franks et al., 1992), the shape of the ant SFZ might also be circular. This new description of the ant colony as a highly spatially structured entity is not simply a new, more objective method. It leads to a new understanding of the actual mechanisms of division of labour, based on much simpler rules than those previously thought to be involved.

Division of labour among workers in colonies of *L. unifasciatus* definitely exists, because the behavioural profiles of workers in the centre of the colony are very different from the behavioural profiles of workers on the periphery. The transition, however, is not discrete but continuous. The nondiscrete character of colony organisation precludes any classification of workers into behavioural classes (castes), since individuals perform an almost unique mixture of tasks. Ants are organised in a meshwork and do the jobs that are spatially within their reach. As a rule this meshwork is intact, because in nature ants build the walls of their nests according to the size of the colony and its activity (Franks et al. 1992). However, the restricted geometry of the laboratory nests revealed that the continuum could break when the size of the colony is small in relation to the size of the artificial nest. Two "centres of attraction" become obvious as a result - the brood pile (or the queen) and the nest exit. In this sense a role can be considered as one such "centre of attraction", where "the centre" is the spatial location of the object involved with a particular set of tasks in similarity to Wilson (1985b). Consequently caste could be defined as a group of workers that are associated with a particular task/role location, a definition, which does not in itself mean that the rules that govern the allocation of individuals to castes are hard-wired. This view is substantiated by the finding that contrary to the predictions of age polyethism the two age cohorts in colony BG90UO are not spatially segregated.

How an individual ant comes to occupy her particular position in the spatial configuration of the colony is a question that will be addressed in Chapter 3. Here I will discuss possible proximate mechanisms for the maintenance of a SFZ and the possible ultimate adaptive significance of this behaviour. Both brood and nestmates are likely to play a role.

Ants appear to move within their SFZs in a cyclical fashion. They could be either oscillating around particular brood items or between other workers from which they rebound off. Different gradients of smell or just the difference in surface area probably provide cues about the brood type and its care requirements. Ants may be able to locate individual brood items both, through learning based on reinforcement by reward (from feeding larvae) and through a feedback mechanism (from following their own trails in the nest). There is evidence that *L. unifasciatus* ants have the ability to learn and to lay individual trails outside the nest (Aron et al 1988), so it is not unreasonable to suppose that they can also learn and lay individual trails inside the nest. For example, an ant may be able to learn the position of an individual larva through the reward of being relieved from a food load or through the reward of food assimilating substances that the larva may provide during the interaction (Brian, 1983). Alternatively an ant may become able to locate a particular brood item through a feedback mechanism based on a preference for the chemical signature of her own trail (Aron et al 1988). Once an ant becomes imprinted on certain brood items, she will oscillate around these items.

Probably there are individual differences in the ability of ants to learn and as a result certain individuals may become imprinted on particular brood items more quickly than others and thus "help" their nestmates by restricting their choice. Ants may also restrict each others movements by bumping into each other.

A totally different argument would be to suggest that ants have a home range like other territorial animals or a territory, i.e. a part of their home range which they actively defend. This seems unlikely, because individuals do not show any overt aggressive behaviour (except after the queen dies, see Chapter 5). Home range is not a plausible explanation for the SFZs either, because the home range of an ant moving outside the nest is tens of times as big as the nest while the home range of the same individual inside the nest is only a fraction of the nest area (see Wilson 1975, Chapter 12). Mainstream literature on the above two subjects (Broom 1981, pp 195-201; Huntingford 1984, pp 188-193; Davies and Houston 1984) is not relevant because in the case of ants we are witnessing a collective phenomenon.

Is the maintenance of a SFZ an adaptive behaviour? Questions about the adaptive significance of a behaviour are always very difficult to answer. The present study shows that the SFZ is present in all of the considered colonies during all of the sessions. There appears to be some individual variation as well. Thus, we can speculate that this behaviour is adaptive, because it is an effective way of dividing labour. Hatcher et al. (1992) argue for the adaptive significance in *Leptothorax* colonies of rhythms of activity, phenomenon in which ants are active together and inactive together. Their argument is that this makes the division of labour more efficient, because when active together ants are unlikely to waste time and resources by, for example, feeding a particular larva twice whilst neglecting others, a wastage that is very likely to occur if different ants were active at different times. Similarly, by partitioning space SFZs make it very unlikely for ants to waste time and resources.

The argument for the adaptive significance of maintaining a SFZ does not imply that this behaviour should be hard-wired and at the same time does not contravene gene level selection theory. If the maintenance of SFZ is adaptive and can be picked up by the forces of natural selection then at least part of this behaviour must be genetically encoded. However, it is very likely that learning and environmental factors also play an important role, since as it appears from the results, this individual behaviour might be only present in a social context. In this sense the maintenance of SFZ could be viewed as a self-organised process, i.e. as an emergent property of the operation of simple behavioural rules on the level of the individual that are amplified by learning and communication. If the environmental factors change, then the simple behavioural rule might not lead to the maintenance of a SFZ. However, such a change is unlikely and by transmitting itself from one generation to another the gene for the simple behavioural rule also transmits the behaviour of maintaining a SFZ.

The next chapter will reveal how the colony spatial order, discussed above, changes with time.

Chapter 3

Change of Spatial Fidelity Zone and Task with Time

3.1 Introduction

This chapter addresses the process of task allocation in ants. Task allocation underlies the division of labour and is therefore central to the organisation and evolution of insect societies. How individual ants change tasks is investigated in colonies observed through most of one active season. The presented approach is new in that it uses the spatial positions of ants (see Chapter 2) and thus, makes their description both simple and objective.

Task allocation and division of labour are widely used as interchangeable terms by those studying social insects. For the purpose of this chapter, however, I wish to emphasise the difference between these terms. **Division of labour** indicates a static picture of the status quo while **task allocation** signifies the underlying process. The reason for their synonymous use lies with the dominating view that ants change tasks according to a predetermined programme of what to do with age, the notion of **age polyethism**. In order to appreciate the meaning and enormous influence of this notion, a brief account of its background is necessary. **Adaptive demography** (Wilson 1968, Wilson 1985a, Oster and Wilson 1978), a well established theory in the study of social insects, maintains that unlike the non-adaptive demography of populations of nonsocial organisms, which is an emergent property based on the schedules of birth and death of individuals, the demography of the eusocial insect colony is directly adaptive. This fundamental difference is due to workers in a social insect colony being sterile. The birth and death schedules of such workers have meaning only with reference to the survival and reproduction of the queen. Hence, the unit of selection is the colony as a whole (Wilson 1985a). This group selectionist view combined with the perspectives of **ergonomic efficiency** and **evolutionary optimisation** (Oster and Wilson 1978) led to the concept of the **caste distribution function (CDF)** which predicts that the number of worker castes and the ratios of their numbers are adaptive and have been selected to approximate an optimal mix for the local environment. The notion of **caste** has its origin in the observation that certain species of ants (only 44 of the 263 living ant genera (Oster and Wilson 1978)) display some prominent degree of polymorphism within the worker population (physical castes). Later on the same term was also used to denote groups of ants of similar age (temporal castes) in monomorphic species (i.e. with no difference in worker morphology). Nevertheless, at present **caste** is defined through what the ants do rather than their morphology or age, i.e. as a group of individuals which perform specialist labour for prolonged periods of time (Oster and Wilson 1978) or as a group of individuals that specialise on particular tasks (Wilson 1985a, p. 309) where **task** is used by the same authors (Oster and Wilson 1978) to denote behavioural acts which achieve some purpose for the colony. This latest definition follows the predictions of ergonomic efficiency and evolutionary optimisation that different age/size groups have different functions. The coupling of caste and task has been confirmed empirically through the method of **random scanning** and the establishing of behavioural profiles for age/size groups by averaging individual behavioural frequencies (see section 2.1). On the foundation of the thus established association between caste and task it is hypothesised that temporal castes (i.e. groups of same age ants doing the same task) are based on a preprogrammed change in behaviour which is a function of aging (Wilson 1985a, p. 307). The implication is the existence of a hard-wired, clock-type mechanism of task allocation, which instructs an ant what to do with age.

However, a growing body of evidence for decoupling of age/size class and task directly contradicts the theory of adaptive demography and thus, renders its notion of **caste** meaningless. Experiments involving the removal of certain age/size groups and observations of individually recognisable workers have demonstrated that (1) remaining castes are able to fill in for missing ones (Lenoir 1979a, McDonald and Topoff 1985) and (2) there is an enormous amount of interindividual behavioural variability (Corbara et al 1986, Calabi and Rosengaus 1988, Calabi and Traniello 1989, Sendova-Franks and Franks 1993). In addition, observations in the field have revealed that there are no differences in the physical and temporal caste structure of colonies living in habitats with significant ecological differences (Calabi and Traniello 1988). In this thesis I will attempt to shed more light on the mechanism of task allocation by applying the novel empirical approach of spatial analysis to the organisation of ant colonies.

Spatial efficiency (Wilson 1976, p. 151, Wilson 1985a, p. 319, Wilson 1985b, p. 1489) is an important consideration from the point of view of ergonomic optimisation theory. In his review on sociogenesis, Wilson (1985b, p. 1492) recognised that tasks are linked into **roles** (see section 2.1) not by the similarity of the behaviours performed but by the proximity of the objects to which they are directed, thus reducing the travel time and energy expenditure of the individual workers. He hypothesises that the changes in the physical position of colony members are programmed according to age and size (Wilson 1985b).

In her work on social wasps West-Eberhard has established that young wasps tend to be in the centre of the colony while older wasps tend to stay on the periphery. West-Eberhard has termed this phenomenon **centrifugal temporal polyethism** and explains it as a product of selection on the level of the individual (West-Eberhard 1979, 1981). She hypothesises that when workers are young and their fertility - highest they maximise their chances of reproduction by staying near the brood, in the centre of the colony. When workers get older, however, and their fertility declines, their best strategy is to do foraging and defence on the outskirts of the colony, thus enhancing colony welfare and the production of siblings rather than personal offspring.

Despite the theoretically predicted importance of positional effects, the relation of caste differentiation to the geometry of labour allocation within the nest has hardly been examined (Wilson 1985b). Task allocation, which is effectively a process of behavioural differentiation, is most commonly realised in the way ants change tasks with time in the absence of any disturbances. The objective description of the patterns which emerge as a result of this process, is essential for any progress in modelling and understanding its rules, and last but not least - for unravelling the evolution of eusociality. The spatial approach facilitates a study of task change over time. It has already been established that tasks are localised (Wilson 1985b, Franks and Sendova-Franks 1992) and that ants are faithful to their own activity orbitals (see Chapter 2). Thus, if it is found that the SFZs of ants shift closer to or further away from the colony centre, this finding would indeed demonstrate that ants are changing tasks, because they cannot do jobs which are topographically out of their reach. Doubtless, spatial results will have to be confirmed by changes in observed behavioural acts - for example, brood care frequency and outside the nest frequency.

The present study addresses the following specific questions: (a) How does the colony's spatial configuration change through the period of observation? How does such change match the change in the colony's behavioural profile? (b) Do ants change positions synchronously or asynchronously? (c) Which are the individuals actively responsible for any changes in the colony's spatial configuration and how? (d) Does the SFZ of an individual ant shift away from the colony centre with time, as would be expected on the basis of the centrifugal model of temporal polyethism? (e) Does the SFZ of any ant ever shift backwards, towards the centre, in conditions of no interference? (f) What is the relationship between the position of an ant and the type of shift? (g) Does the number of individual ant shifts vary with the time in the annual cycle? If this is true, does the greatest number of shifts coincide with the greatest influx of callows? (h) Do external workers ever reverse into internal workers in the absence of perturbations? (i) Do individual ants shift their SFZs after the experimental removal of selected nestmates or the brood?

3.2 Materials and Methods

3.2.1 Colonies and Sessions

Colonies of *L. unifasciatus* were kept and studied as described in sections 1.3.12, 1.4.1, and 1.4.3. This chapter is based on 27 photographic sessions of observation of colonies BG90UO, BG91UL, BG91UM, BG91UN, BG91US, and BG92U1 (see Tables 1.1-1.5).

In order to keep differences between days down to a minimum colonies BG90UO, BG91US and BG92U1 were provided with fresh food each day before commence of the photographic session. Colonies BG91UL, BG91UM, and BG91UN, which were filmed on a long-term basis were fed once per week like the colonies that were not under observation (see end of section 1.3.12).

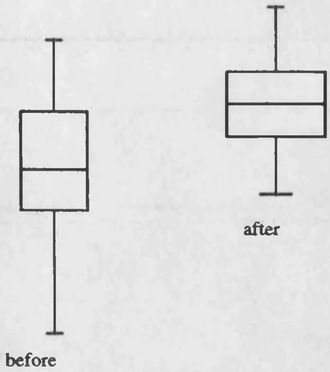
Emigrations of colonies BG90UO, BG91US, and BG92U1 were carried out as described in sections 1.4.2.1 and 4.2.2. The removal of brood in colony BG91US and the removal of workers in colony BG92U1 followed the experimental procedures described in sections 1.4.2.2 and 1.4.2.3. Colony BG90UO was the only one to be disturbed a second time in order to mark the new generation. The second marking followed the procedure described in section 1.4.1.1. Notwithstanding the occasional lost mark, the old ants (distinguishably darker) were transferred directly from the old to the new arena without being anaesthetised or marked a second time.

Only individuals that had survived from the first to the last session were considered in the final analysis. In addition, the ants that had eclosed the same year in colonies BG91UL, BG91UN, and BG91UM were not marked in order to keep disturbance down to a minimum. Colony BG90UO had already acquired a substantial new generation by session 3, but young ants were individually marked later, for sessions 4 and 5 (see Table 1.4).

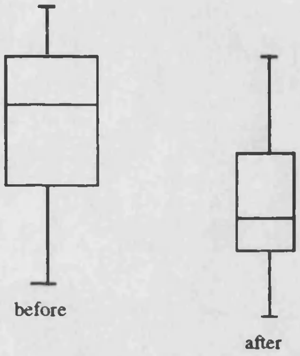
3.2.2 Spearman's Correlation Test

(see section 1.4.4.2)

3.2.3 The Shift Criterion



(a) shift further



(b) shift nearer

Figure 3.1: Illustration of the shift criterion: (a) shift further; (b) shift nearer.

For the purpose of analysing absolute change of location it was postulated that an ant has shifted her position if the difference between her distributions for the two compared sessions was at least half interquartile range (spread).

A shift further away from the centre: when the lower quartile of the second (chronologically) distribution is greater or equal to the median of the first distribution, or when the upper quartile of the first distribution is smaller or equal to the median of the second distribution. **A shift nearer** to the centre: when the upper quartile of the second (chronologically) distribution is smaller or equal to the median of the first distribution, or when the lower quartile of the first distribution is greater or equal to the median of the second distribution (Fig. 3.1).

3.2.3.1 Justification of the Shift Criterion

The chosen criterion is a compromise between first, a meaningful difference and second, sensitivity. For example, the criterion would be more meaningful if the compared distributions are considered different when their interquartile ranges do not overlap (i.e. when the difference between the distributions is at least one interquartile range). However, this criterion is not sensitive enough - when applied only very few individuals are found to have shifted SFZs, while the objective is to have a measure of absolute positional change which at least partially reflects the magnitude of relative positional change in the colony. Another reason why the difference of one interquartile range is unsuitable to be the criterion for a shift is that, though each ant has a unique position, SFZs overlap considerably and the difference between the ant closest to the centre and the ant furthest away rarely amounts to a total spatial separation of their distributions.

3.2.3.2 Comparing Samples of Different Sizes

There is a potential argument that, since it is not always possible to identify all workers on a slide (see section 1.4.1.6) and consequently sample sizes for positions vary among individuals, the two distributions of an ant for two separate sessions might be found to be different according to the chosen criterion (i.e. a false positive result could occur) only because their sample sizes are different.

In order to obtain some estimate of the likelihood a false positive result to occur, I performed the following randomisation test: **Ho** is: Two random samples from a set of consecutive numbers test positive for a shift according to the criterion of at least a half interquartile range difference between their distributions. **Ha** is: Two random samples from a set of consecutive numbers do not test positive for

Colony	No. of ants with diff. of 0 to 24%	No. of ants with diff. of 25 to 49%	No. of ants with diff. of 50 to 74%	No. of ants with diff. of 75 to 100%
BG90UO	77	5	3	0
BG91UI	50	4	2	0
BG91UJ	65	7	0	0
BG91UQ	83	9	2	0
BG91UL	148	6	1	0
BG91UM	300	19	1	0
BG91UN	171	11	2	1
BG91US	40	5	1	0
BG92U1	18	10	3	0
total	952 91.1%	76 7.2%	15 1.4%	1 0.1%

Table 3.1: Sample size differences between the distributions of the same individual for consecutive sessions (incl. the queen).

a shift according to the criterion of at least a half interquartile range difference between their distributions.

The result of the test depends first, on the magnitude of the difference between the sample sizes of the two compared distributions, and second, on the absolute magnitude of the sample sizes. The percentage of individuals whose distributions for successive sessions differ more than 50% in size is negligible - only 1.5% for all analysed individuals in all 9 studied colonies (Table 3.1). Approximately 7% differ by between 25 and 49%, while the overwhelming majority (91%) differ by between 0 and 24%.

At the same time the majority of sample sizes (approx. 95%) are above 24 (Table 3.2). Therefore the test was carried out on 4 couples of samples that differed by 24% and were of size between 24 and 100 (Table 3.3). The conclusion from the altogether 20 tests (each based on 100 simulations) is that for the majority of comparisons between the distributions of the same individual for successive

Colony	**No. of ants with s. size 0 to 24**	**No. of ants with s. size 25 to 49**	**No. of ants with s. size 50 to 74**	**No. of ants with s. size 75 to 100**
BG90UO	59	6	20	210
BG91UI	1	15	26	70
BG91UJ	0	4	44	96
BG91UQ	3	7	51	127
BG91UL	2	61	123	0
BG91UM	3	188	193	0
BG91UN	5	93	124	0
BG91US	6	83	3	0
BG92U1	15	46	1	0
total	94 5.6%	503 29.9%	585 34.7%	503 29.9%

Table 3.2: Sample sizes of ant distributions (incl. the queen).

sessions of observation there is a chance of between 0 and 8% that a false shift could be established.

The test was carried out by using a small procedure written in the language of the S-plus statistical and graphical package (Becker et al 1988).

3.2.4 Strengths and Weaknesses of the Method

The major weaknesses of the used method are first, the small number of colonies, and second, their different sizes. Consequently, comparisons between colonies will not yield any statistically significant inferences. The major advantage is the thorough and long-term description of a maximal number of colony members without disturbance.

Ants were tested for shifts in their SFZs using a programme written in C that was based on the above defined criterions.

24% difference b/n 2 samples	% of false positives for 5 tests	Average % of false positives
s1=100 s2=76	0 0 0 0 0	0
s1=75 s2=57	2 0 3 0 0	1
s1=50 s2=38	3 2 3 2 5	3
s1=32 s2=24	8 10 9 8 7	8

Table 3.3: Results from the test of the importance of sample size magnitude and sample size difference. Each test is based on 100 simulations.

3.3 Results

3.3.1 Relative Change of Location

The number of ants that have changed their position relative to the positions of their nestmates indicates the stability of the colony spatial configuration through the examined period of time. A colony may be considered to have a stable spatial configuration if the ranking of individuals according to their position does not change. Figures 3.2-3.4 follow the stability of colony spatial configuration in three colonies for six successive months.

The following trend emerges for all three colonies: Although the correlations of ranked ant positions are significant for all paired successive months, the spatial configurations of the colonies are most unstable between May and June. Then they stabilise between June and July, July and August, and remain stable until October. There is a minimal reduction of stability between September and October (BG91UL, BG91UN) or August and September (BG91UM). The correlations of ranked brood care frequencies of ants between May and October show that the

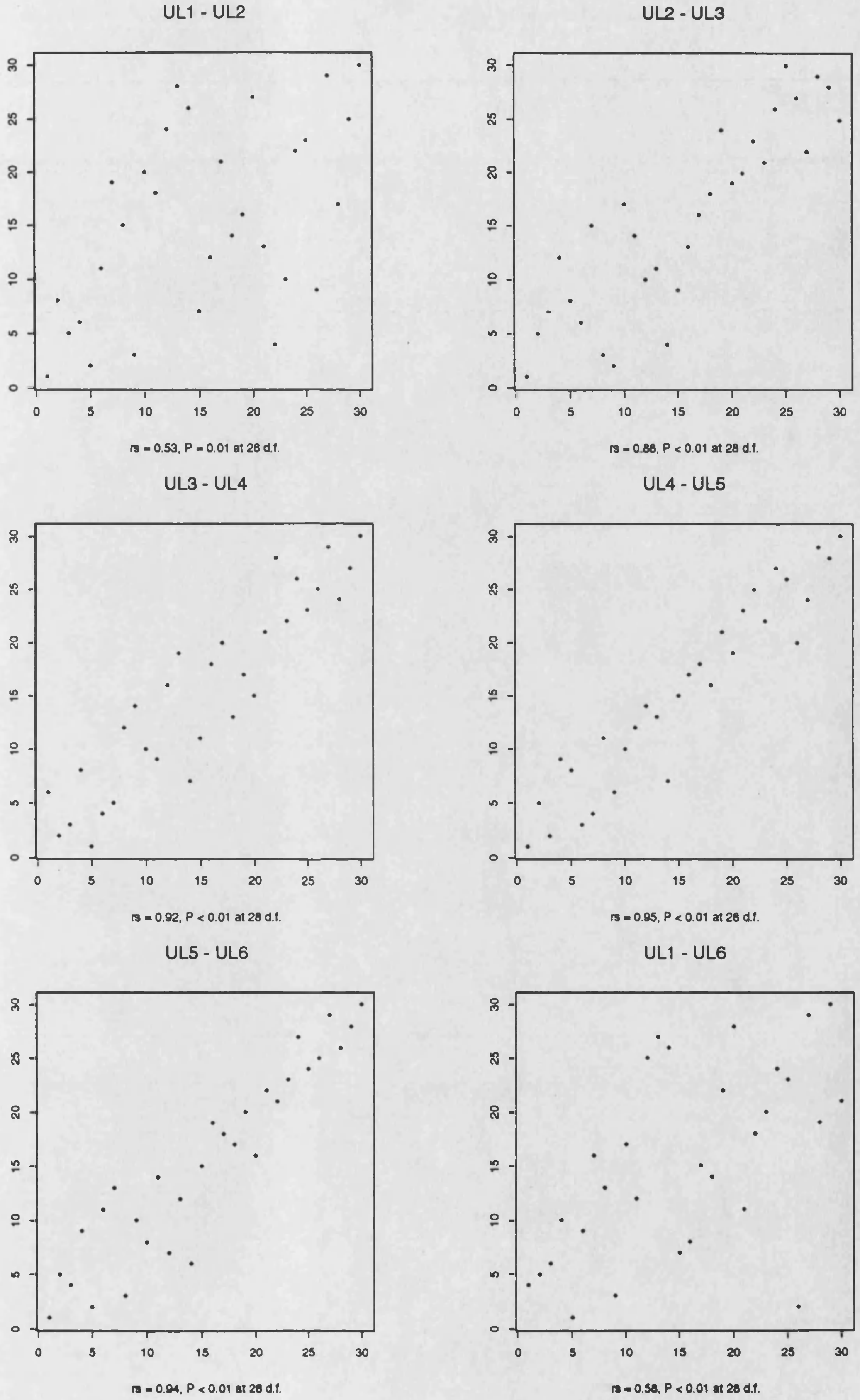


Figure 3.2: Correlations of ranked ant positions for successive sessions. Sessions BG91UL1-6

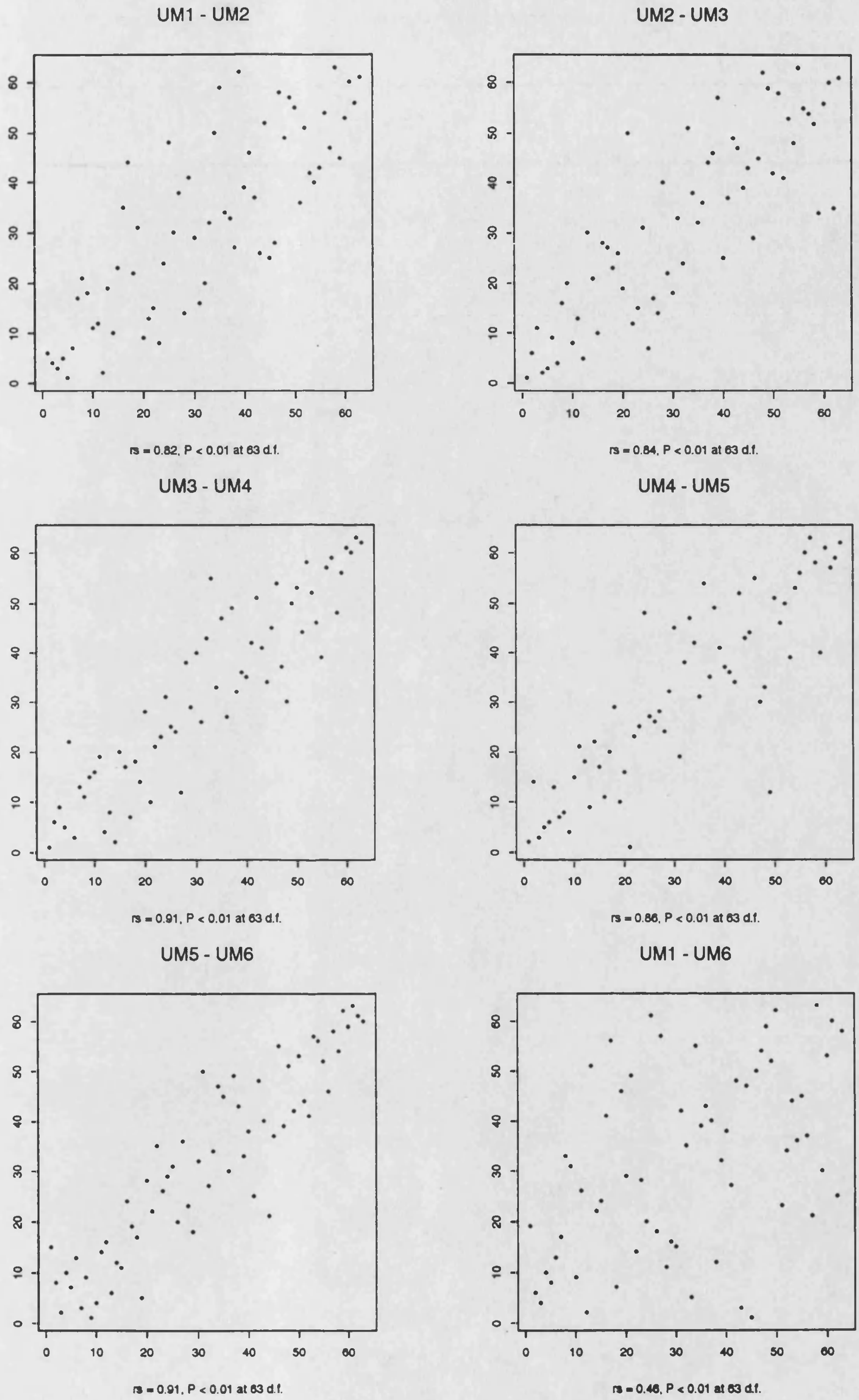


Figure 3.3: Correlations of ranked ant positions for successive sessions. Sessions BG91UM1-6

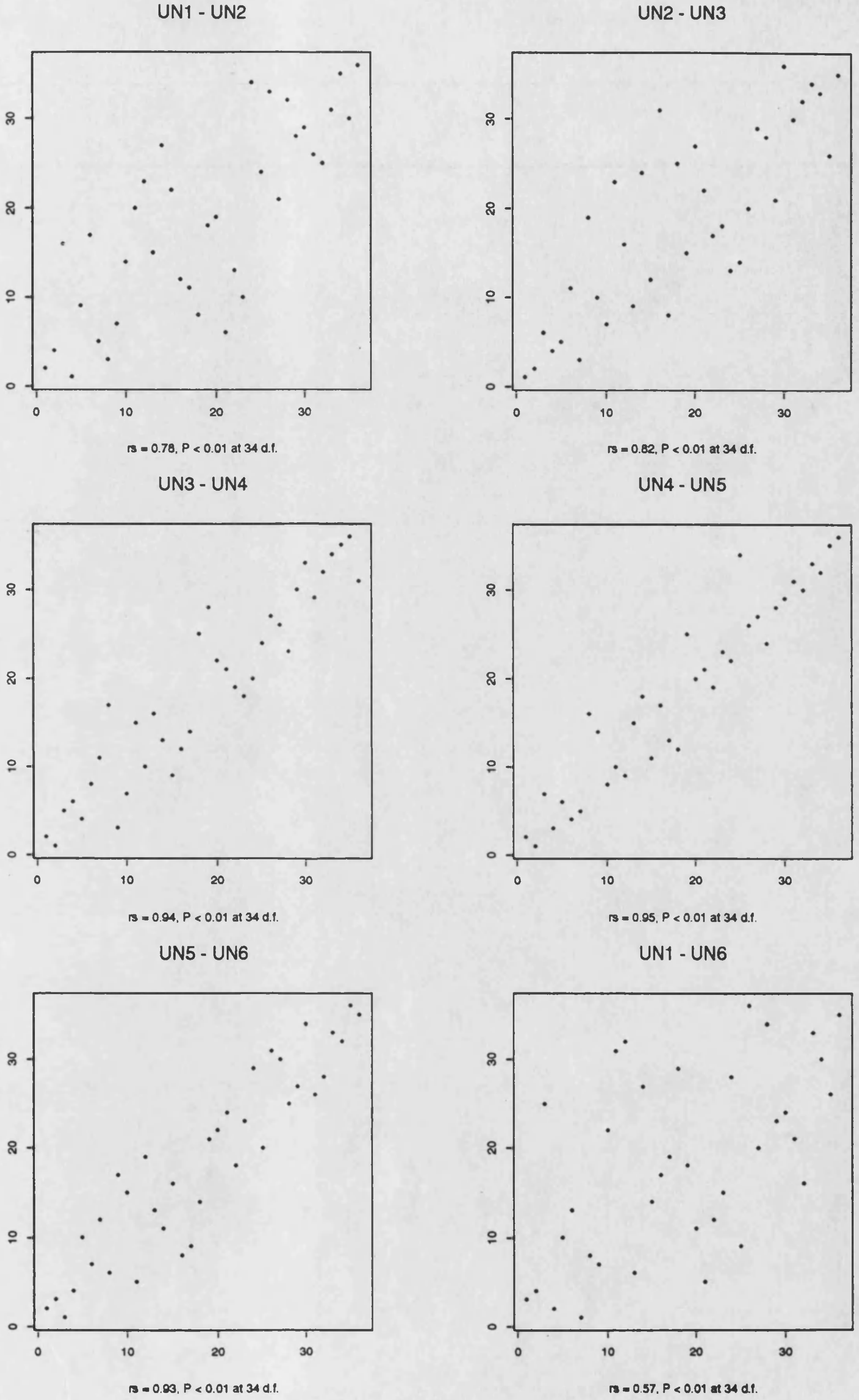


Figure 3.4: Correlations of ranked ant positions for successive sessions. Sessions BG91UN1-6

configurations of the colonies have changed during the six month observation period, though the correlation coefficients are still significant.

This pattern of change in spatial order during May - June, followed by little change in the rest of the summer is in agreement with the previously observed pattern of change in the median size of SFZ (for all the workers in the colony) over time (see Chapter 2). This result is to be expected on the basis of my definition of SFZ and spatial position as respectively the spread and median of a distribution - the bigger the spread (variance) of a distribution, the bigger the variance of the median. The result demonstrates that the annual colony expansion and contraction (see Chapter 2) is accompanied by instability in the colony spatial configuration during the expanded state and stability during the contracted state. What processes could account for this phenomenon? Does the phenomenon in effect represent the annual physiological cycle of the colony - the annual changes in ant activity levels? The annual physiological cycle would then comprise a rising expansion and instability after a colony comes out of hibernation in March, with a peak in May, followed by a gradual contraction and stabilisation of spatial configuration.

This idea should be treated with caution, as is suggested by the results from observations of another colony BG90UO (Fig. 3.5). In this case, the correlations between ranked brood care frequencies of ants for the second week in March and the third week in April as well as between the third week in April and the first week in June are not significant.

However, the median size of SFZ in BG90UO for these two weeks was small (see Chapter 2). A possible explanation for this discrepancy with the established trend is that in the case of colony BG90UO the results are based on compar-

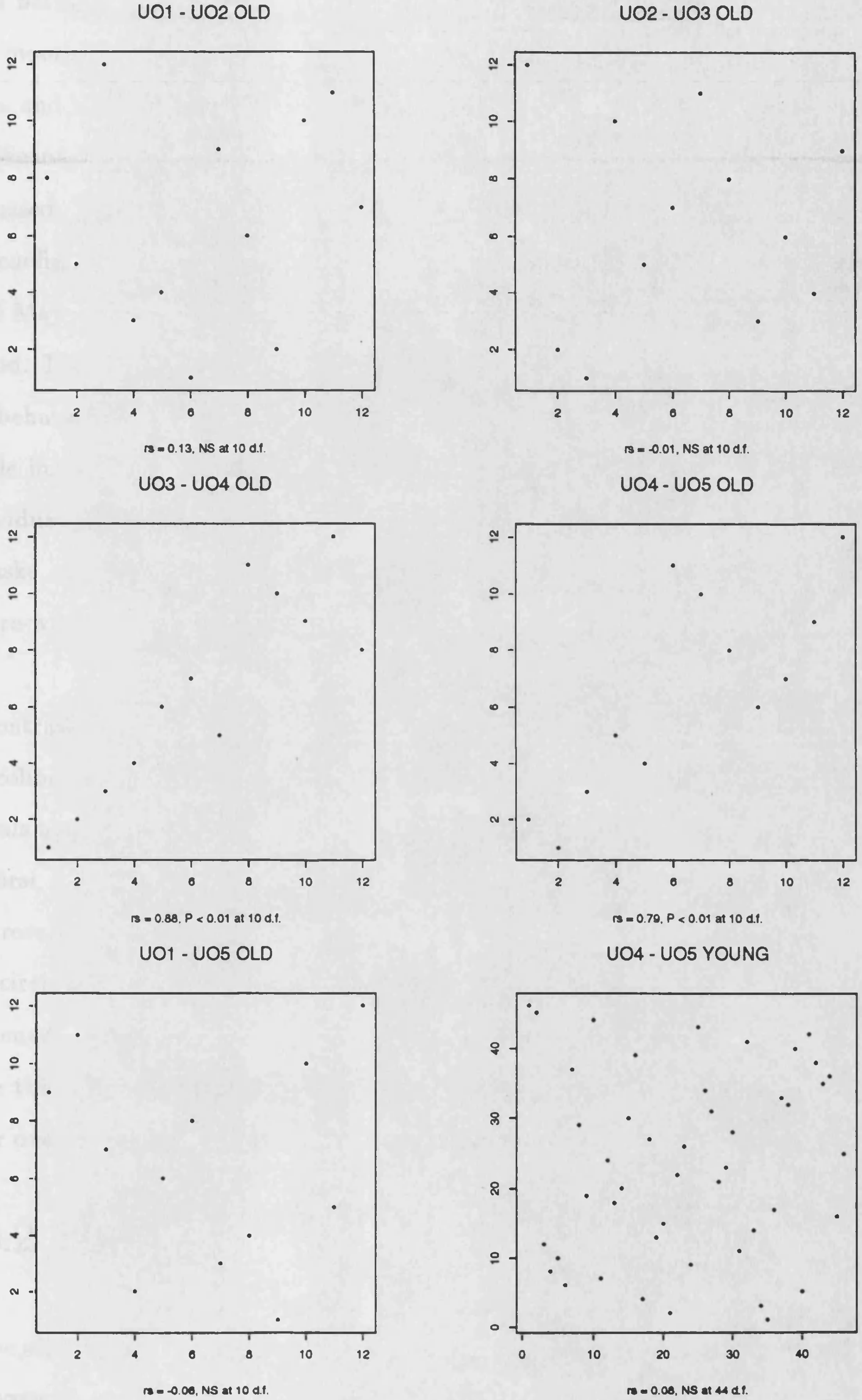


Figure 3.5: Correlations of ranked ant positions for successive sessions. Sessions BG90UO1-5

isons between weeks, which are separated in time, rather than between successive months. However, the correlations between the first and second week in June, and between the second week in June and the first week in September are significant, thus following the trend for the six months observations, previously discussed. The results for colony BG90UO imply that the instability of the spatial configuration begins immediately after hibernation (in March) and persists until May - it can not be judged whether it increases or decreases during this period. This behaviour of the colony spatial configuration then, would not follow the behaviour of the median size of SFZ. In other words, there can be considerable instability in the colony configuration even when the spatial positions of individual ants are not at their most variable. It is possible that a fresh allocation of tasks and positions begins when a colony comes out of hibernation, because all pre-winter experience has been erased (Lenoir 1979b).

In contrast to the cohort of ants that are at least one year old, the same year age cohort in colony BG90UO seemed to undergo a substantial reranking of individuals according to their spatial position between the second week in June and the first week in September. Some caution, however, is necessary in interpreting this result. The reranking may appear sharper than it would have been in normal circumstances, because the marked callows (their cuticle was not completely hardened) had been stressed and their activity thus diminished for a while. Despite this possible effect, the younger generation became intermixed with the older one by the end of September.

3.3.2 Relative Change of Behaviour

I have already established that the general category of ant-to-ant behaviours does not vary in relation to variation in spatial position (Chapter 2). Consequently, here I explore only ant-to-brood behaviours with the purpose of demonstrating that change in spatial position is related to change in behaviour and hence change in task. In colony BG91UL (Fig. 3.6) the rank correlations between all successive months are significant as well as the correlation between May and October, as was the case for correlations of ranked spatial positions (Fig. 3.2). The same is true for colonies BG91UM (Fig. 3.7) and BG91UN (Fig. 3.8) except for the lack of correlation between May and October (BG91UN), and between September and October, May and October (BG91UM). Brood care behaviour in BG90UO (Fig. 3.9) also follows the trend for spatial positions. In all cases the correlations for brood care behaviour ranks are less exact than those for positions. However, it should be stressed that the sample sizes for the behaviours are quite small (see Appendix). Therefore, several ants could have the same rank, the continuum is uneven, and the correlation picture is less clear.

3.3.3 Cumulative Relative Change of Location

Exploring change in the colony spatial configuration has already demonstrated that individual ants do not necessarily finish where they have started relative to their nestmates. To illustrate this point better, in Figures 3.10 and 3.11 the cumulative positional ranks of ants are plotted in order of the ants' initial positions. These plots show how the cumulative positional rank of an ant does not correspond to her initial positional rank. This is important evidence against the hypothesis of age polyethism, because if individual ants change positions unevenly, i.e. with different quantity and at different rate, different age cohorts will eventually intermix.

3.3.4 Absolute Change of Location

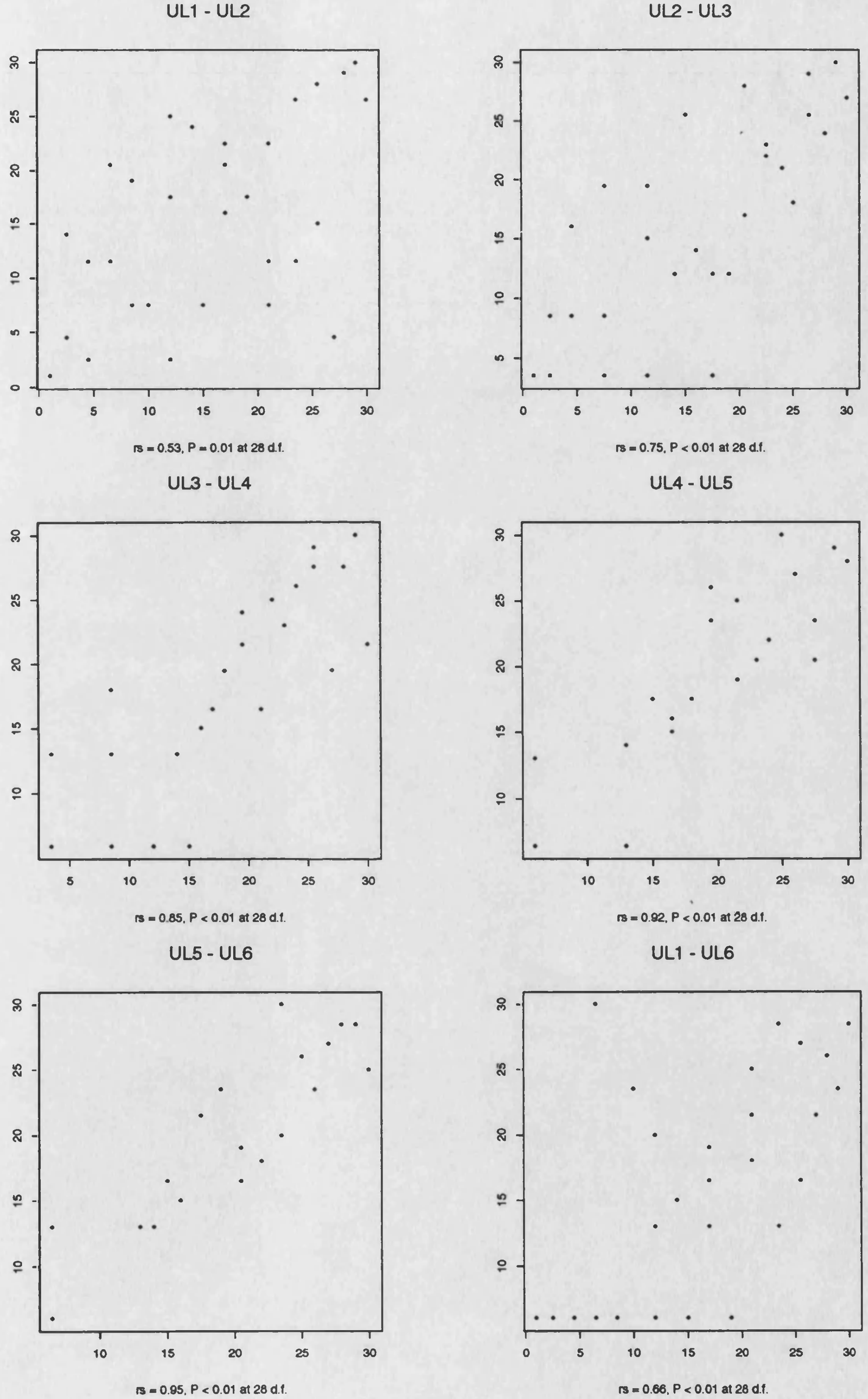


Figure 3.6: Correlations of ranked brood care frequencies of ants for successive sessions. Sessions BG91UL1-6

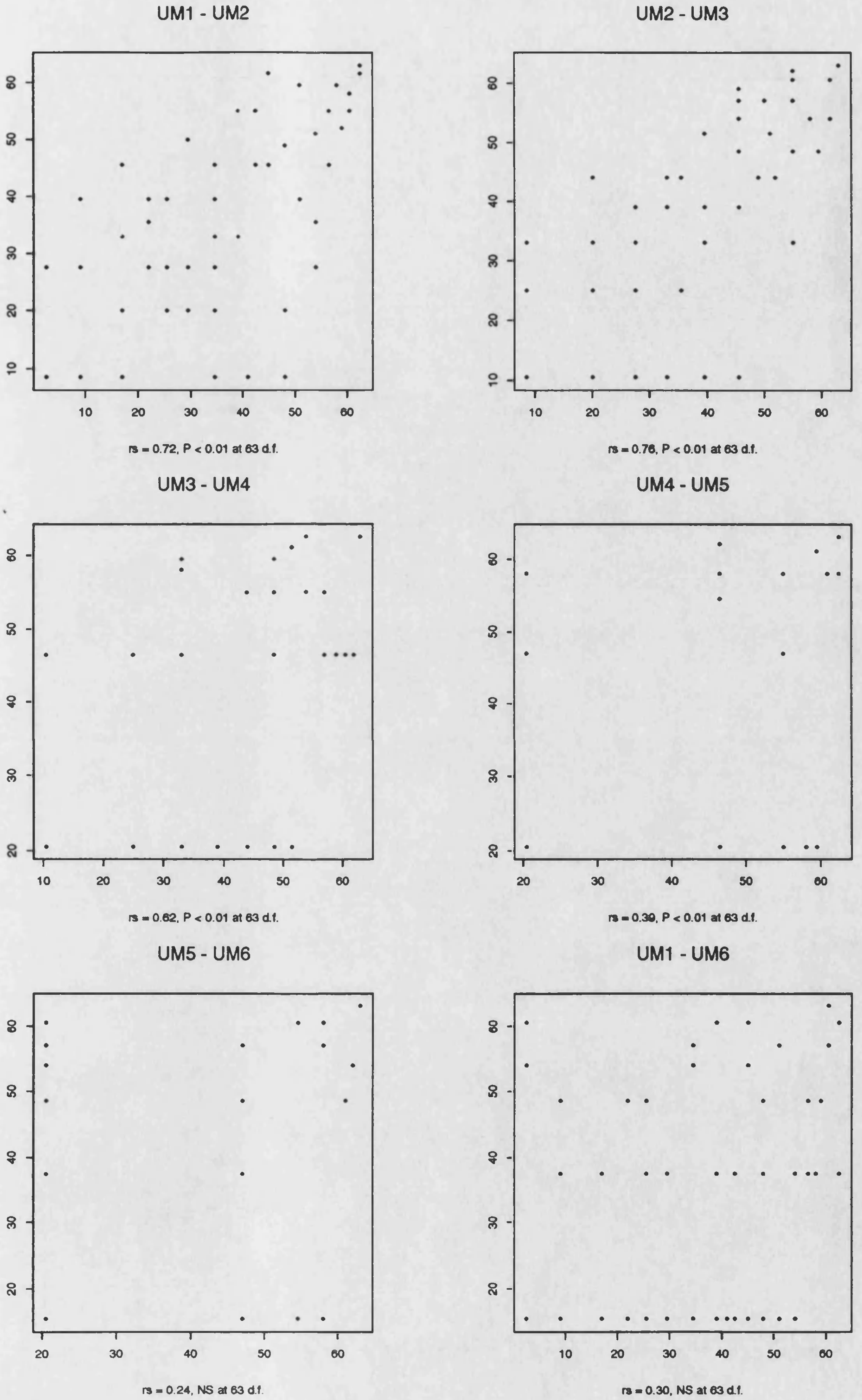


Figure 3.7: Correlations of ranked brood care frequencies of ants for successive sessions. Sessions BG91UM1-6

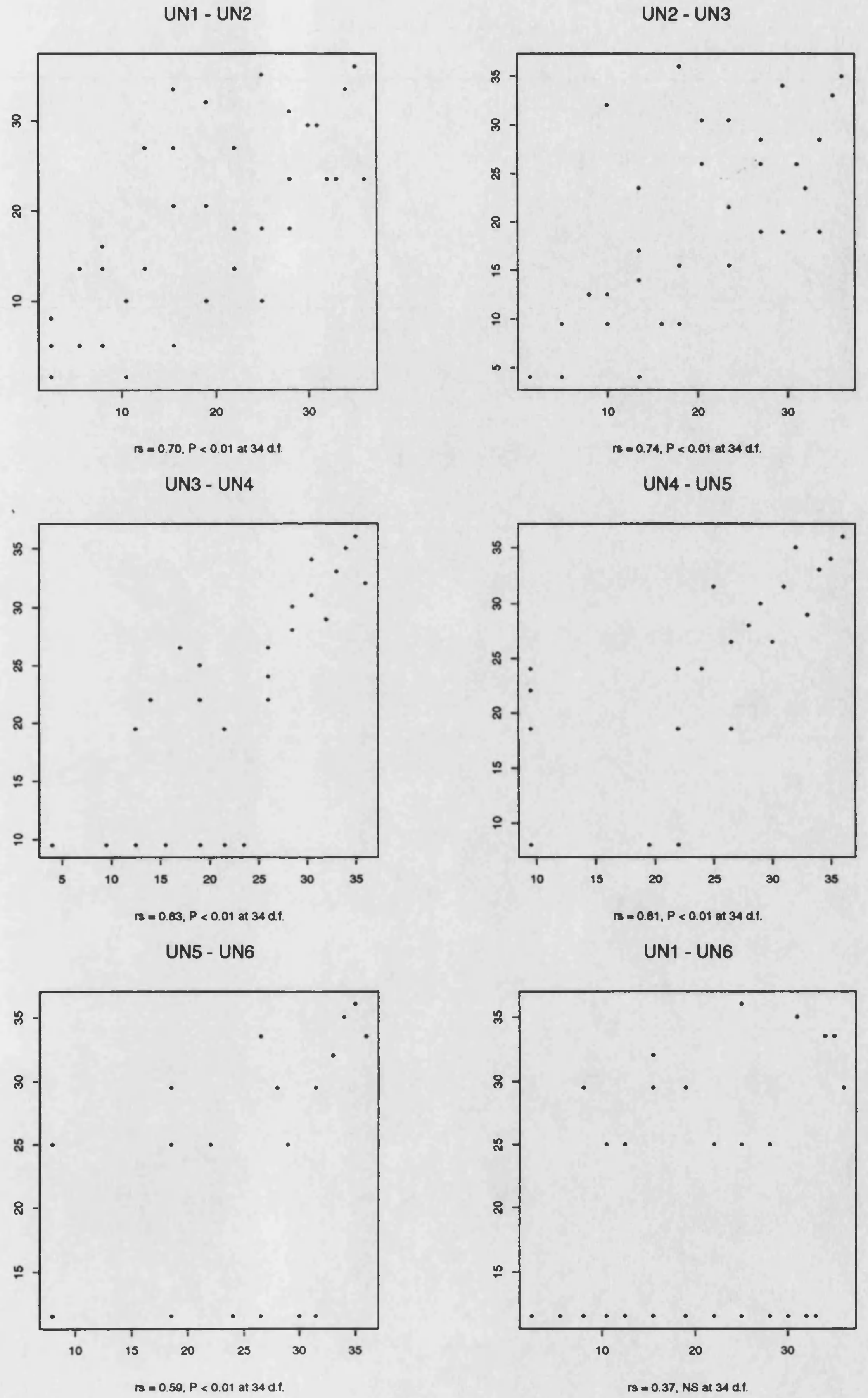


Figure 3.8: Correlations of ranked brood care frequencies of ants for successive sessions. Sessions BG91UN1-6

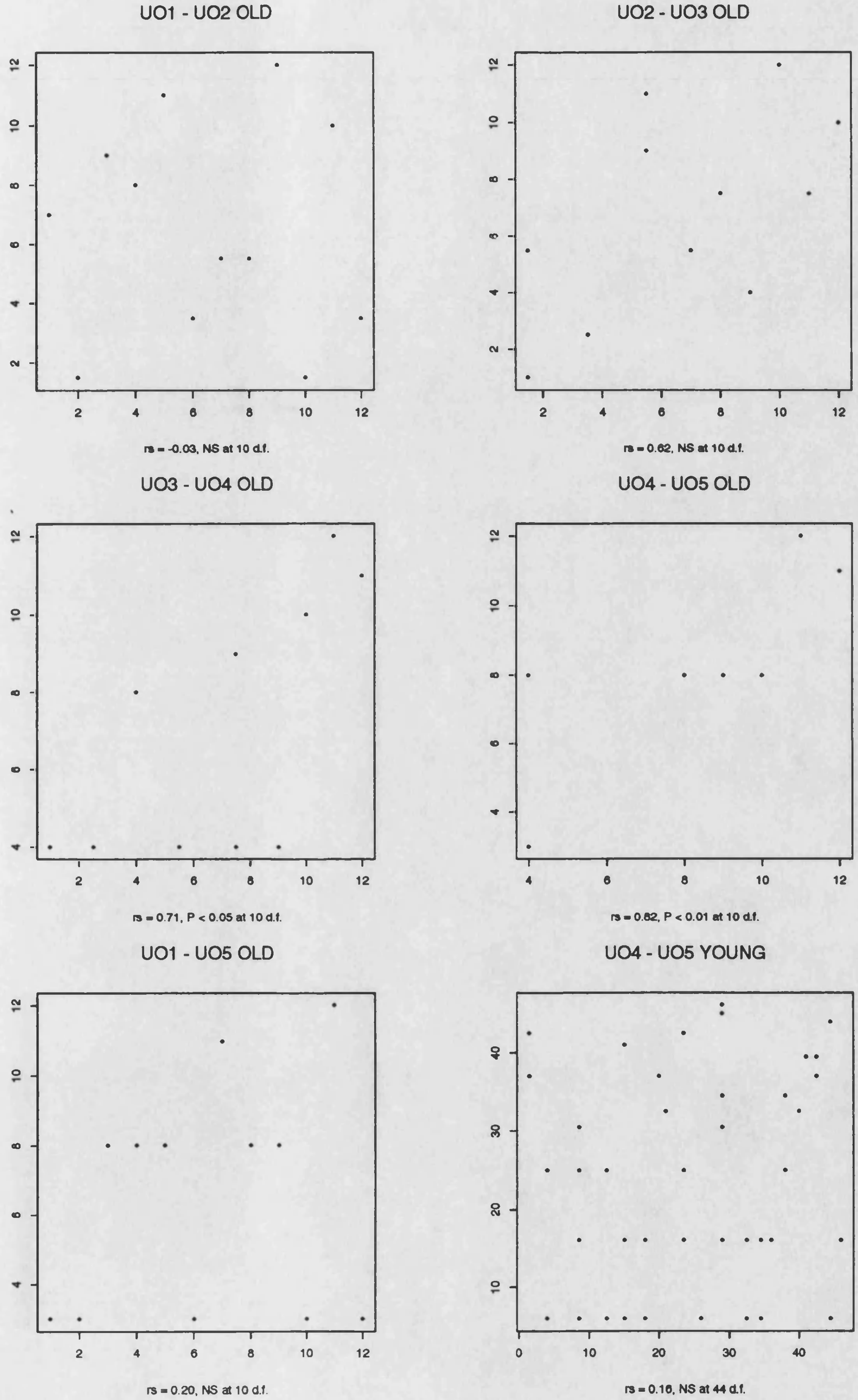


Figure 3.9: Correlations of ranked brood care frequencies of ants for successive sessions. Sessions BG90UO1-5

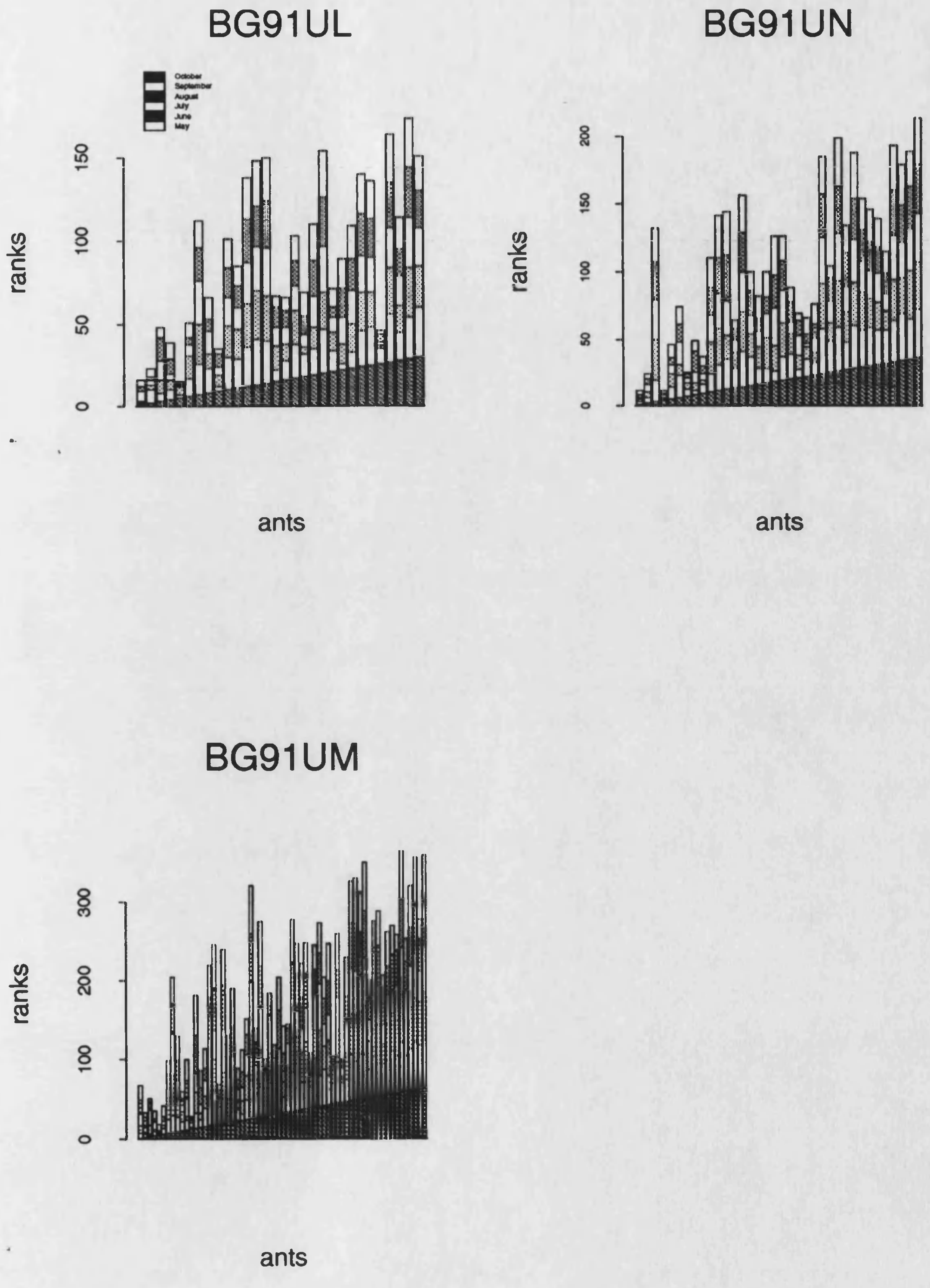


Figure 3.10: Cumulative positional ranks. Ants are ordered according to initial position. Colonies UL, UM, UN

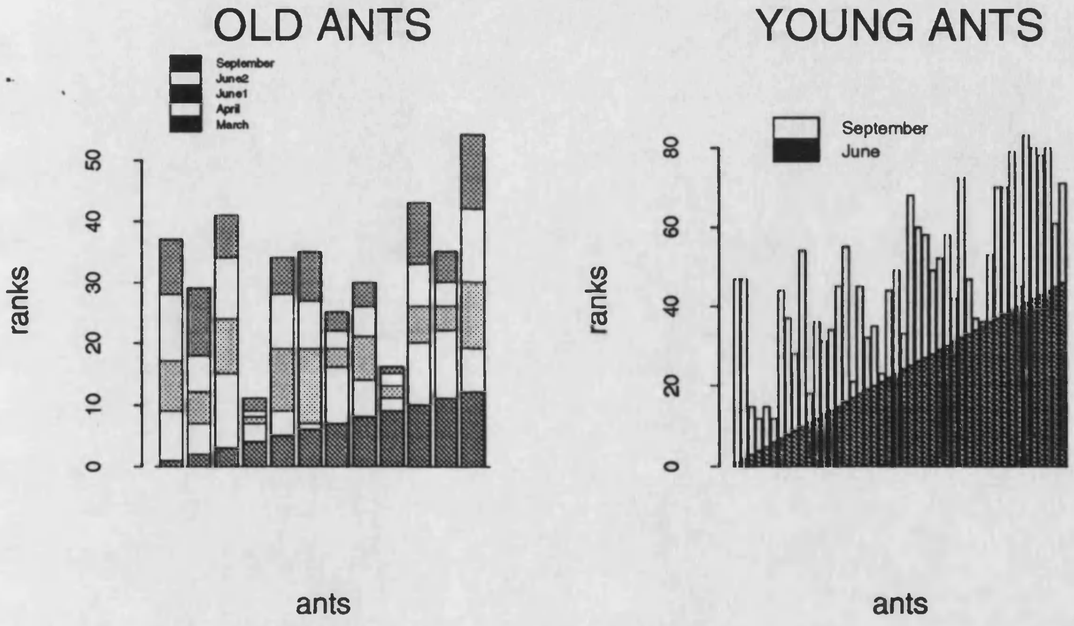


Figure 3.11: Cumulative positional ranks. Ants are ordered according to initial position. Colony BG90UO

BG91UL

Sessions	Further no (%)	Retained Position no (%)	Nearer no (%)	Total no
ul1 - ul2	6 (20)	8 (27)	16 (53)	30
ul2 - ul3	0 (0)	8 (27)	22 (73)	30
ul3 - ul4	0 (0)	18 (60)	12 (40)	30
ul4 - ul5	11 (37)	18 (60)	1 (3)	30
ul5 - ul6	3 (10)	26 (87)	1 (3)	30
ul1 - ul6	0 (0)	3 (10)	27 (90)	30

Table 3.4: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

A. The analysis of relative changes in the locations of workers does not reveal the identities of those ants that are responsible for them. This raises a number of questions: Are the ants that change their positions always the same individuals? Do they always move positions in the same, centrifugal direction? Does a change in position correspond to a change in SFZ? Are annual changes in the colony spatial configuration merely due to an annual trend of variability in spatial position within one and the same SFZ or to a shift of the SFZ itself? In an attempt to explore these problems I have selected a criterion for a shift in an individual's SFZ (see section 3.2.3. for justification). Tables 3.4-3.8 contain the proportions, as well as the numbers of ants that have shifted in either direction or retained their SFZ between respective sessions.

The percentage of "shifts" is more or less as would be expected from the trend for the correlation of relative positions in all four of the investigated colonies, i.e. the percentage of "shifts" is greater when the correlation is smallest. This confirms the supposition that the changes in the colony spatial configuration are not simply a result of the annual expansion and contraction of the SFZs, but are due to shifts of the SFZs of individual ants. There is not any immediately obvious relationship between the shifts in either direction and the time of year. In all of the three

BG91UM

Sessions	**Further** no (%)	**Retained Position** no (%)	**Nearer** no (%)	**Total** no
um1 - um2	12 (19)	37 (59)	14 (22)	63
um2 - um3	14 (22)	42 (67)	7 (11)	63
um3 - um4	7 (11)	46 (73)	10 (16)	63
um4 - um5	1 (2)	34 (54)	28 (44)	63
um5 - um6	0 (0)	41 (65)	22 (35)	63
um1 - um6	13 (21)	14 (22)	36 (57)	63

Table 3.5: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

BG91UN

Sessions	**Further** no (%)	**Retained Position** no (%)	**Nearer** no (%)	**Total** no
un1 - un2	8 (22)	21 (58)	7 (20)	36
un2 - un3	0 (0)	11 (31)	25 (69)	36
un3 - un4	5 (14)	28 (78)	3 (8)	36
un4 - un5	7 (19)	29 (81)	0 (0)	36
un5 - un6	2 (6)	27 (75)	7 (19)	36
un1 - un6	5 (14)	11 (31)	20 (55)	36

Table 3.6: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

BG90UO - OLD ANTS, FULL SESSIONS

Sessions	**Further** no (%)	**Retained Position** no (%)	**Nearer** no (%)	**Total** no
uo1 - uo2	4 (33)	7 (59)	1 (8)	12
uo2 - uo3	7 (58)	3 (25)	2 (17)	12
uo3 - uo4	4 (33)	7 (59)	1 (8)	12
uo4 - uo5	4 (33)	7 (59)	1 (8)	12
uo1 - uo5	9 (75)	1 (8)	2 (17)	12

Table 3.7: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

BG90UO - YOUNG ANTS, FULL SESSIONS

Sessions	Further no (%)	Retained Position no (%)	Nearer no (%)	Total no
uo4 - uo5	28 (60)	9 (20)	9 (20)	46

Table 3.8: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

colonies observed for six months the trend is to have more shifts nearer to the centre as opposed to further from the centre for nearly all of the comparisons. This trend is continued in the comparisons between the first and last months of observation, May and October, where the number of ants that have shifted closer to the centre is bigger than those in the other two categories together. However, the number of ants that have moved further away from the centre between May and October increases with increase in colony size (the colonies in order of increasing colony size are: BG91UL, BG91UN, BG91UM) (see Tables 1.2-1.5). Thus, we can speculate that young workers displace their older nestmates, although it is difficult to establish whether the greatest proportion of shifts in any of the two directions coincides exactly with the greatest influx of callows (see Table 1.4 for colony population censuses).

The results for both the young and the old ants in colony BG90UO are quite different from those discussed above. For all comparisons the percentage of ants that have moved further away from the centre is bigger than the percentage of ants that have moved nearer to the centre. It is difficult to believe that this disparity is due to the difference in the sampling method and at the same time - impossible to estimate its significance and importance.

The long-term positional analysis of these four colonies demonstrates that ants can move inwards as well as outwards. This contradicts the theory of centrifugal age polyethism, which predicts that with time ants will shift from the centre outwards, following a rigidly determined task programme, whatever the global condition of the colony (number of brood, size of the new generation, etc.).

B. The representation of the shifts data in terms of percentages (Tables 3.4-3.8) does not reveal any information about the possible relationship between an ant's position and whether or not it will undergo a shift of SFZ. From the data on successive months (Figs. 3.12-3.15) it appears that in colonies BG91UL, BG91UM, and BG91UN the ants from the half of the colony, that is nearer to the centre, account for all shifts away from it in the comparisons between the first and second months of observation (May-June).

In general, however, it is not the ants from any particular part of the colony that are shifting consistently or shifting in a consistent direction. The comparisons between the first and last month of observation lead to the same conclusion, except for colony BG91UM. Here the ants from the half of the colony that is nearer to the centre 2 for the shifts away from it.

3.3.5 Cumulative Relative Change in Individual Counts Outside the Nest

So far, I have examined only the situation inside the nest. Now I consider the relationship between the internal and the external ants. What governs the event of an ant to become an external worker? It has already been established (Chapter 2) that during short observation periods those ants, with positions beyond certain distance from the centre, are more likely to become external. Figures 3.16-3.20 show that this pattern is maintained over much longer periods of observation, and we see that ants that have already been outside the nest are more likely to leave it

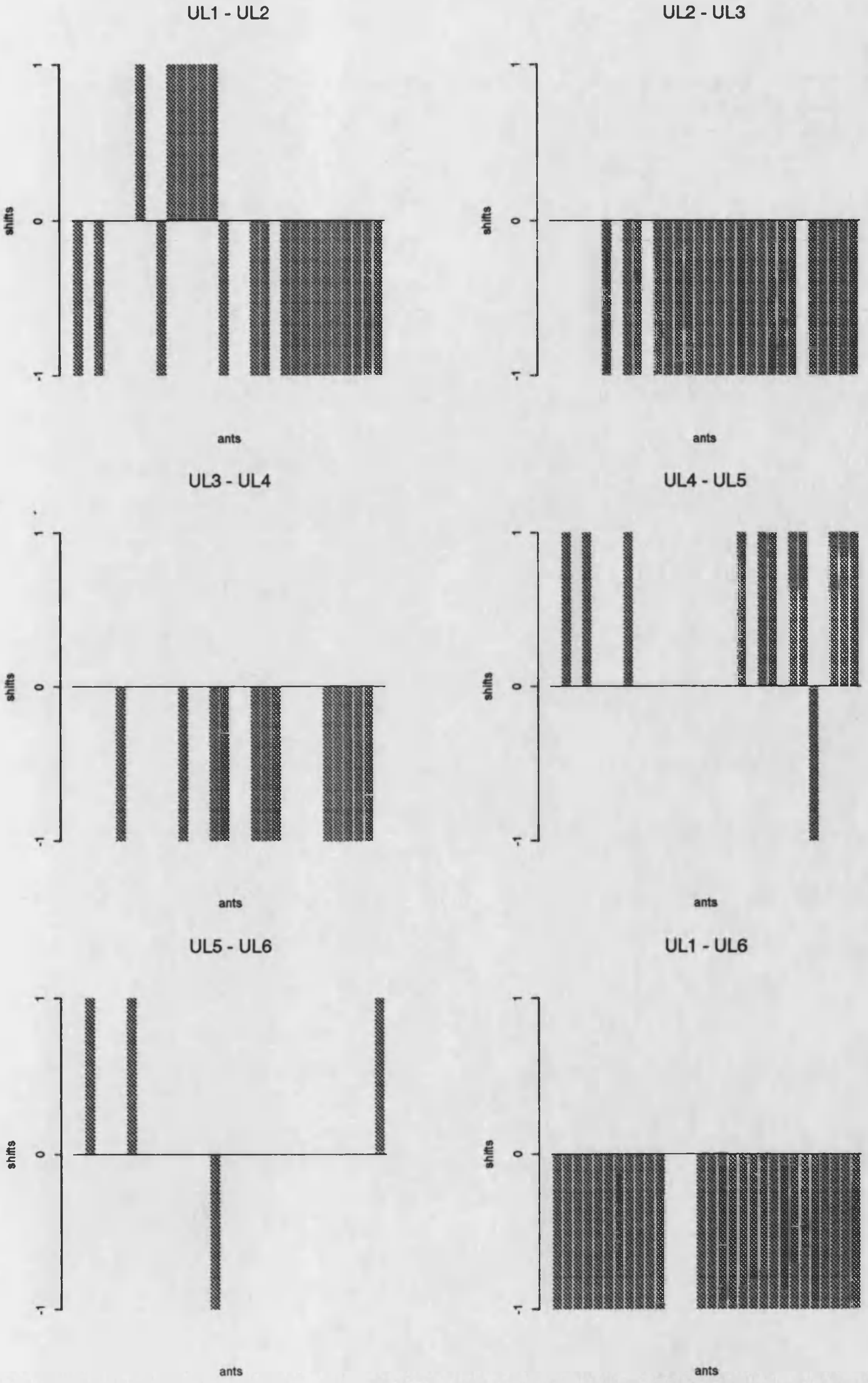


Figure 3.12: Ant shifts for successive sessions. Ants are ordered according to initial position; 1- further, 0 - no shift, -1 - nearer. Colony BG91UL

Figure 3.13: Ant shifts for successive sessions. Ants are ordered according to initial position; 1- further, 0 - no shift, -1 - nearer. Colony BG91UM

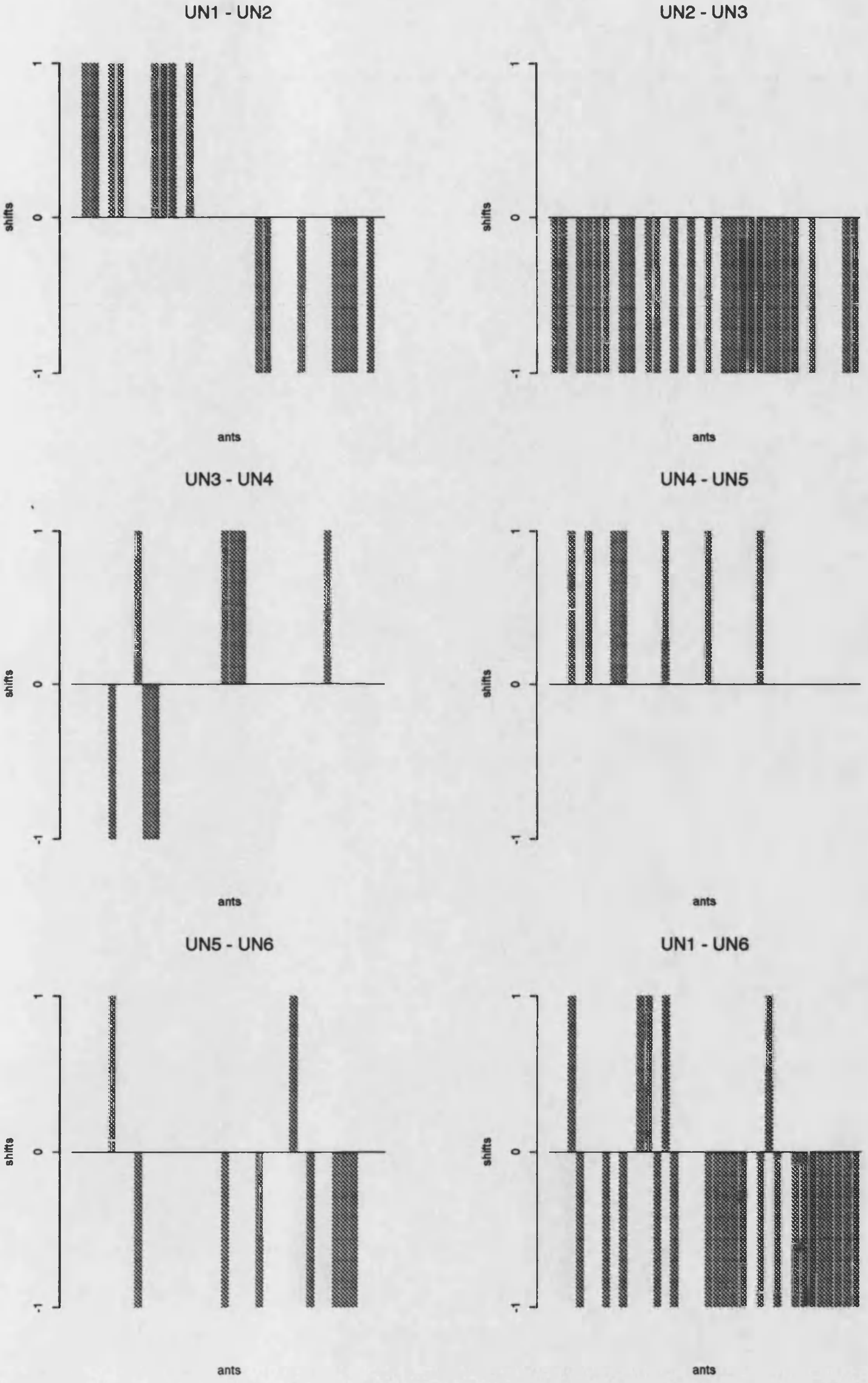


Figure 3.14: Ant shifts for successive sessions. Ants are ordered according to initial position; 1- further, 0 - no shift, -1 - nearer. Colony BG91UN

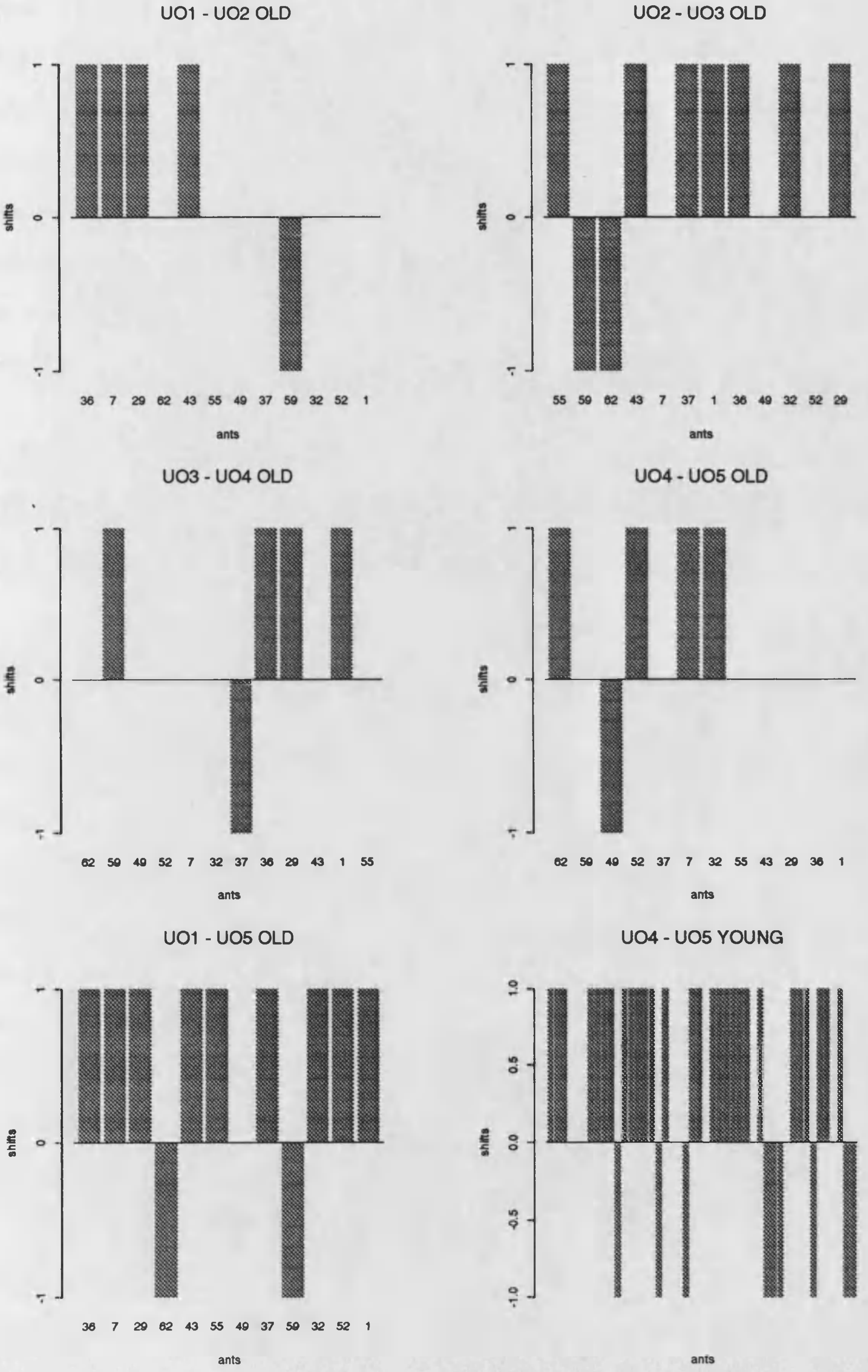


Figure 3.15: Ant shifts for successive sessions. Ants are ordered according to initial position; 1- further, 0 - no shift, -1 - nearer. Colony BG90UO

again. The only exception is made by the old ants in colony BG90UO (Fig. 3.19) where the association between position near the exit and greater likelihood of leaving the nest does not appear to hold. A possible explanation is that the final relative positions of the old ants are quite different from their starting positions in March, even in comparison to the same difference in the three colonies observed from May to October. This explanation is in agreement with the hypothesis that the colony spatial configuration is very unstable immediately after hibernation (see section 3.3.1).

3.3.6 Relative and Absolute Change of Location after the Removal of (a) 1/3 of the Workers and (b) all the Brood except the Eggs

Colony spatial configuration did not change sharply after either type of interference. The change in colony spatial configuration is not substantial because the correlations between the ranked positions of the ants before and after the interference are significant in either case (Fig. 3.21). However, the scatter in the correlation is much greater for colony BG92U1 (removal of ants) than for colony BG91US1 (removal of brood). In this case, the greater scatter corresponds to an expansion in the sizes of the ant SFZs, because as established in Chapter 2, after the removal of a third of the ants in colony BG92U1, there was a significant increase in the median size of SFZ of the remaining nestmates. The removal of brood, on the other hand, led to a greater variance in the size of SFZ among workers.

The significance of the result for absolute change in position (Table 3.9) is impossible to evaluate without replication of the experiments. However, it is possible to compare the single experimental colonies with undisturbed ones. The data for

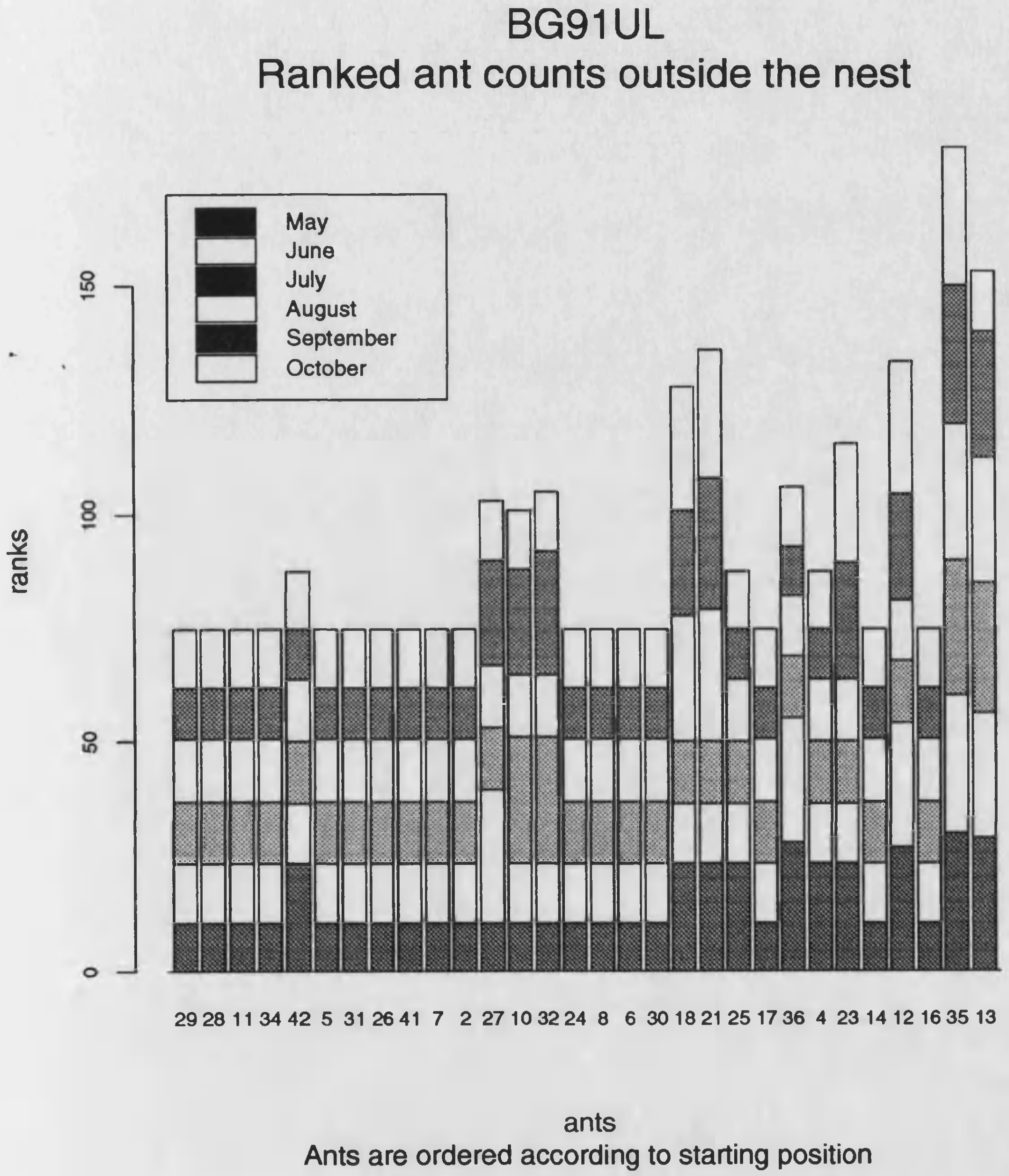


Figure 3.16: Cumulative ant counts outside the nest. Ants are ordered according to initial position. Colony BG91UL

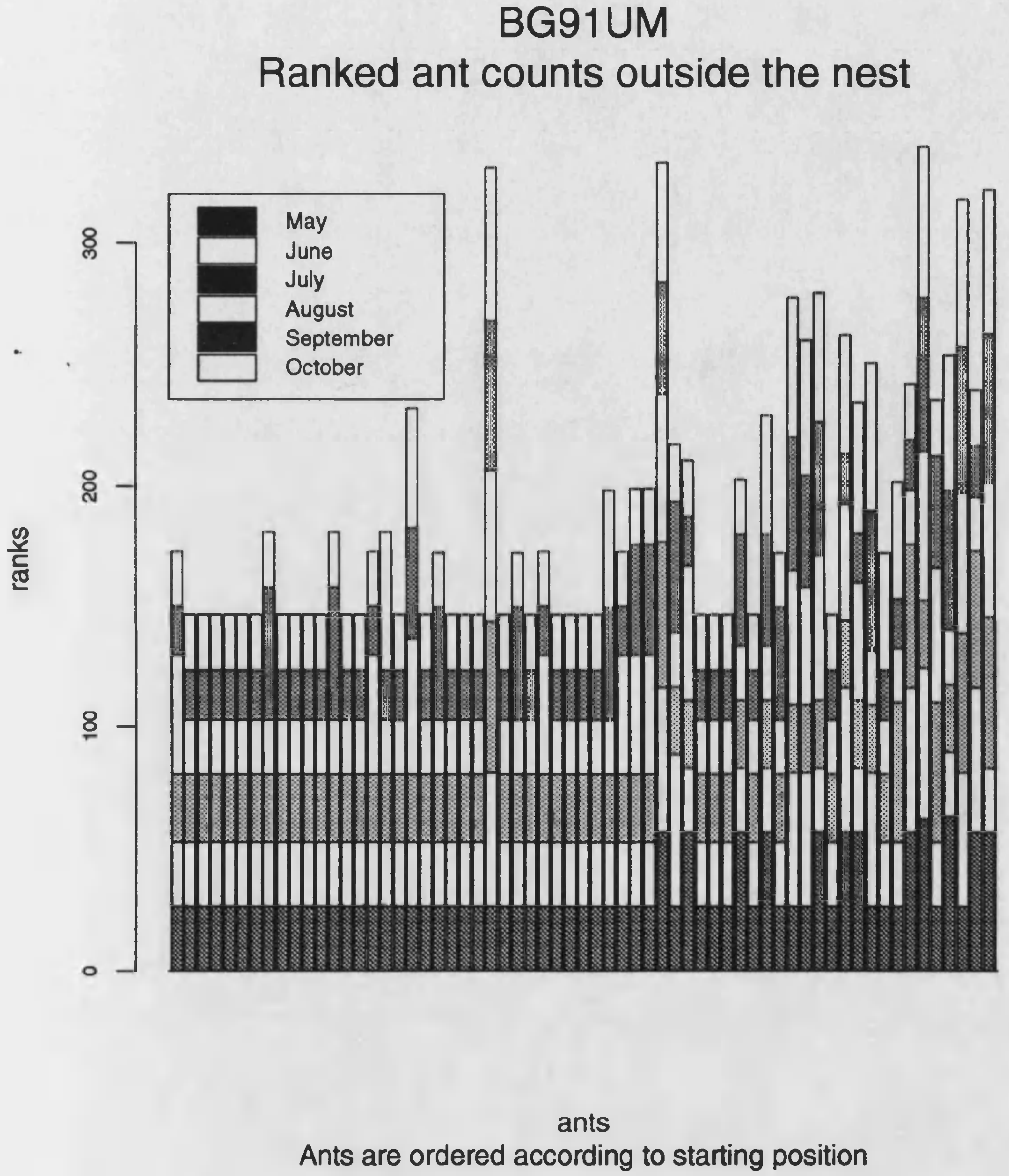


Figure 3.17: Cumulative ant counts outside the nest. Ants are ordered according to initial position. Colony BG91UM

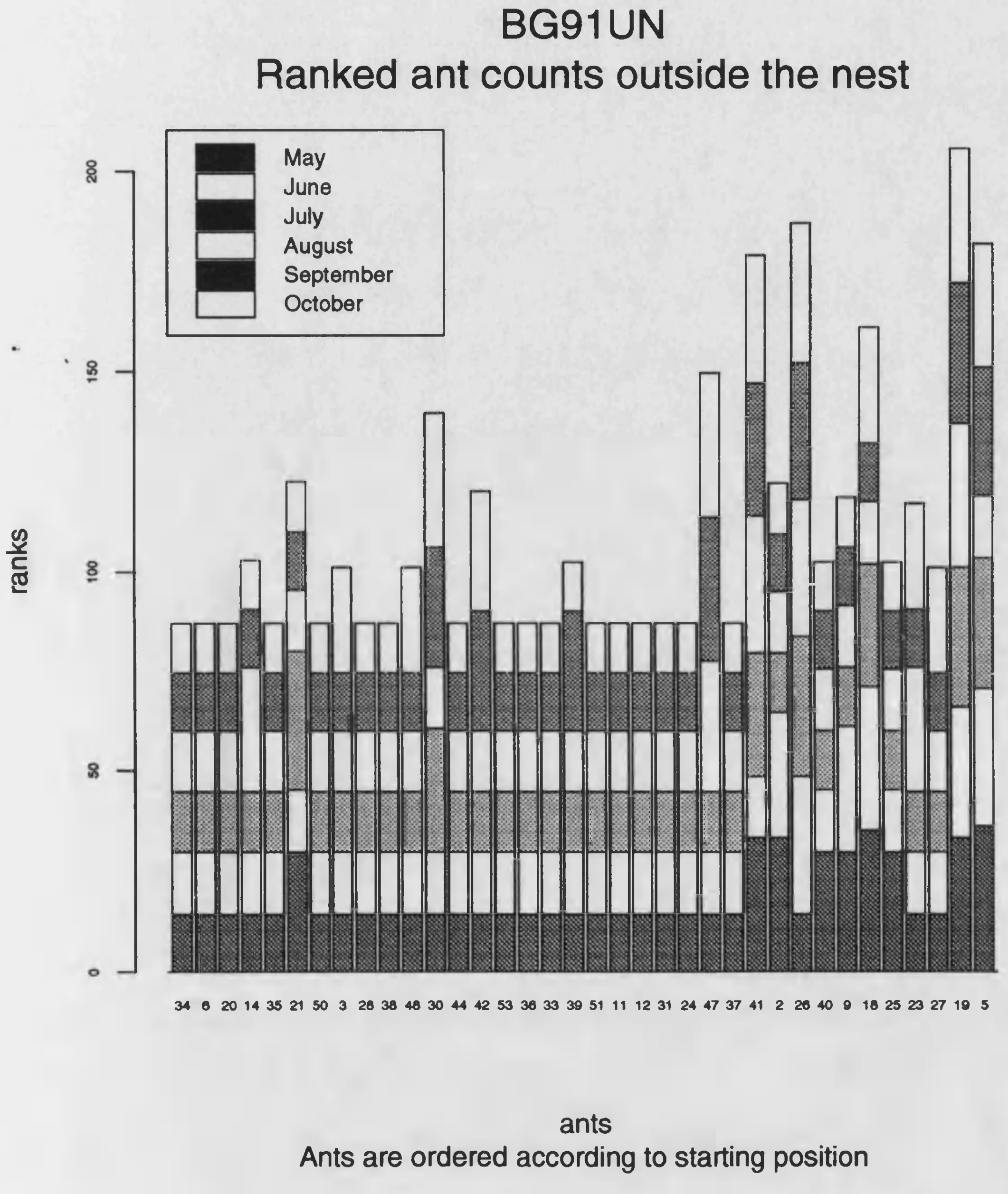


Figure 3.18: Cumulative ant counts outside the nest. Ants are ordered according to initial position. Colony BG91UN

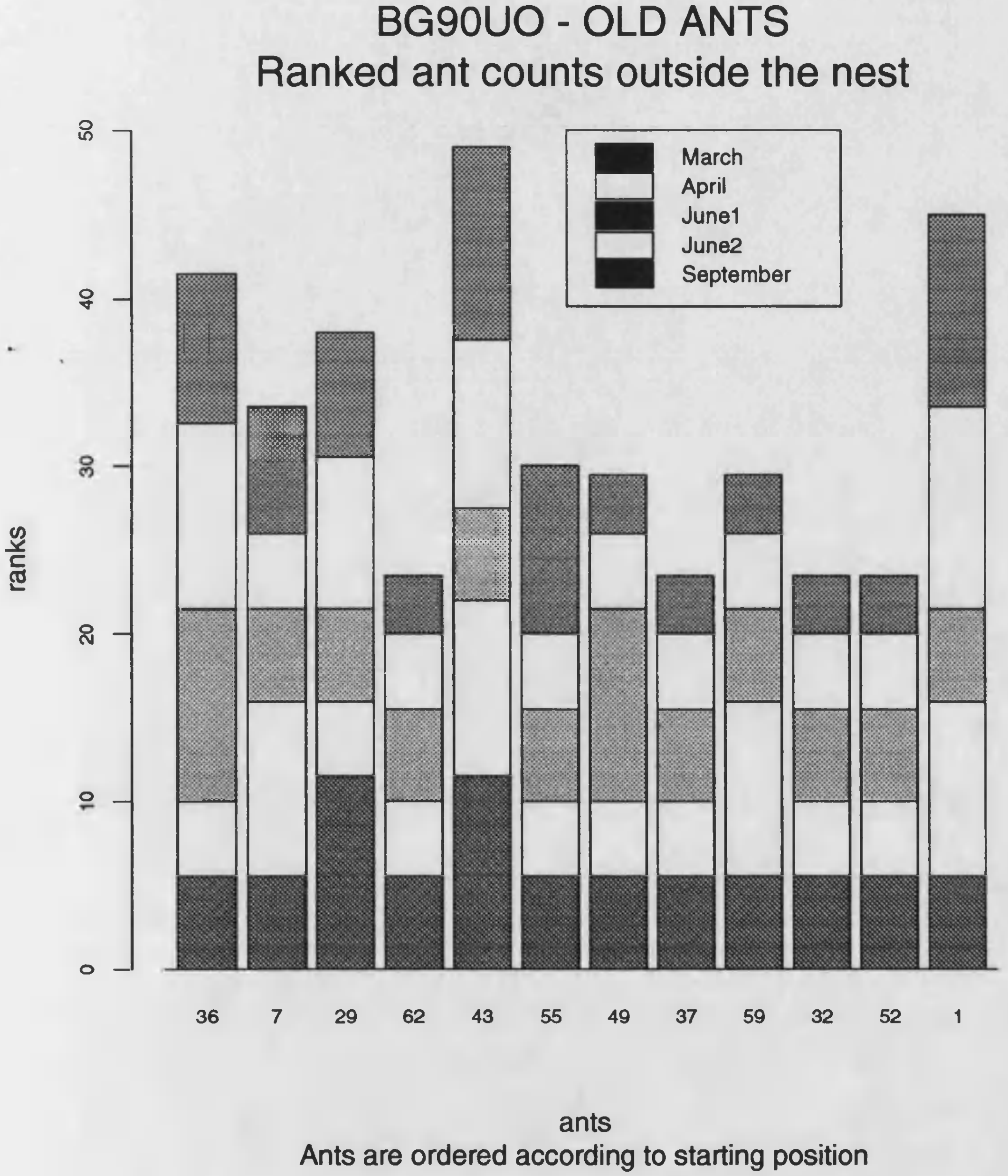


Figure 3.19: Cumulative ant counts outside the nest. Ants are ordered according to initial position. Colony UO - old

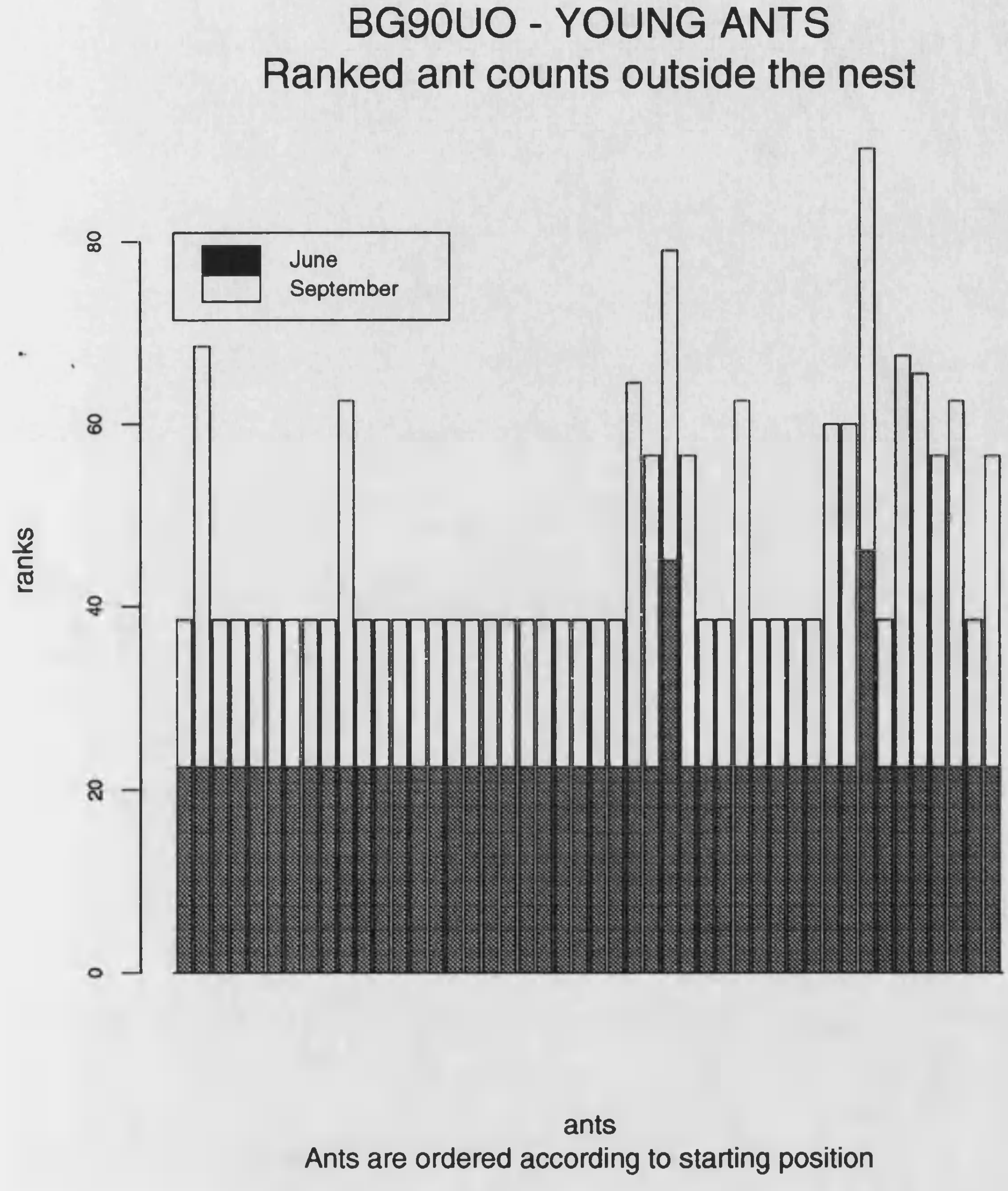
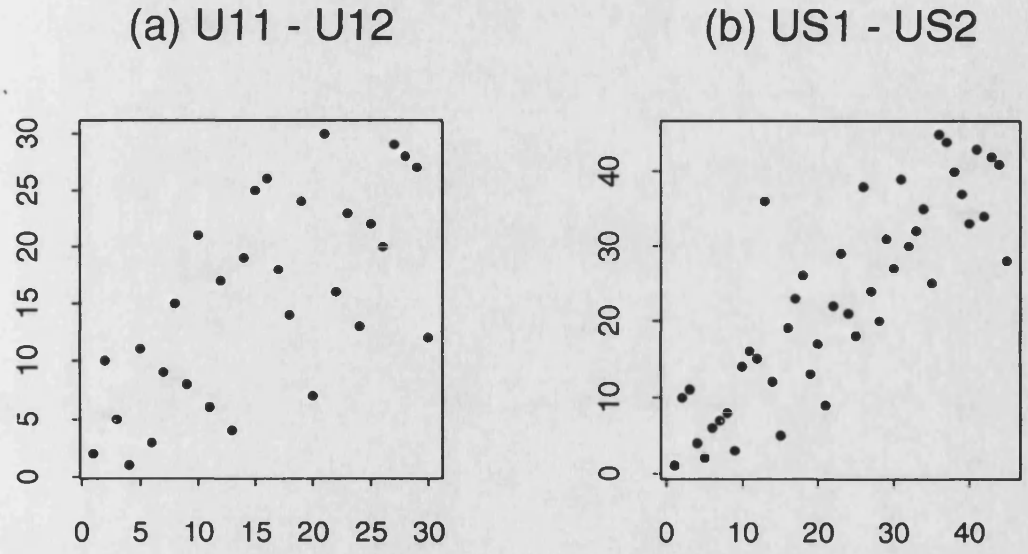


Figure 3.20: Cumulative ant counts outside the nest. Ants are ordered according to initial position. Colony UO - young


(a) U11 - U12

(b) US1 - US2

rs = 0.67, P < 0.01 at 28 d.f.

rs = 0.86, P < 0.01 at 43 d.f.

Figure 3.21: Correlations of ranked ant positions for before and after the removal of (a) ants, (b) brood. Colonies U1, US.

BG92U1 and BG91US

Sessions	**Further** no (%)	**Retained Position** no (%)	**Nearer** no (%)	**Total** no
ul1 - ul2	6 (20)	20 (67)	4 (13)	30
us1 - us2	15 (33)	18 (40)	12 (27)	45

Table 3.9: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

HALF SESSIONS OF TYPE I

Sessions	**Further** %	**Retained Position** %	**Nearer** %
uo1a - uo1b	33	67	0
uo2a - uo2b	8	67	25
uo3a - uo3b	17	50	33
ui1a - ui1b	20	71	9
uj1a - uj1b	17	73	10
uq1a - uq1b	8	78	14
sum total	103	406	91
mean	17.17	67.67	15.16
s.d.	9.24	9.59	11.92

Table 3.10: Comparisons between half sessions for undisturbed colonies

experimental colonies BG92U1 and BG91US was collected in sessions of type one, but for half the time (see 1.4.1.3). Table 3.10 shows the type and number of shifts between the first and second half of sessions of type one for undisturbed colonies. The comparison between the mean percentage and the standard deviation for each type of shift with the results in Table 3.9 shows that the proportion of ants showing shifts for colony BG92U1, after removal of ants, are not very different from those for undisturbed colonies. However, the removal of the brood (colony BG91US) leads to an apparently different result; in this case more individuals shifted either nearer to or away from the centre of the colony.

Previous studies (Lenoir 1979a, McDonald and Topoff 1985) have shown that ants change their behavioural profiles after removal of either ants or brood. This is also the case in *L. unifasciatus.* This change in behaviour is best illustrated for the whole colony by the relative method. Correlations between the ranking of individuals by their frequency of brood care before and after the manipulation are significant for both experiments (Fig. 3.22). Nevertheless, many individuals have conspicuously changed their ranks. When some of the ants were removed, individuals which previously had the lowest rank (relative brood care frequency) moved up to a variety of ranks. Conversely, when brood was removed, ants with a variety of ranks in brood care frequency dropped down to the lowest rank (the lowest relative frequency).

The picture for the correlations of ranks based on outside-the-nest frequencies, is essentially the opposite (Fig. 3.23). Though the correlations are significant for both experiments, in the case of ant removal nestmates with a variety of ranks dropped down to the lowest rank. In the case of brood removal, some ants increased their ranks while the ranks of a few others dropped.

3.4 Discussion

This study demonstrates that when the organisation of the ant colony is examined over a period of time in the light of its spatial configuration and the positional movements of individual ants within it, ants are found to move their positions asynchronously which leads gradually to the intermixing of workers from the same year cohort with their older nestmates. This finding renders the notions of **temporal caste** and **temporal polyethism** meaningless.

3.4.1 Change in the Spatial Organisation of the Colony with Time

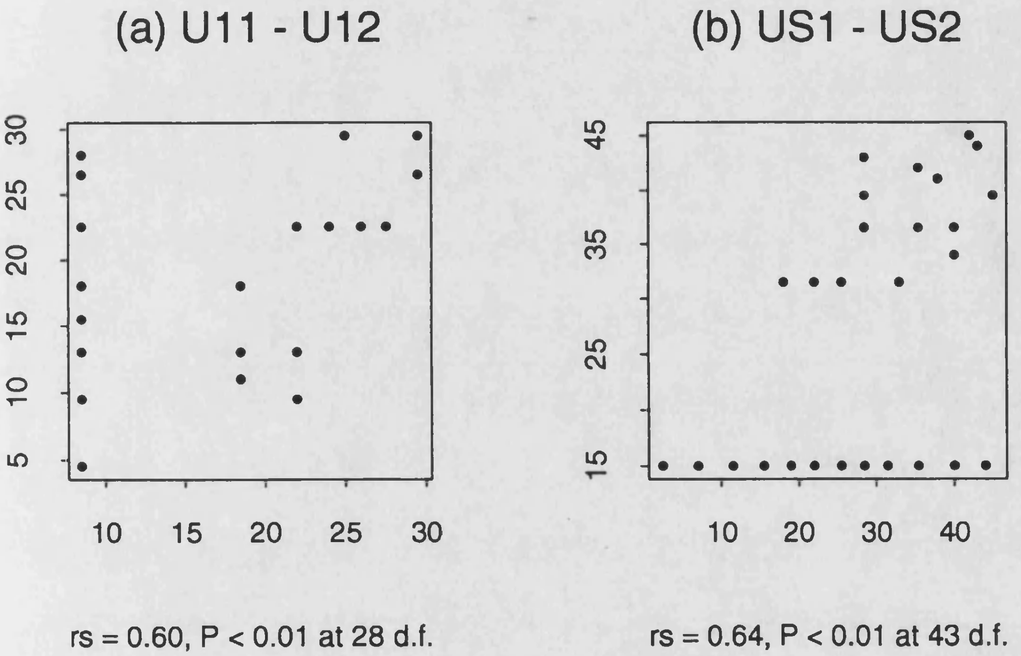


Figure 3.22: Correlations of ranked brood care frequencies of ants for before and after the removal of (a) ants, (b) brood. Colonies BG92U1, BG91US

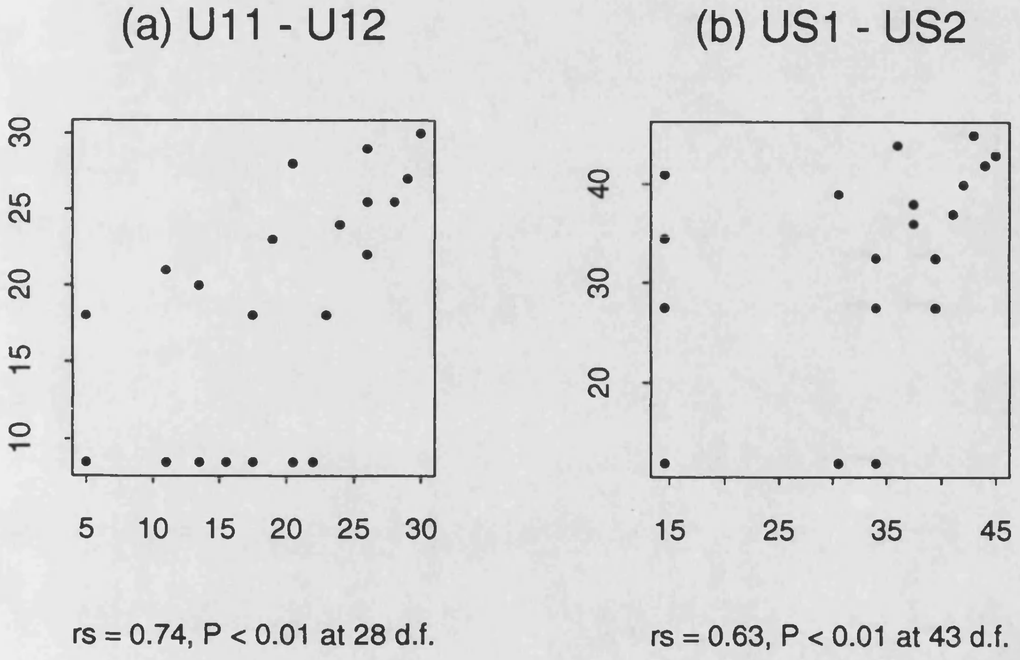


Figure 3.23: Correlations of ranked outside nest frequencies of ants for before and after the removal of (a) ants, (b) brood. Colonies BG92U1, BG91US

It has already been established (Chapter 2) that colonies start to expand soon after coming out of hibernation, probably reaching a maximum diameter sometime in May, and then gradually contract back before going into hibernation. This is a phenomenon that has not previously been documented though it should be obvious to the naked eye. Another characteristic phenomenon which should be anticipated, but has apparently been overlooked (possibly due to the methodology, including the choice of species, nest geometry, and above all the artificial standardisation of colony size for reasons of comparisons) is that the proportion of ants that shift away from the centre is smaller in colonies with a smaller population increase for the year than in colonies with a greater population increase. This finding is in agreement with the experimental results of McDonald and Topoff (1985) that eclosion of callows accelerates the behavioural development of the older workers. Whether this acceleration means a chemical stimulation or a mechanical displacement, or neither, still remains to be resolved.

The changes in colony spatial organisation 2 with changes in behaviour.

3.4.2 Changes in Positions and Behavioural Activities of Individual Ants

This study has demonstrated for the first time that even in undisturbed colonies the positions of some ants move backwards, i.e. towards the centre of the brood pile. It remains possible that the ultimate direction of movement of all ants is centrifugal, as predicted by the theory of centrifugal temporal polyethism (West-Eberhard 1979, 1981). However, continuous observations over a period of several years would be required to falsify or validate this theory. At present what is clear is that the progress of different individuals from the colony centre to outside the nest varies both quantitatively and qualitatively. In the present work, individual ant colonies were studied throughout one season of 6-7 months. Some ants retained their SFZs throughout this time, while others changed their positions. Still others moved and then returned to their original positions. If it is considered that spatial position corresponds to a particular type of behavioural activity, then the results of Calabi and Rosengaus (1988), obtained over much shorter periods of time, and involving only behavioural observations, may be considered similar. They found that individuals did not only differ at any age with respect to proportional performance of behaviours, but they differed absolutely with respect to whether those behaviours were performed at all.

The spatial approach adopted here also sheds new light on the relationship between "internal" and "external" workers - a discrimination first proposed by Otto (1958). Positional analysis makes clear that external workers are a natural extension of the spatial colony continuum. Once the position of an individual reaches a certain distance from the colony centre, it is significantly more likely to go out of the nest. As discussed in Chapter 2, this spatial continuum is not always present when colonies are confined to the geometry of artificial nests. The size of such a nest and the location(s) of its exit(s) are fixed and a colony may prove to be too small for the nest and too far from the exit(s). In such a case the individuals which remain faithful to the exit region are spatially separated from the colony. However, in more natural conditions, when the colony is given the opportunity to build its own nest walls, there is no discontinuity among the colony members. The existence of a spatial continuum explains what is seen to be an inconsistency with Wilson's (Wilson 1985b) notion of **role** as tasks grouped by the spatial proximity of their objects rather than similarity of behaviours. Calabi and Rosengren (1988) found a mix of internal and external behaviours in both internal and external workers, and concluded that being associated with either locale does not in itself constitute a role. If we consider that ants are spatially organised in a continuum of SFZs, then each individual ant will have a unique, spatially defined, behavioural profile. Thus, a worker near the colony centre would care exclusively for eggs and microlarvae, a worker with a position near the colony periphery would care for macrolarvae, but would also occasionally go outside the nest (perhaps just exploring). At the same time, a worker with a position on the colony periphery itself would not care for brood at all but would go outside the nest very frequently, exclusively for foraging.

3.4.3 Responses to Removal of Ants or Brood

The experimental removal of selected ants or the brood, as well as the perturbation caused by the emigration to a new nest involved in the experiment (see also Chapter 4) do not change significantly the spatial configuration of the colony or the relative positions of the remaining workers (in the case of ant removal). It is possible that ants are able to "recognise" their neighbours individually. This could explain how colony configuration can be reconstructed after a perturbation. A possible alternative hypothesis is that ants use the queen (whose SFZ is situated over the eggs, i.e. close to the colony centre) as an olfactory cue and simply remember their respective distances from her. It seems unlikely, however, that an olfactory gradient of this kind would provide a sufficient exact positional cue over such small distances within the nest. Evidently the brood is not essential for the colony reassemblage, because the reassemblage takes place even in its absence (except for the eggs). However, the brood must play a role in the spatial positioning of workers, because when it is not present the SFZs of the "brood workers" diminish in size and also shift.

An even simpler mechanism might be responsible for the restoration of the colony configuration - ants might sort themselves into position according to their activity levels. The most active individuals travel the greatest distances and are most likely to reach the periphery of the colony. The reverse is true for the least active ants which seldom go much further than the centre of the brood pile. At the same time the action of bumping into nestmates and gradual fixation onto a particular area would gradually restrict ants to individual zones. This hypothetical situation is supported by the experimental finding that when two species characterised by different activity levels are mixed together and establish a single colony, the members of the species with higher activity are found on the periphery of the colony (Hölldobler and Carlin, unpublished study, personal communication).

These three hypotheses are not of course mutually exclusive, and it is possible that more than one mechanism is involved in establishing and maintaining positions within the nest.

3.4.4 Implications for the Age Polyethism Hypothesis

Increasing evidence that great individual behavioural variability and flexibility are commonly observed within insect societies has so far led only to the modification but not to the rejection of the theory of age polyethism. Contradictions have been explained with differences in sensitivity thresholds to stimuli, depending on endogenous factors (individual behavioural ontogeny with genetic, hormonal, and learned components) and exogenous factors (colony labour needs) (Calabi 1988, Stuart and Page 1991, O'Donnel and Jeanne 1992, Robinson 1992). Calabi (1988) considers the caste distribution function and flexibility as adaptive expressions along a single continuum, but functioning and under selection on different time scales. However, it is difficult to imagine how this two-tiered selection process could work.

In any case, the notion of individual sensitivity thresholds and the appreciation of the influence of hormonal levels, genetic differences, learning, and environmental necessities (Robinson 1992) do not comprise a new theory for the fundamental rule(s) of how tasks are allocated. Although rarely stated clearly, the basic theoretical rule for task allocation remains the idea of "programmed changes in behaviour as a function of age" (Wilson 1985a, p. 307). Put another way, ants are deterministically allocated to tasks on the basis of some optimal plan for survival rather than tasks being probabilistically allocated to ants according to changing environmental requirements.

It is quite another matter that individual ants will be subject to increasing selection pressure to take on more altruistic tasks as their fertility declines or is totally absent. Leaving aside individual variation in fertility, the altruistic response to this selection pressure could be realised through simple behaviour (with both genetic and learned components). Without exploring simple mechanisms of this kind first, there is no justification in invoking complicated genetical programmes of action.

It is my view that the evidence of behavioural flexibility (an expression coined to imply that a preprogrammed temporal change in behaviour does exist) and great individual behavioural variability directly falsify the deterministic approach to task allocation. The present study goes a step further by demonstrating that there is not only great individual variability, but that this variability has an unexpected order. Every ant has her unique position and unique behavioural profile. If ants are distributed along a behavioural continuum, then attempts to regard individual variability as noise and the averaging of behavioural profiles are meaningless, and the notion of temporal caste has no significance.

3.4.5 The Foraging for Work Model of Task Allocation

The spatial approach adopted in the present work leads naturally to a description of *L. unifasciatus* colonies which is not dependent on the concept of age polyethism. In this view colonies are organised in a stable spatial configuration (or meshwork) that expands and contracts during the annual cycle with individual elements "vibrating" ("oscillating") with bigger or smaller amplitudes around their colony positions. New elements emerge from the centre of the meshwork and old elements are cast off on its periphery. At the same time some of the elements change absolute position within the meshwork moving either forward or away from the centre. This picture of colony organisation is consistent with a new, probabilistic model of task allocation, recently proposed by Tofts and Franks (Tofts 1991, Tofts and Franks 1992, Tofts 1993). In its basic form, this model supposes that ants constantly "forage" for work and that tasks are arranged linearly, like a production line. An ant attempts to take work from the right ("upstream") and to give it to the left ("downstream"). If this succeeds, the ant remains where she is. If it fails, after a certain number of failures for either direction, the ant moves in that direction with some probability. The simulation of this model demonstrates that temporal polyethism may be an emergent property of new ants joining the production line only at one end, rather than an underlying organisational principle.

Chapter 4

Colony Emigration - a Natural Perturbation of the System

4.1 Introduction

The movement of an ant colony from one nest site to another is a fundamental part of ant biology. Colony emigration is frequent in occurrence, vital to survival, and mediated by stereotyped techniques of communication and transportation that vary from one species to the next. So far, the exploration of this widespread phenomenon has been primarily aimed at revealing the functions and mechanisms of the many forms emigration takes, starting from the simple relocations of leptothoracines and finishing with the raids of slave-making and army ants. The stereotyped behaviours involved can even facilitate the determination of phylogenetic relationships among larger taxonomic groups of ants (Hölldobler and Wilson 1990, p. 284). The present chapter, however, is concerned with the division of labour that also occurs during colony emigration.

When the colony moves from one nest site to another some of the workers play an **active** part by transporting brood and nestmates while others are **passive** -

they arrive alone at the new nest or are transported there by a nestmate. It is the generally favoured view that the active participants that organise emigration form a minority and consist of workers who are either "elites", i.e. individuals who work hard at many tasks, or moving specialists - workers with the exclusive task of carrying out the emigration (Hölldobler and Wilson 1990, p. 284). In support of this view Abraham and Pasteels (1980) found that in the genus *Myrmica* transport is performed by a group of old workers. Möglich and Hölldobler (1974) report that in *Formica sanguinea* and *Camponotus sericeus* there exists a group of moving specialists, composed of certain older external workers. The same was found to be true for *Formica fusca* (Möglich and Hölldobler 1975). In order to demonstrate the existence of specialisation the authors took away the moving specialists. As a result, nestmates replaced the removed workers but the time needed for emigration was increased considerably. The removal of this second mover group left the rest of the workers almost unable to achieve an organised nest emigration. Subsequent dissection of all workers in the colony showed that all movers had reduced ovaries, whereas the ovaries of many of the ants being carried were relatively well developed. On the other hand, only a small portion of the external workers (usually considered to have reduced ovaries) acted as mover specialists, while the internal workers, with well developed ovaries, were not able to replace lost mover specialists (Möglich and Hölldobler 1974). Kneitz (1964, from Wilson 1971, p. 261) also found that in *Formica polyctena* most of the workers doing the transporting were older foragers whereas most of the workers being carried were younger individuals. Interpreted in the light of the theory of age polyethsim (see section 3.1.) an association between the age of the workers and their role as transporters or transported during nest moving implies an association between the pre-emigration behaviour of the ants in the nest and their behaviour during emigration.

However, not all studies have supported this deterministic view. In contrast to the above findings, studies of *Formica execta* (Dobrzanska 1973) revealed no age-dependent difference in transport behaviour. No obvious relationship between task in the colony and behaviour during emigration was found either in *Monomorium pharaonis*, *Tapinoma melanocephalum*, *Iridomyrmex humilis* or *Paratrechus longicornus* (Wilson 1971). The carrying of pupae during emigration in *Atta sexdens* was found not to be exclusively performed by a specialised group (Wilson 1980) and brood transport behaviour in *Tapinoma erraticum* was only marginally associated with differences in worker behaviour in the nest (Meudec and Lenoir 1982). Meudec and Lenoir (1982) also found no precise correlation between the activity of the workers in the nest (foragers, nurses, intermediates) and their activity when the brood had to be moved. However, repeated testing did show that there were consistent differences between individuals according to their emigration behaviour.

In a study of emigration in *L. unifasciatus* Lane (1977a, 1977b) described the process as consisting of three stages: initial exploration, period of transport, and final exploration. Recruitment was achieved both through adult transport and tandem running. Unfortunately, the marks used to identify the ants did not persist from one day to the next and it was impossible to verify the hypothesis that the transporters remained the same during a second emigration several days later. Lane (1977a) estimated that the proportion of transporters during a single emigration was around 10-20%, as opposed to the 6% and 11% of ants that were transporters during 85% of 20 emigrations in respectively *Formica sanguinea* and *Camponotus sericeus* (Möglich and Hölldobler 1974).

The approach of the present study is different to those summarised above in that it considers emigration as a perturbation of everyday colony life that sheds light

Emigration no.	**Date**	**Start**	**End**	**First ant to discover the new nest**	**at**
1	06.07.1992	10:50	12:37	2	10:51
2	07.07.1992	8:59	10:27	42	9:03
3	08.07.1992	14:20	15:37	147	14:22
4	09.07.1992	15:30	16:50	blank	15:31
5	10.07.1992	9:15	10:35	113	9:16

Table 4.1: Details of the 5 consecutive emigrations of colony BG92U5

on the mechanisms of colony organisation. The work described in this chapter has three specific objectives. First, to establish whether workers are identical in terms of likelihood to become an active or a passive participant in emigration. Second, to investigate the relationship between an ant's spatial position and behaviour within the nest and the form of her participation in emigration. Third, to examine the effect of provoked emigration on the spatial configuration and behavioural organisation of the colony as a whole.

4.2 Materials and Methods

4.2.1 Colonies and Sessions

Colonies of *L. unifasciatus* were kept and studied as described in sections 1.3.12, 1.4.1, and 1.4.3. The present chapter is based on 6 photographic sessions of colonies BG91UI, BG91UJ, and BG91UQ which were filmed before and after they were emigrated. Colony BG91UI was allowed approximately 24h to settle before being photographed again after the emigration, whereas colonies BG91UJ and BG91UQ were allowed approximately 48h (see Tables 1.1-1.5). In addition colony BG92U5 was emigrated 5 times on 5 consecutive days, from 06.07.1992 to 10.07.1992 (see Table 4.1). This colony was not filmed.

4.2.2 Emigration Procedure

The old nest and an identical new nest are placed 1 - 2cm apart in a petri dish 20cm x 10cm, twice the size of the original nest's arena. In the case of repeated emigrations a new petri dish is used each time. The emigration petri dish is positioned under a dissection microscope. No artificial light sources are used. An emigration is provoked simply by removing the top slide of the old nest. This is considered to be the start of the emigration. Then the identity of each ant that gets into the nest is recorded together with the type or identity of the item she might be carrying. The nest moving is considered to be completed when the queen, all the brood and most of the ants are in the new nest.

4.2.3 Criteria for Arranging Emigration Behaviours into Groups

The records for each emigration were summed up in a matrix giving the identity of each ant followed by the number of times she was recorded (a) arriving alone, (b) carrying a brood item, (c) carrying a nestmate, (d) being carried (see Appendix). Ants were grouped on the basis of the already existing division of ants into **actives** - recruit and transport, and **passives** - arrive alone or are transported (Möglich and Hölldobler 1974) as well as on the findings of Lane (1977a) that carrying behaviour is a means of recruitment in *L. unifasciatus* and therefore being carried is not a sufficient condition to classify an individual as a passive participant. Instead, a single act of carrying an item makes an individual an 0 participant in the emigration effort.

Thus, a **transporter** is an ant that has carried at least one item (brood or ant) during the emigration; a **transported** is an ant that has not carried even a single item and has been carried at least once during the emigration; a **lone arriver** is an ant that has not carried and has not been carried, and has arrived alone at the new nest at least once during the emigration; an **unrecorded** is an ant that has not been recorded even once during the emigration. The result is a set of mutually exclusive and hierarchical conditions which expressed more formally are as follows:

if $b > 0$ or $c > 0$ then **transporter**

if $b = 0$ and $c = 0$ and $d > 0$ then **transported**

if $a > 0$ and $b = 0$ and $c = 0$ and $d = 0$ then **lone arriver**

if $a = 0$ and $b = 0$ and $c = 0$ and $d = 0$ then **unrecorded**

It is important to emphasise here that the above four tasks are not defined functionally in the sense that they fulfil some purpose for the colony. Obviously being **unrecorded** is not a functional task, while being a **transporter** is a task that appears to accomplish a colony purpose. The tasks of being a **transported** and a **lone arriver** could be considered as either, functional or non-functional tasks.

4.2.4 Randomisation Tests

- Randomisation test for task allocation during repeated emigrations

Ho is: All ants are equally likely to be a transporter during an emigration; **Ha** is: Some ants are more likely than others to be a transporter 4 an emigration. **Implementation:** For each of the 5 consecutive emigrations of colony BG92U5 the IDs of ants are permutated against their tasks according to a randomly chosen permutation and the percentage of ants that have been a transporter during (a) at least one of the emigrations and (b) all 5 emigrations, is calculated. This is repeated 100 times and the resulting distribution is compared to the real values for (a) and (b). The same test was also carried out for being a transported, lone arriver, and unrecorded.

- Randomisation test for the association between spatial position in the nest before emigration and membership in a particular behavioural group during the emigration

Ho is: The presence of carrying behaviour (being a transporter) is independent of position; **Ha** is: Individuals at the extremes of the colony's initial spatial distribution (i.e. nearest to the centre and furthermost from the centre) are less likely to be a transporter than expected on a random basis. **Implementation:** The numbers of times individuals have carried a nestmate during the emigration are permutated against their positions in the nest before the emigration according to a randomly chosen permutation. The number of ants that are at (a) the left extreme and (b) the right extreme of the colony's distribution and have not carried a nestmate are counted. The smaller of (a) and (b) is retained. This is repeated 100 times and the resulting distribution is compared to the real value of the statistic. This test was carried out for colony BG91UI, using (a). An analogous test but for the relationship between the carrying of a brood item during emigration and the position of the ant before the emigration was carried out for colony BG91UJ, using both (a) and (b).

- Randomisation test for the association between behaviour before the emigration and membership in a particular behavioural group during

the emigration

Ho is: The presence of carrying behaviour (being a transporter) is independent of initial brood care frequency/outside the nest frequency; **Ha** is: As a group, ants that do not care for brood/go outside the nest have a much greater frequency of carrying items during the emigration than would be expected on a random basis. **Implementation:** The numbers of times individuals have carried a brood item during the emigration are permutated against the numbers of times they performed brood care in the nest before the emigration according to a randomly chosen permutation. The number of ants that did not care for brood before the emigration and carried a brood item during emigration are counted. This is repeated 100 times and the resulting distribution is compared to the real value of the statistic. This test was carried out for colony BG91UJ. An analogous test but for the number of ants that had not been outside the nest before emigration and carried a brood item during emigration, was carried out for colony BG91UQ.

- Randomisation test for association between spatial position in the nest before emigration and the presence of a shift further from the centre after emigration

Ho is: The presence of a shift further away from the centre is independent of the ant's initial position; **Ha** is: Ants with initial positions nearer to the centre than the ant with the median position are more likely to shift further after emigration than expected on a random basis. **Implementation:** The type of shifts individuals have made after the emigration are permutated against their positions before the emigration according to a randomly chosen permutation. The number of shifts of particular type is counted for ants nearer (or further) to the colony centre than the ant with the median position. This is repeated 100 times and the resulting distribution is compared to the real value of the statistic. This test was carried out for colony BG91UQ.

All of the tests described above were performed by procedures written in the language of the S-plus statistical and graphical package (Becker 1988 et al).

4.2.5 Spearman's Correlation Test

(see section 1.4.4.2)

4.2.6 The Shift Criterion

(see section 3.2.3)

4.3 Results

4.3.1 Division of Labour during Emigration

4.3.1.1 Single Emigrations of Different Colonies

The proportion of transporters during the single emigrations of four different colonies are shown in Table 4.2.

A decrease in the proportion of transporters follows the decrease in the brood to ant ratio. In contrast, the percentage of lone arrivers increases with the decrease in the brood to ant ratio. However, the number of observations is limited and it is impossible to make any statistical inference about a possible relationship between the number of brood per capita of the worker population and the number of active

Single emigrations

	BG91UI1	BG91UJ1	BG91UQ1	BG92U51
total no. of ants	80	165	165	150
no. of analysed ants (% of total)	73 (91%)	93 (56%)	106 (64%)	121 (81%)
no. of brood	132	185	83	118
brood/ants	1.65	1.12	0.50	0.79
total no. of transportations	154	229	151	143
no. of brood transportations (% of total)	118 (77%)	126 (55%)	43 (28%)	76 (53%)
no. of ant transportations (% of total)	36 (23%)	103 (45%)	108 (72%)	67 (47%)
no. of transporters (% from marked ants)	41 (56%)	38 (41%)	32 (30%)	39 (32%)
no. of lone arrivers (% from marked ants)	5 (7%)	11 (12%)	20 (19%)	19 (16%)
total no. of transported ants (% from marked ants)	28 (38%)	51 (55%)	55 (52%)	34 (28%)
no. of ants - transported but not transporters (% from marked ants) (% from transported ants)	17 (23%) (61%)	31 (33%) (61%)	39 (37%) (71%)	26 (21%) (72%)
no. of unrecorded ants (% from marked ants)	10 (14%)	13 (14%)	15 (14%)	37 (31%)

Table 4.2: Description of single emigrations of different colonies including the first emigration for colony BG92U5

participants in the emigration effort.

The error in the recording method, as measured by the proportion of unrecorded ants, is at 14% identical for all colonies except colony BG92U5 where it is more than twice as much, 31%. Two factors could account for the greater error in colony BG92U5: it had the greatest number of marked ants and was at the same time the least familiar to the observer.

4.3.1.2 Five Emigrations of the Same Colony on Five Consecutive Days

Multiple emigrations of the same colony (see Table 4.3 for description) are necessary in order to establish whether task allocation among the transporter, transported, lone arriver and unrecorded categories is random. If all workers are equally likely to perform any one of the tasks described by these behavioural groups, then division of labour cannot be deterministic.

On the other hand, if task allocation is not random, that does not necessarily mean a deterministic division of labour. Only the existence of a high proportion of specialists (defined as ants that belong to the same behavioural group during 100% of the emigrations) would favour a deterministic mechanism, otherwise the mechanism could be probabilistic.

56% (68 individuals) were transporters during at least one of the five emigrations. This percentage is significantly smaller than would be expected on the basis of random allocation of score profiles among ants for each emigration (Fig. 4.1). In other words some ants were more likely to be transporters than others.

Colony BG92U5 - 5 emigrations

	First	Second	Third	Fourth	Fifth
no. of ants	150	160	140	150	148
no. of marked ants (% of total)	121 (81%)	121 (76%)	121 (86%)	121 (81%)	121 (%82)
no. of brood	118	143	122	115	119
brood/ants	0.79	0.89	0.87	0.77	0.80
total no. of transportations	143	128	190	142	132
no. of brood transportations (% of total)	76 (53%)	81 (63%)	102 (54%)	63 (44%)	68 (52%)
no. of ant transportations (% of total)	67 (47%)	47 (37%)	88 (46%)	79 (56%)	64 (48%)
no. of transporters (% from marked ants)	39 (32%)	25 (21%)	35 (29%)	24 (20%)	28 (23%)
no. of lone arrivers (% from marked ants)	19 (16%)	26 (21%)	12 (10%)	13 (11%)	10 (8%)
total no. of transported ants (% from marked ants)	34 (28%)	33 (27%)	54 (45%)	50 (41%)	45 (37%)
no. of ants - transported but not transporters (% from marked ants) (% from transported ants)	26 (21%) (76%)	29 (24%) (88%)	44 (36%) (81%)	44 (36%) (88%)	38 (32%) (84%)
no. of unrecorded ants (% from marked ants)	37 (31%)	41 (34%)	30 (25%)	40 (33%)	45 (37%)

Table 4.3: Description of 5 emigrations of the same colony on 5 consecutive days

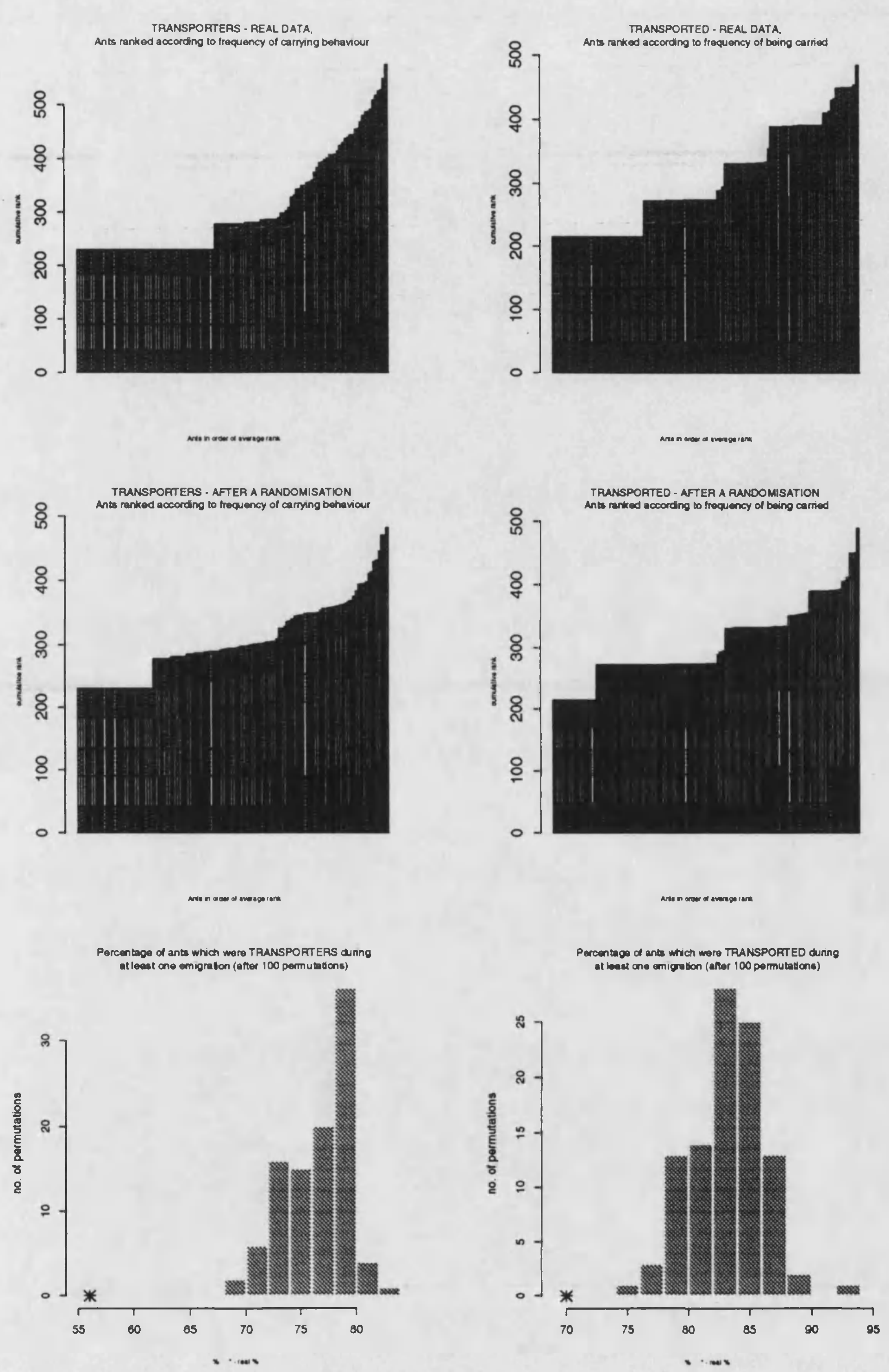


Figure 4.1: Task allocation during 5 emigrations of colony BG92U5; transporters and transported

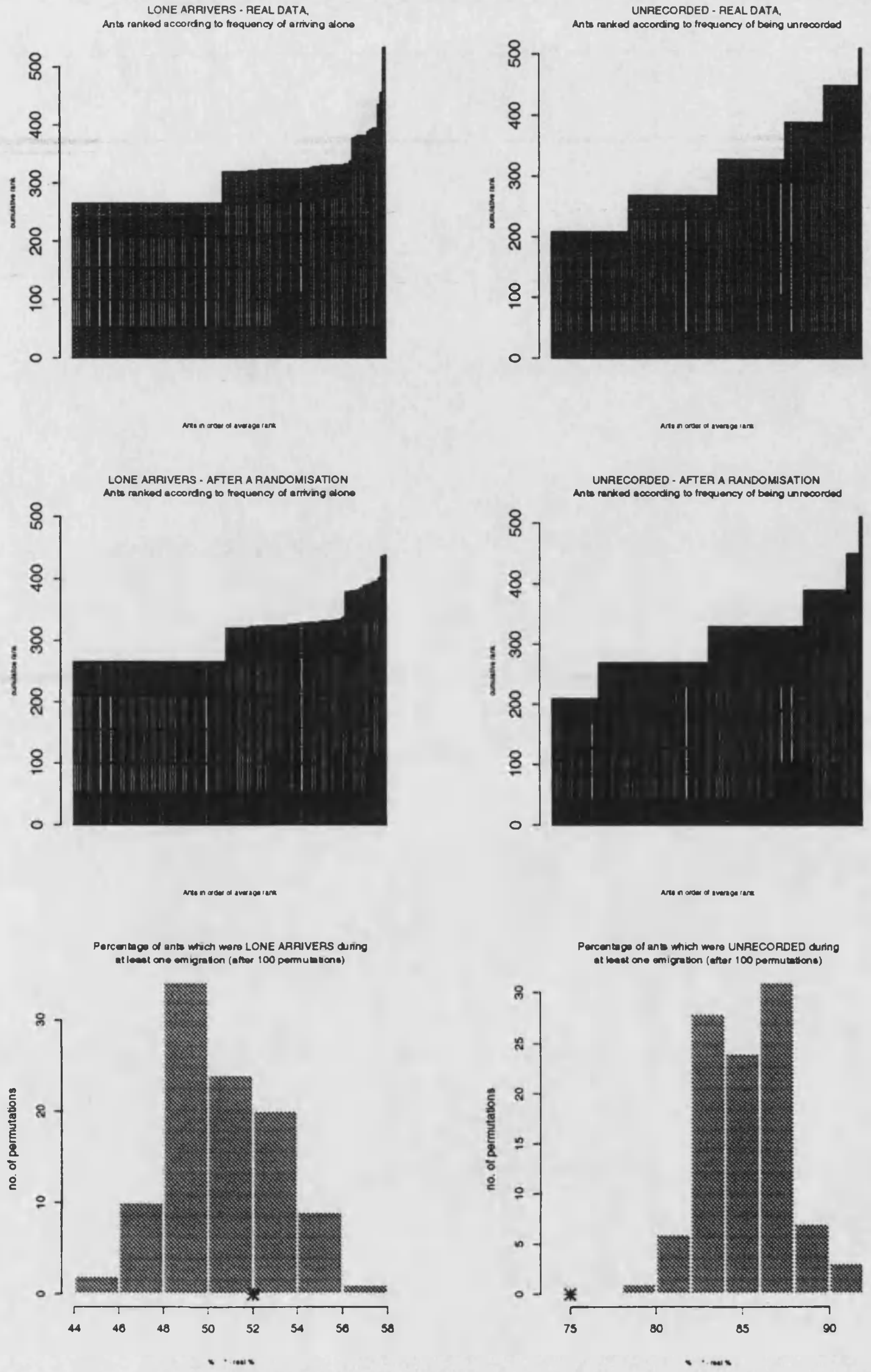


Figure 4.2: Task allocation during 5 emigrations of colony BG92U5; lone arrivers and unrecorded

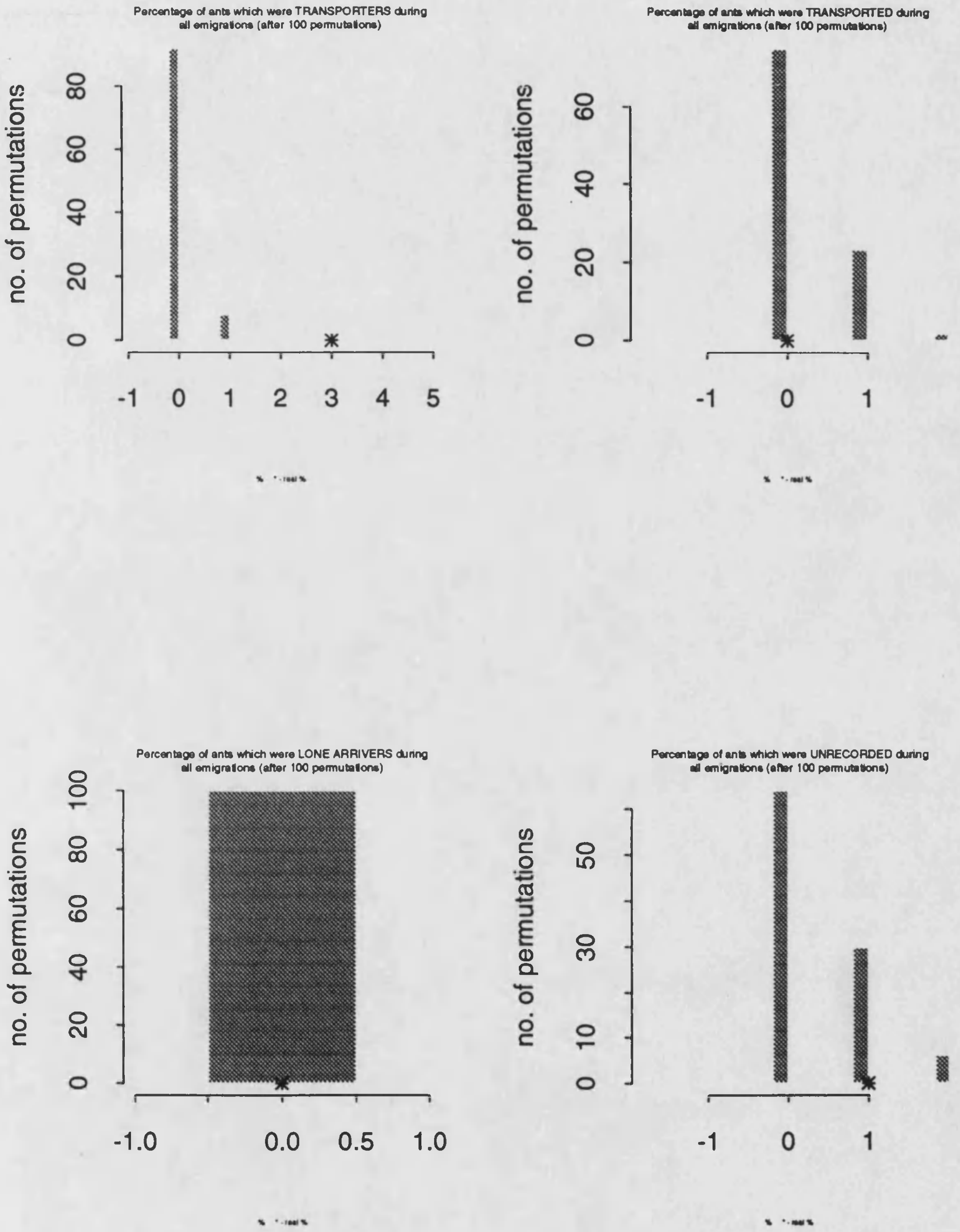


Figure 4.3: Task allocation during 5 emigrations of colony BG92U5; randomisation tests for specialists

From these 56%, 3% (4 individuals) were transporters during all five emigrations, significantly more than would be expected on a random basis (Fig. 4.3). Thus, there is a tendency for specialisation among transporters.

70% (85 individuals) of the workers were transported during at least one of the emigrations. This is a significantly smaller proportion than would be expected on a random basis, which indicates that some ants were more likely to be transported than others (Fig. 4.1). However, as in the rest of the groups, there was no tendency for specialisation in transported (see Fig. 4.3 for results of randomisation tests for percentage of specialists in transported, lone arriver, and unrecorded).

All ants were equally likely to be lone arrivers, because the 52% (63 individuals) of workers that were lone arrivers during at least one of the emigrations are not significantly different from what would be expected on a random basis (Fig. 4.2).

The 75% (91 individuals) of the workers that were unrecorded during at least one of the emigrations, are significantly less than expected on a random basis (Fig. 4.2). This result reveals that the observer was more likely to miss some ants than others. In order to explore what effect this bias might have had on the results for the other behavioural groups, I considered the following three extreme hypothetical cases: (a) every time she was unrecorded, a worker was a transporter; (b) every time she was unrecorded, a worker was a transported; (c) every time she was unrecorded, a worker was a lone arriver. For each of these extreme cases a randomisation test was carried out, measuring in the case of (a) the proportion of ants that would have been transporters during at least one of the emigrations, in the case of (b) - the same for transported, and in the case of (c) - the same for lone arrivers. The proportion was not significantly different from that expected on a random basis for lone arrivers (94%) but was significantly smaller than expected for transported (89%), thus repeating the original results. However, the 98% of workers that were transporters in case (a) during at least one of the emigrations, was not significantly different from expected, in disagreement with the original result. Therefore, if the extreme case (a) was true, the results would have been different. Nevertheless, there is no reason to believe, that the bias in unrecorded was towards individuals belonging to any one behavioural group, and in particular towards transporters. None of the behaviours during emigration was easier to record than the others. It is much more likely that the bias was due to the mark of the individual. During the height of the emigration the traffic in the exit of the nest is intense. It is possible that the ants with less bright and more complicated marks were more likely to be missed, particularly in these conditions. Since the marking was done before the experiments, without any knowledge of the behaviour of the ants, and without any order in picking them up for marking, there should not have been any regularity in how the less bright or more complex marks were assigned among them. Consequently this bias should not have had any impact on the results for any particular behavioural group.

To be a transporter an ant had to transport either a brood item or a nestmate at least once during the emigration (see section 4.2.3). A thorough investigation of the division of labour during emigration requires that it is established whether the group of transporters is homogeneous or divided into two subgroups, of brood transporters and ant transporters. The correlation between the average rank of an ant for transporting brood and her average rank for transporting nestmates is positive and significant (Fig. 4.4). Individuals that tend to be active in one of the transports tend to be active in the other. There is thus no apparent separation of the two types of transport.

4.3.2 Relationship between Spatial Position before

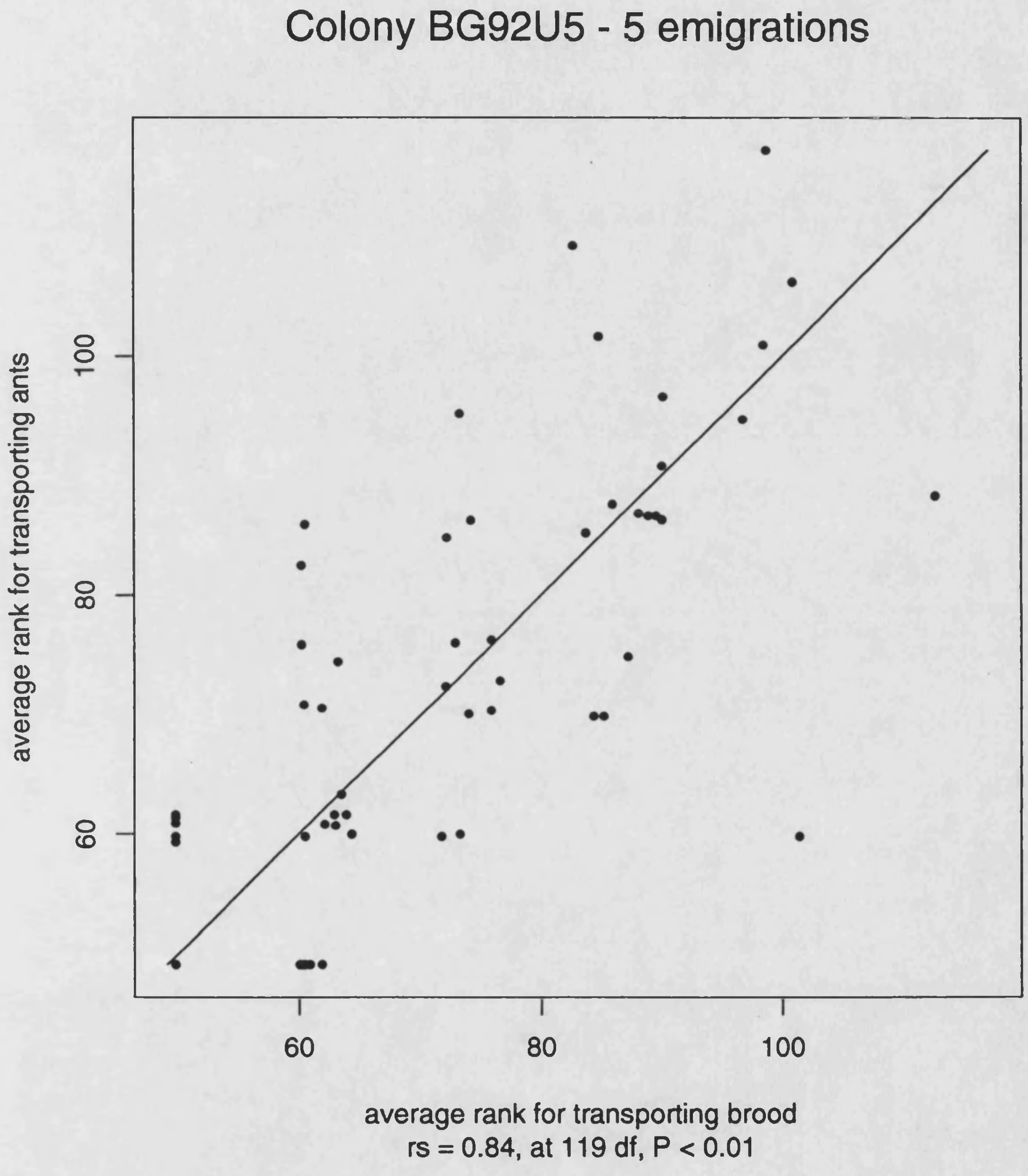


Figure 4.4: Transporters form a homogeneous group

the Emigration and Behaviour during the Emigration

If it is the ants from a particular part of the colony that become transporters or transported during the emigration, then this in itself could be the reason for some workers to be more likely to be transporters or transported than others. Figure 4.5 shows separate plots for the number of brood carried and the number of ants carried by transporters (see definition of a transporter in section 4.2.3) for the three singly emigrated colonies.

There is no significant relation between carrying at least one item to the new nest during emigration and the part of the colony the worker originally comes from. Even the relationship between ants carried and initial position for colony BG91UI and the relationship between brood carried and initial position for colony BG91UJ, both of which seem to show some dependence of carrying at least one item on spatial position, could occur on a random basis (Fig. 4.8). Figures 4.6 and 4.7 show the same result for transported workers, lone arrivers and unrecorded ants.

4.3.3 Relationship between Behaviour before the Emigration and Behaviour during the Emigration

Behavioural results confirm that the behaviour of a worker during emigration does not depend on her initial spatial position and consequently on her spatially related behaviour. Figures 4.9, 4.10 and 4.7 show the relationships between brood care frequency and the frequency of being a transporter, transported, lone arriver or unrecorded respectively, for colonies BG91UI, BG91UJ and BG91UQ.

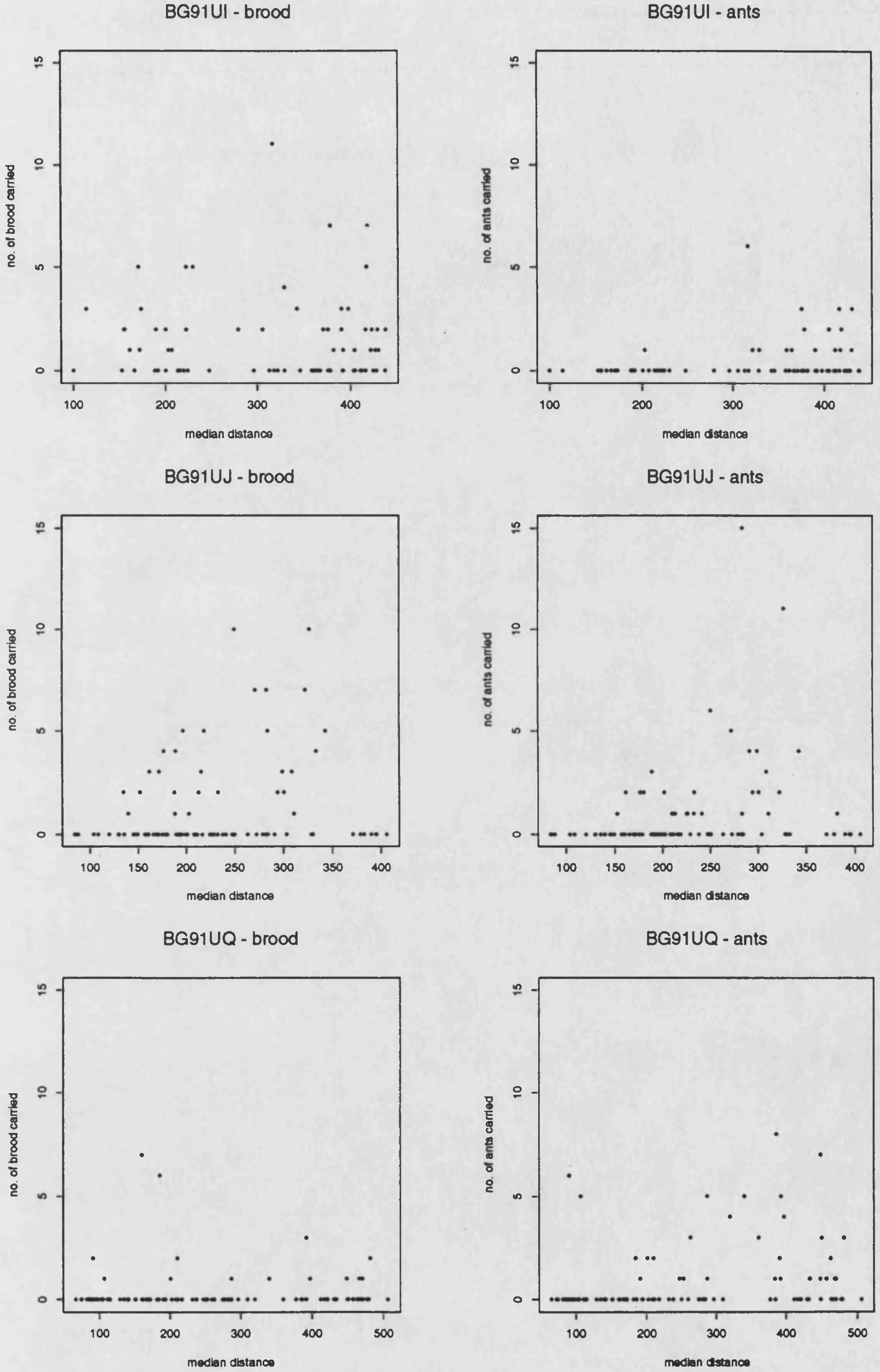


Figure 4.5: Relationship between initial spatial position and the number of items carried by transporters

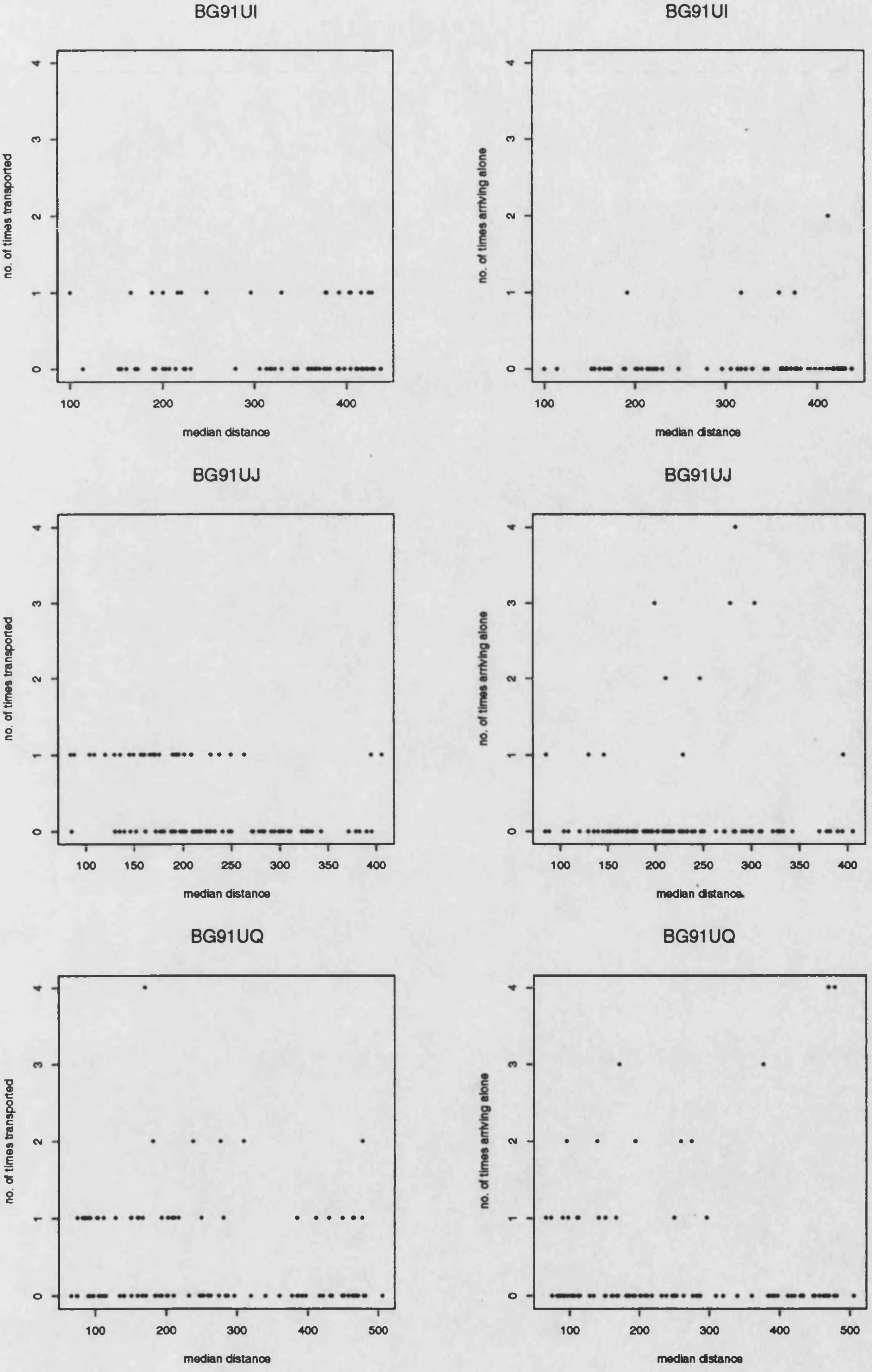


Figure 4.6: Relationship between initial spatial position and being a transported or being a lone arriver

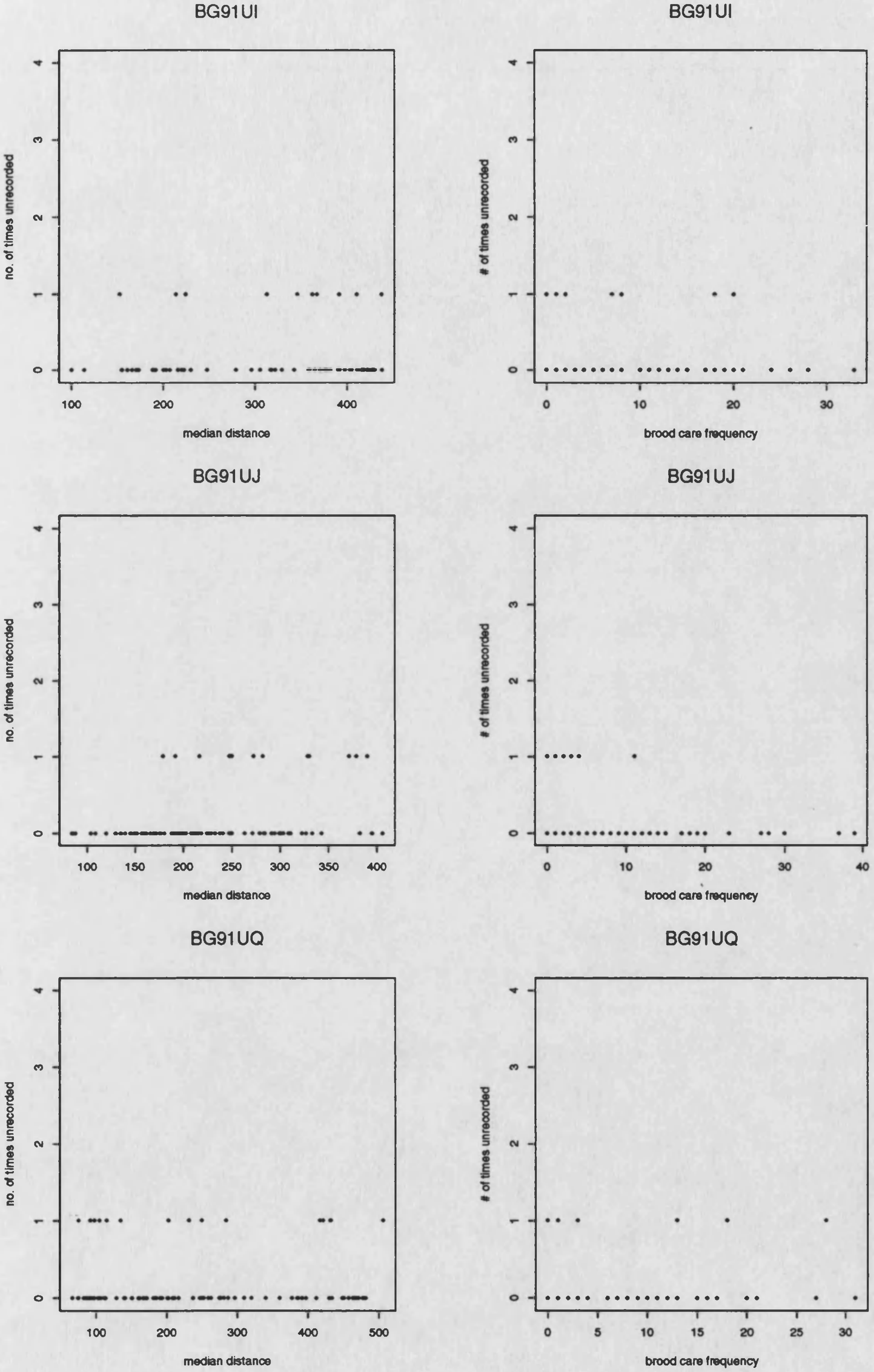


Figure 4.7: Relationship between initial spatial position or brood care frequency and being unrecorded

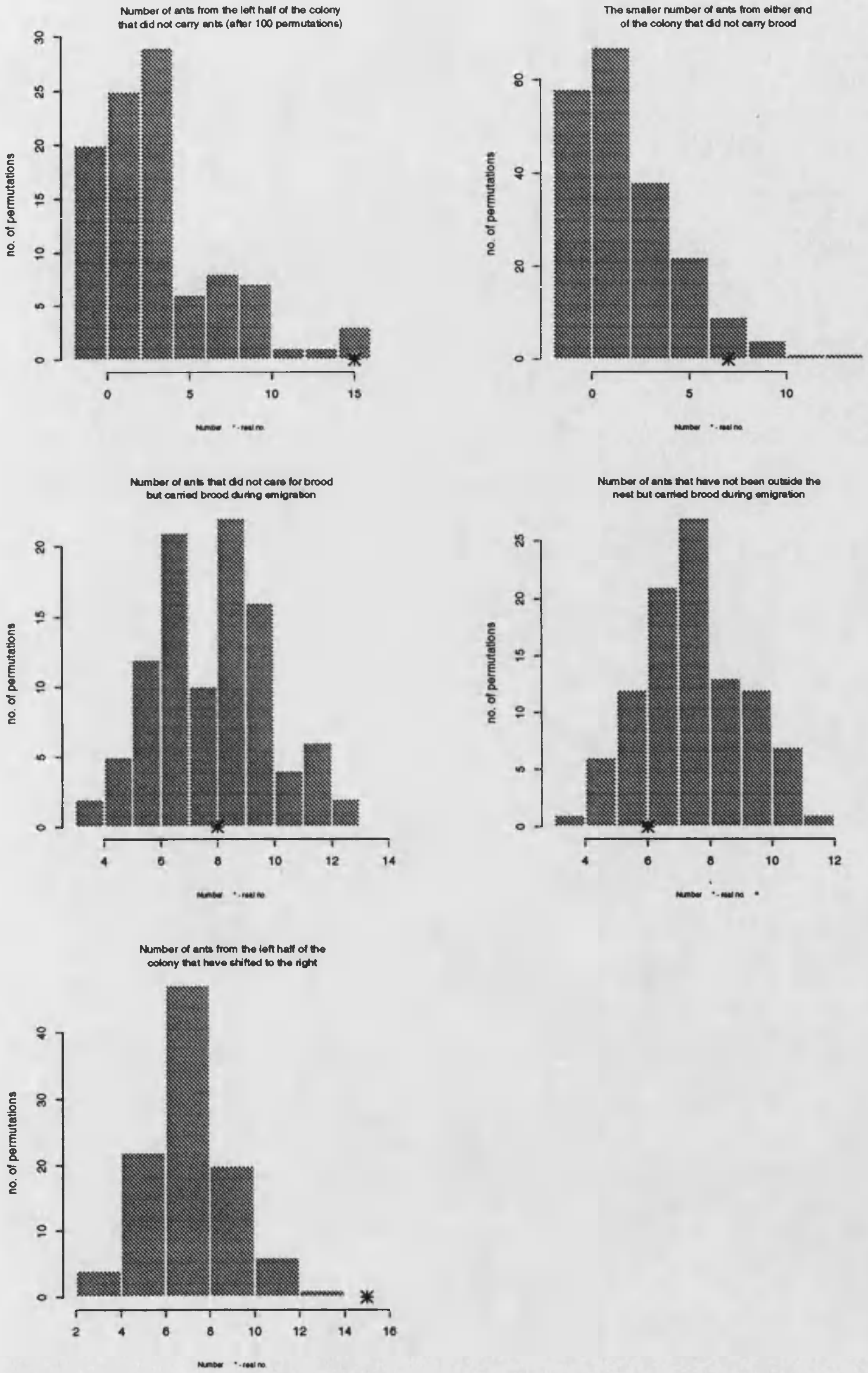


Figure 4.8: Results from randomisation tests. See for (1) Fig. 4.5, 2; (2) Fig. 4.5, 3; (3) Fig. 4.9, 3; (4) Fig. 4.11, 5; (5) Fig. 4.17, 3

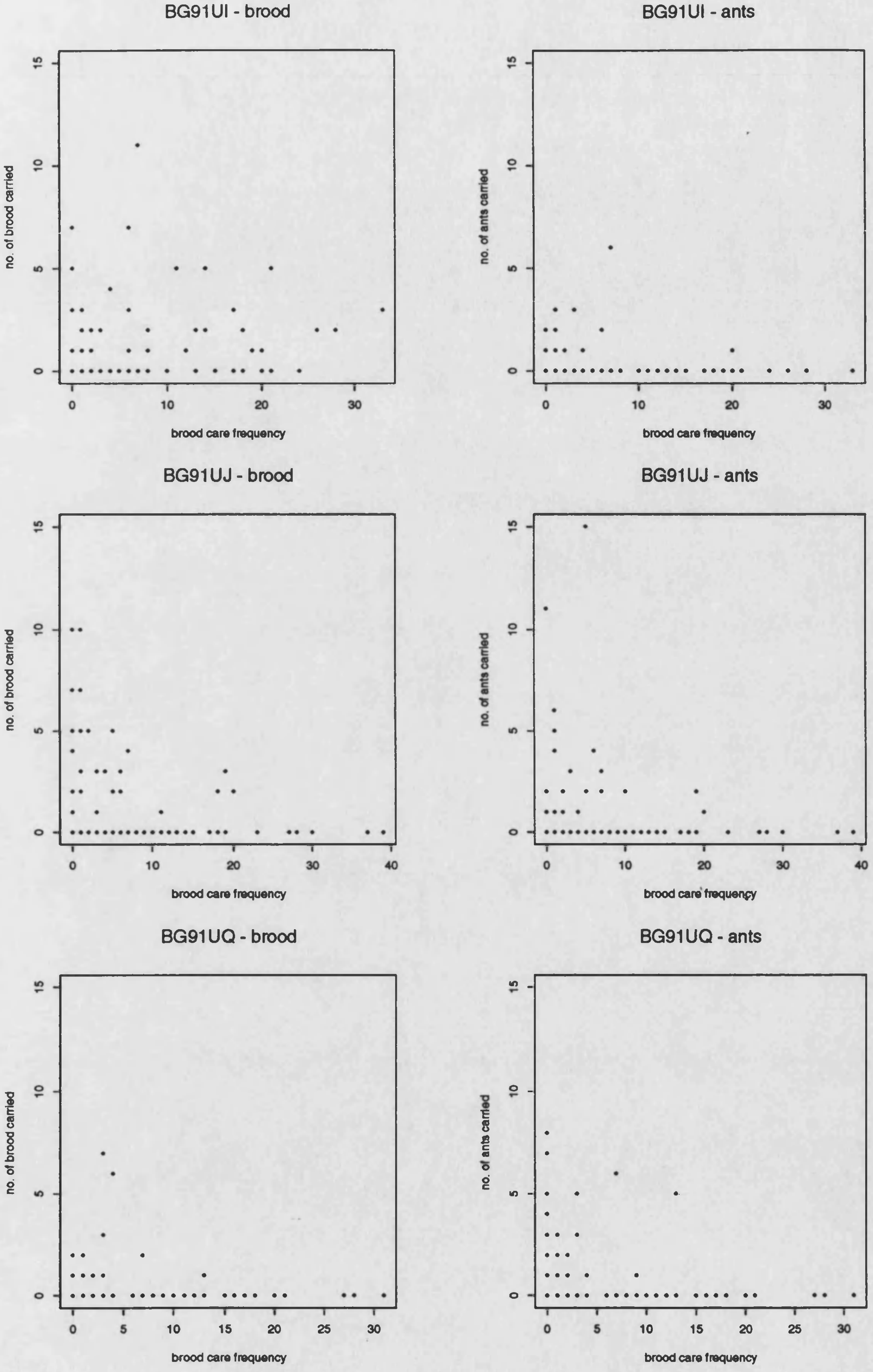


Figure 4.9: Relationship between brood care frequency and the number of items carried by a transporter

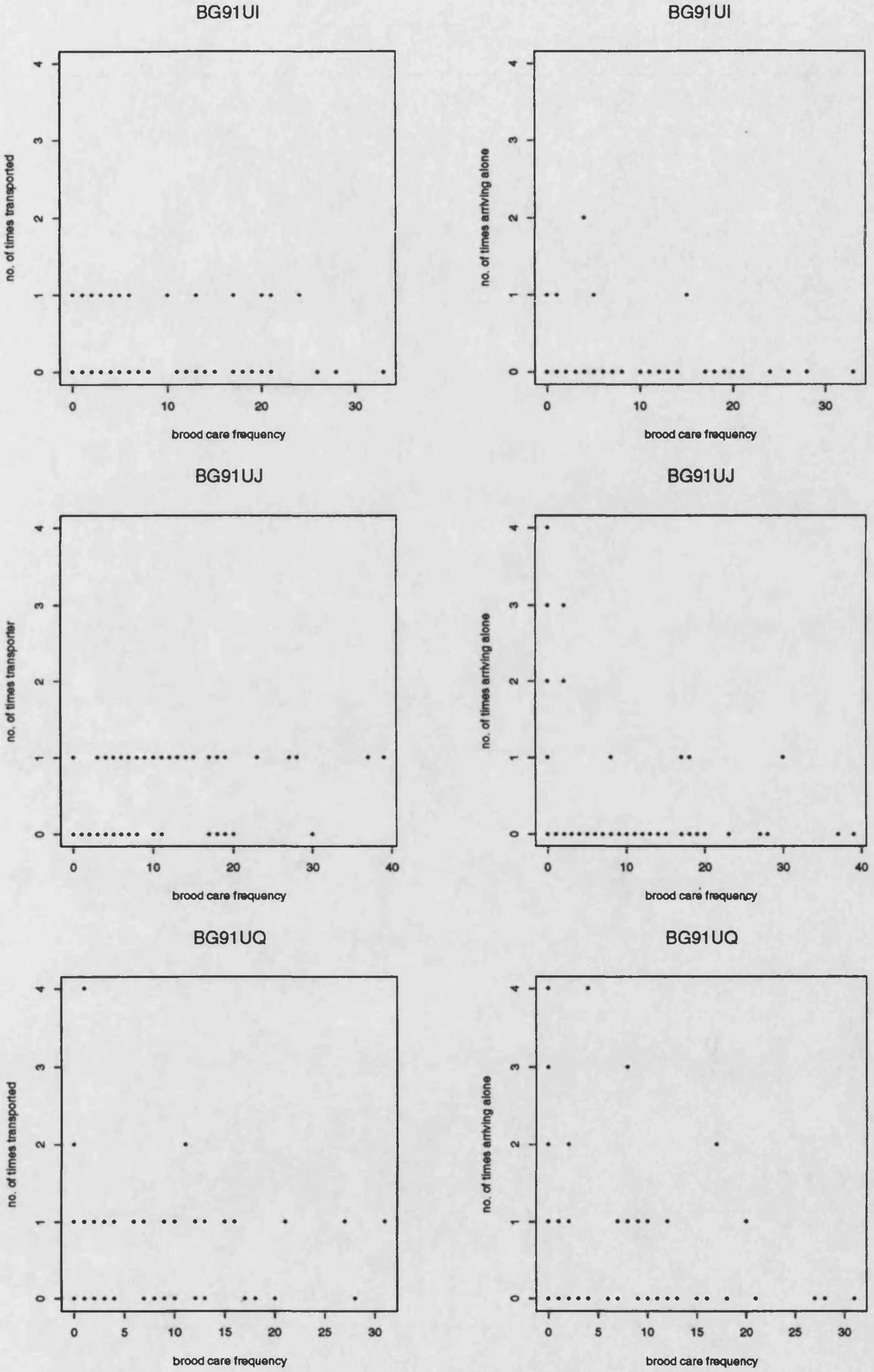


Figure 4.10: Relationship between brood care frequency and being a transported or being a lone arriver

A randomisation test was carried out only for one of the most extreme cases, i.e. the relationship between frequency of brood care and the task of being a brood transporter for colony BG91UJ. Even here, the relationship is not different from the randomised expectation (Fig. 4.8). Any impression that ants, which have done no brood care are more likely to transport brood, is deceptive. It is due to the great number of workers that have done no brood care.

Figures 4.11-4.13 show the relationships between outside the nest frequency and the frequency of being a transporter, transported, lone arriver or unrecorded respectively, for the three singly emigrated colonies. A randomisation test was carried out only for the relationship between outside the nest frequency and the task of being transporter of brood for colony BG91UQ. The result does not depart from expectation (Fig. 4.8), thus following the already established trend.

4.3.4 Relative Change in Spatial Position after the Emigration

The main purpose of the single emigration experiments was to find out the effect of this perturbation on colony everyday organisation - the effect on its spatial configuration and on the distribution of tasks among workers. Figure 4.14 shows the correlations of the ranked ant positions before and after the emigration for colonies BG91UI, BG91UJ and BG91UQ.

All three correlations are highly significant. It is obvious even without an illustration that the results are significantly different from what would be expected on the basis of ants being randomly allocated to positional ranks after the emigration. In other words the spatial configuration of the colony is retained despite the turmoil of a provoked emigration. Further experiments, involving controls will be

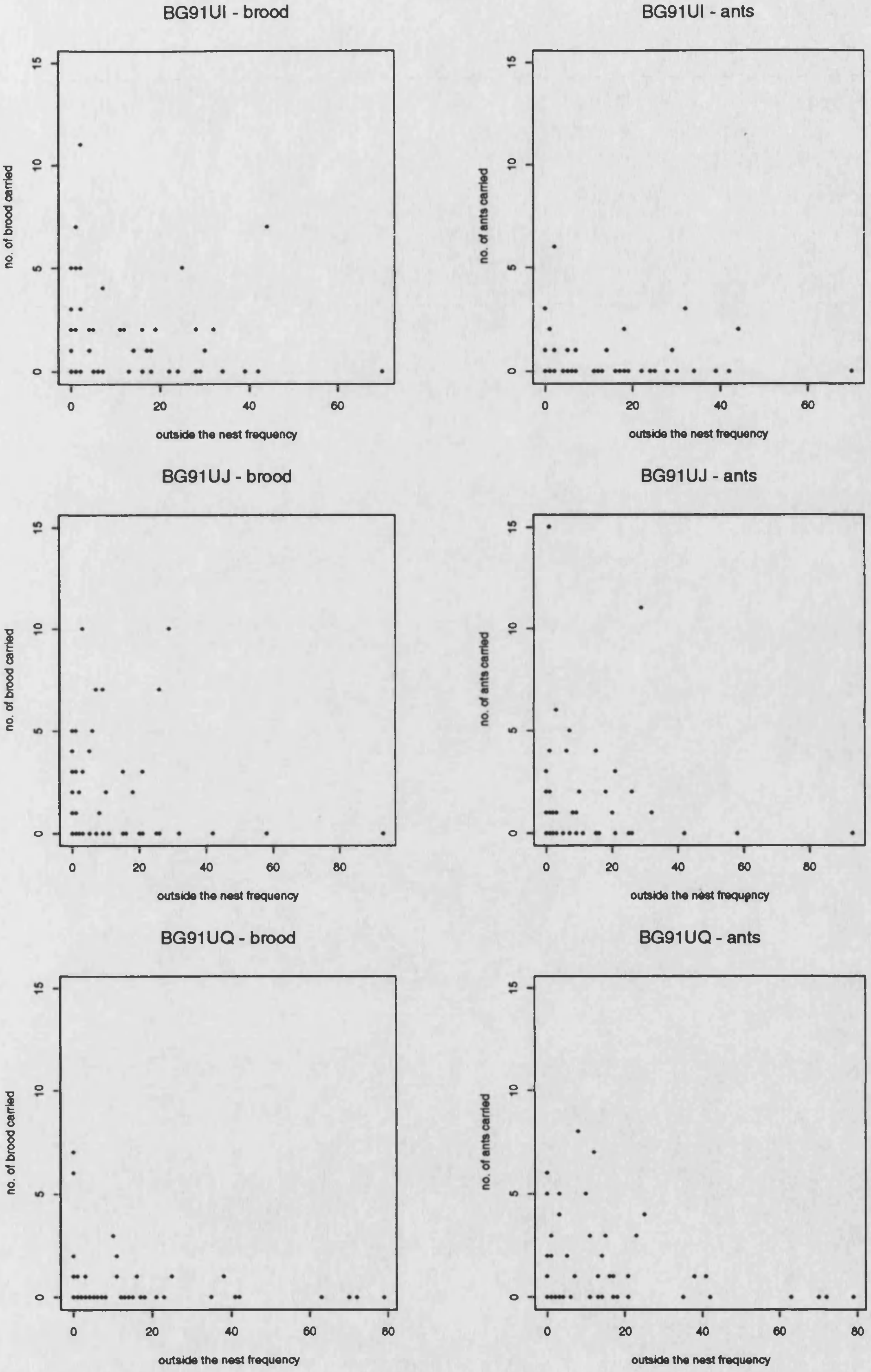


Figure 4.11: Relationship between outside the nest frequency and the number of times carried by a transporter

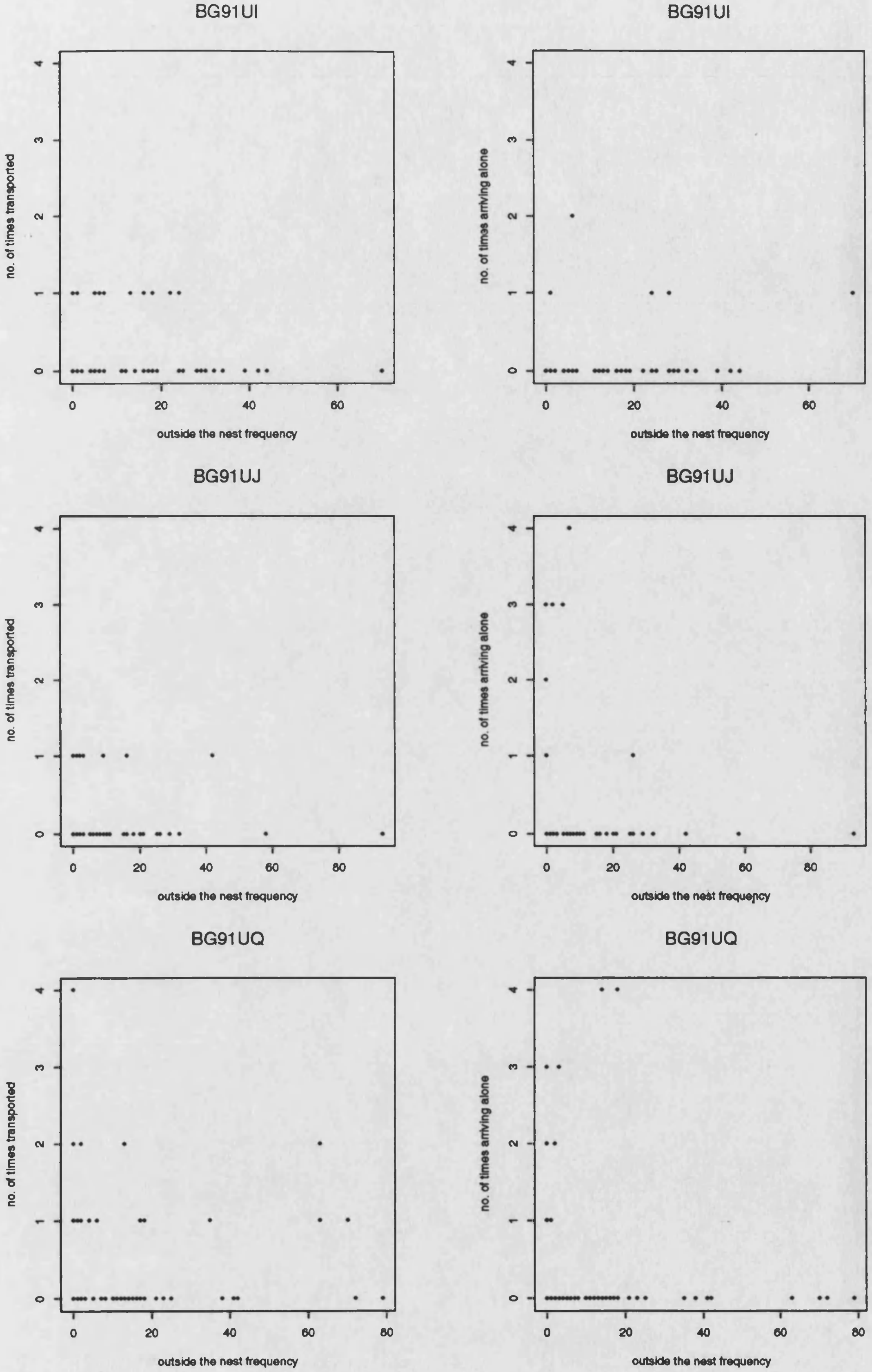


Figure 4.12: Relationship between outside the nest frequency and being a transported or a lone arriver

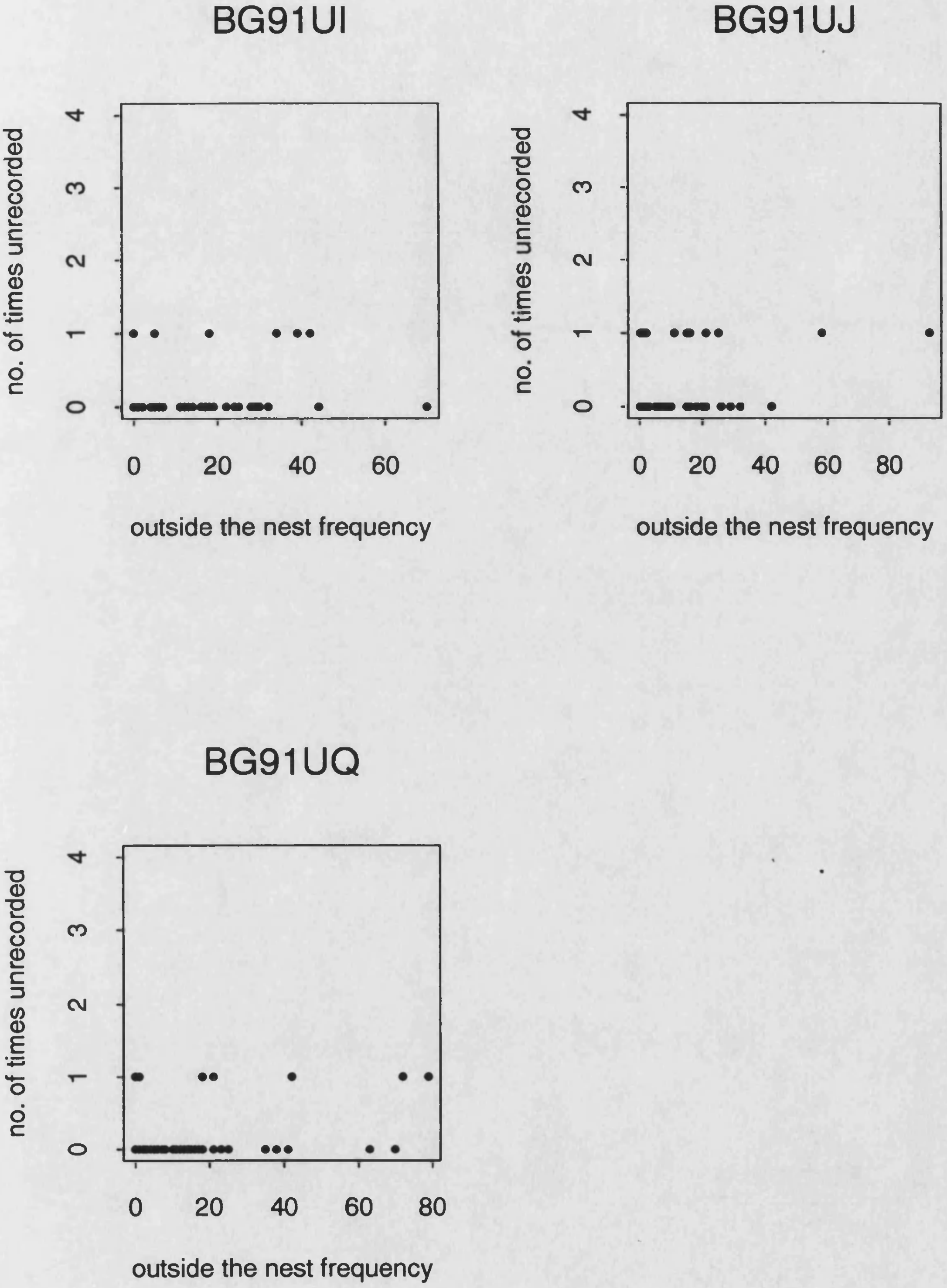
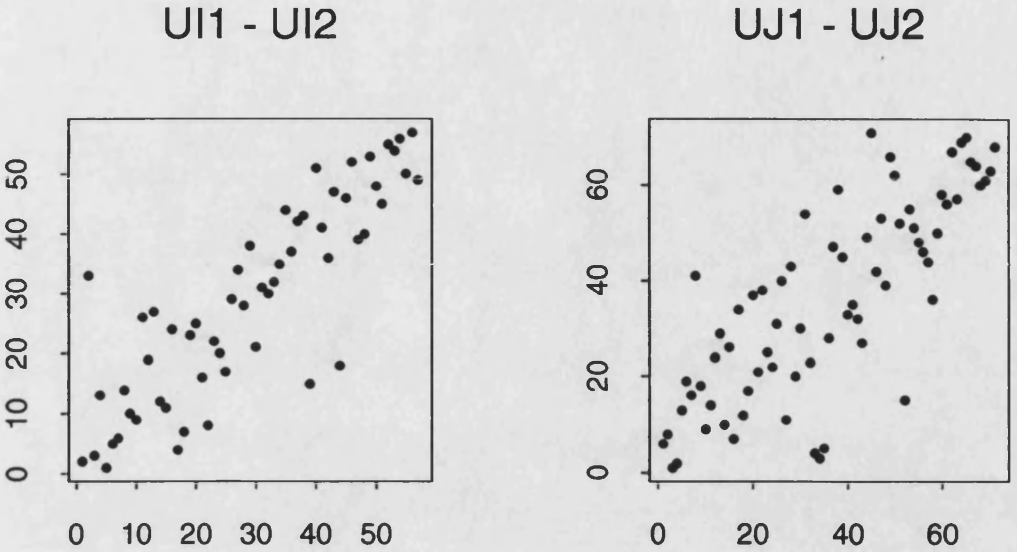


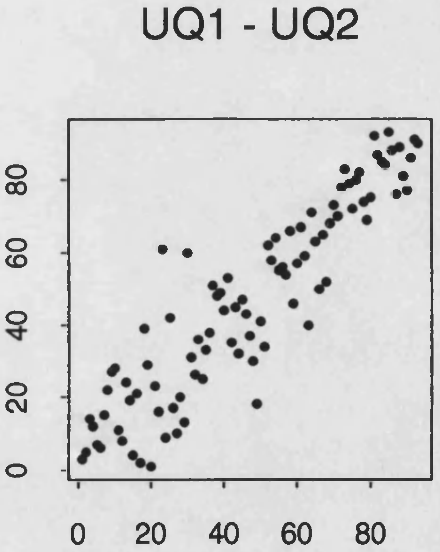
Figure 4.13: Relationship between outside the nest frequency and being unrecorded


UI1 - UI2

UJ1 - UJ2

rs. = 0.86, P < 0.01 at 55 d.f.

rs. = 0.80, P < 0.01 at 69 d.f.


UQ1 - UQ2

rs. = 0.92, P < 0.01 at 91 d.f.

Figure 4.14: Correlations of ranked ant positions for before and after the emigration

necessary in order to establish whether, despite being significant, the correlations are substantially weaker than they would have been between two successive weeks without an emigration.

4.3.5 Relative Change in Behaviour after the Emigration

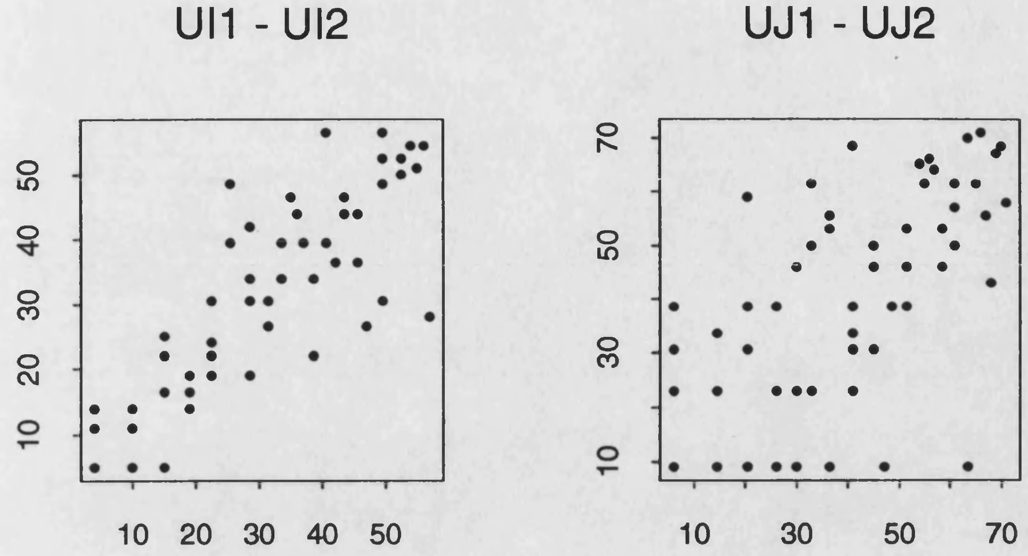
The correlations between ranked brood care frequencies (Fig. 4.15) and ranked outside the nest frequencies (Fig. 4.16) for before and after the emigration are significant in all of the three colonies, although less marked than the correlations of ranked spatial positions.

There are individuals from a range of ranks according to their frequency of brood care or outside the nest frequency which after the emigration are demoted down to the lowest rank, and vice versa, individuals with the lowest rank before the emigration are promoted to a range of higher ranks.

The general conclusion is that a single emigration does not cause a complete rearrangement of the colony's basic structure. On the contrary, the colony's spatial and behavioural order are conserved.

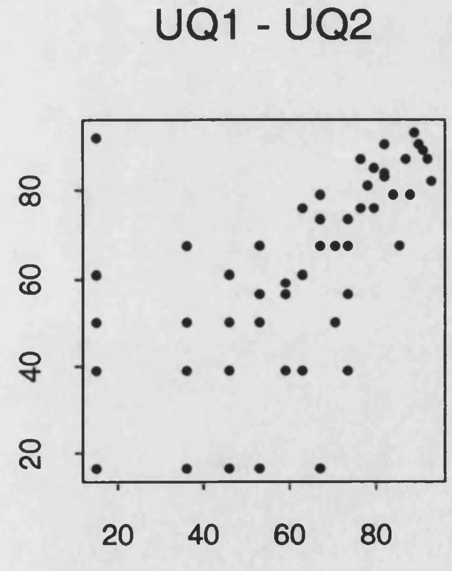
4.3.6 Absolute Change in Spatial Position

The individuals that were actively responsible for the relative change in position could only be revealed through a measure of the absolute change. The concept of shift (see section 3.2.3) is used for this purpose. The proportion of ants that have not shifted during the first half of the session after the emigration is considerably smaller in the emigration colonies than the same proportion for a half session



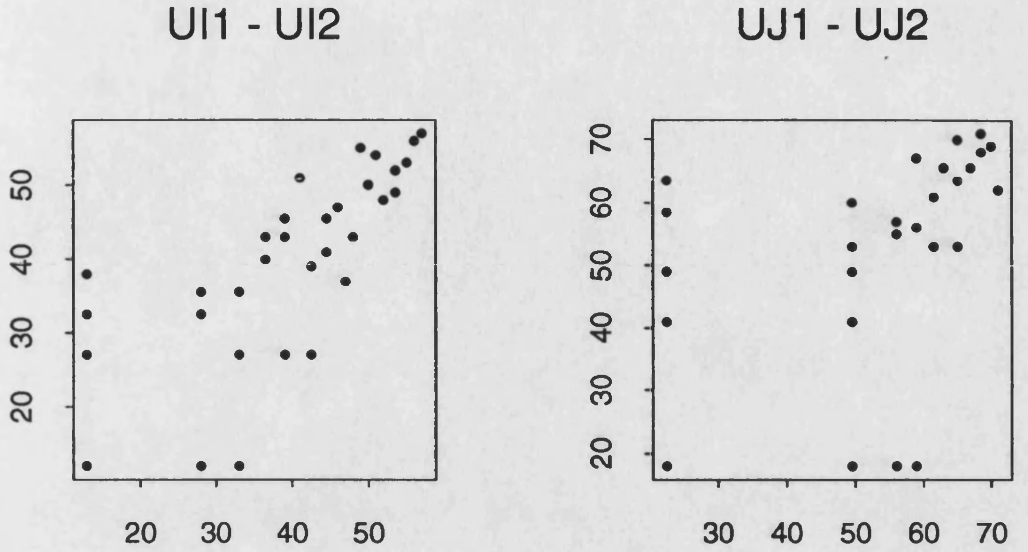
rs. = 0.86, P < 0.01 at 55 d.f.

rs. = 0.67, P < 0.01 at 69 d.f.



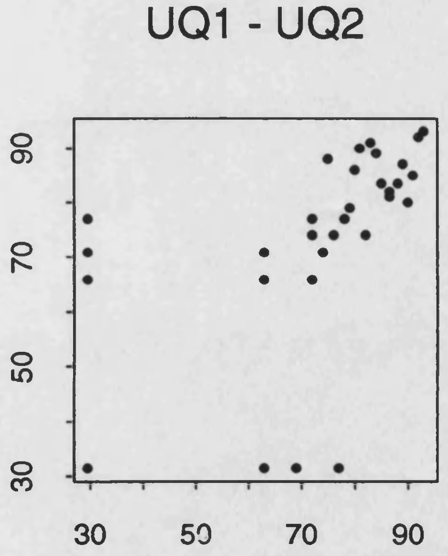
rs. = 0.78, P < 0.01 at 91 d.f.

Figure 4.15: Correlations of ranked brood care frequencies of ants for before and after the emigration



rs. = 0.80, P < 0.01 at 55 d.f.

rs. = 0.60, P < 0.01 at 69 d.f.



rs. = 0.77, P < 0.01 at 91 d.f.

Figure 4.16: Correlations of ranked outside the nest frequencies for ants before and after the emigration

BG91UI - HALF & FULL SESSIONS

Sessions	Further no (%)	Retained Position no (%)	Nearer no (%)	Total no
ui1a - ui1b	11 (20)	39 (71)	5 (9)	55
ui1b - ui2a	14 (26)	34 (61)	7 (13)	55
ui2a - ui2b	7 (13)	43 (78)	5 (9)	55
ui1a - ui2b	22 (40)	28 (51)	5 (9)	55
ui1 - ui2	11 (20)	42 (76)	2 (4)	55

Table 4.4: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

BG91UJ - HALF & FULL SESSIONS

Sessions	Further no (%)	Retained Position no (%)	Nearer no (%)	Total no
uj1a - uj1b	12 (17)	52 (73)	7 (10)	71
uj1b - uj2a	32 (45)	32 (45)	7 (10)	71
uj2a - uj2b	23 (32)	32 (45)	16 (23)	71
uj1a - uj2b	40 (56)	26 (37)	5 (7)	71
uj1 - uj2	35 (49)	32 (45)	4 (6)	71

Table 4.5: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

without disturbance (Tables 4.4-4.6, and 3.10).

The smaller percentage of ants that have not shifted in colony BG91UQ is balanced equally by the percentage of ants that have shifted closer to the centre and ants that have shifted away from the centre. However, in colony BG91UI twice as many ants have shifted further than nearer, while in colony BG91UJ the proportion of ants that have shifted further from the centre is more than four times greater than the proportion of ants that have shifted nearer to the centre.

The presence and type of shift does not appear to depend on the spatial position of the ant before the emigration in colonies BG91UI and BG91UJ (Fig. 4.17).

BG91UQ - HALF & FULL SESSIONS

Sessions	Further no (%)	Retained Position no (%)	Nearer no (%)	Total no
uq1a - uq1b	7 (8)	73 (78)	13 (14)	93
uq1b - uq2a	24 (26)	40 (43)	29 (31)	93
uq2a - uq2b	15 (16)	55 (59)	23 (25)	93
uq1a - uq2a	21 (23)	46 (49)	26 (28)	93
uq1 - uq2	15 (16)	62 (67)	16 (17)	93

Table 4.6: Number of ants that have shifted further from the centre, retained position or shifted nearer to the centre

The same is also true for colony BG91UQ except for the shifts away from the centre.

Compared to what would be expected on a random basis this type of shift occurs significantly more in those ants with a spatial position nearer to the centre than the individual with the median spatial position and significantly less in those ants with a spatial position further from the centre than the median (Fig. 4.8). This is a curious result, but being on its own, difficult to interpret.

4.3.7 Time Necessary for the Colony to Stabilise after Emigration

Colony BG91UI was filmed again approximately 24h after the emigration while colonies BG91UJ and BG91UQ were recorded approximately 48h after the emigration (see section 4.2.2). With the purpose of examining whether these intervals of time were sufficient for a colony to stabilise, distances from the colony centre are plotted against time (the chronological sequence of pictures taken) for three ants in each of colonies BG91UI and BG91UJ (Figures 4.18 and 4.19).

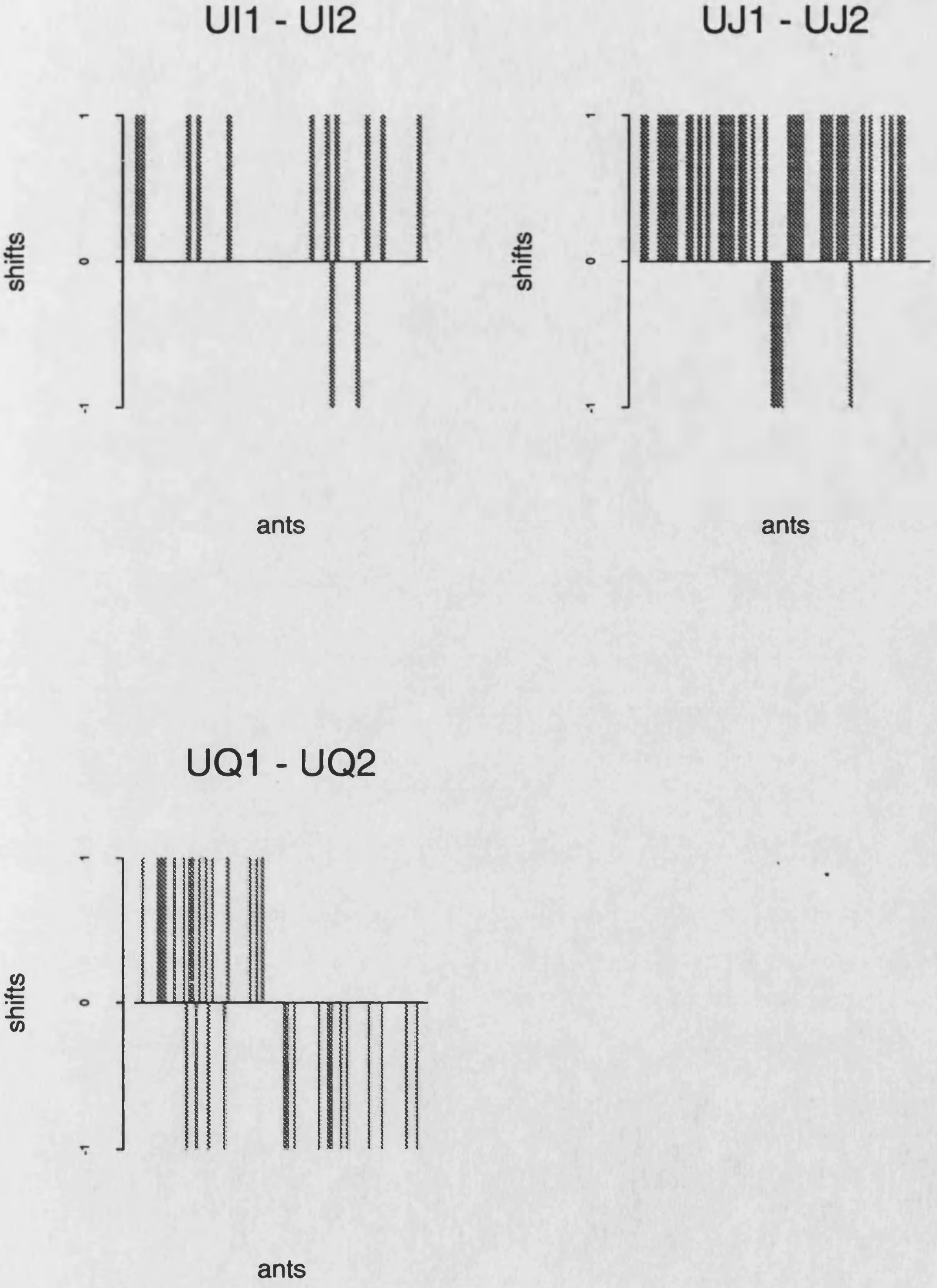


Figure 4.17: Ant shifts after emigration. Ants are ordered according to initial position; 1- further, 0 - no shift, -1 - nearer

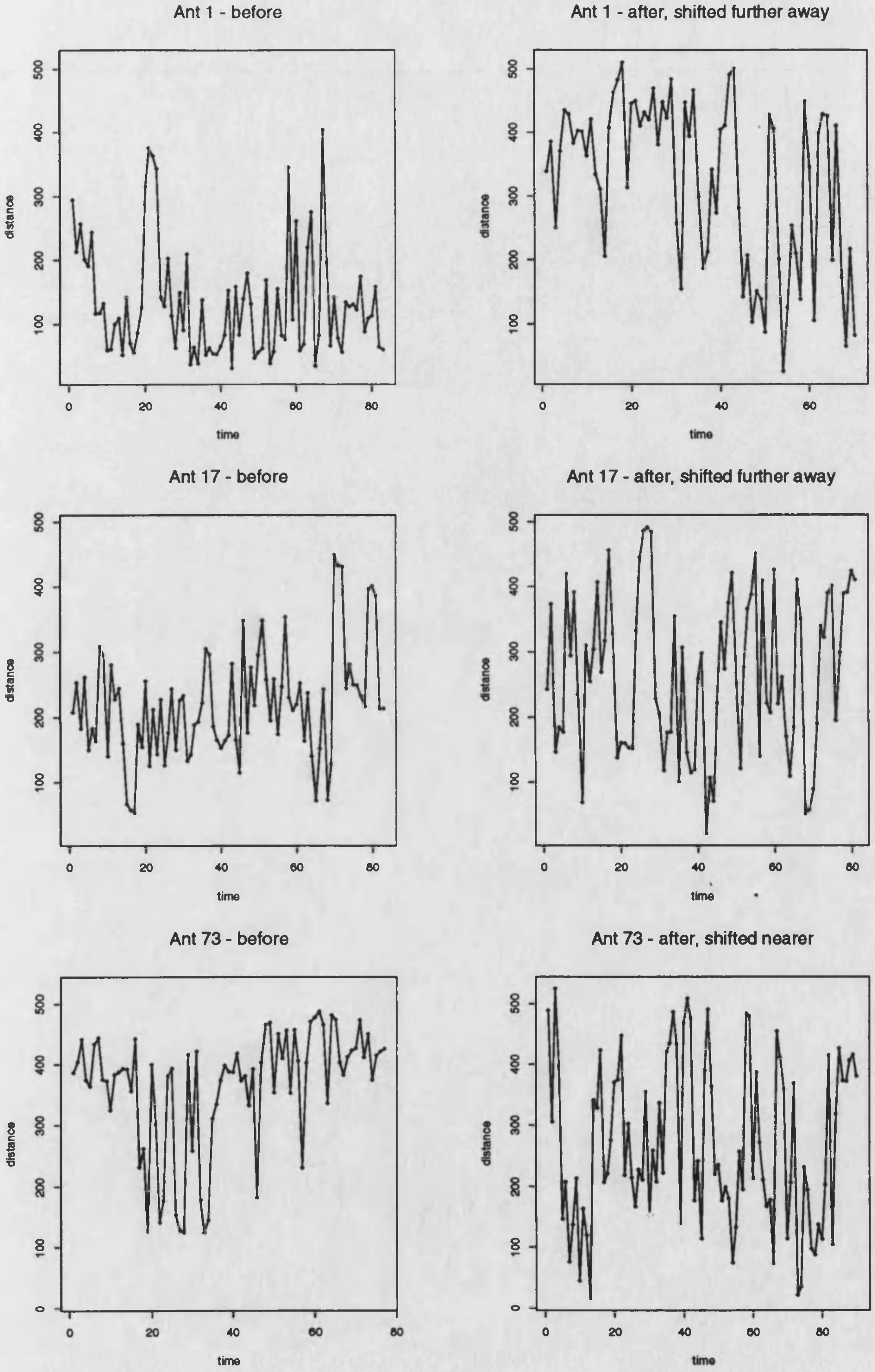
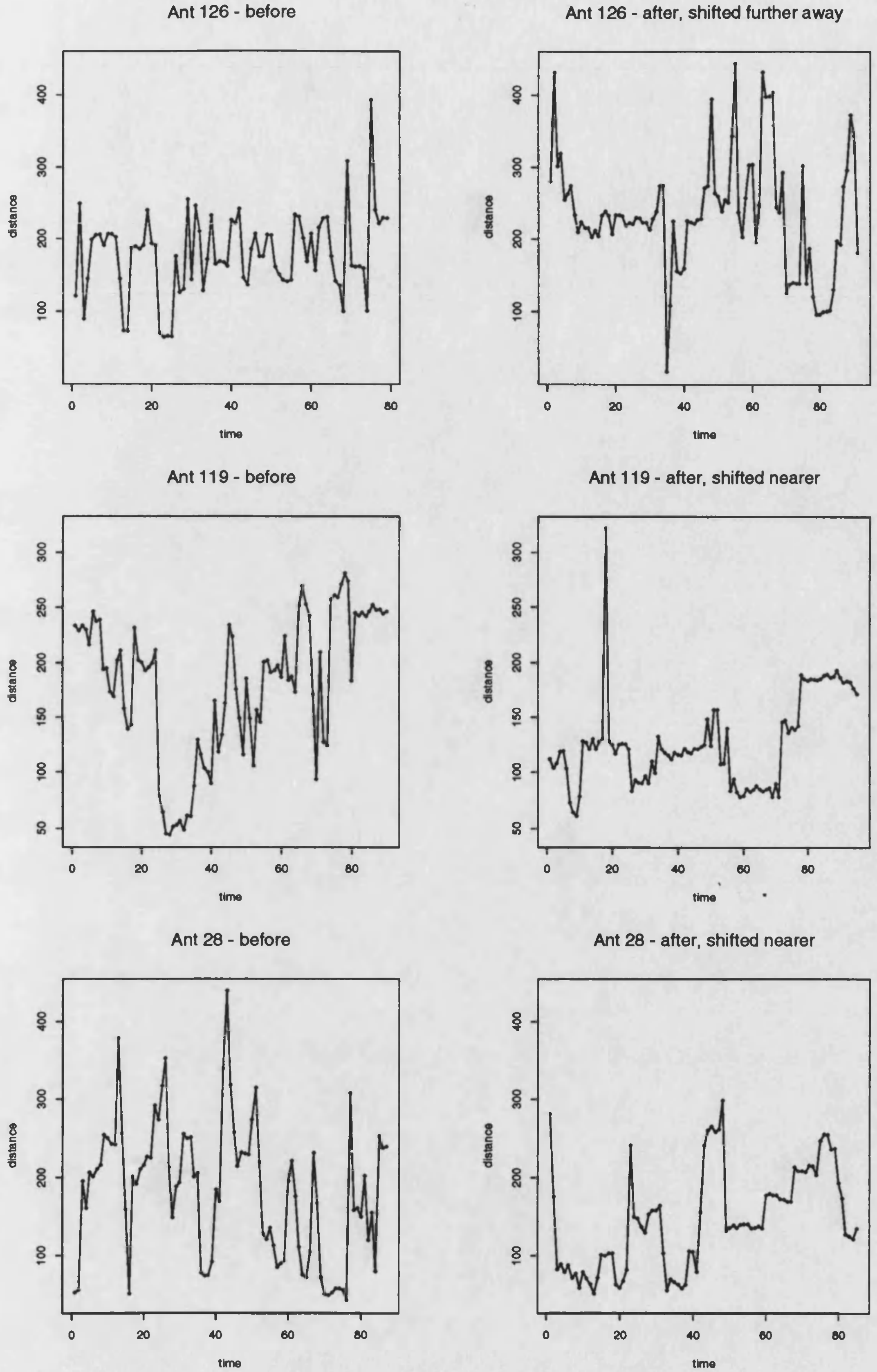


Figure 4.18: Distances from the centre for individual ants for before and after emigration; colony BG91UI


Ant 126 - before

Ant 126 - after, shifted further away

Ant 119 - before

Ant 119 - after, shifted nearer

Ant 28 - before

Ant 28 - after, shifted nearer

Figure 4.19: Distances from the centre for individual ants for before and after emigration; colony BG91UJ

If the allowed period for stabilisation was not long enough, ants that had shifted (nearer or further from the centre) after the emigration would possibly show an initial period of adjustment to their pre-emigration state. There is no clear evidence for such an adjustment. Therefore, the inference is that either the period of observation was too short to reveal any adjustment, or the interval allowed for stabilisation was more than enough. The second case is considered to be more probable.

4.4 Discussion

4.4.1 Task Allocation during Emigration

The present study confirms the existence of a division of labour during emigration. (1) The percentage of active participants during a single nest movement is found to vary among colonies, probably in relation to variance in the ratio between brood and ants, but in all cases this percentage is greater than the one reported for the same species by Lane (1977a). This difference may relate to the differences in the method of observation and recording.

(2) A greater proportion of brood appears to stimulate more ants into action directly or through more recruitment. Conversely, the number of transporters appears to be smaller when the brood to ant ratio is smaller. At the same time the number of transporters appears to decrease more slowly than the brood to ant ratio, because the proportion of transported ants appears to increase with the decrease of the brood to ant ratio. In other words, a smaller percentage of workers transport more nestmates since the number of brood is disproportionately smaller. Further experiments will be necessary to confirm the hypothesis that the proportion of brood is an organising factor in emigration and to evaluate its

interaction with other factors, e.g. distance between the old and the new nest, task allocation.

(3) The process of task allocation is not random. The percentage of ants that have been a transporter during at least one of five successive emigrations is so much smaller than expected that even the percentage of the ants that have been a transporter during the maximal possible number of emigrations is greater than expected. Although not all ants are equally likely to be transported during an emigration, the bias is not as great as it is for transporters. In other words, the differentiating process is less intensive for being a transported than for being a transporter. The group of lone arrivers, on the other hand, is apparently undifferentiated - the probability of any one ant arriving at the new nest by chance is not significantly different from random. Therefore, it is justified to consider only two specialisations during nest moving - those of being a transporter and being a transported. The existence of a neutral (undifferentiated) behavioural group only supports the idea that the differentiation is probabilistic, because if everybody is equally likely to belong to it, then nobody could be completely specialised.

The result that specialisations during nest emigrations do exist does not necessarily mean that some ants are deterministic transporters, i.e. genetically preprogrammed, genetically differentiated. Equally, probability models based on learning, for example, could reveal that environmental nondeterministic differentiation could lead to this level of specialisation. Let us suppose that during the first emigration every ant has the same probability of becoming a transporter, but the more an individual has been a transporter, the more likely she is to become one during the next emigration due to learning (this likelihood of course depends on the period of time between emigrations). Probably the differentiation of the transported group, could also be accounted for by the transporters, who might be capable of learning to recognise individually the items they carry during successive emigrations. When Möglich and Hölldobler (1974) removed the most differentiated transporters in their experiments on *Formica sanguinea* and *Camponotus sericeus*, the time necessary for emigration increased. The removal of the less specialised transporters that carried out the emigration in these new circumstances left the colony almost unable to organise an emigration. It is unfortunately not clear from Möglich and Hölldobler's paper whether these results are based on one or more emigrations. Based on single emigration, their findings do not contradict the learning hypothesis advanced in this chapter. Möglich's and Hölldobler's (1974) results concerning the association between ovarian development and being a transporter or transported are inconclusive. However, if confirmed they might suggest some inequalities among the ants in terms of the initial probability of undertaking a particular emigration task. Ants with bigger ovaries are more likely to be heavier and less mobile and/or similarly very young ants might still not have developed the communication skills necessary for carrying nestmates or laying and recognising trails.

4.4.2 Relationship between Position and Behaviour before Emigration and Form of Participation in the Emigration

The nondeterministic differentiation hypothesis is supported by the lack of association between the spatial position or behaviour of ants before the emigration and their behaviour during emigration. In other words, workers from all parts of the colony and from all behavioural backgrounds are equally likely to become transporters, transported or lone arrivers. In this sense the group of transporters is comprised neither of specialists who do not do anything in the nest, because they are reserved exclusively for carrying out the emigration, nor of "elites" who work hard at many tasks. At the same time ants are not rendered identical by the alert situation which puts the entire colony in a state of alarm (Wilson 1980), since not all ants are performing the same task during emigration, and the original spatial and behavioural structure of the colony is readily re-established in the new nest after the emigration is completed. In addition, the group of transporters does not necessarily form a minority. After all it would be extremely inefficient if all the workers in a colony became transporters during a single emigration. It is important that some ants run away with the brood and hide. What would be the point of transporting nestmates if all of them were transporters themselves?

The learning hypothesis for emigration still remains to be tested with repeated emigrations of colonies whose most differentiated transporters are removed. Assuming that learning increases efficiency, the alternative experiment is to provoke the emigrations of several colonies several times and to find out whether there is a tendency for the proportion of transporters to decrease, whereas the proportion of transported has a tendency to increase and the proportion of lone arrivers - to decrease as well. The data for colony BG92U5, which was emigrated five times (Table 4.3), shows some vague evidence for the existence of such tendencies, but the sample size is not big enough to make any statistically significant inferences.

4.4.3 Effect of the Disturbance of Emigration on the Spatial and Social Organisation of the Colony

Description of the colony before and after the disturbance of emigration reveals that the colony spatial configuration and behavioural distribution are robust and are reconstructed substantially in a matter of hours. It is possible to interpret this result as a confirmation of the theory of age polyethism, since on the basis of this theory after nest movement individuals would be expected to re-sort themselves to their original tasks simply by not being able to perform any other task but the one that they are genetically preprogrammed to do at their age. However, since the theory of age polyethism has already been falsified (Chapter 3), a new theory is necessary to explain how colonies restore their spatial and social order.

The result that ants are restored to their initial spatial positions complements the finding that after emigration the brood is re-sorted into its original spatial pattern, and that this could be achieved by simple rules of thumb on the level of the individual ant (Franks and Sendova-Franks 1992). Neither the brood nor the ants appear to be the sole factor that makes reconstruction of spatial and behavioural order possible. Both of them, and also the queen could play a role in re-establishing structure (see sections 3.3.6. and 3.4).

In conclusion, it is fair to say that similarly to the task allocation process within the nest, task allocation during emigration shows signs that falsify the hypothesis of a deterministic underlying mechanism, whereby rigid, preprogrammed specialisations of workers account for their behavioural differentiation.

Chapter 5

The Queen - her Position and Interactions with Workers

5.1 Introduction

In a colony of *L. unifasciatius* the queen is easy to distinguish from the workers. She is not only morphologically different and much bigger than the rest of the ants, but since she is the mother of all colony members she is usually found in the centre of the colony, over the egg pile. This central spatial and social position of the queen, as well as the term "queen" itself, suggest that she gives out instructions and is in control of the colony. However, whether this is the case is far from clear.

In order to understand the position of the queen in a colony of *L. unifasciatus*, it is necessary to consider the relationship between ant queens and workers in evolutionary terms.

Sex determination in order Hymenoptera is haplodiploid - males are derived from haploid (unfertilised) eggs and females - from diploid (fertilised) eggs. Hence, on average sisters have a coefficient of relatedness equal to 0.75, while the probability of an allele being present in both brother and sister is on average 25%. As in all advanced eusocial Hymenopterans, workers in *L. unifasciatus* are not truly female, because they cannot mate and therefore cannot produce diploid, female offspring. On the other hand, typically for most advanced eusocial Hymenopterans, *L. unifasciatus* workers have ovaries and can lay unfertilised, haploid, male-producing eggs. Although *L. unifasciatus* is a monogynous species, it is not known whether this species is mono- or polyandrous (one or more fathers, which is equivalent to one or more matings).

Theoretically these asymmetries of relatedness lead to potential conflicts of interest over reproduction between queen and workers, and between workers and workers. The most important of these conflicts are: sex allocation - the ratio between the sexes in the produced reproductives, male production - who produces the males, and queen-rearing discrimination - whose females are reared. However, in agreement with a fundamental characteristic of life history strategies (Stearns 1992) ant colonies undergo a period of growth before they start to rear sexuals. The colonies of all known ant species are perennial. The period of colony growth varies with the species, and could take five or more years. Generally, the life cycle of an ant colony is divided into three parts: founding stage - in *L. unifasciatus* the young queen starts a colony on her own after the nuptial flight, ergonomic stage - the period of growth, and reproductive stage - in which sexual offspring are produced (Oster and Wilson 1978, Hölldobler and Wilson 1990, p. 143) Theoretically, conflicts over reproduction are not expected during the ergonomic stage, because there is no reproduction and no options for manipulating it are yet available (Ratnieks and Reeve 1992). Note that workers would be more closely related to their full-sisters (0.75) than to their own sons (0.50) and therefore they are not expected to initiate any production of males before the queen starts to produce sexuals.

On the other hand, subtle manipulations might exist in "anticipation" of the reproductive phase. Consider a phenotypic trait like the "work load" for example. High work load benefits the colony but increases the mortality risk for the individual. Some individuals could "cheat" by being lazy and thus could prolong their lives into the period of colony reproduction (Schmid-Hempel 1990). This would be a likely strategy for a species like *L. unifasciatus* where the life span of individual workers (about 3 years) is roughly comparable to the duration of the ergonomic stage (probably 3-4 years, the longevity of the queen is about 10 years) (Franks et al. 1990). It is considered reasonable to assume that workers could assess the stage that the colony is in by using colony population or the presence of reproductive brood as cues (Ratnieks and Reeve 1992).

Another aspect of the relationship between queen and workers is the empirical evidence of a wide diversity of anatomical structures and behaviours in the queen that may be interpreted as mechanisms by which she controls the workers, in the sense that the conflict between queen and workers is resolved in favour of the queen (Hölldobler and Wilson 1990). Indeed, mated physogastric queens produce pheromones which make them very attractive to the workers (Glancey et al. 1982, Glancey 1986, Edwards and Chambers 1984, through Hölldobler and Wilson 1990). It has been established in a number of cases (Bourke 1988) that a negative association exists between the presence of a reproductive queen and the ability of workers to lay eggs. This applies also to the colonies of *L. unifasciatus* where workers have been observed laying viable, full-size, well-formed eggs in the presence of the queen (unpublished observations); these eggs were apparently only trophic, because no male pupae emerged among the brood as a result. At the same time, most queenless colonies of *L. unifasciatus* produce numerous eggs, some of which survive to produce viable imago males (unpublished observations).

Since aggressive behaviour on the part of the queen is rarely observed and since pheromonal queen control has never conclusively been demonstrated and is evolutionarily difficult to justify (Keller and Nonacs 1993), it could be argued that the workers are not controlled but are simply monitoring the presence of the queen and responding in ways that increase their own inclusive fitness (Hölldobler and Wilson 1990, p. 224).

The objectives of the present chapter are first, to describe the queen in terms of her spatial position in the nest and to compare her position with those of the workers which were described in a similar manner in Chapter 2, and second, to look for interactions between queen and workers that can not be detected through behavioural records, but are possible to demonstrate through randomisation tests on the spatial data. Associations between queen and workers were first demonstrated by McCoy (1991) on the spatial data for the colony studied and described in Sendova-Franks and Franks 1993.

5.2 Materials and Methods

5.2.1 Spatial Description of the Queen

5.2.1.1 Sessions Involved

Colonies of *L. unifasciatus* were kept and studied as described in sections 1.3.12, 1.4.1, and 1.4.3. The spatial description of the queen is based on all 33 photographic sessions of colonies BG90UO, BG91UI, BG91UJ, BG91UQ, BG91UL, BG91UM, BG91UN, BG91US, BG92U1.

5.2.1.2. Randomisation Test for Association between Spatial Position and Number of Interactions with the Queen

Ho is: The presence of an interaction with the queen is independent of ant position. **Ha** is: Ants with positions nearer to the centre than the ant with the median position are more likely to have an interaction with the queen than expected on a random basis. **Implementation:** The types of association between the queen and each worker are permutated against the positions of the workers according to a randomly chosen permutation. The number of associations of particular type is counted for ants nearer (or further) to the colony centre than the ant with the median position. This is repeated 100 times and the resulting distribution is compared to the real value of the statistic. This test was carried out for sessions BG90UO5, BG91UJ1, BG91UJ2, BG91UQ1, and BG91UQ2 by a procedure written in the language of the S-plus statistical and graphical package (Becker 1988 et al).

5.2.2 Associations between Queen and Workers

5.2.2.1 Sessions Used for the Analysis - Justification

Only the 4 colonies observed in full sessions of type one (see section 1.4.1.3) were used for this analysis (sessions BG90U01-5, BG91UI1-2, BG91UJ1-2, BG91UQ1-2). Colonies observed in sessions of type two are not suitable, since the analysis is based on pictures taken at regular intervals during a day period. The two colonies observed in half sessions of type one were also excluded from the final analysis, because the number of queen-worker interactions was insufficient.

5.2.2.2 Background

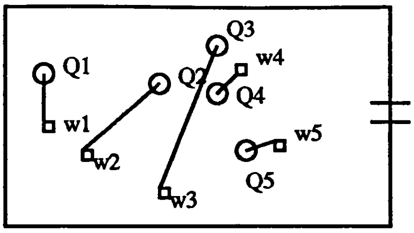
As already demonstrated in Chapter 2, only the workers with positions close to that of the queen make physical contact with her, but even this happens very rarely. The spatial description of the colony where the positions of all its members are known for the same moments in time, allows for another method of checking for any interactions between queen and worker as well as between worker and worker. This method is based on measuring the distance between the two individuals of interest for all the occasions when they were simultaneously recorded and then finding the median value of the resulting distribution.

The idea behind the test is that the distance between two individuals that move in space (which in our case is confined) independently of each other, should not on average be any different whether measured when the sampled positions of the individuals in question are in chronological order or when their chronological order is permutated.

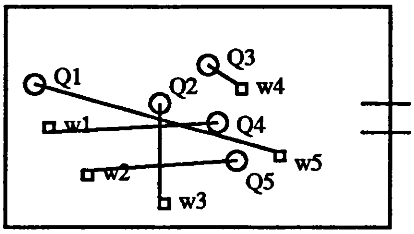
5.2.2.3 Randomisation Test and Clarification of the Terms 'Follow' and 'Avoid'

Ho is: The median distance between the chronologically ordered positions of the two examined individuals (Fig. 5.1, a) falls within the distribution of 100 such medians when the distances between the two individuals for the same day are measured after 100 random permutations of the chronological order of the positions of the second individual (Fig. 5.1, b).

Ha is: The two examined individuals are considered to **follow** each other (or one of them to follow the other) when the experimental median distance between them for the day is smaller than the distribution of 100 simulated (randomised) medians; the two individuals are considered to **avoid** each other (or one of them to



(a) in chronological order



(b) after randomisation

Figure 5.1: Distances between the queen and an ant: (a) in chronological order; (b) after randomisation. Illustration

avoid the other) when the experimental median distance between them is greater than the distribution of 100 simulated (randomised) medians. **Implementation:** (1) Make the individual files (i.e. the secondary type of raw data files - see section 1.4.3.1) of the examined individuals to contain only positions for photographs on which both individuals were identified, (2) divide the resulting files into days, (3) randomise the positions of the second ant for every day against chronological order, (4) find the distances between the chronological positions of the two ants for each day, (5) find the median of these distances for each day. Repeat points (3) to (5) 100 times and compare the resulting distribution for each day to the real mean of distances between the two individuals for that day.

The number of compared positions per day should be not less than 5, since the above test uses 100 permutations and there exist 120 permutations of the numbers from 1 to 5 in groups of 5 ($nPr = n!/(n-r)!$). The probability of the compared positions to be less than 5 per day is negligibly small, however, because the rule for an ant to be considered in the analysis at all is that she has at least 5 positions per session (see section 1.4.3) and this 5 positions are usually for the same day (see also Table 3.2, second column). In the final analysis ants that had less than 5 synchronous positions with the queen were considered as not being in an association with her for that day (see Appendix).

The test was carried out by a programme written in C, which uses the algorithms for generating pseudo-random numbers and for random choice of permutations that are described in sections 1.4.4.4 and 1.4.4.5.

5.2.2.4 Weaknesses of the Method

(1) If the queen (or the investigated worker) has been stationary throughout the explored period, no "avoiding" or "following" could be demonstrated and it is assumed that no association is present for the day in question. At the same time, if "following" or "avoiding" of the queen is demonstrated for at least one queen - worker pair during a given day, then the queen could not have been stationary.

(2) As specified in the description of the **Ho** and **Ha** this randomisation test does not in itself recognise who follows whom or who avoids whom. On days, for example, when the queen is found to be associated in the same way (following or avoiding) with more than one worker, this could mean that: (a) the queen is following/avoiding at most one worker and the rest are following/avoiding her; (b) the queen is following/avoiding more than one of the workers for parts of the day and for the rest of the day the workers are following/avoiding her; (c) every worker is following/avoiding the queen independently; (d) one worker is following/avoiding the queen and the rest are following/avoiding the worker or

each other, in a chain.

The possibility that the queen is following/avoiding more than one worker at a time is very small, since such a manoeuvre is difficult to calculate in one's stride. Therefore, it can be assumed with some certainty that workers must take an active part in the associations while the active participation of the queen is possible, but not indispensable.

Since the present chapter is concerned with the queen, only all possible queen - worker pairs were examined for the presence of any association. Potentially it is possible to examine any pair of workers for such associations.

5.3 Results

5.3.1 Spatial Position of the Queen

Spatial description of the queen confirms the visual impression that she occupies a central position in the colony, near the middle of the egg pile. Figures 2.3 to 2.9 (Chapter 2) demonstrate that the queen is the ant in the nest who is nearest to the centre. However, the positions of the queens for sessions BG91UI1, BG91UI2, BG91UM1, and BG91UM2 make an exception. Since in the majority of cases the position of the queen is in the centre of the colony, it is very likely that where an exception occurs this is an indication of the weakness or the infertility of the queen, or even that she is not a functional queen at all. Nevertheless, it is clear that the queen was functional in both of the colonies involved (BG91UI and BG91UM), because these colonies produced only workers and not a single sexual individual. A more rigorous examination of this idea requires a quantitative investigation of the relationship between spatial position and fertility of the queen.

5.3.2 Size of the Queen's SFZ

The queen is the ant with the smallest size of SFZ for most of the sessions (Figs. 2.11-2.16 (Chapter 2).

5.3.3. Shifts in the SFZ of the Queen between Sessions

According to the shift criterion (see section 3.2.3) there are shifts in the queen's SFZ between the following sessions:

BG90UO3 and BG90UO4 - further away from the centre;

BG91UJ1 and BG91UJ2 - further away from the centre;

BG91UQ1 and BG91UQ2 - further away from the centre;

BG92U11 and BG92U12 - further away from the centre;

BG91US1 and BG91US2 - closer to the centre;

BG91UL1 and BG91UL2 - closer to the centre;

BG91UN1 and BG91UN2 - closer to the centre (Figs. 5.2 and 5.3).

There was an emigration in between each pair of the above sessions with the exception of colonies BG91UL and BG91UN (see Table 1.4).

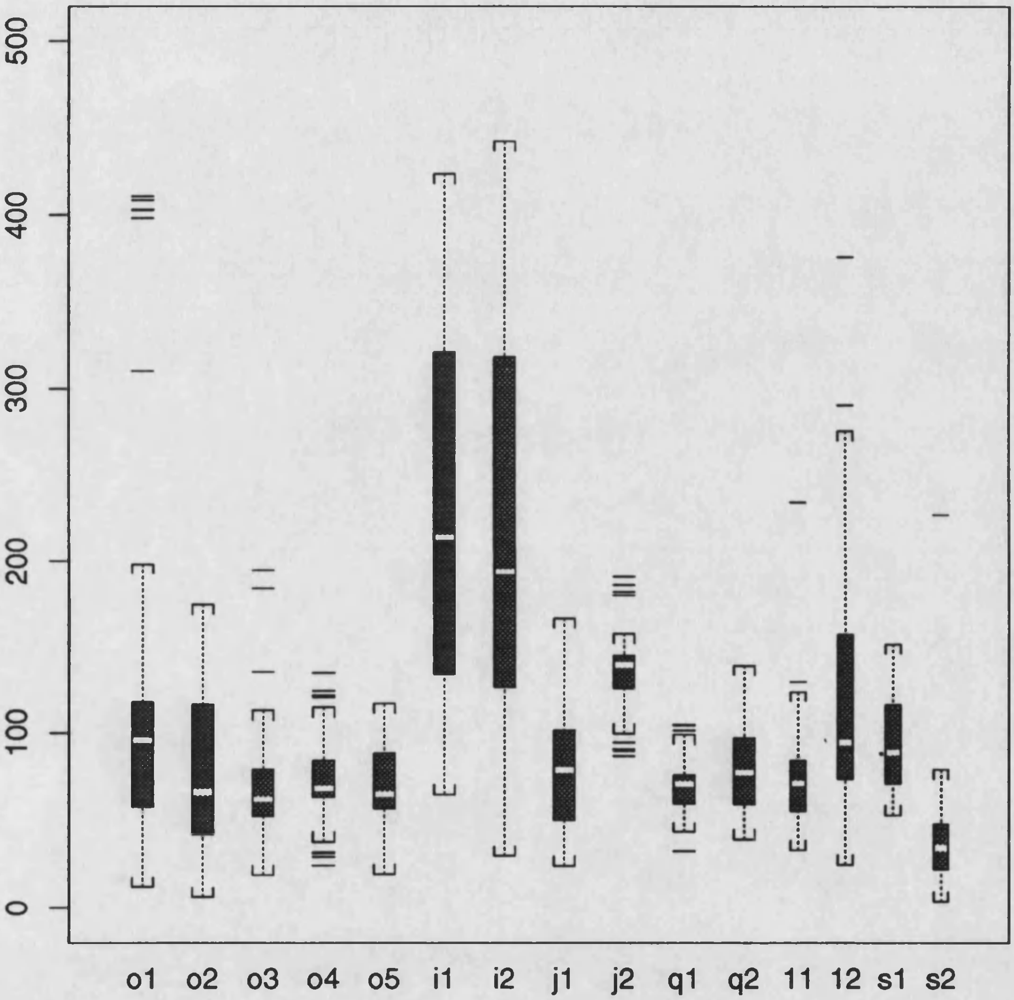


Figure 5.2: Distributions of the queens' distances form the centre; part 1

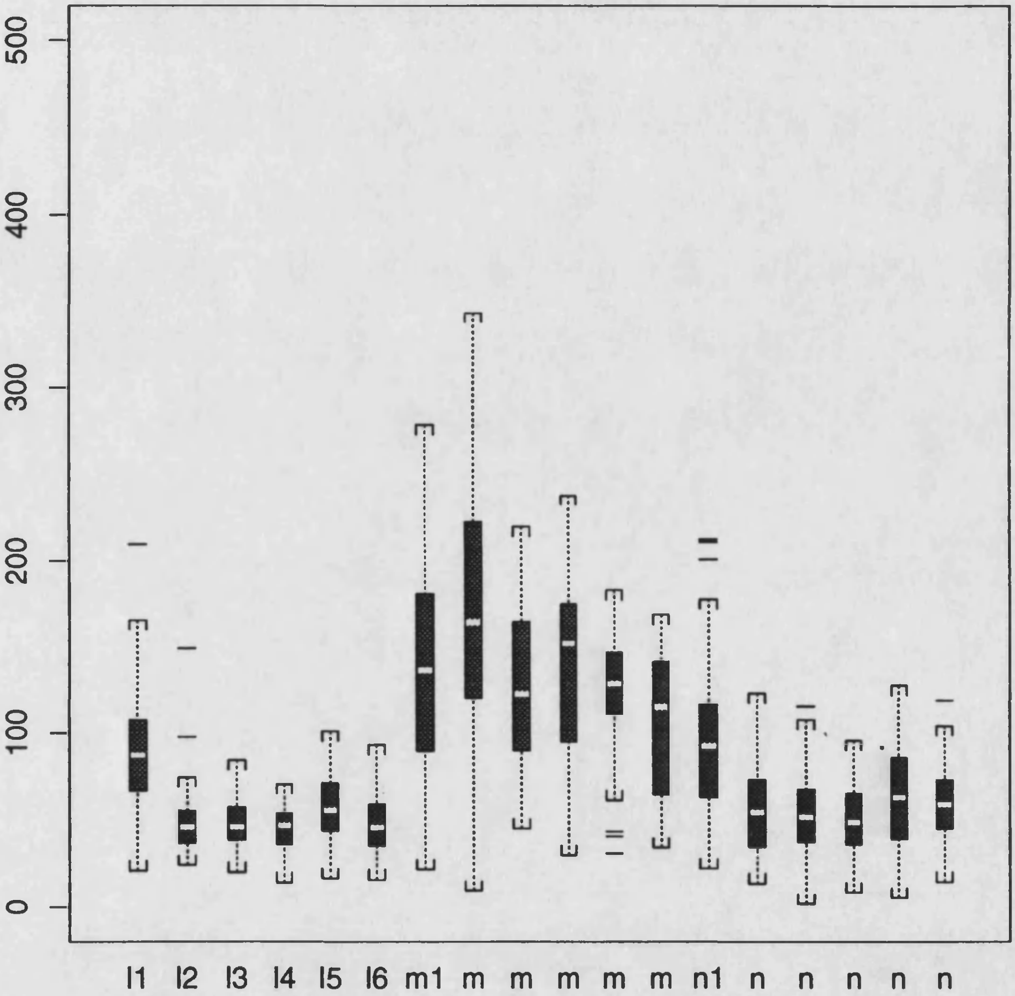


Figure 5.3: Distributions of the queens' distances form the centre; part 2

5.3.4 Relationship between the Position of the Queen and the Size of her SFZ

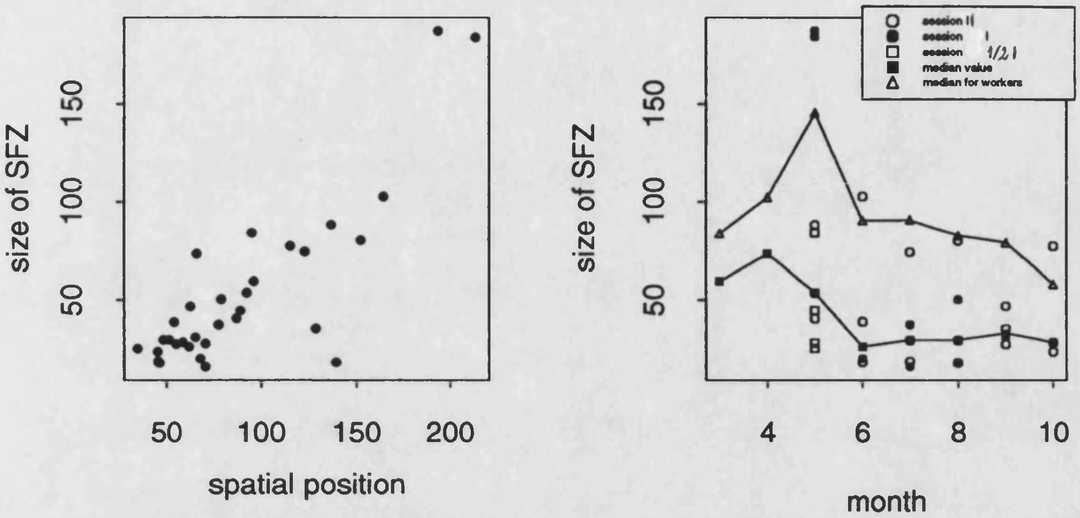
There is a significantly positive correlation between the position of the queen and the size of her SFZ (Fig. 5.4, a). The same relationship is present in workers with positions of up to approximately 400 - 500 pixels relative to the centre. In other words, the more mobile/active the queen is, the further away her position from the centre and the bigger her SFZ.

5.3.5 Relationship between the Size of the Queen's SFZ and the Median Size of Worker SFZ

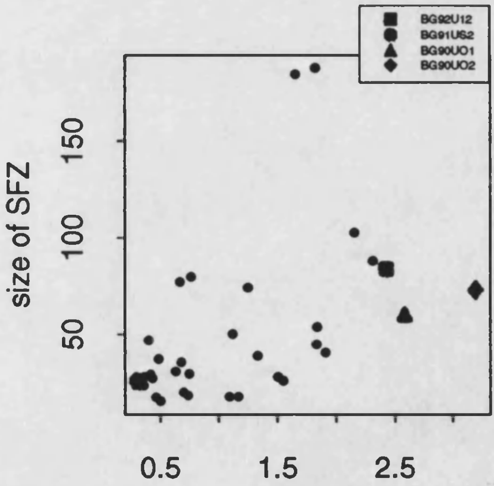
There is no correlation between the size of the queen's SFZ and the median size of the worker SFZ (Fig. 5.5). Why? Does the size of the queen's SFZ not vary with the time of year or with the brood to ant ratio in the same way as the median size of the worker SFZ? Is the maintenance of the queen's SFZ not governed by the same rules as that of the workers?

5.3.6. Variation in the Size of the Queen's SFZ with the Time of Year and with the Change in the Brood to Ant Ratio

A plot of the variation in the size of the queen's SFZ with time does not show the distinct peak in May, characteristic of the variation over time of the median size of worker SFZ (Fig. 5.4, b). One possible explanation is that the queen, like other ants with SFZ over the eggs and microlarvae, does not increase her SFZ in May because the brood and microlarvae do not spread out then as the rest of the brood does (the size of the queen's SFZ for session BG91UL1 on Fig.



rs. = 0.72, P < 0.01 at 31 d.f.



rs. = 0.58, P < 0.01 at 31 d.f.

Figure 5.4: Relationship between the size of the queen SFZ and: (a) her spatial position; (b) month of the year; (c) brood to ant ratio

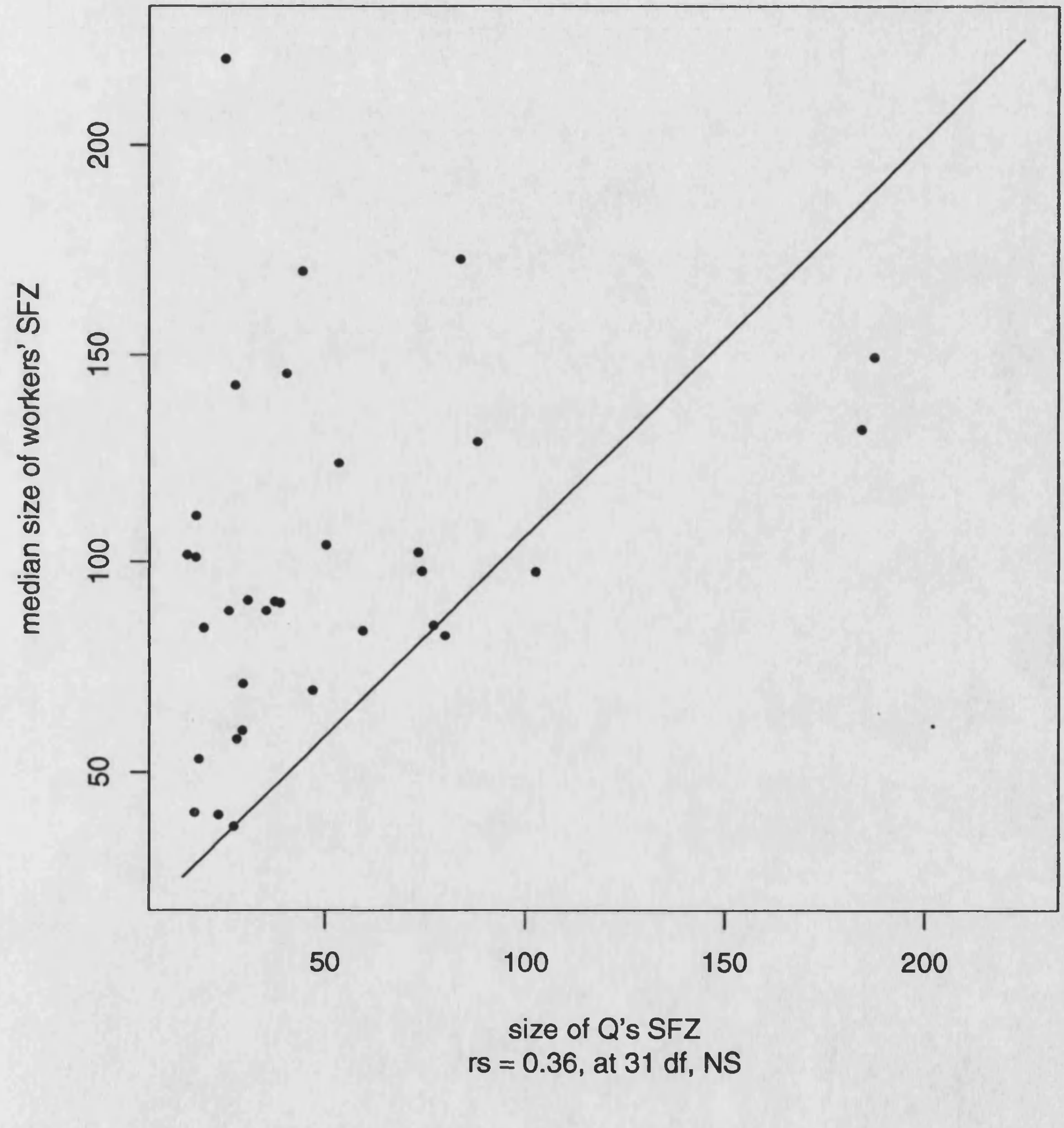


Figure 5.5: Relationship between the size of the queen SFZ and the median size of worker SFZ

5.3 contradicts this explanation). A possible alternative explanation is that the queen is simply different from the rest of the ants in the character and mechanism of maintenance of her SFZ.

There is a significant correlation between brood to ant ratio and size of queen's SFZ (Fig. 5.4, c), as is the case for the median size of worker SFZ (see Fig. 2.17, b). In the case of the workers this correlation makes sense in terms of workload - the greater the number of brood items an ant has to look after on average the greater her SFZ. Recall, however (Chapter 2) that the brood to ant ratio varies with time in a way similar to how brood tile area varies with time. In other words, the reason for the increase in worker SFZ in May may not lie with brood number but with a process similar to that leading to the expansion of the brood tile areas - increase in metabolic rate and activity perhaps. On the other hand, the experiment with the removal of approximately 1/3 of the workers (BG92U11 - BG92U12) led to a dramatic increase in the median size of worker SFZ (note that the experiment itself was carried out in May). Why should the queen increase her SFZ with the increase in brood to ant ratio if she does little or no brood care? Especially after the experiment with the removal of workers? Perhaps similarly to the workers she needs to retain contact with her nestmates.

5.3.7 Description of all Observed Queen - Worker Associations

The null hypothesis that the number of followings is not significantly different from the number of avoidings can not be rejected for any of the 11 sessions explored (Table 5.1)

The number of associations per capita of analysed workers varies between 0.14

QUEEN - WORKER ASSOCIATIONS

Session	Avoid no.	Follow no.	Total no.	Chi2	Associations per capita no.
BG90UO1	3	5	8	0.50	0.30
BG90UO2	3	1	4	1.00	0.17
BG90UO3	5	2	7	1.29	0.37
BG90UO4	16	10	26	1.39	0.38
BG90UO5	10	8	18	0.22	0.31
BG91UI1	5	5	10	0.00	0.14
BG91UI2	6	2	8	2.00	0.14
BG91UJ1	15	24	39	1.04	0.42
BG91UJ2	8	8	16	0.00	0.22
BG91UQ1	16	9	25	0.98	0.24
BG91UQ2	15	16	31	0.03	0.30

Table 5.1: Numbers of queen - ant associations of either type. At 1 df chi2 is not significant for any of the sessions

and 0.42.

5.3.8 Relationship between the Position of a Worker and the Presence or the Type of any Association with the Queen

Figures 5.6 and 5.7 show the relationship between the presence and type of queen - worker association on one hand and the position of the worker in the nest, on the other.

The results from the randomisation test (see section 5.2.2.3) show that workers which have a position in the colony closer to the centre than the worker with median position are more likely to have an association with the queen than expected on a random basis for sessions BG91UJ1, BG91UQ1, BG91UQ2, and BG90UO5. However, when the total number of queen - worker associations is small, as in

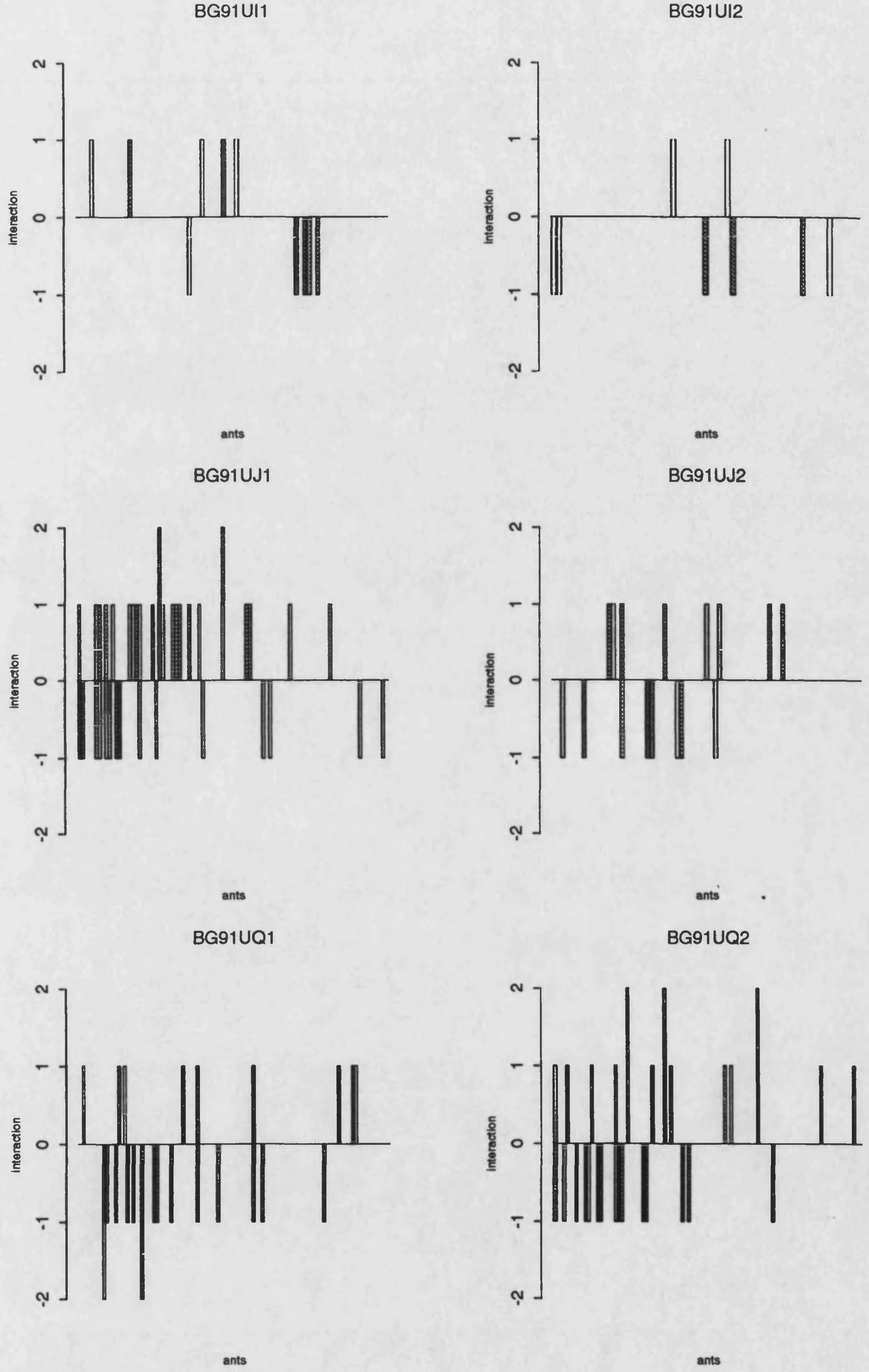


Figure 5.6: Relationship between spatial position and interaction with the queen. Ants are ordered according to position; 1 - avoid, -1 - follow. Colonies I,J,Q

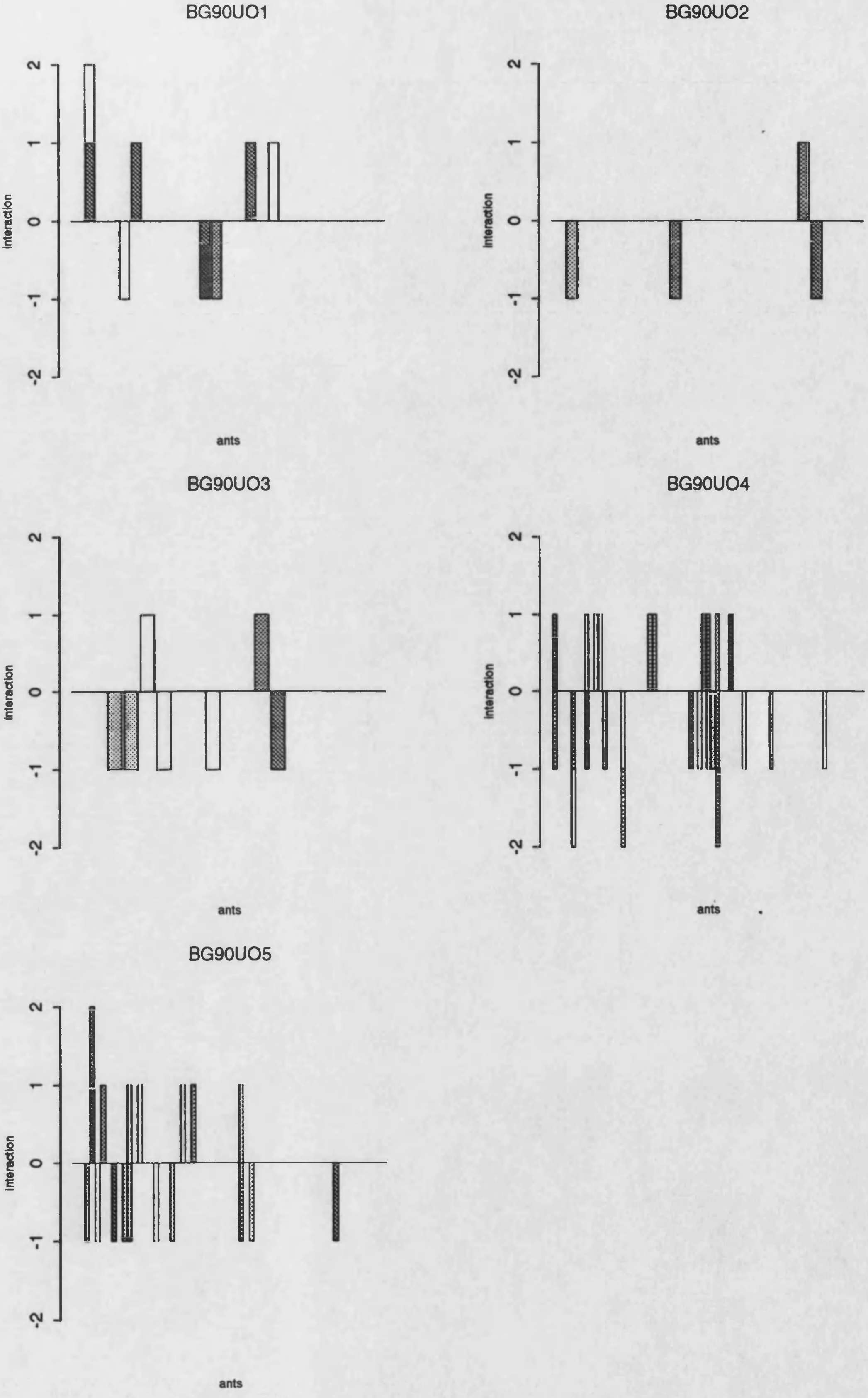


Figure 5.7: Relationship between spatial position and interaction with the queen. Ants are ordered according to position; 1 - avoid, -1 - follow. Colony BG90UO

Session	**Near & avoid**	**Near & follow**	**Total**	**Far & avoid**	**Far & follow**	**Total**
	no.	no.	no.	no.	no.	no.
BG90UO1	3	3	6	0	2	2
BG90UO2	1	0	1	2	1	3
BG90UO3	3	1	4	2	1	3
BG90UO4	9	7	16	7	3	10
BG90UO5	7	7	**14**	3	1	**4**
BG91UI1	1	3	4	4	2	6
BG91UI2	3	2	5	3	0	3
BG91UJ1	11	20	**31**	4	4	**8**
BG91UJ2	7	4	**11**	1	4	**5**
BG91UQ1	13	5	**18**	3	4	**7**
BG91UQ2	14	10	**24**	1	6	**7**

Table 5.2: Relationship between spatial position and presence and type of association with the queen. The numbers that are significantly different from random expectation are highlighted

the remaining 7 sessions (Table 5.2) this bias can not be detected. Perhaps this means that the bias itself is also small.

Such a result suggests that the nature of the queen - ant associations depends on distance, perhaps depending on an olfactory cue.

No association between the type of the queen - worker association and the position of the worker in the colony was found for any of the 11 sessions (chi2 test) (see Table 5.2).

Implications of the following association: (1) the ant is aggressive and the queen is running away; (2) the behaviour of the queen is independent of the behaviour of the worker who is passively following the queen (because she has an attractive smell, for example).

Implications of the avoiding association: (1) the queen is aggressive and the worker is running away; (2) the behaviour of the queen is independent of the behaviour of the worker who is passively avoiding the queen (because she has a repulsive smell, for example).

5.3.9 Comparison between the Distribution of Median Distances from the Queen for Ants that "Follow" her and the Distribution of Median Distances from the Queen for Ants that "Avoid" her

For all except two cases (BG90UO2 - very small sample sizes, and BG91UI1) the median distances for ants that "follow" the queen are considerably smaller than the median distances for ants that "avoid" the queen (Figs. 5.8 and 5.9) At the same time there is no difference between the distributions of median distances from the queen for ants that are in an avoiding association with her and ants that are neutral.

The possible implications of each of the two types of queen - worker association, avoiding and following, can be grouped into four categories: (1) Ants that are near to the queen "follow" her and their positions become closer or do not change with respect to the queen; ants that are away from the queen "avoid" her and their positions get still further from or do not change with respect to the queen. (2) Ants from any part of the colony can "follow" the queen and as a consequence get closer to her; ants from any part of the colony can "avoid" the queen, thus getting further away from her. (3) When close to the queen some ants "follow" her while others - "avoid" her. (4) When away from the queen some ants "follow" her, others - "avoid" her.

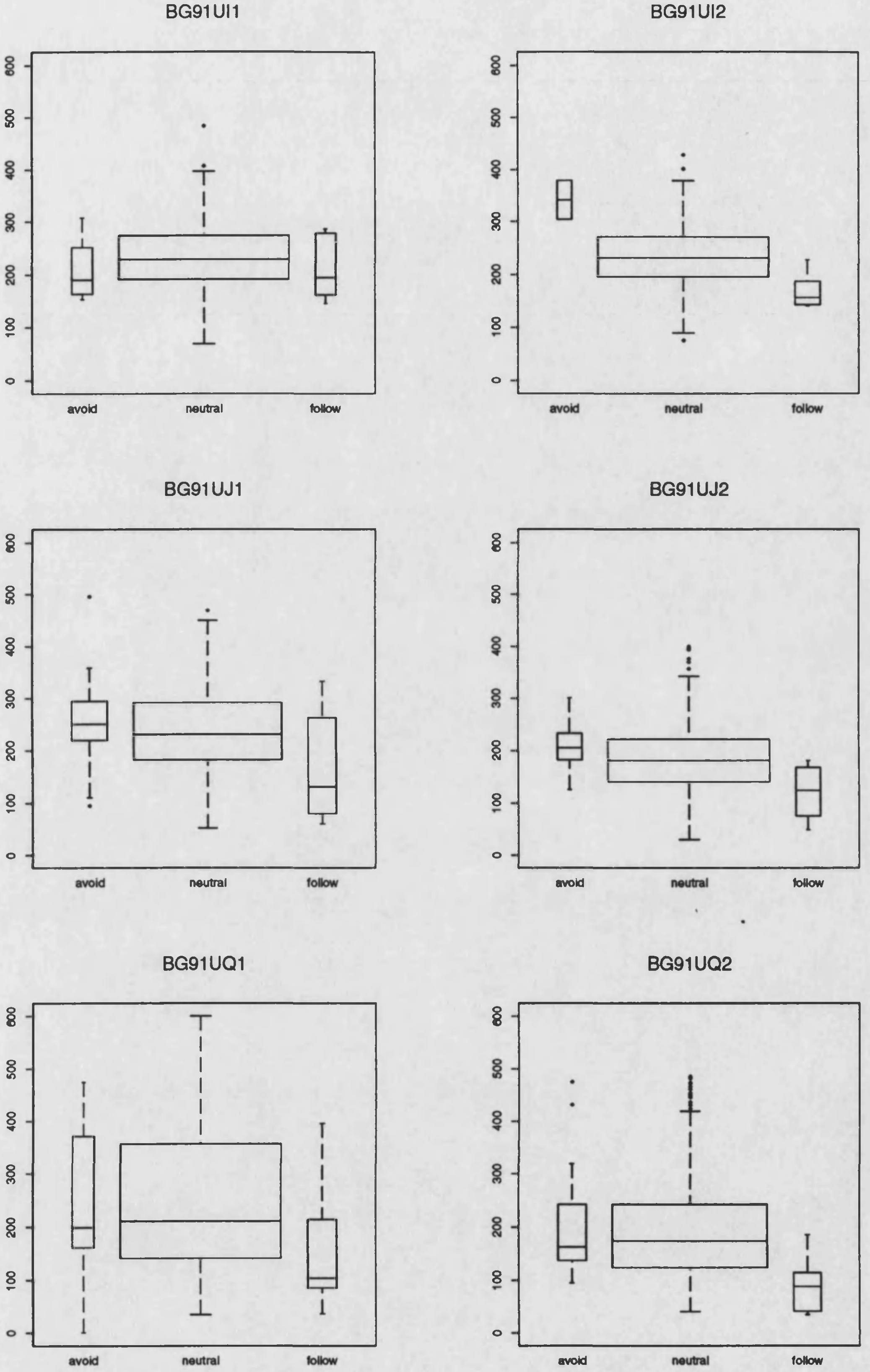


Figure 5.8: Distributions of median queen-ant distances. Colonies BG91UI, BG91UJ, BG91UQ

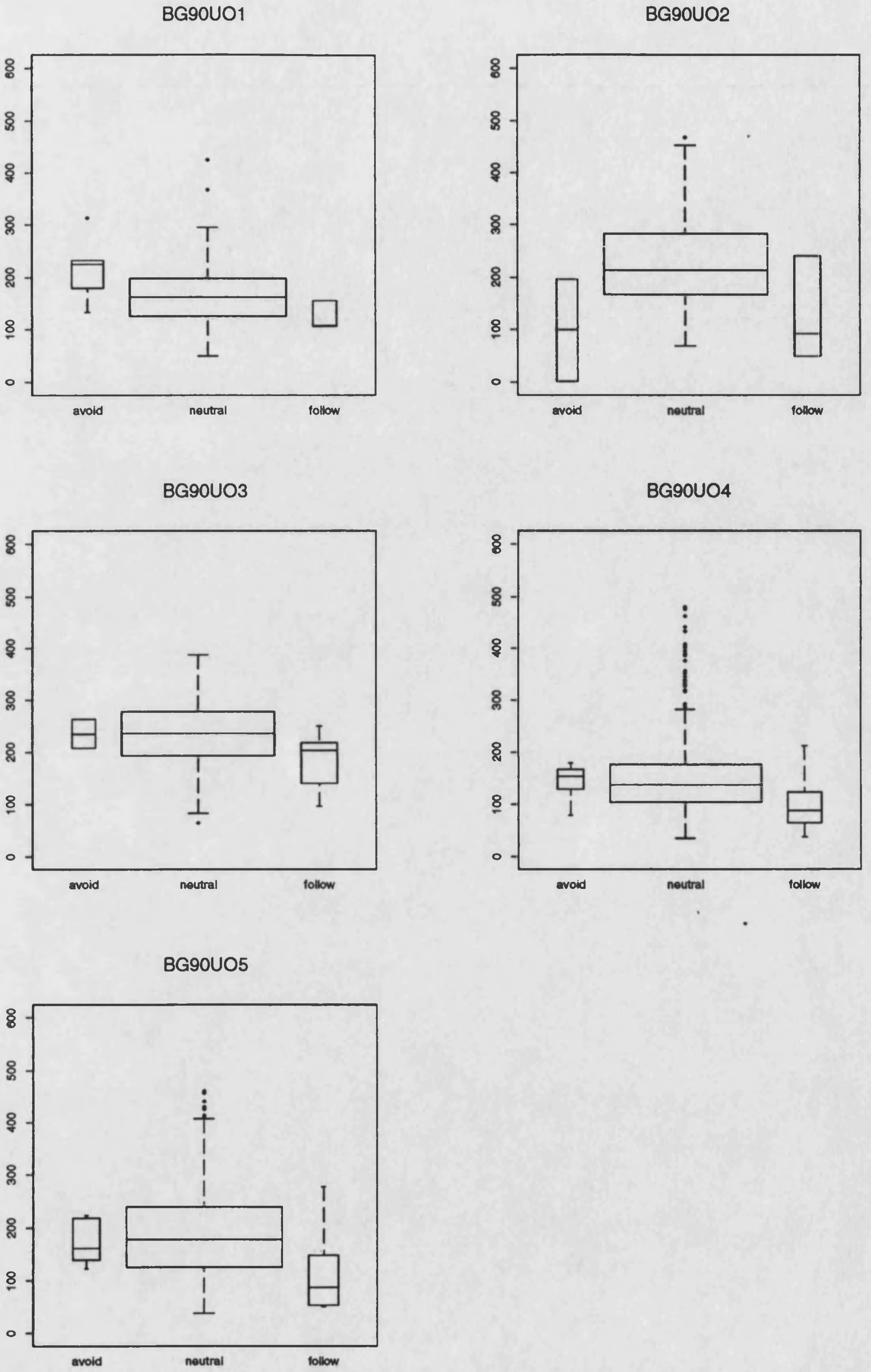


Figure 5.9: Distributions of median queen-ant distances. Colony BG90UO

Ants that "follow" the queen tend to be closer to her and at the same time there is no association between the type of queen - worker interaction and the position of the worker. This suggests that perhaps ants that "follow" the queen get closer to her on the day of interaction, but later resume their usual relative position in the nest (second implication).

5.4 Discussion

The spatial description of the queen confirms that she has a central spatial position in the colony. Like the workers she has a SFZ which follows the general rule that the size of an ant SFZ decreases with the decrease in its distance from the centre. On the other hand, the size of the queen SFZ does not correlate with the median size of the worker SFZ. Her activity does not appear to follow the same annual pattern as the activity of the workers. Further experiments might establish a correlation between the mobility of the queen and her fertility.

The discovery of queen - worker associations, which can not be detected through behavioural methods, has a great potential for unravelling queen - worker interactions. Further analysis that could detect whether any one of the two individuals was "anticipating" the move of the other may be able to establish whether the queen, the worker, or both play an active part in the queen - worker associations. What is clear at present is that the participation of the workers is indispensable. Therefore, we can assume with some certainty that at least some proportion of the queen - ant interactions mean that the workers are monitoring the queen. It seems possible that they are able to follow/avoid her on the basis of olfactory cues provided by the queen's pheromones, as evidenced by the dependence of the number of associations on distance from the centre of the colony. There is no correlation between the size of the queen's SFZ (a measure of her mobility) and the number of associations (both types together and each separately) between her and workers. This result dismisses the hypothesis that queen - worker interactions appear when the queen enters the SFZs of the workers during her bouts of activity.

The alternative hypothesis is that ants monitor the queen from time to time in order to "adjust" their position in the colony and at the same time to update their information about the condition of the queen, because any changes in her fertility alter their interests in terms of what and how much brood should be produced.

Interpretation of the results from the present study will be most certainly aided by future exploration of the frequency of worker - worker interactions in queenright as well as queenless colonies.

Chapter 6

General Discussion and Conclusion

6.1 Summary of the Results

The present study has found that:

(1) The colonies of *L. unifasciatus* are organised so that workers are spatially ordered in a continuum of individual Spatial Fidelity Zones (SFZs) where each ant carries out the work available within her own SFZ. SFZs increase in size from the centre of the colony outwards and also expand or contract in relation to the time of year.

(2) Along with the annual expansions and contractions of the colony, some ants change their absolute spatial positions and behavioural profiles. Ants change their absolute spatial positions at different rates, different age cohorts intermix gradually, and hence no strong relationship between age and task is observed.

(3) A colony's spatial configuration is dynamic, but also flexible and stable. Nei-

ther the removal of workers or brood, nor the disturbance of emigration lead to a new arrangement of the colony structure.

(4) There is no correlation between the spatial position and behavioural profile of an ant before emigration and the form of her participation in the emigration effort.

(5) The queen, of all the ants in the nest, is the one nearest to the centre of the colony and has the smallest SFZ. However, the size of the queen's SFZ does not vary over the year in the same way as the median size of the worker SFZ.

(6) The spatial description of ant colony organisation provides new evidence for queen-worker interactions.

6.2 Implications

The spatial structure that I have discovered constitutes a striking confirmation of the predictions made by the top-down approach of optimisation theory that a social insect colony should be an example of functional and spatial efficiency. As predicted by Wilson (1985b), ants fall into classes on the basis of their role (defined as tasks that are grouped so as to divide labour) and where the role is determined by the spatial locale to which an ant holds her allegiance (for example, the brood pile, the exit). However, the ants that comprise such a class do not belong to the same age cohort. My work demonstrates that age cohorts are spatially and behaviourally intermixed and thus falsifies the concept of temporal caste.

In earlier studies the great behavioural variability in individuals of the same age cohort was alluded to as "noise" or explained within the theory of adaptive demography. This is no longer possible. The changes of colony spatial configuration over time leave no doubt that the underlying process is incompatible with a deterministic, hard-wired mechanism.

The top-down approach of optimisation theory needs to take into account the findings made by the observation of day-to-day colony organisation. The evidence is that the *a priori* efficiency presumed by optimisation models, can be achieved through probabilistic behavioural differentiation. There is now ample evidence that societies of eusocial insects have the capacity through interaction to amplify simple behavioural rules at the individual level into complex behaviours at the colony level (Pasteels et al. 1987, Franks et al. 1992, Camazine 1993). Therefore, natural selection does not need to act on genes that code for all possible eventualities, but rather, on genes that code for simple behavioural patterns.

As demonstrated empirically, hormonal and genetical differences can account for at least some of the observed interindividual behavioural variability and influence the process of task allocation (Robinson 1992). However, the establishment of these differences does not in itself constitute a demonstration of a testable organisational principle. It is helpful to produce abstract models of colony organisation that can be falsified. The 'foraging for work' model of task allocation provides an abstraction that amounts to an organisational principle and strikingly resembles the concept of SFZs arranged in a continuum that was empirically established in the present work. This model and the data from the present study are both simplified to 1-dimension. Further empirical research and new more detailed, 2-dimensional spatial modelling should shed new light on the processes that underly spatial order and make function possible.

The results of the present study also contribute to the view of the social insect colony as a superorganism. The ant colony constitutes an entity that is much more similar to the multicellular organism than previously suspected, because the colony has its own spatial organisation and possibly its own structures for information acquisition and information processing.

The presented approach to the complexity of colony organisation by no means contradicts the theory of evolution by natural selection. On the contrary, it complements evolutionary theory by looking objectively at the mechanisms of day-to-day colony functioning.

6.3 Suggestions for Future Work

The work described in this thesis could develop in two directions. First, it could continue with the detailed investigations of the colony spatial organisation through mathematical modelling of its spatial configuration and the processes that underly the existence of SFZs. The approach of such an investigation would be to follow the idea that flexibility and spatial position do not only matter but are essential to biological differentiation. This idea is well established in embryology (Auerbach 1960) and is also gathering momentum in neurobiology (Rager and Rager 1989, Edelman 1992), despite Edelman's Theory of Neuronal Group Selection (TNGS) being a controversial view of how the brain works. Second, the bottom-up approach of spatial analysis can be used to bridge the gap between self-organisation theory and kin selection theory, which at the moment are successful on very separate grounds. It is clear that workers do not begin their lives as identical entities, as is usually assumed by self-organisation models, instead they are different in size, weight, activity, capability to learn, capability to produce eggs and reproductive interests. A detailed experimental analysis will be required to explore the consequences of such variation for spatial and behavioural parameters.

6.4 Conclusion

Further understanding of colony organisation requires that self-organisation and optimisation theory work in cooperation. In addition, it is my hope that the present work has demonstrated the great potential of the study of social insect colony organisation to contribute to a future general theory of biological organisation (Hölldobler and Wilson 1990, p. 359).

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Appendix

Due to the great amount of data this Appendix provides only examples. All data are available on floppy disks if required for examination.

Raw Data File of Type One for Session BG91UL1

652 422

0 235 265

39 330 227

16 343 205

42 411 183

24 393 235

21 507 254

41 290 306

22 184 357

5 150 305

29 155 256

27 261 186

6 210 91

7 122 107

10 72 204

11 99 234

28 57 317

17 606 101

9 603 314

2 215 233

37 494 235

33 610 203

35 634 289

4 565 375

3 448 282

1 577 146

34 244 306

31 301 75

Raw Data File of Type Two for Session BG91UL1

```
0012 26 36 208
0013 26 248 355
0021 26 412 174
0022 26 62 157
0023 26 310 160
0031 26 329 197
0032 26 63 146
0033 26 29 362
0051 26 194 360
0061 26 116 188
0062 26 380 83
0063 26 395 50
0071 26 237 328
0072 26 147 121
0073 26 107 151
0081 26 278 86
0082 26 161 278
0083 26 199 244
0091 26 371 275
0093 26 148 190
0101 26 328 183
0112 26 202 336
```

0113 26 202 336

0121 26 193 345

0122 26 127 213

0123 26 189 123

0131 26 154 85

0132 26 341 102

0133 26 315 160

0141 26 131 234

0142 26 207 279

0151 26 333 87

0152 26 246 325

0153 26 344 206

0161 26 430 123

0163 26 479 252

0171 26 237 126

0172 26 216 261

0173 26 39 163

0181 26 463 217

0182 26 197 277

0191 26 102 233

0192 26 180 265

0193 26 278 196

0202 26 474 151

0203 26 463 221

0211 26 320 303

0212 26 285 186

0213 26 555 239

0221 26 250 255

0222 26 266 256

0223 26 537 168

0231 26 265 136

0232 26 410 115

0233 26 527 296

Scores for Each Ant for Session BG91UL1

Columns represent: Ant ID, No. inside the nest, No. in the exit, No. outside the nest, Total

0	63	0	0	63
1	38	0	16	54
10	59	0	0	59
11	58	0	0	58
12	50	0	2	52
13	40	0	7	47
14	50	0	0	50
15	35	0	5	40
16	44	0	0	44
17	49	0	0	49
18	45	0	1	46
19	13	0	0	13
2	52	0	0	52
20	18	0	0	18
21	59	0	1	60
22	21	0	7	28
23	45	1	0	46
24	56	0	0	56
25	51	0	1	52
26	55	0	0	55
27	61	0	0	61

```
28 62 0 0 62

29 59 0 0 59

3 44 0 6 50

30 59 0 0 59

31 61 0 0 61

32 53 0 0 53

33 49 0 3 52

34 55 0 0 55

35 32 0 8 40

36 39 0 5 44

37 15 0 0 15

38 29 1 5 35

39 59 0 0 59

4 45 0 1 46

40 28 0 6 34

41 51 0 0 51

42 55 0 1 56

43 2 0 0 2

5 62 0 0 62

6 55 0 0 55

7 60 0 0 60

8 49 0 0 49

9 39 0 15 54
```

999 0 0 1 1

666 0 1 2 3

Raw Data File with Ant Behaviour for Session BG91UL1

Columns represent: Ant ID, No. of contacts with 5 types of brood, No. of contacts with queen, No. of contacts with young or old nestmates, No. of trophallaxes with larvae or ants

Ant C4 C6 C8 C9 C10 CQ CYA COA TWL TWA

1 0 0 1 0 0 0 0 0 0 1(14)

10 4 0 2 0 0 0 0 1(4) 5(4x8,6) 0

11 3 1 2 4 3 1 0 0 4(4x8) 0

12 0 0 1 0 0 0 0 0 0 1(8)

13 0 0 2 1 0 0 0 1(8) 0 0

14 0 0 3 0 1 0 0 0 0 2(1,666)

15 0 0 0 0 0 0 0 1(666) 1(8) 1(28)

16 1 0 1 2 1 1 0 0 0 0

17 2 1 1 2 0 0 0 1(10) 2(2x6) 0

18 0 0 2 0 0 0 0 2(31,23) 2(8,8) 0

19 0 0 1 0 1 0 0 0 0 0

2 6 0 1 3 1 0 0 0 4(9,2x8,6) 0

20 0 0 2 0 0 0 0 0 0 0

21 0 0 2 2 1 0 0 1(666) 2(8,8) 0

22 0 0 2 0 0 0 0 0 0 1(4)

23 0 0 3 3 2 0 0 0 1(9) 2(6,9)

24 4 0 1 1 2 0 0 1(36) 4(9,3x8) 0

25 1 2 4 1 2 0 0 0 2(6,6) 0

26 4 0 0 1 0 0 0 1(31) 2(8,4) 1(6)

27 0 1 2 2 0 1 0 1(15) 0 0

28 20 0 2 2 0 0 0 0 3(8,6,4) 1(15)

29 18 0 0 1 0 0 0 0 1(6) 0

3 1 0 0 0 0 0 0 0 0 0

30 1 1 1 4 2 0 0 1(27) 1(6) 0

31 3 1 0 1 0 2 0 1(30) 2(8,6) 1(6)

32 0 0 2 2 2 0 0 0 7(7x8) 0

33 0 0 3 0 0 0 0 2(666,4) 2(8,6) 1(40)

34 6 0 0 2 0 0 0 0 6(8,3x6,2x4) 0

35 0 0 0 0 0 0 0 2(17,27) 0 2(42,666)

36 0 0 0 1 2 0 0 0 0 0

37 0 0 0 0 0 0 0 1(27) 1(8) 0

38 0 0 0 0 0 0 0 0 0 0

39 0 0 2 4 1 0 0 3(36,27,13) 4(2x8,2x6) 1(666)

4 0 0 0 0 0 0 0 0 1(8) 2(8,22)

40 0 0 1 0 1 0 0 1(666) 1(6) 2(33,16)

41 5 0 1 4 0 0 0 0 4(9,3x8) 0

42 9 1 1 0 0 0 0 1(32) 2(8,8) 1(35)

43 0 0 0 0 0 0 0 0 0 0

5 1 1 2 4 1 0 0 0 3(2x8,6) 0

6 0 0 1 2 2 0 0 0 5(4x8,6) 3(23,31,26)

7 4 0 3 0 0 0 0 0 3(2x8,6) 0

8 0 0 2 1 0 0 0 1(666) 3(3x8) 2(12,4)

9 0 0 0 0 0 0 0 0 0 1(23)

Pooled Data File with Ant Behaviour for Session BG91UL1

Columns represent: Ant ID, No. of contacts with all types of brood, No. of all types of contacts with ants, No. of contacts with queen, Total

Ant allwtbrood allwtants allwtQ total

1 1 0 1 2

10 11 0 1 12

11 17 1 0 18

12 1 0 1 2

13 3 0 1 4

14 4 0 2 6

15 1 0 2 3

16 5 1 0 6

17 8 0 1 9

18 4 0 2 6

19 2 0 0 2

2 15 0 0 15

20 2 0 0 2

21 7 0 1 8

22 2 0 1 3

23 9 0 2 11

24 12 0 1 13

25 12 0 0 12

26 7 0 2 9

27 5 1 1 7

28 27 0 1 28

29 20 0 0 20

3 1 0 0 1

30 10 0 1 11

31 7 2 2 11

32 13 0 0 13

33 5 0 3 8

34 14 0 0 14

35 0 0 4 4

36 3 0 0 3

37 1 0 1 2

38 0 0 0 0

39 11 0 4 15

4 1 0 2 3

40 3 0 3 6

41 14 0 0 14

42 13 0 2 15

43 0 0 0 0

5 12 0 0 12

6 10 0 3 13

7 10 0 0 10

8 6 0 3 9

9 0 0 1 1

Raw Data File with Ant Behaviour during Emigration for Colony BG91UI

Columns represent: Ant ID, No. of times arriving alone, No. of times transporting each of 5 types of brood, No. of times transporting marked ants, No. of times transporting blank ants

Ant alone wtl4 wtl6 wtl8 wt9 wt10 wtmant wtblant

1 2 0 0 1 2 0 0 0 5

10 2 0 0 0 0 0 1(48) 0 3

11 0 0 0 0 0 2 1(52) 0 3

12 0 0 0 0 0 0 0 0 0

13 5 0 0 1 1 1 0 0 8

14 5 0 0 0 0 0 0 0 5

15 0 0 1 0 0 0 0 0 1

16 0 0 0 1 0 0 0 0 1

17 0 0 0 0 0 0 0 0 0

18 3 2 1 1 2 1 2(69,30) 0 12

19 1 0 0 0 0 0 0 0 1

2 1 0 0 0 0 0 1(29) 0 2

20 1 1 0 0 0 1 0 0 3

22 4 0 0 0 0 0 0 0 4

23 1 0 0 0 0 0 0 0 1

24 0 0 0 0 0 0 0 0 0

25 0 0 0 1 4 0 0 0 5

26 1 0 0 1 0 1 0 0 3

27 0 0 0 0 0 0 0 0 0

28 0 0 0 0 0 0 0 0 0

29 3 0 0 0 0 0 0 0 3

3 0 0 0 0 0 0 0 0 0

30 0 0 0 0 0 0 0 0 0

31 0 0 0 0 0 0 0 0 0

32 0 0 0 0 0 0 0 0 0

33 3 0 0 5 0 0 0 0 8

34 0 0 0 0 0 0 0 0 0

35 2 0 0 1 2 0 0 0 5

36 0 0 0 0 0 0 0 0 0

37 0 0 0 0 0 0 0 0 0

38 1 0 0 0 1 1 0 0 3

39 1 0 0 0 1 0 0 0 2

4 1 1 0 1 0 1 0 0 4

40 5 0 3 4 1 3 5(31,48,66,11,82) 1 22

41 0 0 0 0 0 0 0 0 0

42 0 0 0 0 0 0 0 0 0

43 0 1 0 0 0 0 0 0 1

44 0 0 0 0 0 0 0 0 0

45 1 0 1 0 0 0 2(26,57) 1 5

46 2 2 0 3 0 0 1(666) 0 8

47 0 0 1 1 0 0 0 0 2

48 1 1 0 0 0 0 2(69,5) 0 4

49 1 0 0 0 0 1 1(22) 0 3

5 0 0 0 0 0 0 1(18) 0 1

50 1 0 0 0 0 0 0 0 1

51 2 0 0 0 2 0 1(14) 0 5

52 1 0 0 0 0 0 0 0 1

53 0 0 0 0 0 0 0 0 0

54 3 0 0 0 0 0 1(60) 0 4

55 1 0 0 0 0 0 0 0 1

56 0 0 1 0 0 1 3(2,25,48) 0 5

57 1 0 0 0 0 0 0 0 1

58 1 0 0 0 0 0 0 0 1

59 0 0 0 0 0 0 0 0 0

6 0 0 0 0 0 0 0 0 0

60 0 0 0 0 0 0 0 0 0

61 3 1 1 0 0 0 0 0 5

62 1 0 0 0 0 0 0 0 1

63 1 0 1 0 1 0 3(0,40,23) 0 6

64 0 0 0 0 0 0 0 0 0

65 0 0 0 0 0 0 0 0 0

66 0 2 0 0 0 0 0 0 2

67 0 0 0 0 0 0 0 0 0

69 0 0 0 1 0 1 0 0 2

7 2 0 2 2 1 0 0 0 7

70 2 0 0 0 0 0 0 0 0 2

71 0 0 0 1 0 1 0 0 2

72 2 1 0 6 0 0 2(44,17) 0 11

73 0 0 0 1 0 0 0 0 1

74 4 1 0 1 0 1 0 0 7

75 0 0 0 0 0 0 0 0 0

8 1 1 0 0 0 1 0 0 3

81 1 1 0 0 0 0 0 0 2

82 0 0 0 0 0 0 0 0 0

83 2 1 1 0 0 0 0 0 4

84 1 1 0 1 0 2 1(6) 0 6

9 1 0 0 0 0 1 0 0 2

999 7 2 4 7 1 2 6(19,37,56,75,27,64) 0 29

Pooled Data File with Ant Behaviour during Emigration for Colony BG91UI

Columns represent: Ant ID, No. of times arriving alone, No. of times transporting any type of brood, No. of times transporting any type of ant

Ant	alone	carry brood	carry ant
1	2	3	0
10	2	0	1
11	0	2	1
12	0	0	0
13	5	3	0
14	5	0	0
15	0	1	0
16	0	1	0
17	0	0	0
18	3	7	2
19	1	0	0
2	1	0	1
20	1	2	0
22	4	0	0
23	1	0	0
24	0	0	0
25	0	5	0
26	1	2	0
27	0	0	0

28 0 0 0

29 3 0 0

3 0 0 0

30 0 0 0

31 0 0 0

32 0 0 0

33 3 5 0

34 0 0 0

35 2 3 0

36 0 0 0

37 0 0 0

38 1 2 0

39 1 1 0

4 1 3 0

40 5 11 6

41 0 0 0

42 0 0 0

43 0 1 0

44 0 0 0

45 1 1 3

46 2 5 1

47 0 2 0

48 1 1 2

49 1 1 1
5 0 0 1
50 1 0 0
51 2 2 1
52 1 0 0
53 0 0 0
54 3 0 1
55 1 0 0
56 0 2 3
57 1 0 0
58 1 0 0
59 0 0 0
6 0 0 0
60 0 0 0
61 3 2 0
62 1 0 0
63 1 2 3
64 0 0 0
65 0 0 0
66 0 2 0
67 0 0 0
69 0 2 0
7 2 5 0

70 2 0 0

71 0 2 0

72 2 7 2

73 0 1 0

74 4 3 0

75 0 0 0

8 1 2 0

81 1 1 0

82 0 0 0

83 2 2 0

84 1 4 1

9 1 1 0

999 7 16 6

Raw Data File for Colony BG91UI with the No. of Times an Ant was Carried during Emigration

```
1 0
10 0
11 1
12 0
13 0
14 1
15 0
16 0
17 1
18 1
19 1
2 1
20 0
22 1
23 1
25 1
26 1
27 1
28 0
29 1
3 0
30 1
```

```
31 1

32 0

33 0

34 0

35 0

36 0

37 1

38 0

39 0

4 0

40 1

41 0

42 0

43 0

44 1

45 0

46 0

47 0

48 3

49 0

5 1

50 0

51 0
```

52	1
53	0
54	0
55	0
56	1
57	1
58	0
60	1
61	0
62	0
63	0
64	1
66	1
67	0
69	2
7	0
70	0
71	0
72	0
73	0
74	0
75	1
8	0

81 0

82 1

83 0

84 0

9 0

Centroid for Each of 5 Types of Brood for 0 BG91UL1

Columns represent: Brood type, Mean x-coord., Mean y-coord.

10	502.18	175.00
4	184.12	220.14
6	214.78	234.99
8	274.99	205.69
9	321.42	192.76

Description of the Distribution of Distances from the Centre for Each Ant for Session BG91UL1

Columns represent: Ant ID, Upper extreme, Upper quartile, Median, Lower quartile, Lower extreme

1 466.680 395.330 334.715 252.160 96.680

10 381.140 229.110 151.990 113.280 18.320

11 361.090 136.750 103.390 86.010 20.340

12 446.720 388.130 332.490 193.030 96.860

13 463.590 430.825 395.815 299.285 73.470

14 440.370 365.360 304.605 182.860 73.120

15 462.410 412.360 319.890 231.990 110.700

16 462.990 413.715 338.855 161.525 75.780

17 438.380 362.435 266.830 166.635 26.150

18 437.040 310.295 235.920 175.580 99.470

2 388.980 206.190 144.125 109.190 31.170

21 435.930 335.850 244.940 171.870 44.650

22 424.300 385.325 310.410 217.710 114.390

23 422.830 368.055 281.340 186.815 69.800

24 354.640 274.280 175.865 113.205 11.140

25 437.840 330.140 256.900 160.910 48.680

26 371.360 199.730 139.510 96.930 28.120

27 428.070 293.895 147.410 123.720 35.040

28 358.990 128.880 92.900 47.150 22.020

29 374.710 126.790 83.720 50.610 21.360

3 440.350 384.740 338.050 223.550 111.110

30 377.640 240.560 209.520 120.820 17.620

31 349.990 215.135 119.990 69.785 18.970

32 353.450 269.740 164.330 122.970 35.170

33 426.220 362.520 253.500 157.460 48.270

34 339.180 159.420 109.690 74.450 12.260

35 458.120 393.130 340.865 238.655 133.840

36 434.280 338.820 268.860 172.740 40.450

38 475.970 402.060 370.690 244.450 174.460

39 398.020 267.850 191.520 141.320 44.230

4 423.820 370.555 275.100 233.220 102.930

40 442.650 388.570 350.050 289.405 95.210

41 376.150 220.900 142.240 86.650 28.880

42 384.980 163.230 109.990 59.290 18.300

5 354.650 188.640 119.300 94.160 21.650

6 378.490 301.080 194.190 149.470 99.150

7 375.150 229.905 143.200 99.125 41.590

8 445.910 251.730 180.030 142.780 54.790

9 441.320 391.450 330.630 238.350 141.060

Description of the Distribution of Distances from the Centre for Each Ant for Session BG91UL1 - Part 2

Columns represent: Ant ID, Interquartile range (spread)

1	143.170
10	115.830
11	50.740
12	195.100
13	131.540
14	182.500
15	180.370
16	252.190
17	195.800
18	134.715
2	97.000
21	163.980
22	167.615
23	181.240
24	161.075
25	169.230
26	102.800
27	170.175
28	81.730
29	76.180
3	161.190

30	119.740
31	145.350
32	146.770
33	205.060
34	84.970
35	154.475
36	166.080
38	157.610
39	126.530
4	137.335
40	99.165
41	134.250
42	103.940
5	94.480
6	151.610
7	130.780
8	108.950
9	153.100

The Value of the Test Statistic from the Runs Up and Down Test for Each Ant for Session BG91UI1

0	-1.21
1	-0.35
10	-0.56
11	0.51
12	0.34
13	-1.05
14	0.24
15	-1.74
16	-1.22
17	-1.15
18	0.68
19	1.83
2	-0.12
20	-0.17
22	-2.17
23	-0.96
25	1.27
26	0.36
27	-2.65
28	0.40
29	0.19
3	0.54

30	-0.93
31	-1.04
32	-1.91
33	-2.90
34	-2.30
35	-1.71
36	-1.22
37	0.54
38	-0.25
39	-0.09
4	-3.10
40	-1.71
41	-2.01
42	-0.70
43	-1.13
44	-1.78
45	1.47
46	-1.14
47	0.00
48	-0.66
49	0.27
5	0.19
50	1.13

51 -0.88

52 -1.27

53 1.56

54 2.00

55 0.22

56 1.29

57 0.69

58 -2.01

60 -0.23

61 0.94

62 -0.87

63 1.63

64 -0.18

66 1.79

67 -1.56

69 -1.78

7 -3.00

70 -1.17

71 -1.94

72 -0.29

73 -2.02

74 -1.68

75 -2.64

8 -0.17

81 -1.36

82 1.57

83 -0.11

84 -0.58

9 -0.46

Shifts for Each Ant between Sessions BG91UL1 and BG91UL2

Shift away from the centre is 1, Shift closer is -1, No shift is 0

1 -1

10 1

11 -1

12 -1

13 -1

14 -1

15 0

16 -1

17 -1

18 -1

2 1

21 0

22 -1

23 -1

24 -1

25 -1

26 0

27 1

28 0

29 -1

30 -1

31	1
32	1
33	0
34	0
35	-1
36	-1
38	-1
39	0
4	-1
40	-1
41	-1
42	0
5	0
6	0
7	1
8	0
9	-1

Median Distances between the Queen and Each Ant for Each of the 5 Days of Session BG91UI1

Columns represent: Ant ID, Days 1 to 5

1 194.517 329.521 148.366 151.476 196.023

10 166.823 227.736 285.447 308.075 162.028

11 183.330 273.138 340.978 271.827 297.214

12 265.767 243.387 211.606 235.478 219.939

13 181.810 202.356 344.177 221.312 214.805

14 170.206 192.094 325.960 244.546 161.218

15 210.712 271.751 366.879 292.479 216.592

16 297.717 227.264 200.942 150.586 165.579

17 120.301 296.906 215.780 194.920 187.518

18 229.896 274.134 286.421 282.884 199.459

19 180.531 129.285 287.924 292.801 287.631

2 212.572 143.532 343.783 219.823 222.100

20 147.906 216.592 240.527 161.059 282.897

22 114.586 169.047 238.519 212.013 268.248

23 170.857 176.581 274.117 255.760 147.240

25 245.002 257.783 192.083 198.726 248.297

26 176.139 352.797 318.535 198.071 186.304

27 257.232 243.265 114.634 188.875 267.978

28 230.586 205.000 315.879 215.568 179.366

29 247.557 184.211 345.722 248.200 196.637

3 178.992 226.595 262.356 268.307 188.043

30 192.813 216.234 243.456 188.600 195.707

31 207.692 229.532 265.499 256.626 275.087

32 146.386 296.319 226.274 183.807 0.000

33 253.403 248.499 298.498 248.270 282.900

34 153.078 218.119 284.345 262.392 162.982

35 242.002 226.609 199.271 236.614 322.334

36 202.714 300.270 220.000 270.164 0.000

37 209.071 229.965 139.135 310.184 259.873

38 248.944 321.338 104.809 185.599 219.054

39 181.374 212.209 306.095 282.177 227.264

4 140.101 259.050 143.071 153.771 241.868

40 268.496 213.604 268.955 284.905 217.479

41 172.192 156.160 226.962 229.244 224.008

42 262.922 268.888 166.342 228.169 233.094

43 302.333 391.515 153.021 177.079 267.825

44 299.715 294.760 181.177 242.100 171.493

45 257.441 387.594 363.200 277.349 253.941

46 195.489 125.431 360.037 255.539 257.413

47 224.080 347.714 332.488 296.277 242.398

48 150.233 147.027 336.586 265.633 223.017

49 215.433 337.231 190.184 236.209 233.420

5 173.277 235.452 172.230 233.461 227.343

50 125.543 183.349 324.854 264.174 168.518

51	266.017	145.000	214.537	300.601	302.866
52	282.252	162.810	246.916	228.786	198.404
53	184.921	164.479	397.613	484.286	223.790
54	185.401	210.879	329.577	257.856	220.006
55	205.922	221.237	313.672	196.825	218.021
56	194.487	165.952	228.137	235.183	194.091
57	190.325	147.248	377.381	237.794	200.112
58	353.112	336.245	188.733	271.978	360.323
60	193.685	275.211	329.694	306.858	209.690
61	237.645	173.773	257.000	275.952	162.644
62	255.787	240.859	232.550	224.154	195.133
63	152.533	149.694	324.261	241.677	275.577
64	219.082	149.629	179.326	218.892	353.902
66	223.133	69.354	215.058	230.734	183.619
67	169.000	251.247	268.354	0.000	0.000
69	203.907	310.467	225.198	213.265	228.219
7	267.470	92.655	194.201	193.521	285.883
70	277.838	195.870	356.215	308.212	273.425
71	270.622	406.787	190.170	171.560	277.927
72	217.899	182.060	383.557	291.604	306.788
73	159.144	267.095	279.153	283.146	188.043
74	167.171	180.995	340.311	269.818	290.419
75	271.301	316.004	158.808	184.086	248.091

8 255.190 326.768 217.690 264.577 372.634

81 378.809 112.294 292.912 274.089 271.671

82 219.572 209.619 355.820 233.380 197.170

83 263.651 172.003 352.525 241.423 247.421

84 200.302 167.141 267.918 229.811 283.436

9 307.059 391.053 186.936 225.192 202.074

Associations between the Queen and Each Ant for Each of the 5 Days of Session BG91UI1

Columns represent: Ant ID, Days 1 to 5, where 1 stands for "follow", -1 stands for "avoid', and -2 stands for ants that had less than 5 synchronous positions with the queen. -2 was eventually equated to 0 which stands for no association

1 0 0 0 0 0

10 0 0 0 1 0

11 0 0 0 0 0

12 0 0 0 0 0

13 0 0 0 0 0

14 0 0 0 0 -1

15 0 0 0 0 0

16 0 0 0 0 0

17 0 0 0 0 0

18 0 0 0 0 0

19 0 0 0 0 0

2 0 -2 0 0 0

20 0 0 0 0 0

22 0 0 0 0 0

23 0 0 0 0 -2

25 0 0 0 0 0

26 0 0 0 0 0

27 0 0 0 0 0

28 0 0 0 0 0

29 0 0 0 0 0

3 0 -2 0 0 -2

30 0 0 0 0 0

31 0 0 0 0 0

32 0 0 0 -2 -2

33 0 0 0 0 0

34 0 0 0 -2 -2

35 0 0 0 0 1

36 0 0 0 0 -2

37 0 0 0 0 0

38 0 0 0 0 0

39 0 0 0 0 0

4 0 0 0 0 0

40 0 0 0 1 0

41 0 0 0 0 0

42 0 0 0 0 0

43 0 0 0 0 0

44 0 0 0 0 0

45 0 0 0 0 0

46 0 0 0 0 0

47 0 0 0 0 0

48 0 0 0 0 0

49 0 0 0 0 0

5 0 0 0 0 0

50 0 0 0 0 0

51 0 0 0 0 0

52 0 -1 0 0 0

53 -2 -2 -2 -2 -2

54 0 0 0 0 0

55 0 0 0 0 0

56 -2 0 0 0 0

57 -1 0 0 0 0

58 1 0 0 0 0

60 0 0 0 0 0

61 0 0 0 0 0

62 0 0 0 0 0

63 0 0 0 0 0

64 0 0 0 0 0

66 0 0 0 0 0

67 0 -2 -2 -2 -2

69 0 0 0 0 0

7 0 0 0 0 0

70 0 0 0 0 0

71 0 1 0 0 0

72 0 0 0 0 0

73 0 0 0 0 -1

74 -1 0 0 0 0

```
75 0 0 0 0 0
8 0 0 0 0 0
81 -2 0 0 0 0
82 0 0 0 0 0
83 0 0 0 0 0
84 0 -2 0 0 0
9 0 0 0 0 0
```

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Behavioral Ecology and Sociobiology

Brood sorting by ants: distributing the workload over the work-surface

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Summary. *Leptothorax unifasciatus* ant colonies occupy flat crevices in rocks in which their brood is kept in a single cluster. In artificial nests made from two glass plates sandwiched together, designed to mimic the general proportions of their nest sites in the field, such colonies arrange their brood in a distinct pattern. These patterns may influence the priority with which different brood are tended, and may therefore influence both the division of labour and colony demography. Different brood stages are arranged in concentric rings in a single cluster centred around the eggs and micro-larvae. Successively larger larvae are arranged in progressive bands away from the centre of the brood cluster. However, the largest and oldest brood items, the prepupae and pupae, are placed in an intermediate position between the largest and most peripheral larvae and the larvae of medium size. Dirichlet tessellations are used to analyze these patterns and show that the tile areas, the area closer to each item than its neighbours, allocated to each type of item increase with distance from the centre of the brood cluster. There is a significant positive correlation between such tile areas and the estimated metabolic rates of each type of brood item. The ants may be creating a "domain of care" around each brood item proportional to that item's needs. If nurse workers tend to move to the brood item whose tile they happen to be within when they have care to donate, they may apportion such care according to the needs of each type of brood. When colonies emigrate to new nests they rapidly recreate these characteristic brood patterns.

Introduction

One of the key characteristics of eusocial insects is care of brood by adults (Hölldobler and Wilson 1990). This often involves a division of labour with foragers bringing food to nurse workers, who in turn feed and tend the brood. Such a division of labour involves individuals finding, and possibly queuing for, workers or brood items who are willing and able to receive food. The efficiency of the whole process, especially if it occurs in parallel – with large numbers of workers concurrently undertaking each type of task – may be enhanced if different tasks occur predictably in different places.

In this paper we show how certain ants create predictable spatial patterns in their nests by sorting their brood. This is not only of direct interest in terms of spatial organization and ergonomics, but may have a direct bearing on colony demography. Not all the brood items are likely to survive. Food may be a limiting factor so that some larvae may starve and may be fed to others (Hölldobler and Wilson 1990). By sorting their brood into a pattern some brood stages may receive more care than others. This in turn may affect rates of recruitment to adult ant populations and thus influence further the division of labour in the growing society.

Earlier work on brood sorting has concentrated on the ants moving certain types of brood items to different brood chambers possibly in response to changing microclimatic needs (Headley 1941; Le Masne 1953; Peakin 1960; Corbara et al. 1986; see also Wheeler 1913; Sudd 1967; Hölldobler and Wilson 1990). Camazine (1991), using models based on cellular automata, has shown how honey bees may allocate comb cells as larval chambers, and honey or pollen stores, by employing only simple rules to create self-organizing patterns (see also Camazine et al. 1990). Deneubourg et al. (1991) have developed a simple model of brood sorting in ants, similar in philosophy to Camazine's (1991) approach of looking at pattern generation through self-organization.

Here we show how ants sort their brood, without apparent reference to environmental gradients such as temperature or humidity nor to such structures as the fixed lattice provided by the cells of a honey comb or distinct brood chambers within the nest.

We describe how colonies of the ant *Leptothorax unifasciatus* (Latreille) sort their brood. The brood of such Leptothoracines is immobile, i.e. brood items only change location as a result of being carried by workers. *Leptothorax unifasciatus* is an ideal species for this analysis because these ants nest in relatively flat pre-formed crevices in rocks. In the laboratory they readily nest between glass plates, which not only mimic the natural flatness of their nests, but permit straightforward recording of brood patterns by filming (see Franks et al. 1990). Such nests are almost completely uniform with no specific brood chambers: the only non-uniformity is that there is a single nest entrance in one wall. *Leptothorax* colonies will also readily emigrate from one nest site to another. We utilize this phenomenon to examine the ants' ability to re-sort their brood.

Methods

The ant colonies

Colonies of *Leptothorax unifasciatus* were collected in the summer of 1989 and the spring of 1990 from steep wooded hillsides above the village of Thompson in the Balkan mountains, Bulgaria. The colonies were nesting in crevices in rocks formed by the dual action of water and ice. These ants use such natural crevices and do very little nest building other than sometimes arranging a wall of debris at the edge of the cavity. The nest cavities are very thin and often extremely flat. All the ants in the colony live in close proximity to one another, and they rarely seem to use more than one nest chamber. Such colonies are very easy to collect by aspirating all the ants, and to culture by transferring them to artificial nests made from a pair of large microscope slides separated by a perimeter of cardboard to make a cavity 35 × 21 × 1 mm. Colonies were fed an *ad libitum* supply of honey solution (1 part honey in 10 parts water) and *Drosophila* larvae. For the experiments five colonies were selected; population censuses for these are given in Table 1.

Observation techniques

A fine-grain black and white photograph (Ilford Panf 135, ISO/ASA 50/18°) of each nest population was made, using a 35-mm single lens reflex camera mounted on a Zeiss stereo dissection microscope (Fig. 1). The camera was then focused on a new nest site whose entrance was situated 1 or 2 cm away from that of the old nest. The colony was then obliged to emigrate by removing the top microscope slide of the nest it was currently occupying. Photographs were then taken at set intervals, initially every 5 min, starting when the first brood item was transported to the new nest. Filming continued throughout the migration when all the brood items are carried from the old nest to the new and during the brood sorting process.

Each negative, mounted as a slide, was then projected so that the position of the centre of each brood item could be marked on an acetate sheet. Each sheet was then transferred to the screen of a microcomputer, where a programme was used to record the coordinates of each item by moving a mouse-controlled cursor to each item's position, selecting it, and then entering its developmental stage, coded as a numerical value. The brood was classified into five categories: pupae (white and immobile but having the sculptured morphology of adults), pre-pupae (white and opaque but without adult sculpturing), large larvae (as with the other larvae, light yellow and translucent), medium larvae, and last, micro-larvae and eggs. It is hard to distinguish between eggs and newly hatched micro-larvae so these were grouped into the same category. Brood categories were coded numerically as follows; pupae (10), pre-pupae (9), large larvae (8), medium larvae (6), micro-larvae and eggs (4). In our procedure, the larval brood items were allocated to size categories initially by eye. The accuracy of this method is indicated by the distinctly different mean lengths and standard errors of these lengths for each type of item (Table 2). Brood item lengths and widths were measured from the same photographs at a later stage than the classification by eye and show the consistency of the earlier qualitative judgement.

Fig. 1. The fully sorted brood distribution for colony D before it emigrated to a new nest. All of the nest cavity is shown. The *brood* are clustered to the left hand side of the nest away from the single entrance in the middle of the short wall of the nest on the *right hand side* (not shown). The nest was photographed on a black background to highlight the white brood; the dark *workers* and *queen* are hardly visible against this background. A Dirichlet tessellation of this brood distribution is shown in Fig. 2

Analytical and statistical techniques

The resulting computer files with the coordinates and identity of each brood item were then transferred to a mainframe computer where a package of programmes developed by the spatial statistics group of the School of Mathematics at the University of Bath were used to analyse the distribution patterns.

Dirichlet Tessellation. One way to analyse the distribution of items in a 2-dimensional space is to allocate this space among the items in a Dirichlet tessellation. This technique divides the space into a series of polygons or tiles: one for each item. The boundary of each item's tile is delineated by the perpendicular bisectors of the lines joining that item to each of its immediate neighbours. The area within each item's tile includes all the points on the plane that are closer to that item than to any other. Dirichlet tessellations were made for all the brood distributions based on the algorithm of Green and Sibson (1978).

One problem with the use of tessellation is how to draw tiles for peripheral items. Such items by definition do not have other items beyond them to define the outer boundaries of their tiles. One way to allocate a tile to such peripheral items is simply to set a perimeter all around the cluster of items and terminate tile boundaries where they meet that perimeter. This was done by taking the maximum distances across the brood cluster along both the x and y axes and positioning the perimeter 5% beyond these limits in each direction. Such a procedure makes it possible to draw peripheral tiles and calculate their area. Nevertheless, there can be problems associated with either the inclusion or exclusion of peripheral tiles from the analysis. First, the area of peripheral tiles may be unrepresentative. Second, if peripheral items and their tiles are excluded from the analysis there will generally be an underestimate of the space allocated to peripheral items. This will tend to cause biases especially if peripheral items tend to belong to one category as is the case here: almost all of the peripheral items are the largest larvae.

Given these considerations peripheral tiles have been excluded from all quantitative analyses of tile areas. However, we have not excluded peripheral tiles from the graphical analysis of brood pattern formation following emigrations because here we are concerned with qualitative comparisons. Further, the choice of a perimeter 5% larger than the brood cluster in each direction was made to minimize biases caused by including peripheral tiles, and the same rule was used for all comparisons.

Nearest neighbour analysis. Another way to analyse the distribution of items in 2-dimensional space is to compare the observed mean nearest neighbour distance with that expected under the hypothesis of a Poisson point process. Clark and Evans' (1954) test statistic provides an evaluation procedure for this comparison. Clark and Evan's (1954) test statistic is extremely familiar to ecologists concerned with pattern analysis [see for example Sudd and Franks (1987)]. We have also used Donelly's (1978) correction to the Clark and Evans' (1954) test statistic, which minimizes errors caused by edge effects. Nearest neighbour analyses were made for each type of brood item in each of the brood dispersion patterns.

Nearest neighbour distances and identities, and distances of each item from the centroid (i.e. the mean co-ordinates of the centres of all the brood items in each distribution) were also calculated using mainframe programmes written in C. The frequency of contacts between items were also recorded from direct observation of the photographic negatives.

Results

The brood were distributed within each colony in the same distinct pattern: a single cluster with concentric rings each consisting of a different type of brood item (Fig. 1). Such patterns were also seen as we collected colonies in the field. This general pattern was consistently observed even though the colonies differed in worker population size, brood numbers and composition (Table 1). The characteristic brood pattern was rapidly recreated when each colony emigrated to a new nest (see Fig. 2 and below).

Overall the brood tend to be clustered within one part of the available nest space, away from the entrance of the nest. Within this brood area, the eggs and micro-larvae (the smallest brood items) were always tightly clustered as indicated by the spatial dispersion statistics of Clark and Evans (1954) (Table 2). The dispersion patterns of larger brood items within the brood pattern, when analysed type by type, were only rarely significantly under-dispersed (Table 2). Indeed, different brood items are arranged roughly in concentric rings in the brood cluster. The standard pattern is for eggs and micro-larvae to be in the middle, with the larger larvae further from the centre in order of increasing size. Prepupae and pupae are distributed in positions between the outer ring of the largest larvae and those of the next largest size. These positional relationships are seen in Fig. 2 and shown by the analyses of distances of each type of item from the centroid (mean x and y co-ordinates) of the overall cluster (Table 2). That similar types of brood item tend to occur in the same neighbourhood is confirmed by the analyses of nearest neighbour associations. In all cases there was a significant positive association of similar types (Table 2, Fig. 5). Further evidence for sorting is seen in the data for physical contact between brood items (Table 2). Most contacts were between items of the same type.

Table 1. Colony population data (approximate numbers inside the nests: each colony had a single queen)

Colony	No. adult workers	Total brood	Larvae (3 sizes)			Pre-pupae	Pupae
A	60	118	85	22	11	0	0
B	94	164	100	51	13	0	0
C	106	165	56	48	47	8	6
D	152	188	54	54	46	13	21
E	184	171	106	23	32	0	10

The experiments on colonies A through E were conducted on the following dates: 13.03.1990, 20.04.1990, 09.05.1990, 13.06.1990, 07.08.1990, respectively

Larger brood items tended to have larger centre-to-centre nearest neighbour distances. The exception to this positive relationship was for the prepupae and pupae which are among the largest brood items in the nest but are given less space in their intermediate position between the medium and largest larvae. This spacing pattern of neighbours is clearly shown by the analysis of the tile areas in each Dirichlet tessellation (Table 2).

Eggs and micro-larvae have small tile areas, larvae of medium and large size have proportionately larger tile areas, respectively; whereas pre-pupae and pupae have tile areas smaller in general than those of the largest larvae that lie further from the middle of the brood pile.

All the brood items have ellipsoid shapes. The relationship between the lengths of the principal axes of each item can be described thus:

Brood Item Width $= 0.180 + 0.307 \times$ (Brood Item Length) ($n = 150$, $r = 0.834$, $P \ll 0.001$).

Calculating the mean area of each type of item as an ellipse projected onto the plane of the nest floor and subtracting this area from that type of item's mean tile area (for each of the five colonies) to give a boundary zone area, shows that larger items, with the notable exception of pupae and pre-pupae, tend to have larger boundary zone areas. For the larval brood the relationship between the mean boundary zone area (BZA) in mm^2 within each type of item's tile and the mean area of that type of item (A) is best described by:
(BZA) $= 0.703 + 1.65$ (A) ($r = 0.746$, $n = 28$ $P < 0.001$).

This relationship does not hold for pre-pupae and pupae which have been excluded from this equation as they have unusually small tiles (see Table 2). Differences in space allocation for larvae and pupae can be further highlighted by comparing mean tile area for each type of item with the area that would be swept out if such an item was rotated around its centre such that its long axis sets the diameter of the circle. For larval brood, the relationship between tile area (TA) and these circular areas (CA) is best described by: TA $= 0.900 + 1.01$ (CA) ($r = 0.868$, $n = 28$, $P \ll 0.001$). Here again, the data for prepupae and pupae have been excluded because in 8 of the 10 possible cases examined, the mean tile area (TA) for these items is *smaller* than the circular area (CA). Compared with other types of brood item pre-

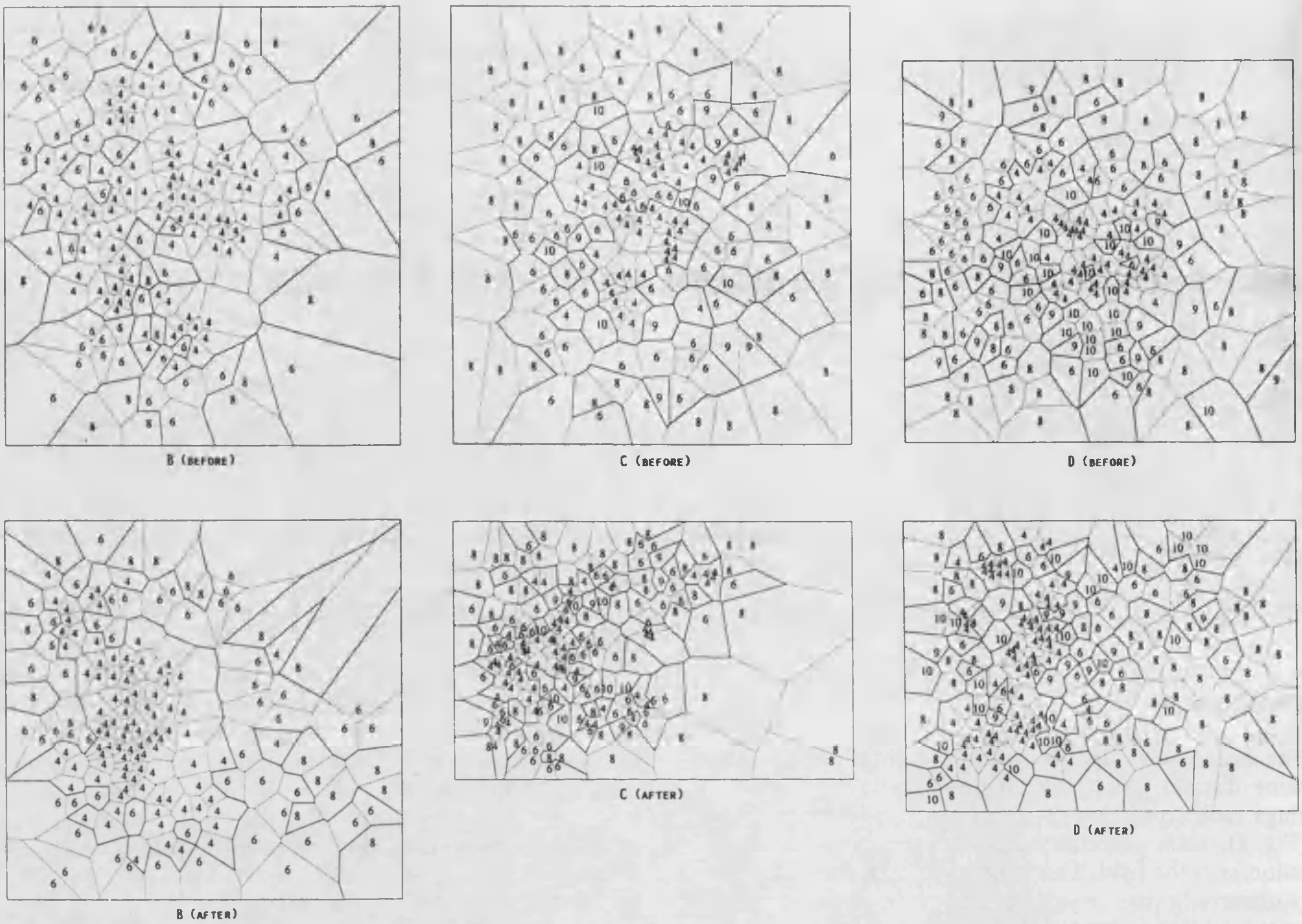

Fig. 2. Dirichlet tessellations of brood distributions for three of the five colonies B, C, D, before and after they emigrated to a new nest. *Dotted lines* and *continuous lines* indicate boundaries between similar and dissimilar items, respectively

pupae and pupae are given very little space for their size (see also Fig. 3).

Assuming that the weight of each item scales as a cubic power of its linear dimensions and assuming that basal metabolic rate scales as a three-quarters power of weight, then basal metabolic should be proportional to brood item length raised to the power 2.25 (Schmidt-Neilson 1984). There is a significant positive relationship between the tile area of each type of item and the predicted metabolic rate (see Fig. 3). Pre-pupae and pupae are not fed by workers, but merely groomed. Nevertheless, they may have a basal metabolic rate that is quite appreciable when compared to larvae of similar size. Therefore, for comparative purposes pupae and pre-pupae have been included in this analysis. It is clear from Fig. 3 that pupae and pre-pupae have relatively smaller tile areas compared to estimated metabolic rates than those of the larvae. This is consistent with the hypothesis that the workers who create the brood patterns may

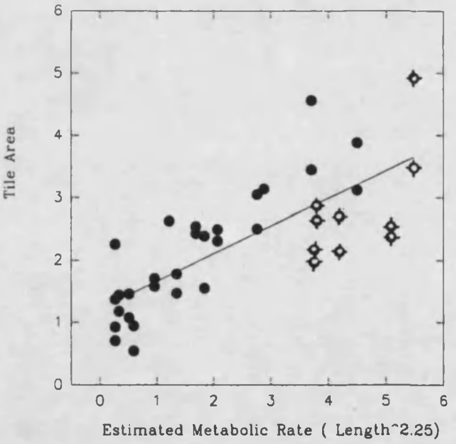

Fig. 3. The mean tile area for each type of item, as recorded from the Dirichlet tessellations (peripheral tiles excluded) of the fully sorted brood distributions before and after the emigration, for each of the five colonies (see Table 2) plotted against estimated metabolic rate. This estimate was made by raising the mean length of each type of item to the power 2.25. The relationship can be described by:
Mean Tile Area $= 1.22(\pm 0.18) + 0.442(\pm 0.06) \times$ (Mean Length $^{2.25}$) ($n = 38$, $r = 0.755$, $P \ll 0.001$, $\pm$SD). Pre-pupae and pupae are marked with *crossed circles*, all other types of brood with *filled circles*. A significant majority of pre-pupae and pupae are below the line (χ^2 6.4, 1 *df*, $P < 0.01$)

Table 2. Data for fully sorted brood before and after each emigration

Colony A before emigration

Brood type	No. of items	Nearest neighbour Type 4	6	8	Distance Mean	SE	Distance to centroid Mean	SE
4	85	75	9	1	0.48	0.01 }*	2.68	0.15 }*
6	22	14	6	2	0.61	0.05 }**	3.83	0.36 }*
8	11	1	4	6	1.35	0.13	7.18	0.36

Colony A before emigration ctd.

Brood item type	Contact type 4	6	8	Item length Mean	SE	Tile area (exc. peripherals) Mean	SE	*n*	Clark and Evans
4	45	11	3	0.79	0.02 }**	0.55	0.06 }*	76	−6.01**
6	9	2	1	1.14	0.02 }**	1.47	0.37 }n.s.	22	−2.76*
8	1	1	2	1.57	0.04	3.05	1.12	4	−1.32
Totals			75						−2.51*

Colony A after emigration

Brood type	No. of items	Nearest neighbour Type 4	6	8	Distance Mean	SE	Distance to centroid Mean	SE
4	83	76	6	1	0.57	0.02 }**	3.14	0.02 }**
6	20	12	5	3	0.91	0.04 }n.s.	5.24	0.48 }n.s.
8	11	4	3	4	1.27	0.23	6.43	0.46

Colony A after emigration ctd.

Brood item type	Contact type 4	6	8	Item length Mean	SE	Tile area (exc. peripherals) Mean	SE	*n*	Clark and Evans
4	29	9	5	0.79	0.02 }**	0.95	0.08 }*	77	−5.01**
6	6	7	3	1.14	0.02 }**	1.78	0.38 }n.s.	13	−1.28
8	4	3	4	1.57	0.04	2.50	0.51	3	0.12
Totals			70						−1.00

Colony B before emigration

Brood type	No. of type	Nearest neighbour Type 4	6	8	Distance Mean	SE	Distance to centroid Mean	SE
4	100	85	15	0	0.57	0.01 }**	3.58	0.15 }**
6	51	20	24	7	0.80	0.04 }*	6.14	0.26 }*
8	13	4	4	5	1.11	0.14	7.32	0.50

Table 2 (continued)

Colony B before emigration ctd.

Brood item type	Contact type			Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	Mean	SE		Mean	SE		*n*	
4	32	13	0	0.55	0.01	**	0.93	0.07	*	98	−4.74**
6	8	13	4	0.98	0.01	**	1.58	0.14	n.s.	39	−0.87
8	0	3	4	1.31	0.06		1.55	0.36		7	0.05
Totals			77								0.44

Colony B after emigration

Brood type	No. of type	Nearest neighbour						Distance to centroid		
		Type			Distance					
		4	6	8	Mean	SE		Mean	SE	
4	94	83	9	2	0.52	0.02	**	3.11	0.18	**
6	54	22	27	5	0.81	0.04	+	5.85	0.21	*
8	16	3	5	8	0.92	0.05		7.27	0.38	

Colony B after emigration ctd.

Brood item type	Contact type			Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	Mean	SE		Mean	SE		*n*	
4	46	8	2	0.55	0.01	**	0.71	0.05	**	92	−4.63**
6	5	18	9	0.98	0.01	**	1.71	0.17	n.s.	39	−1.43
8	2	7	4	1.31	0.06		2.39	0.84		7	−1.44
Totals			101								0.73

Colony C before emigration

Brood type	No. of items	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	56	48	7	0	0	1	0.62	0.03	**	3.58	0.23	**
6	48	11	22	10	4	1	1.00	0.05	**	6.13	0.39	**
8	47	0	14	28	3	2	1.51	0.08	**	9.46	0.30	*
9	8	3	2	2	1	0	0.95	0.07	n.s.	6.93	0.79	n.s.
10	6	1	3	2	0	0	0.99	0.09		5.16	0.67	

Colony C before emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE		*n*	
4	36	8	0	1	1	0.61	0.04	**	1.18	0.10	**	56	−6.80**
6	8	9	4	3	6	1.26	0.02	**	2.54	0.18	**	45	−1.54
8	0	4	4	2	0	1.79	0.03	n.s.	3.45	0.19	+	24	1.43
9	1	2	2	0	0	1.89	0.06	n.s.	2.70	0.36	n.s.	8	−1.25
10	1	4	0	0	0	2.06	0.10		2.54	0.49		6	−0.24
Totals					96								2.35*

Table 2 (continued)

Colony C after emigration

Brood type	No. of items	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	61	36	17	5	2	1	0.64	0.03	**	6.01	0.37	*
6	62	20	20	16	2	4	0.96	0.04	**	7.32	0.39	**
8	43	5	22	13	2	1	1.39	0.10	**	10.17	0.64	*
9	7	1	4	1	0	1	0.99	0.06	n.s.	7.02	1.20	+
10	7	0	6	0	1	0	0.89	0.12		4.70	0.36	

Colony C after emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE	*n*		
4	25	3	5	1	4	0.61	0.04	**	1.44	0.09	60	*	-7.08**
6	3	14	3	3	1	1.26	0.02	**	2.43	0.31	55	n.s.	-3.01*
8	5	3	6	2	0	1.79	0.03	n.s.	4.56	1.40	25	n.s.	-0.11
9	1	2	2	0	2	1.89	0.06	n.s.	2.14	0.29	6	n.s.	-0.61
10	3	1	0	2	2	2.06	0.10		2.37	0.39	7		-2.14*
Totals					93								-2.03*

Colony D before emigration

Brood type	Item no.	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	54	48	2	0	2	6	0.68	0.04	**	3.71	0.24	**
6	54	7	26	12	5	4	1.04	0.03	**	7.70	0.25	**
8	46	0	18	25	2	1	1.43	0.06	*	10.96	0.28	*
9	13	1	6	3	0	3	1.16	0.07	n.s.	8.70	0.96	*
10	21	9	4	1	1	6	1.03	0.06		4.96	0.60	

Colony D before emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE		*n*	
4	22	4	4	2	7	0.74	0.04	**	1.46	0.11	**	54	-6.95*
6	4	20	16	1	3	1.38	0.04	**	2.49	0.16	**	49	-1.94^{+}
8	3	15	14	6	1	1.95	0.03	**	3.88	0.26	**	27	-0.79
9	1	1	7	0	4	1.81	0.02	n.s.	1.16	0.07	**	9	0.17
10	6	6	1	4	14	1.80	0.04		1.97	0.17		20	-2.71*
Totals					160								2.40*

Table 2 (continued)

Colony D after emigration

Brood type	No. of items	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	83	70	5	1	1	6	0.62	0.02	} **	5.69	0.28	} *
6	30	8	4	11	3	4	1.05	0.06	} *	6.98	0.59	} **
8	51	8	11	20	5	7	1.33	0.06	} *	9.53	0.44	} **
9	13	3	4	4	1	1	1.04	0.12	} n.s.	5.36	1.06	} n.s.
10	25	9	4	3	2	7	0.98	0.04		7.75	0.61	

Colony D after emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE		*n*	
4	41	7	0	3	7	0.74	0.04	} **	1.08	0.07	} **	79	−7.02**
6	5	10	5	3	8	1.38	0.04	} **	2.31	0.17	} **	25	−0.70
8	0	5	20	7	6	1.95	0.03	} **	3.12	0.15	} n.s. } +	36	1.72
9	2	3	4	0	4	1.81	0.02	} n.s.	2.64	0.66	} n.s. }	12	−0.16
10	5	6	6	4	12	1.80	0.04		2.17	0.17		19	−0.33
Totals					173								3.09*

Colony E before emigration

Brood type	No. of items	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	106	98	4	1	–	3	0.75	0.03	} **	7.54	0.22	} **
6	23	3	10	8	–	2	1.44	0.13	} n.s.	13.10	0.60	} *
8	32	1	7	22	–	2	1.49	0.11	} *	15.26	0.37	} **
9	–	–	–	–	–	–	–	–		–	–	
10	10	7	1	1	–	1	1.15	0.13		10.46	0.55	

Colony E before emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE	*n*		
4	50	1	0	–	4	0.55	0.04	} **	2.26	0.17	103	} **	−6.06**
6	1	2	3	–	1	1.09	0.04	} **	5.36	0.74	16	} n.s.	−1.89
8	0	3	8	–	1	1.60	0.03	} **	5.64	1.10	13	} n.s.	−0.46
9	–	–	–	–	–	–	–		–	–	–		–
10	2	1	1	4	8	2.13	0.04		4.92	0.95	10		−1.23
Totals					82								−0.71

Table 2 (continued)

Colony E after emigration

Brood type	No. of items	Nearest neighbour								Distance to centroid		
		Type					Distance					
		4	6	8	9	10	Mean	SE		Mean	SE	
4	110	103	3	4	–	0	0.63	0.02	**	6.09	0.24	**
6	21	2	12	6	–	1	1.14	0.07	n.s.	10.60	0.65	n.s.
8	29	11	8	8	–	2	1.30	0.09	n.s.	11.07	0.67	n.s.
9	0	–	–	–	–	–	–	–		–	–	
10	10	5	2	1	–	2	1.43	0.11		9.29	1.10	

Colony E after emigration ctd.

Brood item type	Contact type					Item length			Tile area (exc. peripherals)				Clark and Evans
	4	6	8	9	10	Mean	SE		Mean	SE		*n*	
4	92	0	8	–	0	0.55	0.04	**	1.37	0.09	**	107	−6.81 **
6	0	8	5	–	3	1.09	0.04	**	2.63	0.19	n.s.	13	−2.46 *
8	6	5	2	–	3	1.60	0.03	**	3.14	0.45	n.s.	18	0.92
9	–	–	–	–	–	–	–		–	–		0	–
10	0	2	3	–	0	2.13	0.04		3.48	0.41		9	−0.61
Totals					136								−0.66

In the table eggs and micro-larvae are designated (4), medium larvae (6), large larvae (8), pre-pupae (9) and pupae (10). χ^2 values for the association of nearest neighbours presented in this table are plotted in Fig. 5. The table provides, in addition to other data Clark and Evans' (1954) test statistic [with Donelly's (1978) correction] calculated for each type of item alone and for the overall brood cluster. The Clark and Evans' statistic is a standard normal variable with a critical value of 1.96 at $P=0.05$. Negative values indicate under-dispersion (items more clumped than random) and positive values overdispersion (items more regularly spaced than random).

Contacts between items are also recorded. In this procedure contact, for example, between a 4 and a 6 is recorded once in the 4/6 category and once in the 6/4 category. However, if a 4 was simultaneously touching two 6's only one contact with the 6-category was recorded, but when each of the 6's were surveyed both independently contact a 4, so 2 contacts were recorded. This procedure was adopted so that unitary and binary or higher numbers of contacts were not conflated. Contacts among items other than 4's (eggs and micro-larvae) are relatively rare.

The column headed '*n*' is the number of tiles of each type remaining when peripheral tiles have been removed. Exclusion of peripheral tiles has disproportionately decreased the sample sizes of large larvae and inflated the standard errors of mean tile areas for such items. This has lead to a lack of statistical significance in certain cases. Levels of significance are indicated +, * and ** for significance at 0.05 for a one-tailed test or $P<0.05$ or $P<0.001$ for two-tailed tests, respectively.

be distributing the work load driven by the metabolic demands (i.e. mainly the nutritional requirements) of the brood rather evenly over the brood area within the nest.

When the colonies emigrate the workers rapidly recreate these brood patterns (see Figs. 2, 4). This is shown by the analyses of nearest neighbour associations (Fig. 5), mean distances to the centroid of the cluster (Fig. 6), and tile areas (Fig. 7) based on the analyses of each photograph taken during the emigration.

Initially, brood items are placed randomly within the new nest – but the items transported later in the emigration tend to be placed near other items that are already in the new nest, especially if they belong to the same type (Fig. 4). Even before all the brood items have been carried to the new nest, those already within the new nest may be picked up and relocated, often nearer to larger groups of items of the same type. These growing clusters of items compete with one another to 'capture' other brood items that are being moved about. Items seem to be more frequently removed from small rather than large clusters. In this way, clusters are formed and break up as the standard pattern, of concentric brood rings in a single brood cluster, is finally formed. This process is highly dynamic and leads to rapidly fluctuating nearest neighbour relationships as indicated by the χ^2 association analyses shown in Fig. 5.

The mean distances of brood items from the centroid of the cluster gradually decrease as the ants gradually form their brood into a single cluster (Fig. 6). The mean tile areas for each type of item seem more rapidly to approximate their final values (Fig. 7, especially colonies A and B). These facts suggest that items may be positioned at rather set distances from their nearest neighbours from the outset of the sorting procedure. In 4 of the 5 emigration studies there was a significant tendency for the ants to move items of the same type first.

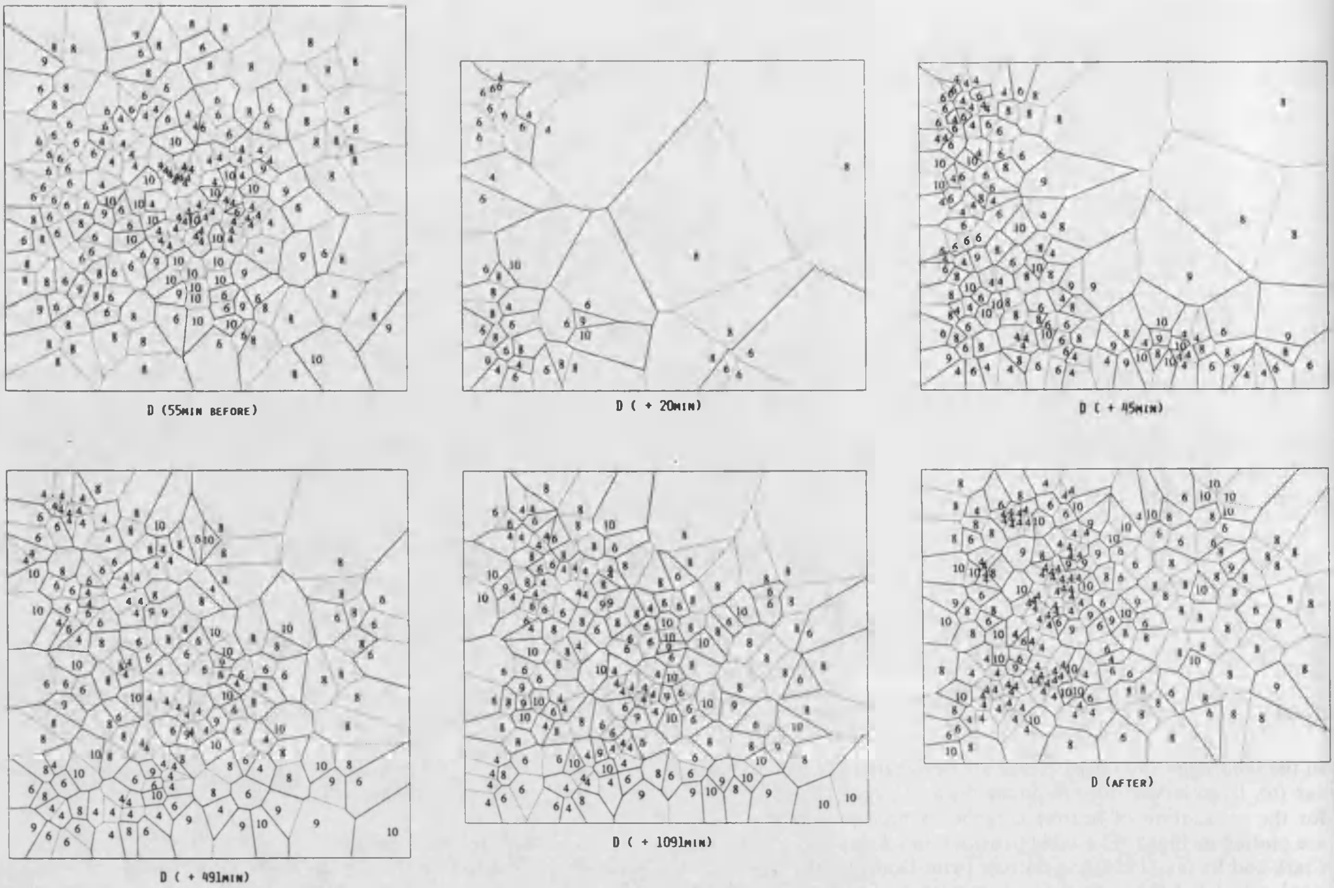

Fig. 4. A sequence of tessellations of the brood pattern of colony D before during and after it emigrated to a new nest. The 6 frames are 55 min before, and 20, 45, 491, 1091 and 7200 min after, the start of the emigration

In two cases the large items tended to be moved first and in two cases small items tended to be moved first (Table 3).

Discussion

Leptothorax unifasciatus colonies arrange their brood in a distinct pattern. In the colonies we have studied there is a single cluster of brood arranged in a two-dimensional pattern on the floor of the nest. The single cluster is made up of concentric rings with eggs and micro-larvae in the middle surrounded by larger larvae in progressively larger rings (Fig. 1). The pupae and pre-pupae tend to be placed in an intermediate ring between the medium and largest larvae.

The Dirichlet tessellation analysis of the brood patterns draws a boundary around each brood item, and thus allocates a tile to each item. Recall that the area within each item's tile includes all the points in the plane that are closer to that item than to any other. For the ants' brood patterns tile areas increase from the centre of the brood cluster outwards. Eggs and micro-larvae have small tile areas, larvae of medium size have tile areas of medium size. Pre-pupae and pupae have tile areas larger than those of medium-sized larvae but smaller than those of the more peripheral largest larvae. Consequently, tile area is not just a function of item size as pre-pupae and pupae are as large as the largest larvae.

Thus it seems that the ants are not merely providing an access corridor around each item, but actively spacing them for some other reason. One possibility is that by creating these spatial patterns they are helping to organize the work of brood care.

The possibility that these brood patterns have some adaptive significance is further suggested by the high priority that the ants give to rapidly recreating such patterns when they emigrate to a new nest. This also shows that the patterns are not simply created by the way the colony develops, as would be the case in the following scenario. Consider, for example, the queen taking a central position in the nest, where she lays eggs. As brood develop younger brood push older brood stages further and further away from the queen's central position in the cluster. This possibility is not only disproved by the re-creation of the characteristic pattern during the emigration (even in the absence of a queen; personal observations) but also by the non-peripheral position of the oldest brood, the pre-pupae and pupae.

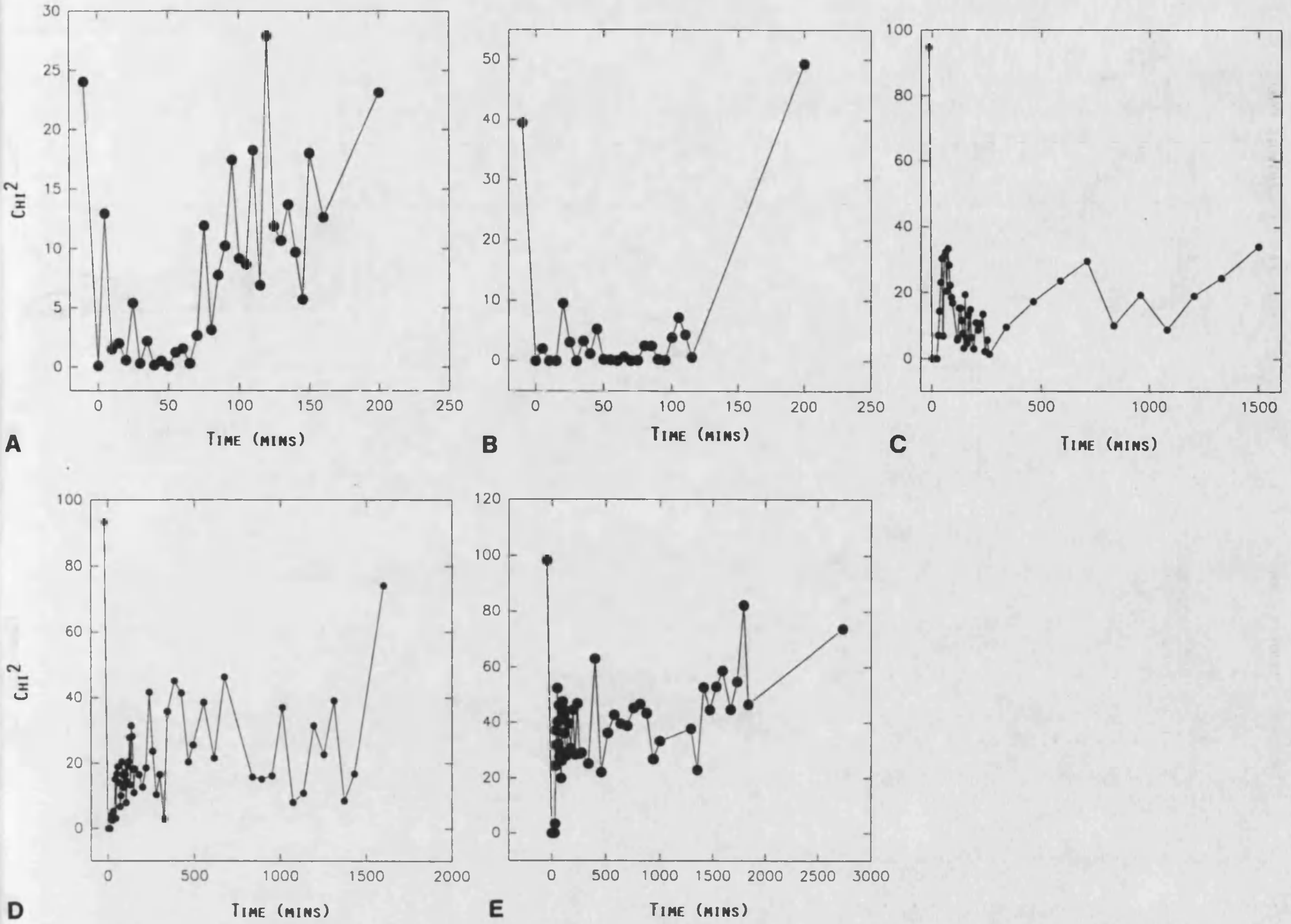

Fig. 5A–E. χ^2 association tests of nearest neighbours for each of the five colonies as a function of time (min) before, during and after each emigration to a new nest. Brood categories had to be grouped so that the data met the assumptions of such a χ^2 analysis (i.e. expected frequencies had to be greater than or equal to five in more than 80% of the cells of each table). Thus for colonies **A** and **B** nearest neighbour associations were examined for the categories 4's and 6's + 8's. For colonies **C** and **D** the categories were 4's, 6's and 8's + 9's + 10's: and for colony **E** they were 4's and 6's + 8's + 10's. Hence for colonies **A**, **B** and **E** two-way tables were constructed, giving 1 *df* and a critical χ^2 value of 3.84 at $P = 0.05$. For colonies **C**, and **D** three-way tables were constructed giving 4 *df* and a critical χ^2 value of 9.49 at $P = 0.05$

The predictable spatial pattern of the brood may enable workers to specialize in caring for certain types of brood whilst remaining in one particular location within the nest. This would mean that workers would not have far to travel to find similar work. Thus the brood patterns may favour and promote the distribution of labour within the nest which in turn may promote brood survival and development. For example, by putting the hungriest larvae at the outside of the brood cluster, the ants may be creating a system in which these most valuable larvae are fed first and only when these brood are satiated do the workers progress further towards the centre of the brood cluster. In short, the brood patterns may create a hierarchy of differential brood care, which under limiting conditions may favour the most valuable or most demanding brood. The intermediate position of the pre-pupae and pupae may be associated with these brood items requiring no food but only grooming.

Figure 3 shows a significant positive relationship between the tile area of items and their estimated basal metabolic rate. This in turn suggests that the workers who determine the brood pattern may be effectively spreading the workload associated with brood care fairly evenly over the brood-nursery area. Each brood item's tile area represents the area closer to it than its neighbours. Thus if the workers within the nursery simply move to the nearest brood item when they have food or care to donate, they will tend to go more often to those items that require more care. This is a similar process to that outlined in Hamilton's (1971) argument about the geometry of the selfish herd in which a predator is most likely to attack the prey whose tessellation tile it happens to be within. Thus prey cluster into a selfish herd because each is trying to diminish its "domain of danger" proportional to its tile area. In these ants, however, the workers might arrange their brood to create a "domain of care" around each brood item proportional to that items needs. The small domains

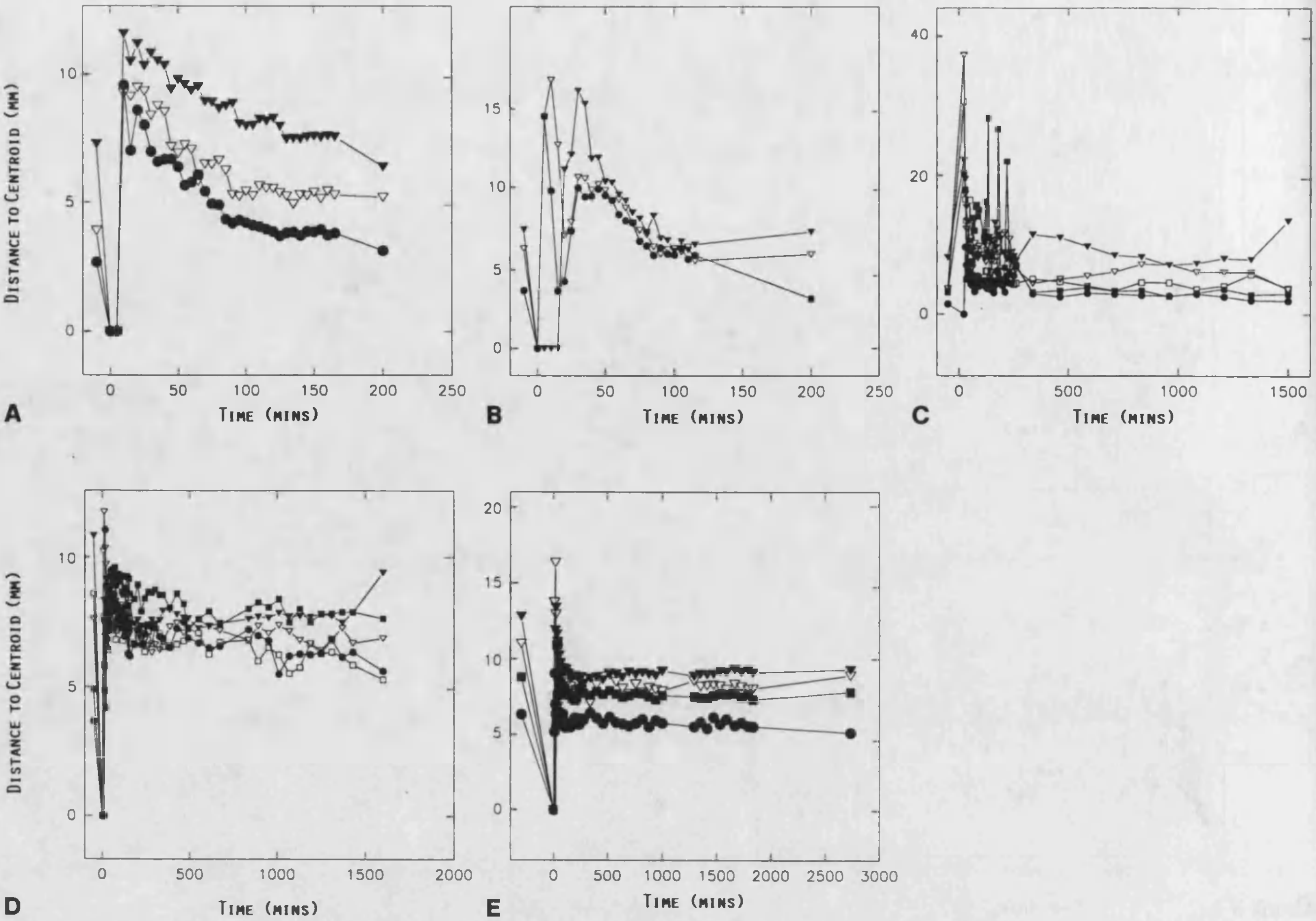

Fig. 6A–E. Mean distances (mm) for each type of brood item from the centroid of the whole brood cluster for each of the five colonies, **A**, **B**, **C**, **D**, **E**, as a function of time (min) before, during and after each emigration to a new nest. Eggs and micro-larvae are represented by *solid circles*, medium larvae by *open triangles*, large larvae by *solid triangles*, pre-pupae by *open squares* and pupae by *solid squares*

of care of pupae and pre-pupae suggest that the main factor involved in the brood patterns are the nutritional requirements rather than the grooming requirements of the brood.

The mechanism that the ants use to re-create these brood patterns when they move to a new nest is not fully known. Part of the mechanism may involve conditional probabilities of picking up and putting down each item which depend on each item's neighbours. Situations in which there is a high probability of putting down a particular item might also be situations in which there is a low probability of picking up such an item. An item may be more likely to be moved if it is either isolated or in a group of dissimilar items and it may be more likely to remain where it is if it is in a large cluster of similar items. Recently, Deneubourg et al. (1991) have shown that such simple rules are sufficient for ants to produce item-specific clusters. Our data on spatial relationships within the brood clusters during emigrations suggest that these rules might apply. The significant association tests show that very soon after the outset of the emigration brood items tend to be placed near brood items of their own type.

The model of Deneubourg et al. (1991), though it may explain the association of like with like, is not, in its present form, sufficient to create concentric brood rings as observed here. However, if the size of brood items, and more importantly the size of the domain of care of each item, are taken into account concentric brood clusters might result from the interaction between the conditional probabilities of moving items and packing rules. The phenomenon of self-sorting by size in which grains of sand form strata with the largest on the top of the pile and the smallest at the bottom is well known. The self-sorting occurs under the influence of shaking and gravity as the smaller particles are able to move down the crevices that lie between the larger particles (see Barker and Grimson 1990).

A similar process may occur when the ants sort their brood. Essentially the tendency of the ants to cluster their brood provides a centripetal force that serves instead of gravity, and the smaller brood items can be manoeuvred through the larger gaps left between the larger items. The tendency of the ants to leave larger gaps between the larger brood items promotes this self-sieving process. Indeed, the empty space surrounding

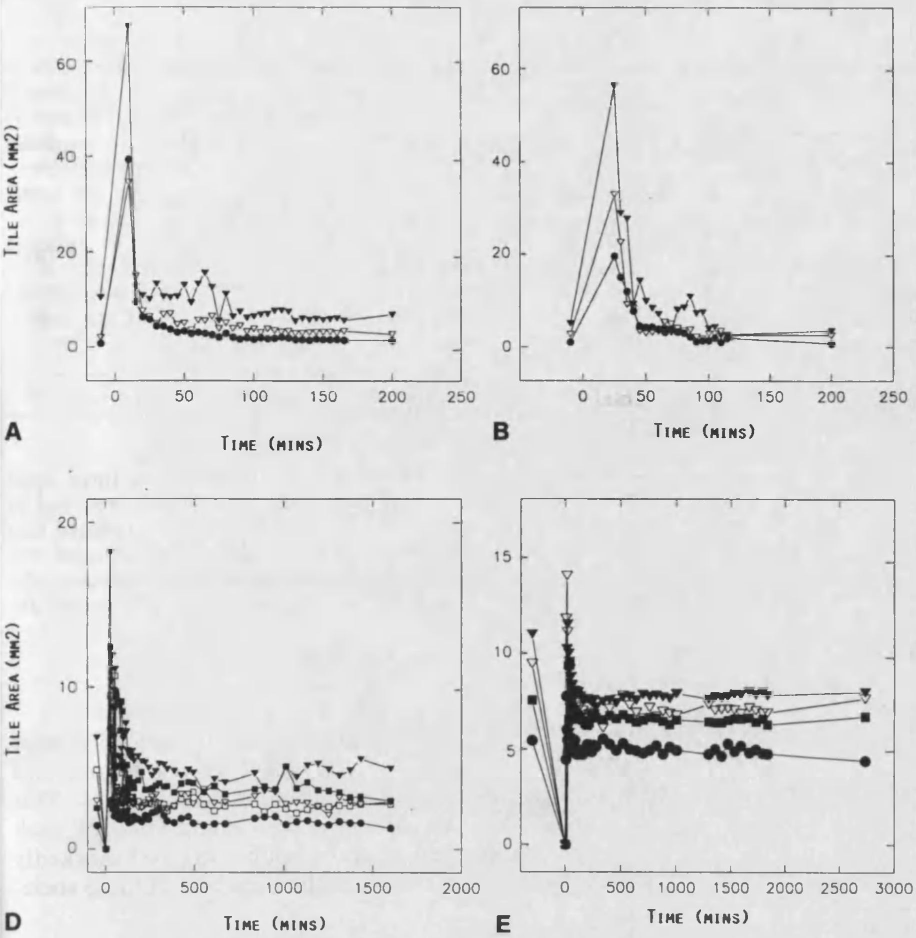

Fig. 7A–E. Shows mean tile areas (mm^2) for each type of item for each of the five colonies, **A**, **B**, **C**, **D**, **E**, as a function of time (min) before, during and after each emigration to a new nest. For this analysis peripheral tiles have not been excluded (see text). Eggs and micro-larvae are represented by *solid circles*, medium larvae by *open triangles*, large larvae by *solid triangles*, pre-pupae by *open squares* and pupae by *solid squares*

each item – i.e. its domain of care – may be a critical aspect of the process, as larger items represent very large objects when their boundary zone is taken into account. This may explain why the pre-pupae and pupae tend to occupy intermediate positions. If these pupal stages have a smaller boundary zone than those of large larvae, they may effectively be smaller objects that are naturally displaced more towards the centre of the cluster.

The mechanisms that set the distance to an item's neighbour are unknown. They may be pheromones that the brood produce and which tend to diffuse over rather predictable distances, or they may simply be metabolic waste products which would give a direct indication of metabolic activity.

In four of the five emigrations, the workers showed a significant tendency to move the same type of item at the same stage in the emigration. Thus workers may recognize different types of item or simply tend to return to the same site in the old nest to pick up the next item. There is no evidence that the brood patterns serve to help the workers move the most valuable items first during an emergency emigration, since in two cases the workers tended to move the larger items first and in the other two cases they tended to move the smaller items first.

Our work, in progress, is showing that the ants are constantly re-sorting their developing brood from day to day to maintain the standard pattern of concentric rings. Furthermore, as the colonies prepare for overwintering the brood are progressively more tightly clustered only achieving maximum spacing again at the height of the summer when temperatures and metabolic rates are at a zenith. This work also suggests how the absence of brood combs in ants, compared with those used by wasps and bees, favours the constant repositioning of their brood so that ants can maintain an even distribution of work over the brood-nursery area.

The brood patterns we have discovered may be created by simple rules that enable the ants rapidly to sort their brood with very large numbers of workers, operating concurrently, in the dark, with no central administration (Deneubourg et al. 1991). Such brood patterns, notwithstanding the simple rules for self-organization upon which they may be based, may have profound conse-

Table 3. Sequence of brood transfer during colony migration

Colony A

Stage in emigration	Item type		
	4's	6+8's	Total
Beginning	21 34.99	33 19.01	54
End	60 46.01	11 24.99	71
Total	81	44	125

$\chi^2 = 27.983$; $df = 1$; $P < 0.001$

Colony B

Stage in emigration	Item type		
	4's	6+8's	Total
Beginning	39 36.30	22 24.70	61
End	58 60.70	44 41.30	102
Total	97	66	163

$\chi^2 = 0.792$; $df = 1$; $P > 0.5$

Colony C

Stage in emigration	Item type			
	4's	6's	8+9+10's	Total
Beginning	0 8.13	6 7.64	20 10.24	26
Middle	27 26.25	31 24.68	26 33.07	84
End	23 15.63	10 14.69	17 19.69	50
Total	50	47	63	160

$\chi^2 = 26.286$; $df = 4$; $P < 0.001$

Colony D

Stage in emigration	Item type			
	4's	6's	8+9+10's	Total
Beginning	7 12.96	20 10.50	15 18.54	42
Middle	37 30.23	22 24.50	39 43.27	98
End	14 14.81	5 12.00	29 21.19	48
Total	58	47	83	188

$\chi^2 = 21.206$; $df = 4$; $P < 0.001$

Table 3 (continued)

Colony E

Stage in emigration	Item type		
	4's	6+8+10's	Total
Beginning	6 5.77	3 3.23	9
Middle	80 65.38	22 36.62	102
End	14 28.85	31 16.15	45
Total	100	56	156

$\chi^2 = 30.412$; $df = 2$; $P < 0.001$

Each emigration has been divided into either two or three equal periods of time, representing the beginning, (middle) and end of the process. The number and type of brood items introduced into the new nest during each period is recorded. In certain cases two classes of brood have been put together to permit chi-square analysis (see legend to Fig. 4). Expected counts are printed below observed counts

quences for the organization of the division of labour and task allocation, the efficiency of brood care, and the differential survival of different brood stages. This in turn is likely to influence the demography of each colony's worker and sexual populations and markedly affect the inclusive fitness of the members of these societies.

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TASK ALLOCATION IN ANT COLONIES WITHIN VARIABLE ENVIRONMENTS (A STUDY OF TEMPORAL POLYETHISM: EXPERIMENTAL)

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We briefly review the literature on the division of labour in ant colonies with monomorphic worker populations, and show that there are anomalies in current theories and in the interpretation of existing data sets. Most ant colonies are likely to be in unstable situations and therefore we doubt if an age-based division of labour can be sufficiently flexible. We present data for a type of small ant colony in a highly seasonal environment, concentrating on individually marked older workers. We show that contrary to expectation such workers undertake a wide variety of tasks and can even retain their ability to reproduce, even whilst younger workers are actively foraging. Our analysis shows that old workers occupy four distinct spatial stations within the nest and that these are related to the tasks they perform. We suggest that correlations between age and task in many ant colonies might simply be based on ants foraging for work, i.e. actively seeking tasks to perform and remaining faithful to these as long as they are profitably employed. For this reason, employed older workers effectively displace unemployed younger workers into other tasks. In a companion paper, Tofts 1993, *Bull. math. Biol.* develops an algorithm that shows how foraging for work can be an efficient and flexible mechanism for the division of labour in social insects. The algorithm creates a correlation between age and task purely as a by-product of its *modus operandi*.

Introduction. One reason for the success of social insects is that social life permits a division of labour which can greatly enhance efficiency (Hölldobler and Wilson, 1990; Wilson 1990). Tasks are divided among the members of the society so that individuals specialize for considerable periods in certain roles. Such specialization might mean that individuals can gain particular skills through practice or simply seek work in certain spatial locations so that time and energy consuming movement between widely distributed tasks is minimized. But specialization carries with it the possible cost of a lack of flexibility, which might be important especially in unstable environments.

An age-based division of labour would appear to be inherently inflexible. Nevertheless, the concept of an age-based division of labour dominates current interpretations of how tasks are allocated among ants of one and the same physical caste (i.e. in monomorphic work forces). This concept is central both to West-Eberhard's hypothesis (1979, 1981) for the origin of a centrifugal system of task allocation and to Wilson's (1985; see also Oster and Wilson,

1978) theory of adaptive demography. In a centrifugal system young workers stay in the safe confines of the nest and as they age they do tasks that are further and further from the centre of the nest. In this way the oldest workers, who are closest to the end of their physiological lives specialize in the dangerous task of foraging, where life expectancy is short due to encounters with predators and a hazardous environment. Wilson's (1985) hypothesis of adaptive demography states that since the demographic structure of worker population determines a colony's division of labour and hence the inclusive fitness of its members, it is likely to be directly influenced by selection. By contrast, in populations that are not highly social, demographic structure is more likely to be an epiphenomenon of selection acting on individuals (Wilson, 1985). West-Eberhard (1979, 1981) has suggested that the origins of the centrifugal system of temporal castes are associated with selection at the level of the individual. Younger workers, who may be able to lay their own viable eggs and have greater reproductive value than their older sisters, stay near the brood in the safety of the nest. In Hymenoptera, males develop from unfertilized, haploid eggs—so certain uninseminated workers can produce sons (see Bourke, 1988). Only when worker fertility declines, as they age, do they switch to serving their kin by undertaking dangerous foraging tasks.

These hypotheses of West-Eberhard and Wilson, both based on the notion that there is a tight association between age and task, may have some explanatory value for colonies in relatively stable social and ecological environments. However a tight association between age and task is more likely to be a problem than a solution in unstable situations, such as might occur in highly seasonal or unpredictable environments or within small colonies.

Consider a small colony producing one generation per year. The brood profile will change throughout the active season and so will the tasks. In the case of a crisis, losses of 10 workers might be 10% or more of the total worker population. In addition, worker reproduction is common in small colonies (Bourke, 1988; Franks *et al.*, 1990a), but as we show here it is not always tightly coupled to age. For all these reasons, it would seem a necessity that ants respond flexibily to the changing situation and needs of their society.

Much recent evidence shows that the division of labour in a variety of social insects has some flexibility. Certain individuals can change their tasks in response to the changing needs of the society (Calabi, 1988; Gordon, 1989). However, this flexibility is still mostly interpreted as a mechanism for fine tuning an age-based division of labour. The fundamental problem is that observed relationships between age and task are correlations and do not establish cause and effect.

Here we begin to address the question: are the observed (and often weak) associations between age and task simply the result of systems for task allocation that are fundamentaly flexible and not based on aging? Such systems might generate a correlation between age and task simply as a by-product (Tofts, 1993).

We suspect that in small social insect colonies, in highly seasonal environments, task allocation is likely to be based on a far more flexible and robust system than could be the case if there is a deterministic age-based division of labour.

We have undertaken an intensive study of task allocation in small colonies of the ant *Leptothroax unifasciatus* (Latr.), collected from highly seasonal, mountain habitats. In such situations even mature colonies have less than 200 workers, some of whom are able to produce their own sons. We have individually marked workers that were at least 1 year old and recorded their spatial locations in the society and the tasks they undertook.

Methods. Colonies of *Leptothorax unifasciatus* were collected in the summer of 1989 and the spring of 1990, from steep wooded hillsides above the village of Thompson in the Balkan mountains, Bulgaria. Such colonies nest in thin and often extremely flat crevices in rocks. All the ants in the colony live in close proximity to one another, and they rarely seem to use more than one nest chamber. Such colonies are very easy to collect by aspirating all the ants, and to culture in artificial nests made from a pair of large microscope slides separated by a perimeter of cardboard to make a cavity 35 mm × 21 mm × 1 mm. A single nest exit passage of 4 mm × 2 mm was provided in one of the short walls of the nest. Colonies were fed on an *ad libitum* supply of honey solution (one part in 10 parts of water) and *Drosophila* larvae.

One experimental colony was selected; a population census for this, both at the time when all the older workers were marked and at the time of the observations when a new generation of young workers had just eclosed, is given below. Altogether the colony was observed, at intervals, over a period from May 1990 to May 1991.

The ants were individually marked using the following technique. A small slit was made in the top surface of a small block of foam plastic, which was placed in a crystallization dish. Carbon dioxide was piped, at a low rate, into the bottom of the dish to anaesthetize the ant which was placed into the slit so that only its gaster was visible. This technique holds the limp and anaesthetized ant without causing it any injury. Tiny droplets of paint (PACTRA R/C polycarbonate, ketone-soluble, model paint) were applied to the ants' gasters with a very thin entomological pin set in a match stick. Several drops of paint could be applied to each ant so that each had its own colour code. The ant was then taken out of the restraining block and left in a new petri dish with a new nest. The paint dried in a minute or two, while the ant recovered in at most 10 min. The complete workforce of a colony was processed by opening up their old nest and taking each worker in turn, and then later allowing the whole colony (with their brood and queen) to emigrate into a new nest. Although such marked ants seem to groom one another more often than usual for the first few days after they have been marked, such paint marks often persist for many months. The colony was marked on 4 May 1990, at which time it had one queen, 60 workers, 41 pupae, 19 prepupae, 49 larvae and 18 eggs. Following this marking, 27 hr of direct observations were made of the colony, including recording the numbers of foraging bouts by marked ants (see Table 1).

Photographic, mapping and digitization techniques. The colony was filmed between 30 July and 3 August 1990, at which time it had one queen, 111

Table 1. Data records for the marked ants in the colony

Ant name	No. of positions recorded in nest	No. of times in the exit	No. of times out of nest in July	Total times recorded	No. of times out of nest in May
2	85	0	0	85	0
5	47	13	1	61	0
9	6	0	8	14	14
10	83	0	0	83	1
11	84	0	0	84	0
12	80	4	4	88	0
13	89	0	0	89	21
14	38	1	37	76	26
15	81	0	1	82	0
18	78	0	0	78	2
20	77	0	1	78	13
23	9	3	0	12	1
26	60	6	1	67	9
27	91	0	0	91	0
29	92	0	0	92	0
31	65	1	15	81	0
32	29	14	39	82	0
33	39	0	0	39	2
38	99	0	0	99	2
39	81	0	0	81	0
41	75	0	0	75	0
42	87	0	0	87	0
44	92	0	1	93	0
48	91	1	1	93	0
49	96	0	0	96	0
51	89	0	0	89	0
52	74	8	8	90	0
53	96	0	0	96	0
56	83	0	0	83	2
58	89	0	0	89	0
59	100	0	0	100	0
60	83	0	0	83	2

workers (32 old and marked, 13 old with lost marks, 66 young), nine pupae, eight prepupae, 108 larvae and one egg. The colony was photographed every day between 09:05 and 18:36 hr. During this time it was maintained at a temperature between 21 and 24°C.

A fine-grain colour slide (Kodak, Kodachrome 64 colour transparency film) of the nest population was made every half hour for 9.5 hr on each of five consecutive days, using a 35 mm single lens reflex camera mounted on a Zeiss stereo dissection microscope. All photographs were taken against a black background. At the end of each day the brood distribution within the nest was photographed. This involved turning the nest over. Doing this slowly and gently caused no noticeable disturbance to the ants. In this way 100 photographs of the adult ants and five photographs of their brood distributions were made. In addition, the identity of the individually marked ants out of the nest and in the 4 mm by 2 mm exit passage was also recorded at the time of each photograph, as were the numbers of young ants outside the nest. Our method of using stills photography for analysing ant social behaviour is similar in principle to that of Corbara *et al.* (1986, 1990).

Each photographic slide was then projected so that the position of each ant's petiole (i.e. the ant's narrow "waist"—the centre of its body) or the centre of each brood item could be marked on an acetate sheet. Each sheet was then transferred to the screen of a microcomputer, which was used to record the coordinates of each recognizable ant (or in the case of the photographs of the brood each brood item) by moving a mouse controlled cursor to each item's position, selecting it, and then entering the ant's identifying number or for each brood item its developmental stage, coded as a numerical value. The brood was classified into five categories: pupae (white and immobile but having the sculptured morphology of adults); prepupae (white and opaque but without adult sculpturing); large larvae (as with the other larvae light yellow and translucent); medium larvae; and lastly microlarvae and eggs. It is hard to distinguish between eggs and newly hatched microlarvae so these were grouped into the same category. Brood categories were coded numerically as follows; pupae (10), prepupae (9), large larvae (8), medium larvae (6), microlarvae and eggs (4).

The resulting computer files with the coordinates and identity of each individually recognizable ant or each of the brood items were then transferred to a Mainframe computer. A package of programmes developed by the Spatial Statistics Group of the School of Mathematics at the University of Bath and based on the algorithm of Green and Sibson (1978) was used to make Dirichlet tessellations for the brood distributions.

The sets of coordinates for each of the individually marked ants pooled over the 5 days (100 photographs) were depicted using a technique for showing their probability density function (Silverman, 1986) over the area of the nest. For

Table 2. Spatial data for all marked ants in the colony

Ant name	Mean *x* (AU)	Mean *y* (AU)	CandE (z)	Dist4 (AU)	Dist6 (AU)	Dist8 (AU)	Dist9 (AU)	Dist10 (AU)	DistQ (AU)
2	9.35	13.14	−6.43	10.07	5.55	9.44	7.00	5.22	10.15
5*	35.57	17.05	−1.62	29.74	21.42	17.67	19.51	25.10	16.87
9†	28.68	18.36	1.82	22.73	15.33	10.85	13.07	18.19	11.15
10	28.73	15.28	−4.00	23.57	14.36	11.00	12.52	18.48	9.81
11	23.16	16.45	−4.66	17.92	9.53	5.31	7.23	12.79	5.72
12	35.04	15.95	−2.51	29.45	20.67	17.18	18.86	24.65	16.06
13	29.33	15.83	−3.35	23.97	15.07	11.51	13.17	18.99	10.56
14*	28.33	19.39	−0.08	22.20	15.48	10.67	13.10	17.87	11.49
15	33.35	17.86	−2.41	27.41	19.51	15.47	17.46	22.86	15.05
18	28.40	18.21	−2.72	22.48	15.01	10.55	12.75	17.90	10.84
20	32.58	16.90	−2.48	26.84	18.49	14.68	16.53	22.13	13.98
23†	36.37	14.93	1.09	30.99	21.81	18.61	20.13	26.09	17.16
26*	36.50	16.94	−1.21	30.67	22.30	18.60	20.42	26.03	17.72
27	21.78	21.13	−5.17	15.48	11.57	5.55	8.75	11.65	9.32
29	20.85	18.57	−4.05	15.06	9.01	3.27	6.26	10.36	6.62
31*	33.44	20.01	−1.08	27.19	20.34	15.80	18.10	23.01	16.07
32*	36.69	15.81	−1.15	31.09	22.26	18.84	20.50	26.31	17.64
33†	29.49	17.77	−0.72	23.64	15.84	11.61	13.68	19.00	11.53
38	20.58	17.18	−4.57	15.25	7.83	2.68	5.18	10.14	5.19
39	25.20	16.53	−3.60	19.82	11.40	7.33	9.22	14.81	7.26
41	29.85	16.52	−2.81	24.28	15.77	11.97	13.78	19.43	11.30
42	23.45	20.87	−5.46	17.17	12.47	6.68	9.73	13.22	9.64
44	29.19	14.87	−4.14	24.13	14.72	11.52	12.96	19.00	10.13
48	29.41	20.40	−2.65	23.15	16.92	11.96	14.50	19.04	12.97
49	21.86	11.99	−7.28	18.86	7.09	6.51	6.08	12.97	2.42
51	11.42	14.24	−4.15	9.90	4.07	7.11	4.83	4.10	8.31
52*	34.80	17.19	−1.55	28.96	20.71	16.90	18.77	24.33	16.17
53	14.90	13.38	−5.49	12.67	1.47	4.82	1.65	6.55	4.72
56	29.32	21.13	−4.32	22.99	17.22	12.09	14.73	19.05	13.38
58	20.60	18.26	−4.19	14.91	8.61	2.92	5.86	10.11	6.26
59	20.07	18.84	−4.95	14.23	8.71	2.74	5.91	9.59	6.76
60	23.68	15.51	−3.78	18.76	9.60	6.01	7.52	13.46	5.43

(AU—abstract units, 1 AU approx. = 1.196 mm, nest length = 41 AU (35 mm), nest width = 26 AU (21 mm); $z < -1.96$—clustered, $z > 1.96$—overdispersed. * Ants with $z > -1.96$. † Ants with $z > -1.96$ which have died or lost the mark during the experiment. Mean *x* and Mean *y* are the mean coordinates, i.e. the centroid for each ant. CandE stands for the Clark and Evans (1954) statistic with Donelly's correction (1978). Dist4, 6, 8, 9, 10 and Q are distances from the ant's centroid to the centroid of each type of brood item or the Queen.

each of the ants, the centroid (i.e. the mean *x* and *y* coordinates of their positions) and Clark and Evans' (1954) statistics (with Donelly's, 1978, correction for edge effects) for their spatial dispersion patterns were also calculated. In addition, the distance of such centroids from the centroid for each type of brood item and that of the queen were also calculated. These data (Table 2) were then used as the basis for a similarity matrix. In order to represent this matrix in the plane and find groups of similar individuals

Table 3. Behavioural data for inside the nest (4, eggs and microlarvae; 6, medium size larvae; 8, macrolarvae; 9, prepupae; 10, pupae; Q, queen)

Ant name	Contacts with brood of type 4	6	8	9	10	Q	Young ants	Marked ants	Tropholaxis wt larvae	Tropholaxis wt ants
2	28	1	0	0	4	1	0	0	0	0
5	0	0	1	0	0	0	2	0	0	0
9	0	0	0	0	0	0	0	1	0	0
10	0	0	3	0	2	11	0	2	0	0
11	0	0	5	6	5	9	0	2	0	0
12	0	0	1	0	0	12	0	1	0	2
13	0	0	2	0	0	14	0	3	1	2
14	0	0	2	0	0	0	0	0	0	8
15	0	0	0	0	0	0	0	3	1	1
18	0	0	2	2	2	5	0	1	0	1
20	0	0	3	1	0	4	1	2	1	1
23	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	2	0	0
27	4	0	2	1	0	0	0	1	0	0
29	0	1	7	1	5	0	0	2	4	1
31	0	0	1	1	0	0	1	2	0	1
32	0	0	1	0	0	0	0	1	0	1
33	0	0	0	0	0	0	0	1	0	0
38	9	1	1	4	1	6	1	1	0	0
39	0	1	2	1	2	13	2	0	5	0
41	0	0	2	0	0	0	0	2	0	0
42	3	0	1	1	0	0	2	1	0	2
44	2	0	1	1	1	12	0	1	1	0
48	0	0	2	1	0	0	0	4	4	1
49	19	0	0	1	0	26	1	1	4	0
51	25	0	0	0	5	1	0	1	0	0
52	0	0	0	0	0	0	1	3	0	0
53	36	5	1	1	0	6	0	1	0	0
56	0	0	1	1	0	0	1	0	3	2
58	4	1	9	3	4	0	0	1	4	1
59	3	0	1	2	3	0	0	4	0	0
60	4	1	3	1	0	14	0	1	1	0

principal coordinate analyses combined with average link hierarchical cluster analyses (using the statistical package GENSTAT) were performed.

The similarity matrix was obtained by applying the city block measure, i.e.:

$$s_{ij} = 1 - \sum_{k=1}^{p} |x_{ik} - x_{jk}| / \text{range of } k$$

where i and j are the two connected individuals and 1 to p are the variables.

A principal coordinate analysis was also undertaken for the individually marked ants, based on observations from the photographs of each of the following 12 behaviours: contacts with: the brood (types 4, 6, 8, 9, 10), the queen, a young nestmate, a marked nestmate; tropholoaxis with a larva or with an adult nestmate (Table 3), staying in the exit passage, and last, going out of the nest (see Table 1). The colony was also observed, on a more casual basis, after it had overwintered between the 5 November 1990 and 5 March 1991.

Probability density functions. In order to illustrate the spatial location of every individual we have used density graphs (Silverman, 1986). Contours joining points of similar density have been drawn using the computational procedures developed by Sibson and Thompson (1981), and Silverman (1986).

These procedures enable us rapidly and efficiently to draw contour maps for the distribution of each set of points. The contour lines designate areas of similar density, and serve pictorially the same function as isobars on weather maps or altitudinal contours on topographic maps. The beauty of such methods is that they enable the eye to determine very quickly if a distribution is highly centred on one or more "peaks" and the relative magnitude of such peaks. Most importantly, all the raw data points appear in such diagrams as well as the constructed contour lines; both together give a clear picture of the variance in the spatial distributions.

Dirichlet tessellation. To give a picture of the amount of space allocated to each type of brood item we have employed Dirichlet tessellation using the algorithm of Green and Sibson (1978). This technique divides the plane into polygons or tiles: one for each item. The boundary of each item's tile is delineated by the perpendicular bisectors of the lines joining that item to each of its immediate neighbours. The area within each item's tile includes all the points in the plane that are closer to that item than to any other.

Results

Distribution and behaviour of older, individually marked ants within the nest. Analysis of the spatial data indicates that the old marked ants appear to fall into four distinct clusters, as shown by principal coordinate analysis coupled with the 75% similarity groups from the average link hierarchical cluster analysis (Table 2, Fig. 1). (The groups were delineated on the basis of thresholding the cluster analysis dendrogram at 75% similarity.) These four clusters entirely overlap with the four groups obtained simply by plotting the mean x and y coordinates of each of the marked individuals. This suggests that there are four distinct spatial locations or stations within the nest. The overlap between the results from the similarity matrix, on one hand, and the simple plotting of the mean coordinates, on the other, confirms once again the existence of an enormous amount of spatial structure in ant organization (Franks and Sendova-Franks, 1992).

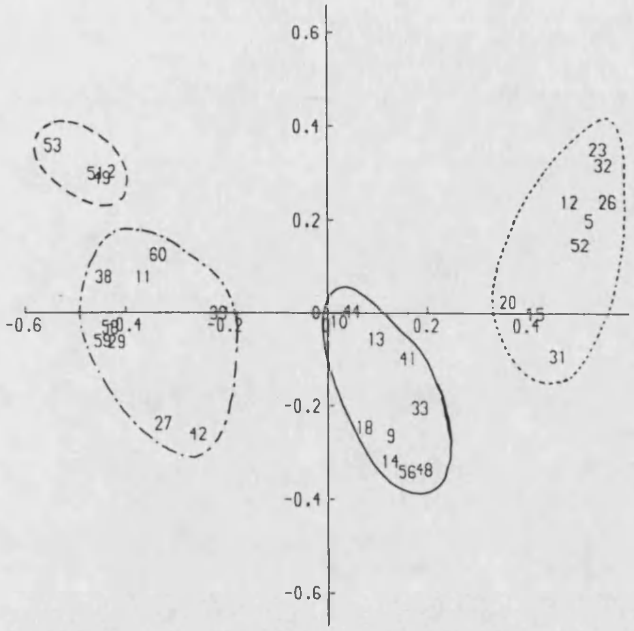

Figure 1. Principal coordinate analysis for the spatial data as in Table 2. Groups were delineated on the basis of thresholding the cluster analysis dendrogram at 75% similarity. The dashed line shows the similarity cluster for station 1. The dots and dashes line shows the similarity cluster for station 2. The solid line shows the similarity cluster for station 3. The dotted line shows the similarity cluster for station 4. (See text for further explanation.)

In the principal coordinate analysis graph for the behavioural data (Fig. 2), four groups have been delineated again on the basis of the 75%-similarity clusters for the spatial data in order to facilitate comparison.

In the two graphs (Figs 1 and 2), there is total overlap between the group of four ants (2, 49, 51 and 53), which occupy the area furthermost from the exit, forming station 1, and the group of four ants which, behaviourally, can be described as nurses of microlarvae and eggs. The group which behaviourally corresponds to station 2 is more spread out and contains two "foreign" individuals (18 and 20). This group is characterized by care for macrobrood. The behavioural group delineated by the same line as station 3 in Fig. 1 can hardly be called a cluster and indeed, these are ants which are almost complete generalists. Such generalists may also provide a living conduit for the flow of food from the more specialized foragers to the brood nurses. The last group corresponds to station 4 and consists of foragers and exit guards.

In general, there is considerable overlap between spatial location and task but it is not perfect. This result might be explained by smaller sample sizes for observed behaviours than for mapped locations, because these ants, in

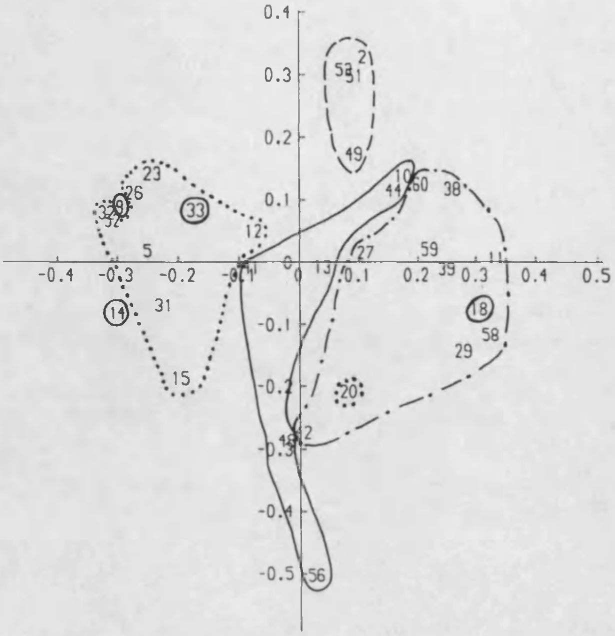

Figure 2. Principal coordinate analysis for the behavioural data as in Table 3 and in columns 2 and 3 of Table 1. Corresponding groups are delineated by the same type of line as in Fig. 2. They can be described behaviourally as follows: dashed line—nurses of young brood, dots and dashes—nurses of old brood, solid line—generalists, dotted line—foragers and exit guards.

common with many others, spend approximately 70% of their time resting when they are within the nest (Franks *et al.*, 1990b).

We have grouped in Fig. 3 (a, b, c and d) the probability density functions for representatives of: (a) station 1; (b) station 2; (c) station 3; and (d) station 4; respectively. Inspection of these figures shows the highly concentrated distributions for station 1 (the nurses of young brood), centred on the distribution of these brood stages (see brood tessellations in Fig. 4) and the clustering of station 4 (the foragers) in the area near the nest entrance. Clearly the distributions of the foragers and egg nurses hardly overlap and indeed hardly touch one another. The distributions of ants in station 2 (the nurses of old brood) rarely meet the short wall of the nest, in which there is the nest entrance, or the opposite short wall of the nest, nearest to the main cluster of young brood. By contrast, the generalists in station 3 are displaced into the front half of the nest, in the intermediate zone between the foragers and the nurses of old brood. Considered together, the locations of all individually marked ants fall into four quite distinct bands, occupying positions along the long axis of the nest from the nest entrance to the rear wall of the nest.

Distribution of young ants within the nest. The positions of all the young, newly eclosed adult ants, recognizable by their pale yellow cuticle, were recorded from the mid-day photograph for each of the 5 days of observation. It is clear from inspection of Fig. 3e that such young ants tend to populate fairly evenly almost all of the area of the nest with the exception of the area close to the nest entrance, which they generally seem to avoid. Most of the young adult ants in the nest are found distributed over the region inhabitated by the brood and the queen (compare Fig. 3e with a–d).

Distribution of foraging activity among old and young workers. The colony, at the time of observation, had one queen, 45 old workers, of which 32 were marked (the rest having lost their marks), and 66 young unmarked workers with ages ranging from 1 day to 3 months. At the time of each photograph a census was made of all the ants that were foraging, i.e. outside the nest, and whether they were old or young, marked or unmarked. Three hundred and forty seven counts were made of foraging bouts, of these 167 were made by old ants and 180 by young ants. One hundred and seventeen of the observations of old ants foraging could be attributed to just 12 of the marked ants (Table 1). The majority of the foraging was conducted by young ants but *per capita* it was mostly undertaken by older ants ($\chi^2 = 8.1$, $P < 0.01$).

Distribution of brood within the nest. The different brood stages are distributed within the *L. unifasciatus* nest (Fig. 4) in the standard pattern. Such brood patterns have been described and analysed in detail by Franks and Sendova-Franks (1992). The microlarvae and eggs are tightly clustered

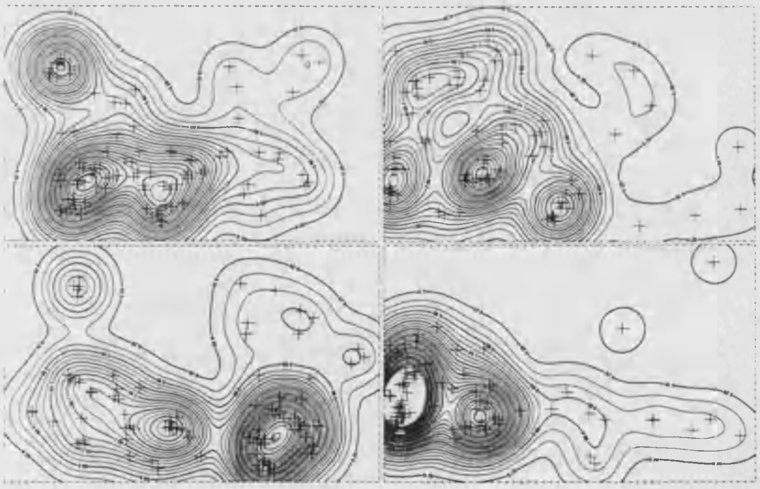

Figure 3 (a).

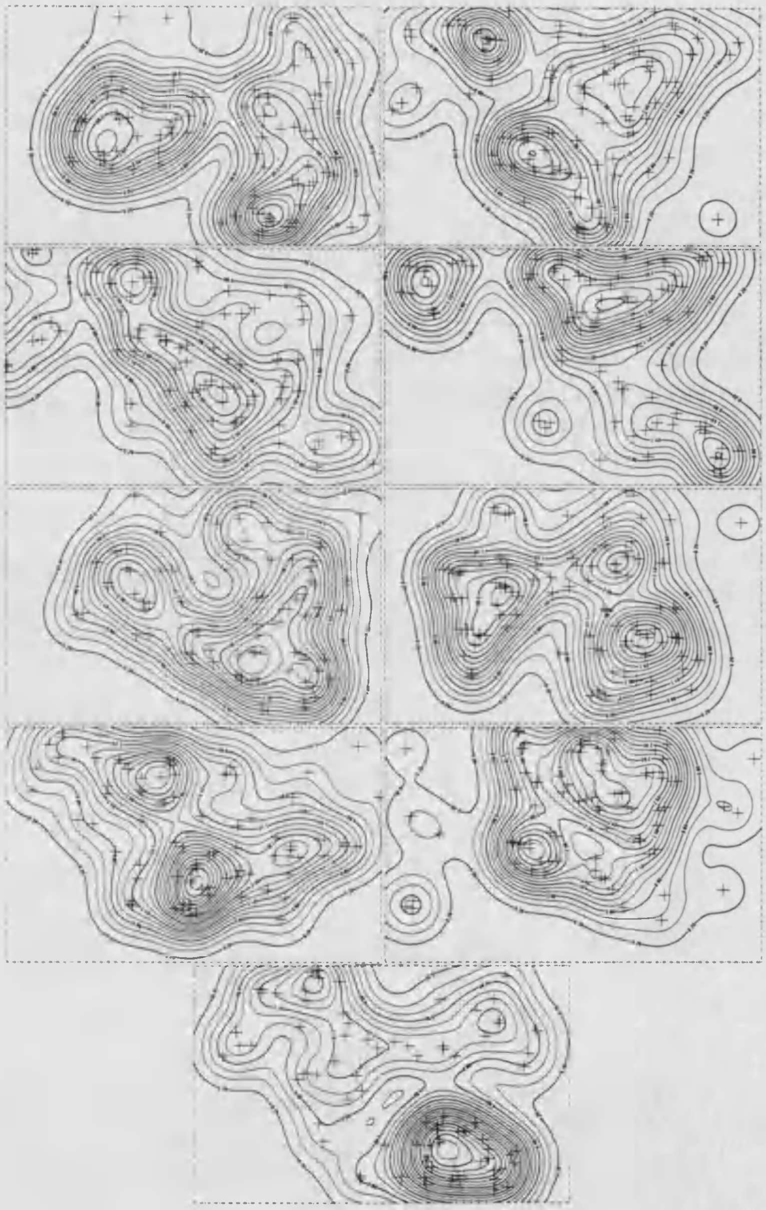

Figure 3 (b).

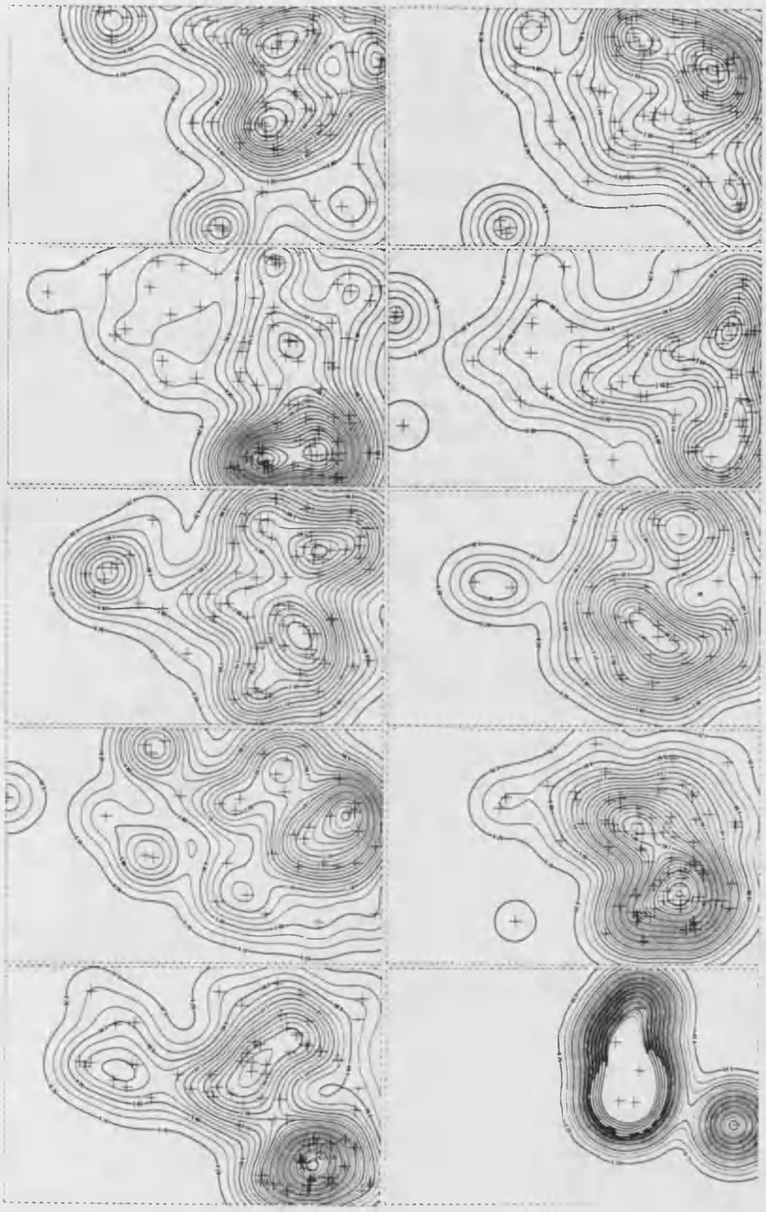

Figure 3 (c).

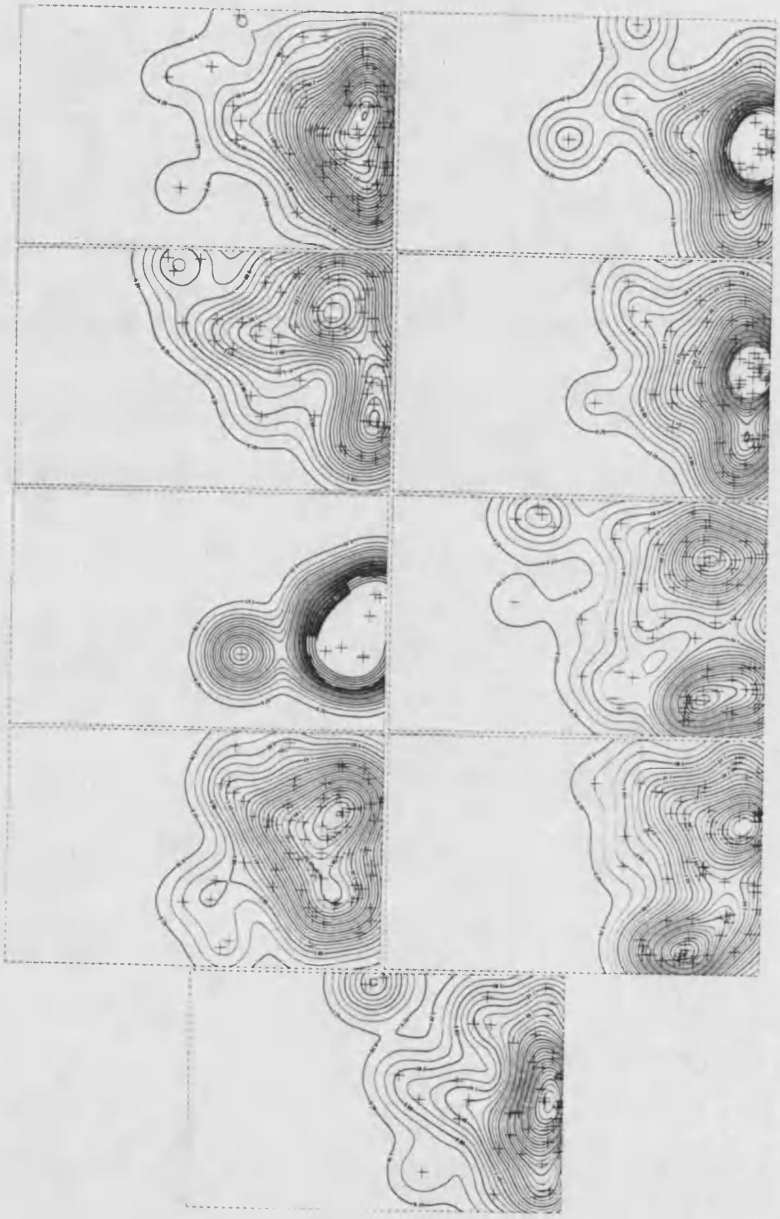

Figure 3 (d).

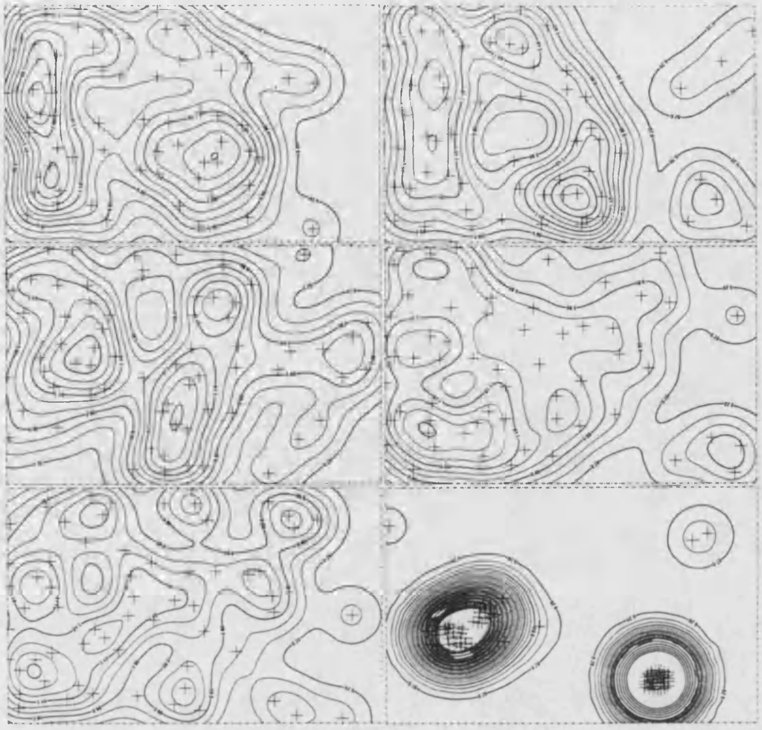

Figure 3. Probability density function graphs (see text for further explanation). (a) For the nurses of young brood (ants 53, 51, 49, 2) (i.e. station 1); (b) for the nurses of old brood (ants 60, 59, 58, 42, 39, 38, 29, 27, 11) (i.e. station 2); (c) for the generalists (ants 56, 48, 44, 41, 18, 33, 14, 13, 10, 9) (i.e. station 3); (d) for the exit guards and foragers (ants 52, 32, 31, 26, 23, 20, 15, 12, 5) (i.e. station 4); (e) for the young, unmarked workers and the queen.

about as far away from the nest entrance as possible. Each of these small brood items occupies a very small polygon "tile" as delineated by the Dirichlet tessellation. Larger larvae are spaced so that they have proportionately larger tile areas, and positioned further away from the eggs and microlarvae in proportion to their size. The prepupae and pupae are placed in an intermediate zone between the largest larvae and those of smaller sizes. The pattern is roughly one of concentric rings of different types of brood more or less centred on the microlarvae. Tile areas increase from the microlarvae outwards, but are not a direct function of item size. Pupae and prepupae (together with the largest larvae) are the biggest items in the nest but they are given rather small tile areas for their size.

The only slightly unusual thing about the brood patterns observed here is the growing secondary cluster of eggs close to the middle of one of the long walls of

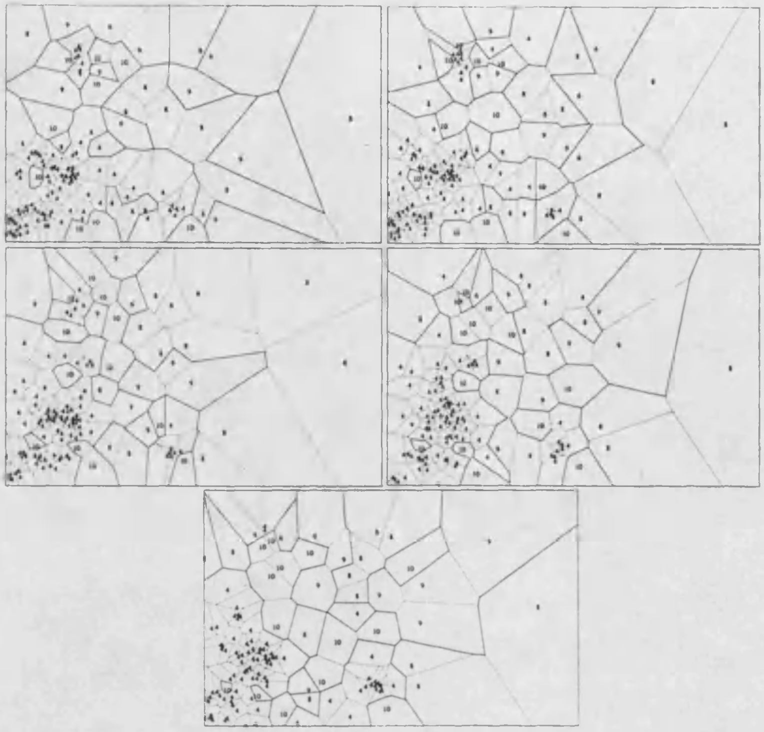

Figure 4. Dirichelet tessellations for the brood distributions at midday on days 1, 2, 3, 4 and 5 of the photographic recordings.

the nest (see Fig. 4). This secondary cluster of eggs is almost certainly associated with laying by the workers. The queen died soon after the photographic observations were complete, and worker egg-laying occurs both in queenright and even more commonly in queenless *L. unifaciatus* colonies (personal observations, see also Bourke, 1988). In addition, observations made after the colony had overwintered show that even certain of the older ants had preserved their ability to lay eggs. Within 1 day of the colony being returned to the laboratory after overwintering eight eggs had been layed. Ant 2 was then observed handling these new eggs with her mandibles and laying a trophic egg which she directly placed on the side of a macrolarva. Within 1 min the egg was fed to this same macrolarva by an unmarked young worker. Ant 53 was also observed handling the new eggs with her mandibles and showed aggressive behaviour, drumming her antennae on the head and biting two unmarked "young" ants. We suspect that many of the newly layed eggs, which were clearly not trophic (because of their large size, elongate shape, turgidity, longer period of oviposition, and longer term survival), were laid by one or both of the ants, 2 and 53, both of whom had distended gasters, typical of egg-laying workers.

Indeed, on 15 March 1991, ant 53 was directly observed laying a reproductive egg which she immediately carried to the centre of the egg cluster. On 23 May 1991 ant 2 was observed laying a reproductive egg.

Such worker-layed eggs can almost certainly develop into males as queenless colonies collected in the field often produce extremely male biased, or entirely male, broods. We have monitored a number of queenless colonies and have recorded worker layed eggs developing successfully into larvae of medium size. All these observations contradict the standard view that only very young ants are able to lay eggs (Hölldobler and Wilson, 1990).

Discussion. The data we have collected show an extremely weak association between age and task. The 32 individually marked workers whose behaviour and locations we have recorded were all at least 1 year old, yet some of them were specialist nurses of young brood whilst others were dedicated foragers and indeed some of them have even retained the ability to produce their own eggs.

The majority of the young (unmarked) workers (who were 1 day to 3 months old at the time of the photographic recording) were fairly evenly distributed within the nest, except that they seemed to avoid the area in the immediate vicinity of the nest entrance. This area was dominated by older individuals, three of whom were specialist foragers, the rest being generalists or possibly exit guards or even part-time foragers. Excluding such old exit guards and foragers, there was very little difference, overall, between the locations of the young workers and those older individually marked workers who mostly stayed within the nest. Furthermore, even though more foraging bouts were undertaken *per capita* by older workers rather than young ones, callow workers did accomplish a very high proportion of all the foraging. Thus our data do show a correlation between age and task. But this correlation is extremely weak. Simultanelously, within the same colony, some old workers are nurses (and indeed producers) of the youngest brood and some callows are foragers.

Many of the individually marked older workers show extremely strong spatial fidelity. The older individually marked ants were divided among four stations/spatial zones within the nest. The first station, deepest into the nest, i.e. furthest from the nest entrance, was the location for the nurses of eggs and microlarvae. The second station, between the first station and the middle of the nest, was populated by the nurses of older brood. The third station, between the middle of the nest and the area around the nest entrance, was where the generalists could be found. The forth station, in the vicinity of the nest entrance, was where some of the foragers occurred between foraging bouts, together with the exit guards.

The four spatial stations roughly correspond to the tasks performed, i.e. the clusters for the spatial and behavioural data (Figs 1 and 2) contain the same sets of individuals. However, some individuals are anomalous. For example, ants 9 and 14 were dedicated foragers, but inhabited the generalist station; and ant 38, who mainly tended eggs and microlarvae, was nevertheless mostly seen at station 2 where the nurses of older brood usually occurred. A proximate explanation for these anomalies is that such workers may spend 70% of their time resting (see Franks *et al.*, 1990b), for this reason the data on active tasks obtained from the still photographs is much less abundant than that for spatial location. Thus the less clear separation of the role clusters (Fig. 2), compared to the spatial clusters (Fig. 1), might be attributable to smaller sample sizes. Additionally, workers might rest in different areas of the nest from those in which they work; possibly because such workers are in transit from one role to another. If this is true, fidelity to a certain area in the nest might either lag behind or proceed role fildelity.

Spatial efficiency, the hypothesis that ants may do a set of tasks that are close to one another to minimize time and energy wastage in intermittent movement (Wilson, 1976), may play a significant role in the division of labour in these ants. In addition, workers that do not find employment in their immediate neighbourhood may actively look for work in the closest neighbouring area within the nest. Certainly many ants are faithful both to certain stations within the nest and a subset of tasks that occur within those areas. The only ants that regularly seek work beyond their home stations are, of course, the foragers, some of whom, appropriately, mostly live in the nest entrance area when not foraging.

The main cues to the position for the worker in the nest are probably the nest entrance and the brood cluster. In a recent paper, Franks and Sendova-Franks (1992) have intensively analysed the distribution of brood within the brood cluster in colonies of these ants. They have shown that not only are the brood sorted but the ants tend predictably to allocate different amounts of space to different types of brood. The eggs and microlarvae are tightly clustered at the centre of the brood area and each is given relatively little individual space, as indicated by the tile areas allocated to them in the Dirichlet tessellations (Fig. 4). Such tile areas increase from the centre of the cluster outwards so that the largest larvae which are the most peripheral of the brood are given individually more space. Intriguingly, the pupae and prepupae, when they are present, are mostly situated in an intermediate zone between the outermost large larvae and the more central larvae of medium size. Thus, space is not allocated purely on the basis of item size since prepupae and pupae are at least as large as the largest larvae. Instead, Franks and Sendova-Franks (1992) have shown that there is a significant positive relationship between the estimated metabolic rate of each type of item and its tile area. For this reason, the prepupae and pupae may be resorted towards the centre of the brood cluster because they do not require feeding but only grooming. Since the tile area of each item is the area closer to that item than any other, it is further possible that the ants are not only spacing each item according to its needs, but creating a pattern that establishes a domain of care around each item that helps to allocate nursing effort where it is most needed. Recall that the ants within the nest are working entirely in the dark, so if, when they have care (for example food) to donate, they simply go to the item whose tile they happen to be within they will tend to apportion their care according to the needs of each type of item. This is a rather a precise reversal of Hamilton's (1971) notion of domains of danger in a selfish herd, where a predator attacks the nearest prey, i.e. the one whose "tile" it happens to be within.

The intricacy of the brood patterns in these Leptothoracine ants may be of considerable importance in spatial efficiency and the division of labour within these nests. It seems that the workers first sort their brood into a pattern (possibily using varying simple rules for self organization; Deneubourg *et al.*, 1991; Camazine, 1990, 1991; Camazine *et al.*, 1990) and then use these patterns to help to sort themselves into different roles.

The only unusual aspect of the brood distribution in the study colony is the presence of a second egg cluster associated with certain workers laying their own eggs possibly associated with a decline in the queen's reproductive control.

Our observations suggest the possibility that there is large variation in the behavioural and physiological ontogeny of individual workers which may lead to significant differences in their life history strategies. Bourke (1988) and Franks *et al.* (1990b) have emphasized the importance of egg laying by workers in ants especially in the monogynous colonies, which may have extended periods of orphanage after the single queen has died. Egg laying by workers during the orphanage period can be important for the inclusive fitness of all members of the society (Franks *et al.*, 1990b). In *L. unifasciatus* egg laying by workers is seen both in the presence and after the death of the queen (Sendova-Franks, unpublished observations).

That older workers still have the ability to lay eggs is an important finding, in itself, and tends to contradict the generality of West-Eberhard's (1981) hypothesis of certifugal polyethism. This hypothesis is based on the assumption that only young workers can reproduce and so tend to stay in the nest to lay eggs; old workers, by contrast, tend to be sterile and undertake dangerous foraging duties.

Amongst the older, individually marked workers there are both specialists and generalists. The diversity of locations and roles among the older ants might be explained by constraints imposed first, by such older ants belonging to a number of different age cohorts; (for example, they may be 1, 2 or perhaps even 3 years old), and second, the colony producing only one large cohort of new workers per year. For these reasons workers of the same age group might have to undertake a variety of tasks as their cohort floods on to the labour market.

For example, our observations show that a number of the callows immediately become foragers, and such flexibility in what each ant does at a particular age, might also be long lasting. For example, some workers (e.g. ants 13, 20 and 26), which had been active foragers in the spring, did not forage at all during the period of photographic recording later in the summer of the same year. Lenoir (1987) provides other examples of older workers retaining flexibility in responding to experimental removal of the younger ants from the colony labour force. Our observations show such flexibility may naturally occur without major experimental manipulation of the colony.

These observations flatly contradict the traditional hypothesis of a deterministic, causal relationship between age and task (Wilson, 1968, 1971, 1985; Calabi, 1988; Calabi and Traniello, 1989).

We believe that existing theories are not sufficient to explain an efficient division of labour in highly variable environments. Such unpredictable variability might be associated with small worker populations, fluctuating worker longevity, highly seasonal environments so that necessary tasks change rapidly, and worker reproduction so that parts of the labour force abscond from, or never take up, certain altruistic duties.

One possibility for the organization of a division of labour in unpredictable environments is that most of the workers actively "forage for work". That is they actively seek tasks to perform. But this raises the question what prevents total pandemonium if workers are able to change tasks and displace one another? This question is answered in a paper by Tofts (1992).

By foraging for work we mean that both old and young ants seek work where they can find it, perhaps moving into roles that are not already fully occupied by older, more experienced workers who have had time to practice, and to learn and acquire specialist skills. Thus, even though most of the young workers stay in the nest and even avoid the nest entrance area, a significant number of them did undertake foraging, perhaps because nothing else was available.

Our "foraging-for-work" hypothesis also helps to explain why among the cohorts of old ants there are specialists and generalists. For example, task fixation [the idea that ants become increasingly entrained on certain tasks they practice and that they seek out such tasks in preference to others (Wilson, 1976; and refs in Hölldobler and Wilson, 1990)], and other forms of learning may play an important role in the division of labour among these ants. Both of these forms of positive feedback will cause some ants progressively to specialize whilst others are left to flexibily fill in the gaps in the production lines of the society (Tofts, 1992). Deneubourg *et al.* (1987) have also suggested that learning and task fixation may be a key aspect of a temporal division of labour in certain ant societies.

Because relatively small social insect colonies, especially in highly seasonal climates, are likely to have sporadic and, in terms of size, unpredictable waves of recruitment to their worker populations; they do represent much more unpredictable environments than is often assumed. For this reason, the notion of adaptive demography (Wilson, 1985), that the age structure of the worker population is a direct adaption that governs the division of labour of the society, is unlikely to be a sufficient explanation for the structure of colony labour profiles. Instead, the role of learning and long term flexibility will be at a premium in the relatively unpredictable social environment of a small colony in a highly seasonal environment.

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