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Ants through the looking-glass

Blanchard, Guy B.

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Ants through the Looking-Glass

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Guy B. Blanchard

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

In this dim place
The creeping Nidhogg, with his sooty scales
Gnaws at the great Tree's root, and makes his nest,
Curled in the knotted maze on which he feeds.
(A.S.Byatt 1990 Ch. 3)

Summary

The ants are a uniquely tractable example of a biological system where emergent behaviours at the colony-level can be understood in terms of the behaviours of individuals, their interactions with each other and with their environment. While colony-level patterns have been well described, the mechanisms, rooted in individual behaviour, remain poorly understood in the majority of cases.

A research program is identified that addresses individual behaviour in detail. A large amount of behavioural information, combined with non-behavioural information about individual ants, is required to disentangle the correlates from the causes of ant behaviour through natural experiments. With this knowledge, realistic (individual-based) models can be parameterised and used to explore the relationship between individual behaviour and colony-level behaviour, and to explore the evolutionary aspects of colony-level functions.

This study presents the first part of this research program. A novel automated tracking technique using computer image analysis is described which revolutionises the amount of behavioural data that can be gleaned from ant nests. The technique tracks the paths of all ants within two-dimensional nests. The paths are then analysed in parallel by computer to classify the movements of ants as behaviours.

Behavioural data from all the individuals of three colonies are summarised, and the implications of the new technique are explored.

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Contents

1. Introduction

A group of tourists moves *en masse* from portico to vista at the beck and call of the tour guide. The raised red umbrella co-ordinates the movements of the group, which is either stationary, hearing about the conflicts between the Roman gods, or following the guide's beacon.

The pattern of group activity over time in the nests of Leptothoracine ants is superficially similar in that it is often synchronised (Franks & Bryant 1987), with most individuals in the nest being either active or inactive together. However this phenomenon is not organised by any one individual, rather it is the combination of an autocatalytic process, where active individuals activate inactive individuals, and a subsequent dampening of activity as ants that become inactive and remain inactive (Goss & Deneubourg 1988, Cole & Cheshire 1996).

In these two examples co-ordinated activity amongst a group of individuals is achieved in two strikingly different ways. In the first the tour guide has an overview of the group and controls the subsequent activity of the group by sending out signals to which all individuals in the group can respond. The leader is of sufficient visual, auditory and neural complexity to have a global view of the group's situation. In ant nests individuals are not presumed to be aware of the state of activity of individuals in other parts of the nest. Decisions about the timing and duration of activity are assumed to be made in response to the cues within an individual's tactile range, and possibly to a chemical or pheromonal gradient in the nest. An ant's sensory and neural complexity can therefore be very much less, requiring in principle only a relatively simple set of rules of thumb in response to tactile cues.

Exemplified are two poles of a continuum of the possible ways of organising group activity. The organisation of activity is one of many features of the organisation and function of groups that can be organised along this continuum (e.g. foraging/hunting, division of labour, hierarchy formation, building). At one extreme the pattern is prescribed or dictated by one or a small number of individuals, and at the other it is generated through the interactions of the individuals in the group, or is self-organised.

The process of pattern-generation is very different in these two extremes. In the former, the prescriptions of the 'leaders' map simply, or linearly, onto the pattern that they are orchestrating. This type of organisation is very familiar to us humans. In the latter extreme the mapping of the behaviour of the individuals in a group onto the emergent pattern is more complicated, or non-linear, if there is some feedback involved such as the autocatalysis in the above ant example.

Two of the greatest challenges in current biology are to improve our understanding of the generation of pattern through self-organisation, and to understand the impact of the mechanism of self-organisation on the evolution of pattern.

1.1 Self-organisation

Co-ordinated ant activity is one of an increasing number of documented examples of a pattern being generated by a self-organised mechanism in the social insects (e.g. building - Franks et al. 1992, Theraulaz & Bonabeau 1995; brood sorting - Franks & Sendova-Franks 1992; foraging - Deneubourg et al. 1989, Edelstein-Keshet et al. 1995, Stickland et al. 1995; collective decision-making in bees - Seeley et al. 1991; task allocation - Tofts & Franks 1992, Sendova-Franks & Franks 1993; and hierarchy formation - Theraulaz et al. 1995) and in other areas of biology (ecology - May 1976; fish shoaling - Huth & Wissel 1992, herd front patterns - Gueron & Levin 1993, cell organisation - Hess & Mikhailov 1994, the growth of Acetabularia - Goodwin 1994 Ch. 4, the aggregation of slime moulds - Keller & Segel 1970).

The clearest definition of self-organisation from within the social insect literature is that of Camazine and Deneubourg (1994):

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

The term was earlier used by Nicolis and Prigogine (1977) to describe the process of the formation of 'dissipative structures'. A self-organised structure is dissipative because it releases (dissipates) energy into its surroundings and must take on energy to maintain itself. This contextual notion of a structure requiring a flow of energy from and to its surroundings is missing from the above definition which considers an isolated system of components interacting amongst themselves. The definition needs to be expanded to include interactions between the components and their medium and to acknowledge that the ordering of components and the maintenance of pattern requires energy which must ultimately come from without the collective.

The success of the literature on self-organisation in biology has been in finding mechanistic explanations for patterns which were either not explained or were assumed to have been prescribed in a hierarchical manner. This is analogous to the change Darwin instigated when he proposed natural selection as a mechanism for the evolution of species, which previously had been considered designed by a creator and immutable.

1.2 Problems

However, it is suggested that there are a number of teething problems with the study of self-organisation in the social insects. These problems are set out below, followed by a description of a framework of research designed to overcome these problems. The problems are illustrated initially by recourse to two test-cases. These are task allocation in social insects and the mechanism for the generation of synchronised activity in ant nests adumbrated above.

1.2.1 Test case 1: Task allocation in the social insects

The generation of a division of labour through task allocation has recently been the subject of two extensive reviews (Gordon 1996, Bourke & Franks 1995 Ch. 12). Both catalogue the shift in emphasis from the determination of individual behaviour through intrinsic differences (genetic or age-based) following Caste Theory (Oster & Wilson 1978), to a division of labour that could arise in the absence of intrinsic differences between individuals (Tofts & Franks 1992, Gordon et al. 1992). Both offer an ingredient of the mechanism of task allocation, and there is good evidence to suppose that while neither is sufficient, both occur in the social insects (Robinson 1992, Tofts & Franks 1992, Franks & Tofts 1994, Robinson et al. 1994).

Empirical work stimulated by Caste Theory using time budgets and behaviour transition probability matrices (e.g. Herbers & Cunningham 1983) makes the implicit assumption that the behavioural transition probabilities of an ant, and therefore its behavioural profile, is an intrinsic property of an ant. That is, the behavioural profile was the inevitable and only possible expression of the genes (*via* age) of the ant. It should be noted that, given this assumption, a full description of a colony in terms of the genetically determined behavioural tendencies of its members was both theoretically straightforward (Oster & Wilson 1978 Ch. 5) and empirically tractable, as Herbers and Cunningham (1983) demonstrated.

The 'foraging for work' algorithm (Tofts 1993) is the clearest articulation of the opposing view that a division of labour can arise without intrinsic differences between individuals. Under this formulation a full description of a colony takes the form of a number of intrinsically identical individuals whose behaviour is determined by a common set of rules of thumb, but who are moulded by experience. Ants respond to contextual cues, such as the amount of work needing to be done locally. An individual's behavioural profile is thus relative, dependent on the behaviour of the other ants in the nest. When iterated in a manner that reproduces some of the complexity of ant nests (e.g. with a centrifugal arrangement of tasks, and with new individuals being born in the centre of the nest) this results in a division of labour similar to that found in some real ant nests (Tofts 1993).

This algorithm was published expressly as a caricature to make it simple, and therefore general. It was not presented as an exclusive mechanism - "Of course, developmental and physiological constraints are likely to exist in real colonies" (Franks & Tofts 1994 p.471). However there has been little subsequent theoretical and empirical work to tackle the pivotal question:

> What is the relative role of intrinsic factors and contextual factors in task allocation in social insects?

There is a seemingly impenetrable web of factors that may influence behaviour; memory, learning, age, physiological state (e.g. hunger level) and the genetic basis of an individual's rules of thumb. Disentangling the correlates of behaviour from its causal factors seems a long way off.

1.2.2 Test case 2: Synchronised activity in ant nests

Ants are assumed to become active in the nest either through becoming active spontaneously, or because they are activated by active neighbours. The probability of being activated will be related to the number of ants currently active in the nest; presumably it will increase with the number of active ants. However, there must be a counteracting mechanism which stops or reduces the rate of ants becoming activated, or else colony activity will not dampen and there will be no synchronised fluctuations in colony activity. A brief history of how this dampening effect has been encoded in models of ant activity is detailed below.

Goss and Deneubourg (1988) opened with a simple non-spatial simulation model where ants could become active with a fixed probability or could be activated by other active ants with a probability increasing in linear proportion to the number of active ants in the nest. This crudely simulates the assumed spatial spread of activity in the nest. Active ants become inactive with a fixed probability but remain 'unwakeable' for a fixed period on becoming inactive. The dampening effect has therefore been incorporated in the 'unwakeable' period, and without this there is no way for colony activity to dampen.

Hemerik et al. (1990) followed with a model based on food levels in the colony. Hunger caused activity, and the dampening of activity in a colony was caused by "collective satiety", reflecting the physiological state of the individuals. Hatcher (1992), however, later reported that synchrony was little affected by starvation.

Tofts' (1992) paper essentially replicated the model of Goss and Deneubourg (1988), removing the spatial aspect completely without any qualitative change in the behaviour of the model. Cole (1991) found that the activity profile of isolated individual ants had no periodicity and that the pattern of spontaneous activation was chaotic. Cole (1992) then produced a similar model to Goss & Deneubourg (1988), confirming again that a refractive or 'unwakeable' period was essential to generate synchrony.

Empirical work was driven by the following line of argument (e.g. Franks et al. 1990): A test of the models can be found in the shape of the frequency distribution of inactive bout lengths. An 'unwakeable' period will manifest itself as a reduction in the number of short inactive bouts than would be expected if activation was a random process. Therefore an 'elbowed' frequency distribution with few short bout lengths would show that ants tend to be 'unwakeable' through the early period of inactive bouts. This would confirm the models suggesting that the 'unwakeable' period is the mechanism that is driving the synchronicity of individual activity.

This line of reasoning is circular. Considered from the point of view of the frequency distribution of inactive bout lengths that would be expected from a synchronised colony, it is highly likely that there will be fewer short bout lengths simply because of the high number of long inactive bouts spanning the periods of colony inactivity. This will be true irrespective of the mechanism generating the synchrony. It is not therefore valid to claim that such a pattern proves that individuals have an 'unwakeable' period. An 'unwakeable' period is only one of a number of ways of encoding the dampening of colony activity.

New ground was broken when Solé et al. (1993) introduced two novel features. Firstly the model was explicitly spatial, modelled at first as a mobile cellular automata (Miramontes et al. 1993) and subsequently renamed a fluid neural network (Solé & Miramontes 1995). Secondly inactive 'individuals' were allowed to decrease the activity of active individuals. In the cellular automata model (Miramontes et al. 1993) individuals occupy one grid-square on a square two-dimensional lattice and move according to a random walk if they are active. An ant has an activity level that decays exponentially over time. Above a threshold activity level an ant is said to be active. Inactive ants become spontaneously active with a constant low probability and are assigned an intermediate activity level. The activity level of each individual at each time step is calculated as a function of its previous activity level and those of its eight neighbours on the lattice. This function is non-linear, with high activity individuals inducing a disproportionately high increase in the activity of neighbours. Interestingly, the opposite of an 'unwakeable' period is encoded - individuals that have recently become inactive are more likely to be woken by other individuals than those whose activity level has decayed way below the threshold value above which individuals become active.

Cole and Cheshire (1996) performed a parallel theoretical and empirical exploration of this model. They found that the model generated synchronised activity for parameter values taken from behavioural observations on the activity of individuals in small colony fragments. However, an intuitive explanation is not given in any of the above papers for how synchrony occurs in the model. A spatial summary of activity is given in Miramontes et al. (1993), but this does not reveal the spatial spread of activity over time.

It is suggested that the following occurs: During periods of high colony activity, individuals become over-dispersed as they bounce off each other. This will in turn reduce the contact rate of individuals, and activity levels will decay. As activity levels become low, some will become inactive. Active individuals will then become inactive on encountering one or a number of inactive ants. The active individual becomes inactive while not raising the activity level of the inactive individuals sufficiently to activate them because at this stage most individuals will have reasonably low activity levels. Aggregates of inactive individuals will therefore form during periods of low colony activity. Once one or a number of highly active individuals appears in an aggregate through spontaneous activation, activity will spread quickly through the 'island' of inactive individuals because highly active individuals cause a disproportionate increase in each others' activity levels. The spread of activity is therefore suggested to be highly dependent on the spatial organisation of active and inactive individuals. The predictions from this line of argument, that during high colony activity ants will be over-dispersed, and that during low colony activity individuals will aggregate, has not been explored empirically or in these models.

The two types of model sketched above are good examples of how a self-organised pattern can be generated by at least two different mechanisms. However, neither of the model types have explored much of the complexity of real ant nests. It would surely be reasonable to extend the line of argument that if by adding the spatial component and a negative influence on activity an 'unwakeable' period becomes unnecessary, then adding further complexity may make the spatial component redundant. Indeed one could not be sure of the true mechanism until the full complexity of the nest had been explored. Aspects of real colonies that remain to be investigated are:

1. Individual differences such as overall rate of activity, proportion of time inactive and rule of thumb thresholds.

2. Foragers (high activity individuals (Franks et al. 1990)) leaving and returning to the nest.

3. The spatial organisation of different types of individual (Sendova-Franks & Franks 1995).

4. Further spatial complexity such as the central brood pile, 'pillars' of large brood items, and the spatial organisation of activity in relation to the nest entrance.

5. Realistic ant movements and proportions.

6. The different tasks of active ants and their respective movement patterns.

The problem of locating the real mechanism for colony synchrony remains. This needs to be approached with more complicated models supported by more detailed empirical work.

1.2.3 Generalisation in self-organisation

The study of self-organisation in social insects remains at the moment a 'stamp collecting' exercise. No rigorous attempt has been made to generalise across the remarkable and seemingly varied examples of self-organisation in the social insects from the pattern of cell use in the honey bee (Camazine 1991) to the foraging of army ants (Deneubourg et al. 1989). Each self-organised pattern has been studied in isolation.

There are two types of generalisation that can be made; functional and mechanistic. The ecological literature on the status of functional generalisation in ecology (e.g. Judson 1994, Uchmanski &

Grimm 1996) concludes that there is an increasing realisation amongst ecologists that simple or general functional statements in ecology are unlikely to be usefully predictive. General statements about ecology are therefore restricted to blanket statements, such as the ubiquity of parasitism and kin selection. Ecologists are turning to explicit individual-based models to explore the behaviour of particular systems (Uchmanski & Grimm 1996).

However, there is much work to be done on mechanistic generalisations about pattern formation through self-organisation that would apply in ecology, to the social insects and to any other biological pattern formation. One well known example of an abstract generalisation with broad predictive power is the dynamic behaviour of the logistic equation (May 1976). The logistic equation is a very good example of a model that can generate temporal pattern for some parameter values (e.g. regularly cyclic behaviour), which are the result of the asynchronous counter-action of positive and negative feedback. These seem to be essential ingredients of self-organised pattern in time or space.

1.2.4 Adaptive explanations

A further serious problem with the study of self-organisation in biology is the process of establishing whether such patterns are adaptive, or whether they are epiphenomena which have evolved on the back of other features. While this is a general problem afflicting all of evolutionary biology, it is particularly pertinent in the study of self-organisation because of the non-linear nature of pattern-generation through self-organisation.

Some interactions between individuals, and their emergent colony-level patterns, are inevitable consequences of group living. Imagine the nest of a primitively social ant such as *Amplyopone pallipes* (Traniello 1978) where there is little co-operative communication between individuals. There will be some spatial structure or pattern simply because brood items and ant individuals cannot be in the same place at the same time. A division of labour might also be inevitable given the natural variation in behavioural response thresholds amongst individuals within a nest, for example if certain individuals consistently respond more quickly or at a lower threshold level to a stimulus such as food deprivation.

There is a sense, therefore, in which self-organised pattern can appear to be inevitable, or may appear as a by-product of the interactions of the parts of a collective. As Kauffman (1993 p. 16) puts it, "In sufficiently complex systems, selection cannot avoid the order exhibited by most members of the ensemble." The crucial point is that we remain ignorant of the alternative patterns or lack of pattern that could have evolved. It may be, for example, that some self-organised patterns are so robust that all biologically reasonable variation in the rules of thumb that generate the pattern, and contexts of the rules of thumb, will generate the same pattern. The constraints on the patterns that can be expressed by collective systems remain under-explored.

The mechanistic derivation of patterns is also so poorly understood, as the two test cases show, that we have very little understanding of how pattern expression is ensured. It is possible that an advantageous pattern could be secured by multiple mechanisms, each of which can generate the pattern on its own, but in different contexts. The above example of the activity cycles suggests that this is at least theoretically possible.

The problem lies in insufficient exploration in models of self-organisation of the alternatives that evolution has to play with. This problem is more deeply rooted in a lack of empirical data that has prevented us creating the sufficiently realistic models in which rule-variation and context-variation can be explored.

1.2.5 Quantitative self-organisation

A related limitation of the current literature on self-organisation in the social insects is the lack of quantitative assessment of the generation of pattern. In general this is because there is only a qualitative understanding of the phenomena in relatively abstract models.

There is a reluctance to explore the effects of slight changes in the process of pattern formation. This sort of analysis is essential for an understanding of the evolution of the individual traits that generate the pattern. Given the non-linearity of pattern formation through self-organisation, the effect on the pattern of small changes in the rules of behaviour of the constituent parts can in theory be anything from negligible to catastrophic. There are good examples in catastrophe theory of how the changes can be very large (Thom 1975).

A quantitative understanding of the influence of a supposedly adaptive trait (in this case a self-organised pattern) on some currency (such as resource acquisition, reproductive success or disease prevention) is required to understand self-organisation in an adaptive context.

1.2.6 Summary of problems

The first test case has highlighted the lack of an empirical program for separating the intrinsic from the contextual causes of behaviour. The second test case has highlighted the problem of how to ensure that the correct mechanism or mechanisms have been used in relatively abstract models. Assumptions are often made on incorrect theoretical grounds and without empirical justification.

The problem inherent in this confusion can be generalised to the following: the link between a good fit between a summary of behavioural observations and a proposed rule of thumb is only correlative. To claim causation assumes that there is no contextual component involved in generating the observed behaviour and that all alternative mechanisms have been eliminated.

This means that rules of thumb cannot be inferred from log-survivorship plots (Haccou & Meelis 1992 Ch. 4) alone or from information on bouts of behaviour that does not account for the reasons why bouts were stopped, unless contextual factors involved in the causal process are fully understood. The problem then remains of how to break the chain of correlation and locate the intrinsic and external influences on an ant's behaviour. This returns us to the problem of the first test case.

There is no articulated method of dealing with this problem of where the complexity of the system is encoded. Age-polyethism encodes this complexity exclusively in the individual, whereas the 'foraging for work' algorithm encodes it in part in the context of an individual. In most early models of synchronised activity, complexity was encoded exclusively in the individual - in an ant's 'unwakeable' period. It is paradoxical that in these decentralised models, whose ethos is to show how simply pattern can be generated, too much complexity was still being attributed to the intrinsic properties of individuals. Only the model first introduced by Solé et al. (1993) and explored empirically be Cole and Cheshire (1996) considers that some complexity might be encoded in the context of the nest.

The second main problem is an inadequate exploration of the parameter space of pattern formation, and hence a lack of understanding of the impact of the types of mechanism on the evolution of pattern.

1.3 Solution

The development of an empirical methodology and modelling framework that addresses the causes of behaviour must begin by identifying all the possible influences on behaviour. Figure 1-1 shows simplistically the determinants of behavioural transitions according to Caste Theory and pure self-organisation. Caste Theory emphasises the genetic determination of behaviour: self-organisation emphasises the contextual cues involved. A synthesis must incorporate both types of factor.

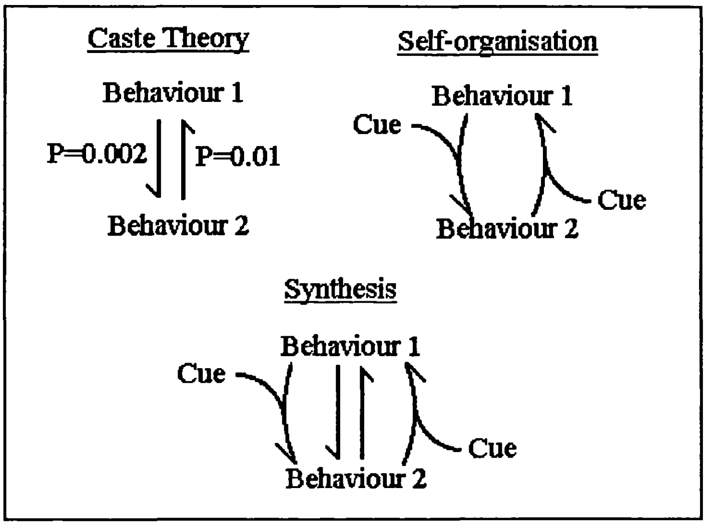


Figure 1-1 Behaviour transition assumptions

Note that in Figure 1-1 the schemata distinguish between intrinsic and contextual determinants in an exclusive manner. In reality the distinction is somewhat blurred. For example if an ant has a memory of the cues it has recently encountered, then the external cues have been internalised, albeit temporarily.

Table 1-1 shows a list of some factors that may influence the behaviour of individuals. The list may well include some factors which are correlates and not causes of behaviour, however at this stage the net must be cast widely.

Table 1-1 The possible influences on behaviour of ants in the nest

Intrinsic Influences

Permanent	Temporary
Skeletal size	Age-determined behaviour
Larval nutrition and other developmental influences prior to eclosion	Nutritional factors (hunger, hydration level)
Physical caste	Ovarian development
Genetic determinants of behaviour (assumed to be in the form of rules of thumb)	Protein and lipid stores
	Memory and learning

Contextual Influences

Tactile cues	Other contextual information
Ants (workers, sexuals, queen(s))	Signals from other ants (e.g. alarm pheromone)
Brood items	Gradients in the nest (CO_2 or pheromones from the brood pile)
Nest architecture	Queen pheromones
Miscelaneous waste material in the nest	Foreign particles on the surface of an ant

All these potentially causal features need to be investigated experimentally and considered in models if the correct mechanisms for pattern formation are to be understood. There is convincing evidence that some of these factors are at least correlated with certain behaviours or behavioural profiles:

1. A correlation has been found between ant size and task in monomorphic ants (Herbers & Cunningham 1983), though this has not been separated from age-related effects of cohorts of workers. Speed of movement and metabolic rate might be expected to vary between individuals of different size (Calabi & Porter 1989) which may in turn affect the speed at which jobs get done and hence affect the dynamics of task allocation.

2. Larval nutrition has been shown to influence skeletal size which in turn may influence behavioural profiles (Elmes & Wardlaw 1981).

3. Robinson and Page (1995) showed that there is likely to be a genetic basis to variation in the propensity to corpse-carrying in the honey bee.

4. There is much literature on the correlation between age and task (see Robinson 1992), although it is not clear that this is causal.

5. Hunger is known to increase colony activity (Wallis 1962, Howard & Tschinkel 1980), but its impact on individual behaviour is less well understood.

6. Hölldobler and Wilson (1990 p. 317) note that there is a close correspondence between the spatial occupation of ants in the nest and the state of their ovaries in a number of studies of *Formica* ants.

7. Lipid stores increase the weight of an ant, and in the extreme case of *Myrmecocystus* repletes do nothing but store honey-dew (Hölldobler & Wilson 1990 p. 333).

8. There has been little work on the effect of recent experience on subsequent behaviour in the nest (Hölldobler & Wilson 1990 pp. 365-370). Most learning studies focus on the use of learning in foraging and learning colony odour. The nature of the internalisation of external cues is of crucial importance in understanding the causes of behaviour.

9. Tactile cues have repeatedly been suggested as being important in determining ant behaviour in the nest (brood items Franks & Sendova-Franks 1992, nest material Franks et al. 1992, workers Gordon et al 1993, Cole & Cheshire 1996).

10. No gradients have been shown to be important in the nest, although the problem has not been addressed empirically. There is likely to be a CO_2 gradient between the nest entrance and the brood pile. This may effect the activity of ants that spend most of their time in the CO_2-rich interior of the nest (Nicolas & Sillans 1989).

11. The behaviour of workers changes in the presence of queens (reviewed in Keller & Nonacs 1993).

12. Foragers self-groom and are groomed more often than workers that stay in the nest (Franks et al. 1990) suggesting that ants are trying to remove foreign particles that have been picked up outside the nest.

1.3.1 Empirical solution

An empirical approach is explored here that uses natural experiments in observational data to examine the causes of behaviour. Given sufficient behavioural data over a sufficiently long time period, information on an individual's response to a variety of cues in a variety of temporal and spatial contexts will be available. The reasons for the duration of behavioural bouts and the reasons for behavioural transitions can be explored. For example the influence of active ants bumping into an inactive ant at different durations of inactivity can be compared to assess the influence of time inactive on the 'wakefulness' of an ant. Perturbations to the colony can be used to alter the contexts of behaviour (e.g. starvation).

The alternative to using natural experiments from observational data is to break the nest apart and perform experiments with individuals or on parts of the nest. For example Cole (1992) isolated individual ants to observe the timing of active and inactive bouts. While this is an interesting exercise, this is depriving the ant of its natural context. It is not at all clear that the behaviour seen in isolated ants will bear any relation to the behaviour of ants in the nest environment. There is the very real risk that ants will display pathological behaviour in reductionistic experiments that deny the ant its normal context.

Natural experiments require huge amounts of detailed observational data. This limitation has precluded their general use thus far. Three novel types of information are required:

1 Quantity of data

The amount of behavioural observation required to determine the likely response of an individual to a certain cue in different situations and for different values of the temporary or permanent intrinsic features of the ant is enormous. It is certainly much greater than the tens, occasionally hundreds of hours of observational data that are currently used in ethological studies in the social insects. A methodology is therefore required that can collect a large amount of information on individual behavioural responses.

2 Non-behavioural information

On top of this behavioural information, the intrinsic features of individuals need to be overlaid, so that the internal context of an ant can be known. It should be possible to calculate individual size and a crude measure of the fat reserves non-intrusively from live ants before or during behavioural observation. Some features can be found retrospectively, for example genetic relatedness or gene presence and ovarian development can be assessed from the corpses of ants after behavioural observation. Measuring other features is problematic. For example there is no established method of calculating the age of an individual in a colony collected from the field. Studying the influence of both age and larval nutrition require long term studies.

3 Contextual Information

The behavioural data must include information about the cues to which an individual can potentially respond. This means following individuals intensively, recording the features of the environment that ants encounter, and observing the response. Of the contextual influences listed in Table 1-1, all tactile cues can be recorded through behavioural observation, though are extremely laborious to collect by hand from real colonies (single individuals) or from video tape (multiple individuals). Gradients of pheromone are more difficult to detect but may be inferred tentatively from spatial trends in behaviour in the nest.

This study explores a methodology for collecting behavioural and non-behavioural data that can be combined to conduct natural experiments on behavioural causation and to parameterise realistic models of social insect colonies. Non-behavioural and contextual information have rarely been incorporated into behavioural studies of social insects.

1.3.2 Modelling solution

Individual-based models in ecology are those that

> "describe a population made up of individuals that may differ from one another, take into account the complexity of the individual's life-cycle, describe changes in numbers of individuals rather than in the population density, and also take resource dynamics explicitly into account." (Uchmanski & Grimm 1996 p. 439)

This can be translated to apply to social insects as models that describe a colony of individuals that may have intrinsic differences which may change over time, that describe individual behaviour explicitly and that describe changes in the environment of an individual explicitly. In short, this means realistic spatially explicit models. The object in such models is to parameterise them directly from detailed observational data, thereby limiting the assumptions and averages that are encoded into the model.

However, many would argue that such complex and explicit models become mere descriptions. They are not useful because they have no generality and are not testable because they make no predictions, they just describe. "It is not sufficient to explain a phenomenon by subjective comparison to a particular result generated by simulation" (Tofts et al. 1992). This point of view betrays a lack of imagination, an unreasonable dedication to analytical methods, and a lack of understanding of the modelling implications of new technologies of data capture. The argument in support of individual-based models can be broken down as follows:

1. Individual-based modelling forces one to be explicit about the causal mechanisms of pattern formation, thereby cutting through the chain of correlations that cannot be addressed in more abstract modelling. The underlying factors have to be encoded either into the individual or left to the interactions of individuals with their context.

2. Individual-based models are easily testable since parallel experiments can be performed simultaneously in the model and empirically.

3. The sorts of pattern generated by variants of the model can be explored quantitatively by modifying the explicit parameters. This is analogous to exploring the effect of mutations, an

exploration of life as it could be (Langton 1989). More abstract modelling can often only make more qualitative predictions.

4. Adaptive explanations for self-organised pattern can be explored through an understanding of the types of pattern expressed in different areas of parameter space. The link between small changes in parameter values and the magnitude of their effect on pattern formation for different regions of parameter space will reveal the types of pattern mutations that are possible.

5. There is a big difference between the prescription and description of a pattern generated by a non-linear process. The parameter values of individual-based models are not a description of a system. As Wolpert puts it, "it seems simpler to specify how to make complex shapes than describe them" (quoted in Bourke & Franks 1995 p. 408). Individual-based models are not therefore mere descriptions.

The main limitation of individual-based models thus far has been that a prohibitively large amount of empirical data is required for adequate parameterisation.

1.3.3 Generalisation

What are the implications for identifying general principles about pattern formation of full explorations of individual-based models? We do not yet know. A better understanding of the mechanistic bases of pattern formation and the mechanistic similarities of patterns will surely improve our understanding of the evolution of pattern. The whole gamut of potential patterns generated by the various forms of a system could be generated through a full investigation of an individual-based model. Unseen patterns or previously unrelated patterns could be found in this way.

More abstract and general models can be produced by 'pruning' or successively abstracting a complex model. This bottom-up method of finding the essential features of a system would ensure that the assumptions made are valid. Each stage of simplification can be checked by comparing the behaviour of the model before and after simplification. While this road will be considerably more lengthy than employing abstract models *ab initio*, fewer assumptions are made about the mechanisms involved. As Uchmanski and Grimm put it (1996 p.12),

> "individual-based models may be able to point the way to any simpler principles, although this is likely to be a lengthy process".

A further prospect afforded by a fuller understanding of the mechanisms employed in biological pattern formation is a comparison of the complexity of different systems, for example using an Information Theoretic (Shannon & Weaver 1949) measure.

1.4 Conclusion

Large amounts of data are required to perform natural experiments on individual ant behaviour in their natural context, and to parameterise individual-based models of pattern formation in ant colonies. A fusion of the systematic exploration of explicit models, such as those considered in Artificial Life studies (Kawata & Toquenaga 1994), with realistic parameterisation from detailed biological data represents one of the most exciting and unexplored areas of social insect work, and indeed of the study of biological pattern formation in general.

This study presents a novel automated technique of behavioural data capture that is designed to capture the quality and quantity of information that can be used to perform natural experiments and to construct realistic models. The technique is presented in two stages: Firstly the paths of all the ants in a nest of ants are tracked using computer image analysis. Secondly the paths are analysed by a computer in parallel to classify the behavioural occupation of each ant. Intrinsic feature of ants are overlaid on the behavioural data. The technique is then assessed, and preliminary results are summarised.

2. Tracking Methodology

2.1 Introduction

The amount of data required to establish a coherent set of conditional behavioural rules used by ants is beyond the scope of any previous methodology. In this chapter, an automated method of behavioural data capture is presented that can track the movements of many ants at the same time and for extended periods using a new image analysis technique. Ants are individually marked and non-behavioural information can be overlaid onto the behavioural data. In chapter 4, the paths and movements of the ants are translated into behaviours.

The methodology is based on the assumption that if we, as ethologists, can distinguish and follow ants as they move about, and interpret these movements as distinct behaviours, then we should be able to make explicit the processes whereby we interpret moving shapes as behaving ants. If we can break this process down into an explicit set of rules, then it should be possible to encode these into a computer and allow it to ask ethological questions of a series of camera images of an ant colony.

The method of tracking used in the automated tracking technique is different from any published method of animal tracking (e.g. Varley et al. 1993, Fourcassié & Traniello 1995). It is also different from the tracking techniques available in some commercial image analysis packages (e.g. Visilog, Noesis Vision Inc.). In such packages, each image is analysed to detect certain shapes or patterns, then an algorithm or the user is employed to link objects in successive images. The method used here does the opposite. It uses the links to explore for recognisable shapes in each image i.e. the previous position is used as the starting point to locate the new position of the object in the next image. There are two reasons why such a technique is not provided in common image analysis packages: the position of the objects needs to be initialised at the start of tracking, and the size of the jump that objects make between images crucially effects the type of algorithm that is required to locate the new positions. These two features are difficult to generalise in an all-purpose image analysis tool kit or package. The first depends very much on the user. The second is difficult because objects tend to move at a variety of speeds, and the number of images required in order to make all the jumps small is normally prohibitive.

A brief summary of the algorithm developed to track ants is outlined in the next section. The constraints imposed by the algorithm on what can be filmed are then discussed. The experimental species is then introduced, and the collection of non-behavioural information is described. Finally the tracking system is described in detail.

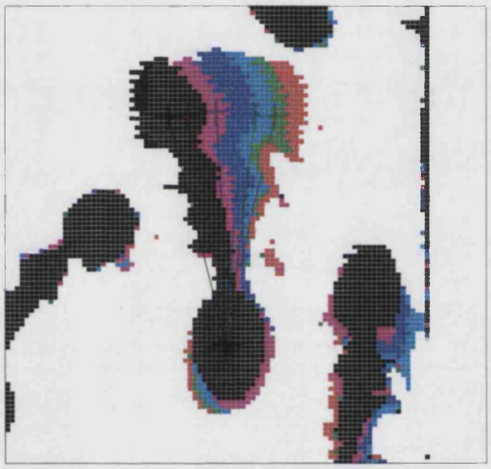


Figure 2-1 Overlaid movement of an ant between 6 successive images 2/25 seconds apart. Coloured crosses show the positions of the centre of the head and gaster of the ant in each image. Images are coloured in the sequence red, green, cyan, blue, magenta, black

2.1.1 Precis of the Automated Tracking Technique for Ants (ATTA)

Silhouettes of ants seen from above, have three distinct sections; the head, thorax and gaster. The head and gaster are bulbous whereas the thorax is thin, and there are constrictions at the neck and waist.

The ATTA makes use of these features of ants to follow the two bulbs, the head and gaster, between subsequent images. The computer can ask questions about the extent of each bulb in order to calculate its centre. If the time interval between images is short, there will be significant overlap between each bulb in subsequent images. If so, the position of each old centre can be updated to the centre of the nearest bulb in the new image. In this way the path of an ant, described by the centre of the head and gaster can be collected.

2.1.2 Implementation issues

Five requirements for such a tracking system to work are detailed below.

Image detail

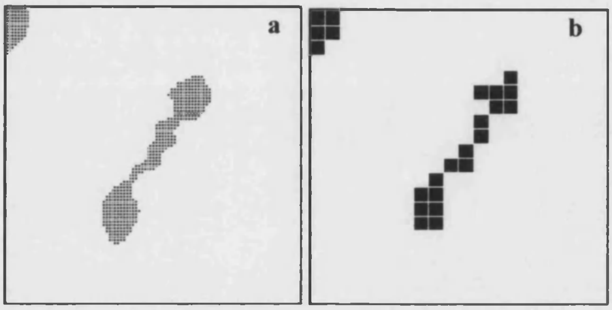


Figure 2-2 Digital images of an ant at **a** high (65 pixels long) and **b** low (13 pixels long) detail.

An ant must occupy a certain minimum number of pixels in each image. This minimum length of an ant has been set at 40 pixels, after much trial and error. This is equivalent to a minimum head diameter of approximately 10 pixels. The required resolution sets considerable limitations on the total area that can be filmed. Computer frame-grabbers are used to capture images (frames) from a camera and convert them to digital information. The dimensions of the converted image in most reasonably priced frame-grabbers is around 700x500 pixels, which is similar to a standard computer screen. Using a single frame-grabber and camera to film ants 40 pixels long, it will only be possible to film an area of approximately 17x12 ant lengths, which is equal to the nest area of a colony of approximately 200 ants. It will not therefore be possible to view both the nest area and foraging arena of an ant colony using the ATTA presented here.

In this study only the nest area is filmed and analysed. Information on the behaviours of ants outwith the nest is lost. The nest was chosen for three reasons:

1. The nest is the centre for much of a colony's information processing.

2. Foraging and other behaviour outwith the nest has been well studied compared to within nest behaviour.

3. Ants are more concentrated in the nest, and so more ants can be followed at any one time.

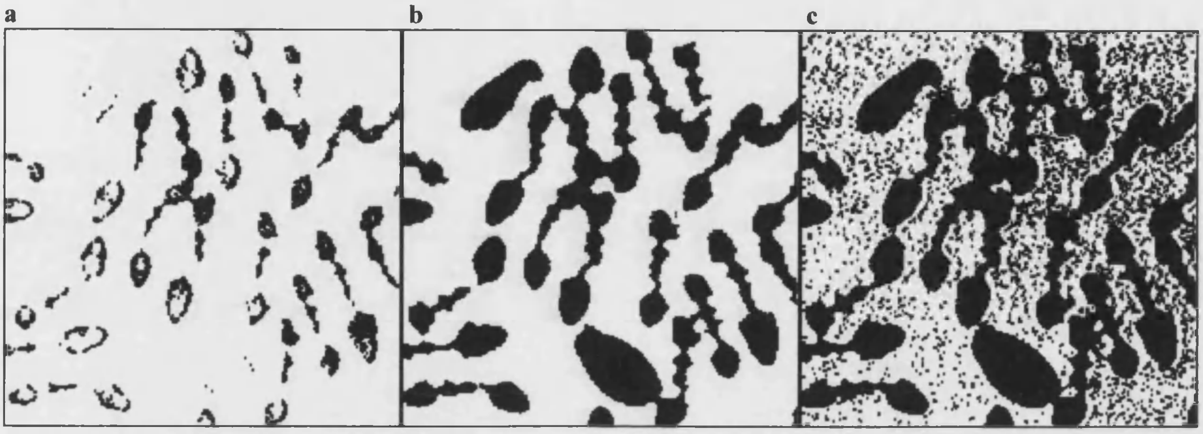


Figure 2-3 Thresholding greyscale images. Black and white images taken from a greyscale image with a threshold value of **a** 75 (too low), **b** 136 and **c** 160 (too high).

Image quality and contrast

Filming ants illuminated from the same side as the camera (top-lit) produces problems of reflection from the bodies of the ants. This is solved by back-lighting the ants, so that they show up as dark silhouettes. This means that both floor and roof of the nest housing must be clear. Even opaque side walls can cause distortion of ant silhouettes near them, so the nest structure must be completely transparent.

Images are required where ants are distinct from the background i.e. a black silhouette on a white background. A greyscale computer (digital) image is made up of a rectangular grid of pixels, each of which has a value of between 0 (black) and 255 (white). Given a threshold value, either calculated using some form of adaptive thresholding or supplied by the user, the computer can interpret each pixel as either background (above the threshold value) or as part of an ant (below the threshold value)

The difficulty of overlapping ants

If the head of one ant merges with the head or gaster of another ant by climbing on top of it, then the algorithm sketched above will not be able to follow the ants since there will be confusion as to which bulb belongs to which ant. The ants can be prevented from climbing over each other by housing them in a low-roofed nest. For strongly dimorphic species of ant, with a large size difference between queen and worker, this will not be completely effective. Allowing the larger (taller) queen freedom of movement will mean that there will be room for workers to climb over each other. However the ATTA can be designed to accommodate limited overlap.

The natural geometry of the nests of many ants is nearly two-dimensional. Even the chambers of nests in large multi-chambered colonies are flat, with ants preferring to walk over nest surfaces than over each other (with the exception of ants that build structures out of their own bodies). Ants have been kept successfully in flat nests in the laboratory for years, where they have produced sexuals each year and maintained a viable worker population. Containing the movement of ants to two-dimensions in the nest therefore does not seem to interfere with the vitality and growth of laboratory colonies.

Nest entrance

It has been concluded above that it will only be possible to film ants in the nest. The problem then arises of how to follow ants that come into the nest or leave the nest. This can be simplified somewhat by building a single thin entrance tunnel which allows only single file traffic. The ATTA stops following ants that leave the nest and monitors the entrance tunnel every image for ants entering the nest in order to start tracking ants as soon as they enter.

Individual identity

Knowledge of the identity of all individuals at all times in the nest is vital for four reasons:

1. Identities are required in order to match non-behavioural data, such as individual size, weight and ovarian development, to the behaviour of each ant.

2. The identities of ants that leave and enter the nest need to be established to compare different periods of in-nest behaviour.

3. Individual behaviour in different periods of tracking (e.g. on different days) can be compared if the identity of each nest ant is known.

4. The algorithm needs to know the expected distance between the head and gaster of each ant, and the expected extent of a bulb when bulbs of neighbouring ants are touching (see later for details). Considerable size variation between workers means that an accurate knowledge of the dimensions of each individual ant is required in order for the ATTA to function in complicated situations.

Figure 2-4 Worker size variation

2.1.3 Summary of requirements

A colony of up to 200 ants must be housed in a thin glass nest with a single-file entrance tunnel. The nest must be back-lit and during filming each ant must be identified at least once each time it is in the nest. Each ant must therefore be uniquely identifiable, and its bodily dimensions known prior to filming.

2.2 Colony collection and measurement

2.2.1 Experimental species

Leptothorax tuberointerruptus (Bondroit) was chosen for this study because much work has been done on this species (Franks et al. 1992, Partridge 1993, Stickland et al. 1995, Orledge 1995) and on other *Leptothorax* species (Sendova-Franks & Franks 1995, Bryant et al. 1990) in the Antlab at Bath University, where culture methods have been well established. It lives in small colonies of about 200 individuals, with occasionally as many as 400 workers (Partridge 1993), and along the British coast it frequently nests between rock laminae. Colonies of *L. tuberointerruptus* are therefore of suitable size for the ATTA, and the ant naturally nests in two dimensions.

L. tuberointerruptus is strongly dimorphic, the queen being at least one and a half times the length and twice as heavy as the average worker. Since the roof of any artificial nest must allow the queen freedom of movement, it is difficult to constrain the workers to moving exclusively in two-dimensions, a requirement of the tracking technique. This disadvantage is offset in part by dimorphic species tending to be monogynous, and hence colonies having a simple genetic structure. This is important for controlling the number of variables affecting behaviour in a colony. Partridge (1993) notes that most *L. tuberointerruptus* queens (79%) are singly mated, so all workers in a nest of this species are likely to be full sisters.

Individuals of this species are small, workers being on average 2mm long. A nest of 100 workers will occupy about 5 cm^2 and is easy to handle. However some form of magnification is required for viewing, and paint-marking individual ants is a delicate process.

2.2.2 Collection

Four colonies of *L. tuberointerruptus* were collected from a disused limestone quarry on the Isle of Portland, Dorset, in February 1995, though only three have been analysed in this study (see 2.3.1). Only queenright colonies with less than 100 workers were taken. According to Partridge's data

(1993), these were relatively small colonies, the average for February being around 200. They were probably in their second or third year. Partridge (1993) found evidence for polydomy in the summer months, but the colonies were monodomous in February. The collected nests are therefore likely to be complete colonies.

The colonies were found by splitting fractured rocks. They were pootered into collecting tubes on discovery. No foraging activity was seen but each nest site was revisited 30 minutes after initial pootering to collect returning foragers or missed ants. However, none were found.

From the time they were collected until their individuals had been weighed and painted, the colonies were kept in an insulated box maintained at between 6°C and 10°C by bags of crushed ice. The collecting tubes containing the ants were separated from the ice by layers of newspaper. This temperature minimised activity (and hence trophalaxis) ensuring that the weights obtained on return to the laboratory would as closely as possible represent field weights. Each ant was weighed and marked uniquely with paint within 24 hours of collection.

2.2.3 Colony summaries

The three analysed colonies were of different sizes, K being the smallest and L the largest. The

Table 2-1 Colony summaries

Colony	K		L		M	
Collection date	22/02/95		22/02/95		28/02/95	
Nest-site aspect	North-facing		North-facing		West-facing	
Termination Date	14/05/95		16/05/95		15/05/95	
Laboratory Nest						
Area mm.^{2}	175		450		345	
Dimensions mm.	19.0 x 9.2		28.1 x 16.0		25.0 x 13.8	
Number of Items	22/02/95	14/05/95	23/02/95	16/05/95	28/02/95	15/05/95
Queens	1	1	1	1	1	1
Workers	33	21	88	83	67	66
Callow workers	-	-	-	5	-	7
Males	-	-	-	3	-	-
Large larvae	1	5	3	-	-	5
Medium larvae	9	-	41	7	13	8
Small larvae	10	-	5	2	14	17
Eggs and microlarvae	21	4	-	32	>80	>90

number of workers remained relatively constant throughout the tracking period since there were few deaths, except for 10 ants from colony K that died within two weeks of collection, probably through dehydration. A total of 12 callows had been born in colonies L and M by the end of the filming period. Brood numbers varied between the colonies. L was collected with a large number of medium larvae, M with many small and micro-larvae. Colony M increased the number of brood items slightly over the filming period, though in L and K brood numbers dropped.

2.2.4 Weighing & Painting

Each colony was transferred from its collecting tube to a petri dish. The base of this dish was separated from a layer of crushed ice by a pad of newspaper. This maintained a dish temperature of between 6°C and 10°C. Individuals were anaesthetised with CO_2 and weighed by Glenda Orledge on a Mettler UM2 microbalance (see Orledge 1995 for protocol). Immediately after having been weighed, the immobile individuals were painted.

Each ant was wedged in sponge foam with either the thorax or gaster protruding and was kept immobile with additional 5 second exposures to CO_2 as necessary. Three spots of modelling paint (Pactra, Medina Ohio) were applied using a thin needle mounted on the end of a matchstick; one on the dorsum of the thorax and one each on the left and right sides of the dorsum of the first gastral tergite. The ants recovered within a few minutes of being released. No deaths were incurred during weighing and painting. The queen of each colony was not marked since her size made her unique.

A colour scheme for marking the ants was employed which maximised the likelihood of individuals being uniquely marked, even if some paint marks fell off. Using eight different colours, the number of unique combinations of eight paints in three positions is $8^3=512$. However, for 64 ants or less, it is possible to paint each ant so that no pair of marks on any two ants is the same. If all ants were to lose one mark, they would all still be uniquely marked. For the next 64 ants, i.e. 65 to 128, some pairs of spots will be duplicated on one other ant only, for 129 to 192 ants some pairs will be triplicated, and so on. Figure 2-5 shows the colour scheme used to mark all colonies.

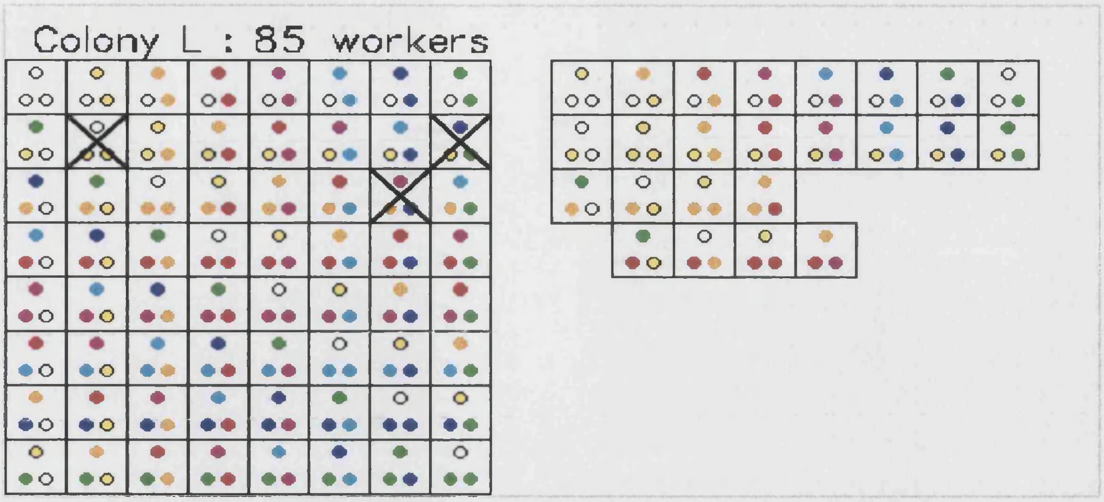


Figure 2-5 Colour scheme for colony L Each box represents one ant and contains the colour of the three paint marks; thorax (top), left gaster and right gaster (bottom left and right). Each row of eight ants has the same left gaster colour and each column of eight has the same right gaster colour. Each different row (and each column) has eight different thorax colours in the same order, but in each subsequent row the colours are shifted along one ant to the right. In each subsequent group of 64 ants, the colours are exactly the same, except that the thorax colours are shifted along to the right by one position. Black crosses indicate deceased ants.

In addition, sketches were made of every ant (Figure 2-6), showing the exact position and shape of each paint spot to assist the identification of individuals during filming, especially those that had lost one or two marks.

Ants were identified by number according to their colour marks, where each colour was assigned a number from 1 to 8. Numbering followed the ordering of colours in Figure 2-5, so number 1 codes for white and 8 for green. Marks were given in the order; thorax, left gaster, right gaster, so ant 461 had a red thorax mark, a light blue left gaster and a white right gaster, as shown in the left-most portrait of Figure 2-6.

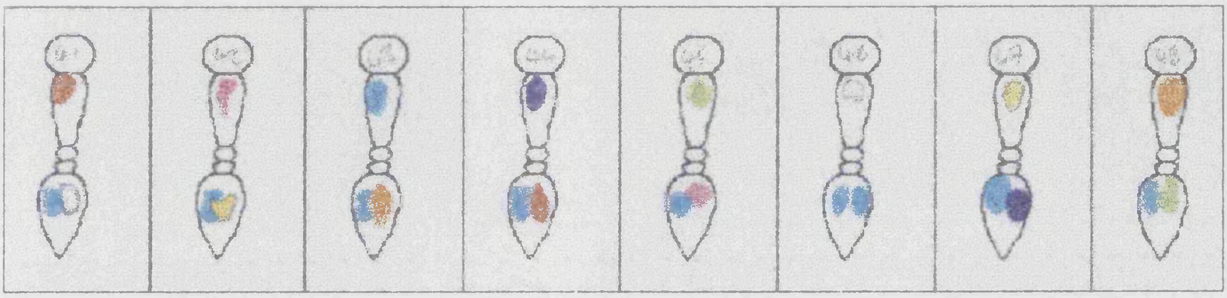


Figure 2-6 Ant portraits showing the variation in mark position and shape The row of eight ants shown is the sixth row of Figure 2-5. (The faint numbers on the heads of these ants should be ignored.)

After painting, each colony was left in a petri dish for a week with a test-tube of water before being housed in its experimental nest. This interim period was essential for monitoring the loss of paint spots, which normally occurred within the first few days, and rarely thereafter. If ants lost spots during this period they were repainted, kept in a holding dish for a few hours until the paint had dried fully, and then returned to their nest-mates.

Figure 2-7 Photographs of painted ants The queen and one of the workers in the upper picture are upside down. The upper picture shows some head-head overlap by the workers.

After initial grooming, ants lost interest in the paint marks. During the filming period ants that had lost two paint marks were removed from the foraging arena or from the nest (by sliding back the nest roof), repainted and replaced in the foraging arena. At the end of the filming period the colonies were frozen and all individuals re-weighed. No weight adjustment was necessary to allow for the weight of the paint spots since the additional weight of these was found to be negligible when they were originally applied (several specimens were weighed both before and after they were painted).

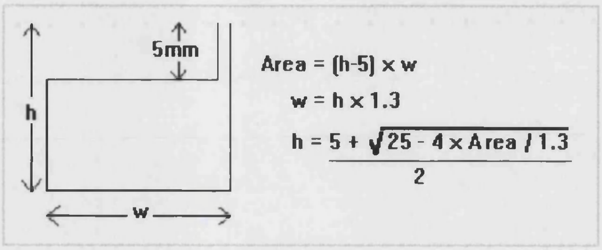


Figure 2-8 Nest size calculations 'h' is a root of the equation for 'Area' after substituting 'h'x1.3 for 'w'.

2.2.5 Housing

The area of each nest was tailored to the number of ants in each colony. Franks and Sendova-Franks (1992) calculated that on average a similarly sized and closely related species *Leptothorax unifasciatus* (Latreille) built nests allowing 5mm^2 per ant, and this figure was used to proportion the experimental nests. The length and width proportions were calculated to match the proportions of the frame-grabber, which were approximately 1.3 : 1. A strip 5mm wide was first set aside on one side of the nest for the entrance tunnel (see Table 2-1 for nest dimensions and area, and Figure 2-8). The height of the nest was a fraction taller than the thickness of the perspex sheet (0.7mm). This was found to be the minimum height under which the queens were still mobile.

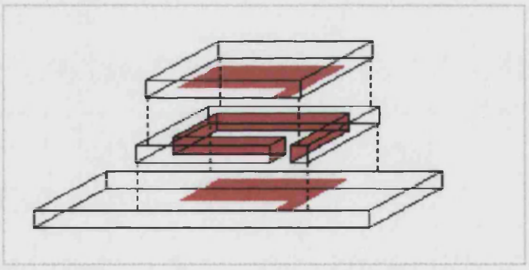


Figure 2-9 Nest chamber design The surfaces painted red highlight the inside walls, floor and ceiling of the nest chamber.

The nests were built out of glass and perspex as shown in Figure 2-9. The floor was a microscope slide, the side-walls were part of a small piece of perspex sheet out of which the shape of the nest and entrance tunnel had been cut. The roof was a portion of a microscope slide just larger than the nest area so that it could be slid back, without too much disturbance, for repainting ants that had lost two or more paint marks. The nest was stuck together with Scotch 3M tape.

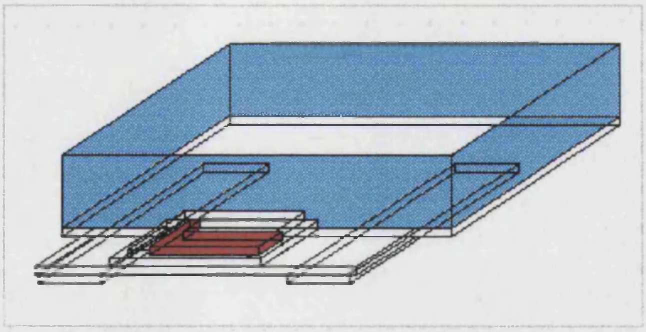


Figure 2-10 Nest chamber and foraging arena assembly

The foraging arena was a petri-dish with a small hole drilled into the base of one of the walls. The inside of the walls were painted with Fluon (Whitford Plastics Ltd.) so that the ants could not climb up the walls. The nest was then mounted outside the petri-dish so that the entrance tunnel opened into the petri-dish through the drilled hole. The nest was placed outside the petri-dish so that ants could not climb over the nest roof and interfere with the filming of the nest. It was attached onto the petri-dish using two microscope slides that had been stuck to the underside of the dish and which projected out under the nest (see Figure 2-10).

The entrance tunnel was narrow to ensure single file traffic (0.7mm high x 0.5mm wide x 5mm long). This was too narrow for the queen and the largest brood items. These were therefore installed in the nest cavity by removing the nest roof plate. The rest of the colony was introduced into the petri-dish arena and allowed to immigrate into the nest. The nest was initially covered with black card to encourage this immigration. Although the queens could not leave the nest, they were never seen trying to do so.

The nests were not completely airtight. Minute imperfections on the edges of the walls of the perspex middle layer allowed limited airflow and diffusion in the nests chamber.

Every two days each nest was given fresh tap water, 10% honey solution and 2-4 wingless *Drosophila subobscura* larvae depending on the size of the colony. These were placed in the petri-dish foraging arena.

2.2.6 Measuring ant dimensions

The dimensions of each ant are required by the ATTA and must therefore be collected before tracking. A non-intrusive method was developed using a video camera attached to a Zeiss dissecting microscope and a MicroEye 2C frame-grabber. Highly magnified images of ants were shown on a PC screen. The dimensions of each part of the ant were marked with a mouse and were calculated by the computer. Each image was taken when an ant was flat and square to the camera. The head, thorax and gaster widths were taken at the widest points. For the head this meant the distance between the outer edge of each eye.

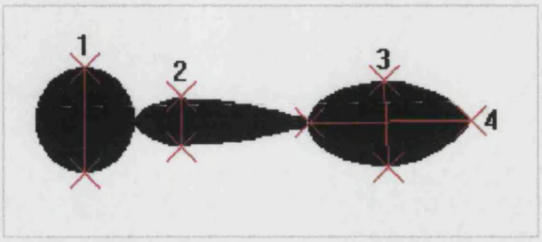


Figure 2-11 Stylised ant measurements 1 Head width, 2 Thorax width, 3 Gaster width, 4 Gaster length

2.3 Filming

2.3.1 Filming the nests

The methodological part of this project is an attempt to automate features of the collation of behavioural data from ant colonies so as to make a significant advance in the amount, quality and type of information that can be gathered. A large part of the time investment in this project is therefore in developing the automated system. The system is then tested on some video tapes of ant nests and the biological information gathered is summarised later. The main purpose of this project is to establish the automated methodology.

Once the system had been developed and tested on some video-tapes of ant nests, it was estimated that it would be possible to film and analyse approximately 20 four hour video tapes. This number of tapes can be used either to film a few colonies a number of times, or a number of colonies a small number of times. The former option was chosen because an emphasis on characterising individuals and assessing the variation between individuals within a colony would be more rewarding than inter-colony comparison. It was not clear how large a sample size would be required for the latter. Furthermore the latter needs to be preceded by an understanding of individual behaviour in order to establish how long individual colonies need to be observed before a representative sample of behaviour has been seen. However, in order to get at least some feel for the similarities between colonies, and in case the focal colony was not representative, more than one colony would be preferable.

A compromise of filming four colonies six times was chosen. Of the 24 tapes filmed, the tapes of three of the colonies, K,L and M have so far been analysed, a total of 18 tapes or 72 hours of nest footage. Colonies K and L were the smallest and largest colonies respectively. The fourth colony, J, which was of similar size to colony M has not yet been analysed because of a lack of time. Colony J has therefore been left out of this report.

2.3.2 Experiment vs observation

Six tapes per colony offers limited scope for experimental manipulation. Indeed it was not clear that experimentation would be appropriate at this stage, for the simple reason that the detail and novelty of the behavioural data captured by such a system would afford many opportunities to examine natural experiments within a colony, for example, the ways that individuals react in different parts of the nest, in different circumstances and to different cues. There would be more than enough information to be gleaned from the analysis of unperturbed nests. Furthermore, if the automation produced a significantly novel data set, it may afford different ways of looking at ant colonies, and facilitate the development of entirely new approaches and questions. It would be a pity to prejudice the potential originality of this study by asking very specific questions at the outset, and by performing too many experiments based on previous knowledge.

2.3.3 Manipulations

Two simple manipulations on whole colonies were performed, both of which are assumed to befall colonies in the wild. The colonies were starved of honey solution for a period of ten days before the third video-tape of each colony, and honey solution was introduced one hour into this four hour filmed session. Of interest in this manipulation was how sugar solution is distributed around the colony. A major hurdle for a society with a division of labour is how to ensure that individuals have access to information or food that is carried initially by a minority of the work-force.

Secondly, the colonies were emigrated into new nests before tape number 5 for colonies K and L, and after tape number 5 for colony M. Sendova-Franks and Franks (1995) reported that individuals occupy distinct radial zones in the nest, and that these zones are retained after emigration. Of interest in this manipulation are both the two-dimensional nature of the one-dimensional spatial fidelity zones reported previously, and the reconstruction of the two-dimensional structure of tasks and individual occupation in the nest after emigration.

2.3.4 Emigration protocol

Identical nests and foraging arenas were made for the colonies to move into. The nest compartment is detached from the old nest and put in the middle of the new foraging arena. Foragers in the old arena are added to the new foraging arena. The roof of the old nest is then removed and the queen and the larger items of brood are lifted with a fine brush into the new nest, which is then closed. Card is then placed over the new nest compartment to keep it dark for 24 hours as the rest of the colony is allowed to emigrate into the new nest. The new foraging arena is kept open so that air currents encourage the ants to move out of the roofless old nest.

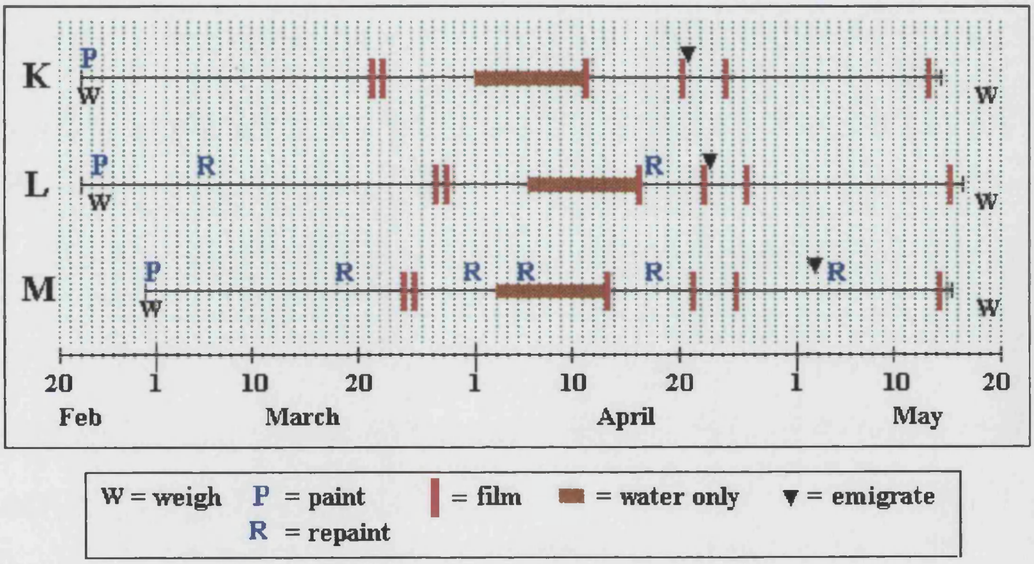


Figure 2-12 Laboratory history of colonies

2.3.5 History of colonies in the Laboratory

Figure 2-12 shows the treatment history of the colonies from collection in February 1995 to termination in the middle of May 1995. All colonies were subjected to the same treatment at the same time, subject to a few days stagger because colonies could only be filmed one at a time. The first two filming sessions for each colony were on consecutive days, so that two four hour periods of colonies in the same condition could be compared. The third filming session followed a 10 day period of sugar and protein deprivation. Session 4 was filmed on the morning before colonies K and L were emigrated, and they were filmed on the third day after emigration to assess the new spatial and task structure of the nest. Colony M was kept as a tentative control and was emigrated after session 5. The colonies were then filmed once more after 20 days to extend the time period over which they were filmed. The colonies were culled immediately after this last filming session. Colony M was repainted 5 times, colony L twice (see Appendix V for details).

2.3.6 Film setup

The experimental set-up is shown in Figure 2-13. The nest is filmed from above and back-lit from below. A light-box covered with a piece of paper to diffuse the light is used to back-light the nest. The light is 50 centimetres below the nest and so does not over-heat the nest. The filming room is kept permanently at 20°C. A Panasonic wv-GL500 CCTV colour camera with a 25mm lens was coupled as tightly as possible to the nest to minimise vibration.

The 25 mm lens on the camera was fitted with an extension tube of approximately 0.5 mm to achieve the correct magnification. The diaphragm was adjusted so that the image was over-exposed. This meant that the silhouettes of ants were reduced to a head, thorax and gaster, the legs not being visible.

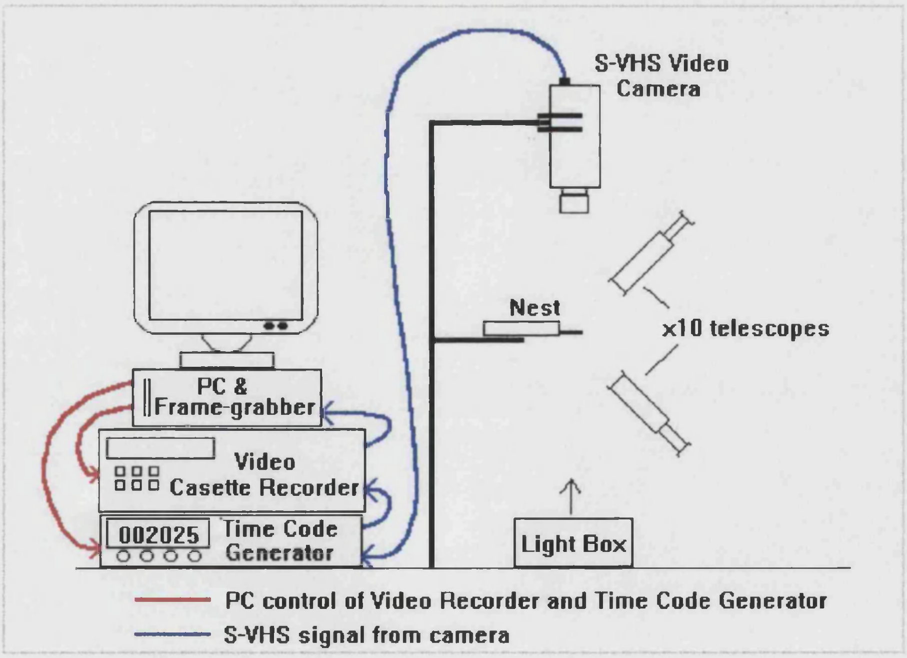


Figure 2-13 Film set-up

The area of each of the ants was made as large as possible (not too over-exposed) and with sharp edges. The focal arc of the lens is quite strongly bowed at this magnification however (the camera is 15 cms from the nest), and the ants on the edge of the image tended to be a out of focus compared to the ants in the centre. The area of out of focus ants is eroded, and the edges of their silhouettes are consequently less sharp. This can be a problem for the tracking program which requires the ants to be the same shape throughout. However the crucial measurement, the length of the ant, is hardly affected, even though the width can be severely eroded.

The signal from the camera was sent through a time-code generator (Alpermann+Velte TC 15) which added a unique time code to each frame of the video-tapes, so that each image on the video tape could be repeatedly and reliably located for analysis.

Nest images were recorded onto four hour S-VHS video tapes (Maxell XP super VHS or TDK XP pro) using a Panasonic AG-7330 Video Cassette Recorder, in the morning between 9AM and 1PM. The colonies were left for a minimum of 20 hours on the filming apparatus before being filmed, and their food was renewed 12 hours before being mounted onto the filming apparatus. Each colony was dismounted immediately after filming and returned to a holding apparatus with identical lighting conditions

Because the ants are so small, they are identified during filming using two binocular x15 eyepieces that are focused on the nest from above and from below (ants often walk upside down in the nest) without interfering with filming. Diffuse light from a window in the filming room was sufficient for recognising the colours of paints from above. From below, ants were illuminated by the light-box.

During filming a record of the identity and position of each ant in the nest and each entering ant needs to be recorded at least once. Ants can enter the nest very quickly in succession, and remain impossible to identify for extended periods, creating problems for identifying entering ants. The identification procedure is therefore organised using FILM, a program developed for this purpose. Non-automated methods, such as using a Dictaphone and pen and paper would have needed to be transcribed into the computer for use by the ATTA. The automated method circumvents this lengthy and error-prone step, and yields the timing accuracy required by the tracking system.

2.3.7 The FILM program

The FILM program has three functions:

1. It allows the user to set up the nest within the field of view of the camera and to specify the positions of the edges of the nest, and of the tunnel.

2. The program starts the video-recorder recording and simultaneously starts the time-code generator running from zero.

3. During filming, it allows the user to record in a computer file the identities and location of all ants in the nest at least once and all ants that entered the nest during the filming period.

Figure 2-14 Binary picture of the nest of colony M The picture contains about 50 ants, some large items of brood and a central brood pile of smaller items. The entrance tunnel is at the top right-hand corner.

2.3.8 The field of view

The nest is set up as in Figure 2-14 with the largest possible magnification of the nest that allowed at least an ant's length of tunnel at the top edge.

2.3.9 Ants in the nest

The live image being recorded on the video recorder is also fed through to the frame-grabber in the PC so that it can be displayed on the PC monitor. The frame-grabber (MicroEye 2C Card) is a video-overlay card, which means that the computer can write or draw on top of the live image which continues in the background. The user clicks with the computer mouse on the head of an ant that has just been visually identified by its paint markings using the x15 eyepieces. At this point the computer stores the current time-code, the pixel co-ordinates of the head of the ant, and the identity of the ant supplied by the user. For example, a record of '133 240 419 090425' specifies that the head of ant 133 (1=white paint mark, 3=orange) was at position [240,419] in the 90425th image (i.e. after 1 hour 17 seconds). These records are used by the tracking program at a later stage to identify the paths that it is following.

2.3.10 Entering ants

The FILM program constantly monitors the tunnel on the camera image for black pixels, which correspond to an ant entering or leaving the nest. It looks at two strips of pixels across the tunnel, the strips being 2/3 of an ant length apart. When some pixels in both strips are black, corresponding to the head and gaster of an ant straddling the entrance tunnel, the computer emits a 'beep' and the user must specify the identity of the ant in the entrance tunnel.

The rate of traffic can become problematic through the entrance tunnel, especially since it can take a little time to get a good view of each entering ant for identification, for example if the ant climbs sideways along the side-wall with its paint marks facing away from the viewer. The visual record of the recent history of ant occupation of both tunnel marks shown in Figure 2-15 and kept on the

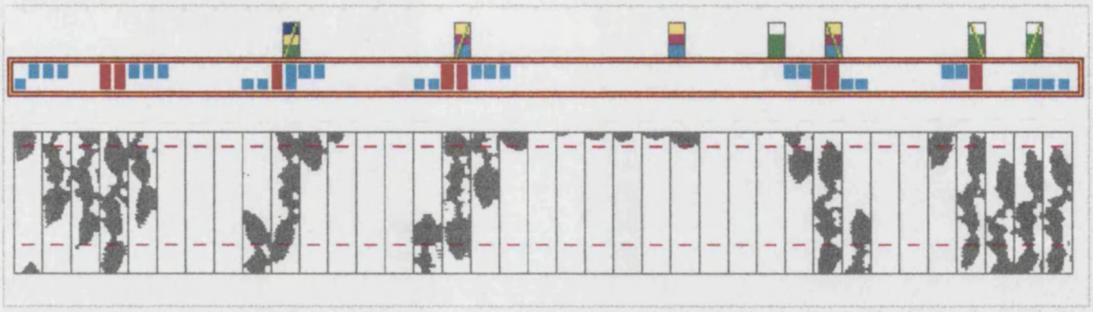


Figure 2-15 Tunnel traffic history The upper part of the figure shows an example of the schematic representation of the recent history of tunnel activity which is constantly displayed and updated by the FILM program during recording. A period of 75 seconds is shown, progressing from left to right. The lower part of the figure shows images of the tunnel for the same time period corresponding to the schematic representation. Ants entering the nest are moving upwards. (See text for further explanation)

computer screen is used to identify entering ants retrospectively. The identity of ants exiting the nest can also be recorded. This is a safeguard against not having identified a nest ant that leaves for the first time, or one that was not identified on entering the nest.

In Figure 2-15, the lower part of the figure shows images of the tunnel in a time sequence from left to right, sampled approximately every 2 seconds. The 2 red lines per image are the tunnel markers which are monitored for ant activity. For example in the first (left-most) image on the left, the pixels of the upper (inner) tunnel marker are black because an ant is entering the exit tunnel to leave the nest. By the eighth second (fourth image) both markers are black as the ant straddles the markers. However the ant does not leave the nest, but retreats back into the nest.

+h 728 017529
+h 256 017834
256 626 368 018201
188 571 357 018379
-h 256 018493
-h 188 018742
+g 188 018825

Figure 2-16 Identification record for the period shown in Figure 2-15.

The upper part of the picture shows the occupation of the tunnel markers for the same time period. This part is displayed on screen during filming below the live image of the nest. The light blue squares signify that either the inner or outer tunnel marker is black. If both are simultaneously black, then a red bar is shown. The user uses this visual display to locate the timing of entering ants, live or retrospectively, by attaching an identity label (3-coloured rectangles) above the red box at the appropriate second. For example the first ant to enter the nest was identified as dark blue, yellow, green, and hence ant number 728, and the second ant to enter had marks yellow, pink, light blue (ant 256). The latter ant left the nest soon after. While it was in the nest however, the user confirmed its identity by clicking on its head and noting its identity. Figure 2-16 shows the records that the computer stored for the period shown in Figure 2-15.

The orientation of each ant in the tunnel and whether it is entering or exiting is specified by the user and is recorded along with a time-code reading and the identity of the ant. For example a record of '+h 425 046450' specifies that ant 425 entered head first at image number 46450. An entry '-g 818 0016400' relates that ant green-white-green reversed gaster first out of the nest at image number 16400.

The tunnel markers are positioned so that there is at least half an ant's length beyond the outer tunnel marker (OTM) so that tracking can be stopped when the ant is sufficiently far out so as not to be caught entering immediately. The distance between the markers has to be small enough to straddle the smallest ant in the nest.

Three sorts of error are made. Firstly, the times of entering ants can be inaccurate if there is a lot of traffic and the user is forced to record the sequence of entering ants at the expense of the exact times of entry. Secondly, entering ants may be unidentified because they are lost in the nest before they can be identified. This is possible if a number of recently entered ants are in the process of being identified. Thirdly, user-error such as specifying an ant entering forwards when it reversed up the tunnel can occur.

A checking program was therefore written to allow the user to run through the recorded video-tape overlaid with a display of all the identification records. With a mouse, the user can adjust the times of entering ants, rectify errors, and can retrospectively identify entering ants if they are identified in the nest at a later stage, or on exit.

2.4 Image management

Ideally, the PC with the frame-grabber should grab images from a running tape, analyse and track the ants in the images in real time. However, there are two reasons why a distinct TRANSFER program was needed to manage image acquisition.

1. In practise the time required for the frame-grabber to grab an image and load it into memory is greater than the time interval between images, so some form of image capture and management was required to present the tracking program with uninterrupted sequences of consecutive images.

2. Workstations are better software development platforms than PCs so the development of the tracking program was done on a Hewlett-Packard series 300 workstation. Images therefore needed to be transferred from the PC to the HP workstation where they were analysed.

2.4.1 The TRANSFER program

This program controls the video-recorder, grabs images in sequence from the video-tape, and transfers them across to the workstation where they are written to disk and then analysed in sequence with the TRAC program.

The TRANSFER program asks the user for a greyscale threshold value to use in converting all the images from a video-tape to black and white binary images. Pixels below the threshold value are interpreted as being part of an ant or an item of brood, pixels above the threshold are background (see Figure 2-14). Binary black and white images are then transferred in compressed format to the workstation. The images are compressed with a run-length encoding algorithm. In essence, the image is scanned from top to bottom and the bout lengths of series of pixels of the same colour are stored, white alternating with black. An uncompressed image of 600x500 pixels would occupy 300 kilobytes of disk-space. A run-length encoded version of the nest images used in this study occupied between 4 and 11 kilobytes depending on the number of ants in the image, a reduction of about 30 times.

During recording, the time-code generator writes a time-code onto each video image 25 times a second, so there are potentially 25 images to be analysed each second, every second for four hours. However it was found that ants did not move quickly enough in the nest to justify this frequency of images. One in every two images were transferred. This means a total of 12.5 images per second x 60 seconds x 60 minutes x 4 hours = 180,000 images per video-tape.

Transferring one image to the workstation took between one and two seconds, or 100 hours to transfer four hours worth of images. The major rate-limiting step was the time it took to read the image from the frame-grabber. The TRANSFER program therefore had to replay the same piece of tape at least 12 times to pick out every image 2/25th of a second apart. The program kept a record of the images that it had transferred in order to transfer blocks of 4000 images (320 seconds) at a time.

The workstation onto which the images were written had a capacity of 100 megabytes, only sufficient for 10,000 images at a time, so the TRAC program deleted files of images that it had analysed in order to free up space for images to be continually transferred. TRAC and TRANSFER were therefore run simultaneously.

2.5 Tracking: The TRAC program

This program takes a series of consecutive black and white images produced by the TRANSFER program and tracks the position of every item in the nest between each image. It is a large program of some 40 different subroutines and about 7,000 lines of code. Part of this is of little interest here, in that it is concerned with the efficient management of the large amounts of information and with making the program 'user-friendly'. The rest of the code is the tracking algorithm and this will be dealt with in this section.

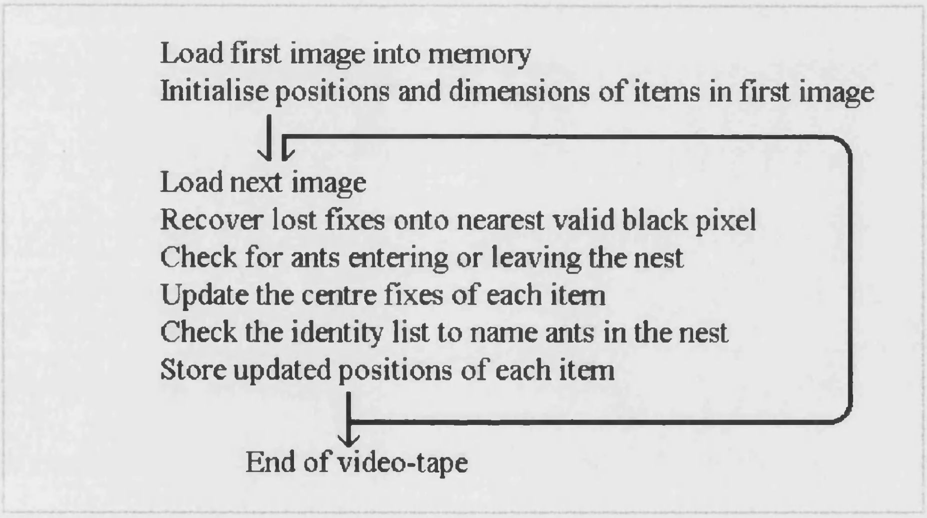


Figure 2-17 Flow diagram of the TRAC program

The exact manner in which the tracking has been encoded in this study is one of a number of possible ways of encoding similar ideas. It is therefore of little use either showing the code that was written, nor the algorithms in detail. The program is presented as a set of visual and textual descriptions of the algorithms that have been used. Some example coding is presented in Appendix I. First a general outline of the core of the program is given, then each part is broken apart and described in greater detail.

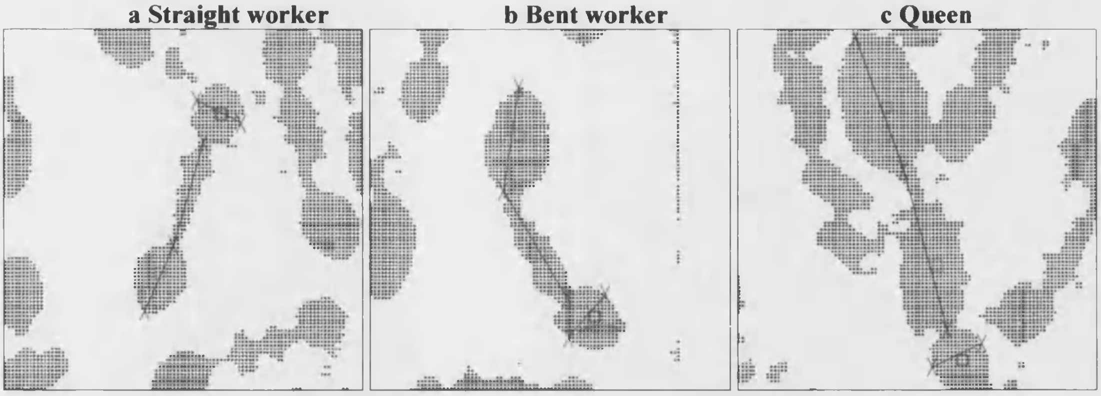
The program begins by loading the first image of a four hour tape into memory. The positions and dimensions of the head and gaster of each ant, and of the centres of all brood items are specified manually by the user using a mouse. The program then takes over and loops through loading and analysing subsequent images. For each image, the program first checks to see if the centres of the bulbs of the previous image are still coloured black. If they are not, it recovers the centre fix to the appropriate nearby bulb. Then the program runs through each item, updating the centre of the head and gaster of each ant and the centre of each item of brood.

For each image the identity records from the FILM program is checked to see if any individual was identified in this image. The tunnel is checked in the same way that it was checked in the FILM program. If an individual comes into the nest, the head and tail are picked up at the tunnel marker. The tracking of ants that leave the nest is discontinued. Finally, the new co-ordinates for items in the current image are stored in memory and are flushed periodically to disk.

2.5.1 First image

The program displays the first image on screen (as in Figure 2-14) and prompts the user to identify and mark the position of each object in the nest. The identities of the objects are as yet unknown, so the dimensions of each item have to be specified manually. Once the position of an item has been marked, for example by the centre of the head and gaster for a worker ant, a magnified image of the item is shown on which the user specifies the dimensions of the item.

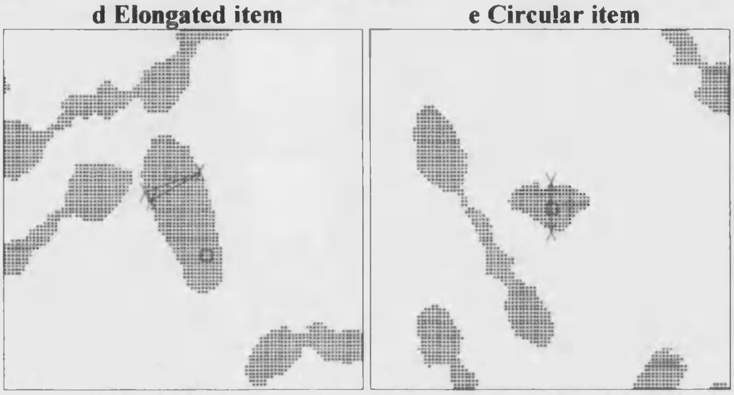
All areas of black on each image must be accounted for and tracked. Non-ant areas, such as pupae, larvae, eggs, solid food material, and waste are tracked as circular or elliptical objects with one or two centres respectively. In total the program recognises five types of item based on shape and treats them in different ways. These are straight ants, bent ants, queens, elongated non-ant items and circular items. The centres and dimensions must all be initialised in the first image, and the dimensions are assumed not to change over the course of each 4 hour tracking period. The dimensions that define each type of item and how they are specified are described below.



a Straight worker

b Bent worker

c Queen



d Elongated item

e Circular item

Figure 2-18 Measuring ants Examples of the five types of item are shown with green (head) and yellow (non-head) boxes marking the centres of each bulb to be tracked. Blue lines, denoted by the manually specified red crosses measure the relevant dimensions of each item.

Straight ants

These are defined by the centre of the head and of the gaster. For an ant, the head width, thorax length and gaster length are specified by clicking the mouse on either side of the head, on the neck, waist and at the tip of the tail. The thorax is assumed to appear as a narrow strip of black directly between the head and gaster (Figure 2-18**a**). The thorax width is assumed to be 2/3rds of the head width.

Bent ants

When an ant curls sideways into a ball, or is at all bent the assumption that the thorax is in between the head and the gaster is violated. It then becomes necessary to explicitly follow the centre of the thorax. A bent ant is therefore specified by three points, and its dimensions are specified in the same way as for a straight ant (Figure 2-18**b**).

Queens

The queen has a unique morphology, being proportionately much wider in the thorax and longer in the gaster. As a result, the tracking program treats the queen a little differently, tracking the thorax permanently. The user must therefore use the mouse to specify the position of the queen's thorax during initialisation. Her dimensions are defined again by head width, thorax length and gaster length, but within the program the thorax width is calculated as 4/3rds of the head width, wider than that of a worker ant (Figure 2-18**c**).

Brood items and other non-ant items

Elongated non-ant items are defined by two points, roughly the foci of the elliptical shape of the object. The size of the item is described by the widths of the item at each foci. Both dimensions are specified by the user across one or other of the foci, as shown in Figure 2-18**d**.

Circular items are described by their centre and diameter (Figure 2-18**e**).

There is a slight discrepancy in the inter-pixel distance in the x and y directions on the frame-grabber. The length in pixels of an ant if measured when aligned parallel to the x-axis is 14/15th of the length

Figure 2-19 First image with crosses on the bulbs of each item

of the same ant were it aligned parallel to the y-axis. Although this discrepancy is slight, it can make a four pixel difference to an item 60 pixels long. This is found to be a large enough error to cause errors in the tracking program. The program allows for this by reducing the dimensions of each item by 14/15 while the gradient of the head-gaster line is more horizontal than vertical i.e. if the modulus of the gradient is less than unity. This approximate method of allowing for the discrepancy is the result of a trade-off between exactitude and the speed at which the program can run. Throughout the program many simple and efficient algorithms have been used in place of more computationally demanding but more exact algorithms.

Each item is assigned an arbitrary number to begin with. The identities of the ants are updated as tracking continues.

2.5.2 Lost fixes

The tracking algorithm relies on the ants not having moved too far between consecutive images. More specifically, it assumes that the ant will not have moved more than half a head width in the time interval between images (2/25th of a second). If this is the case, then the previous centre will still overlap the black area of the head in the next image. Occasionally, ants do move faster than this and lost centres must be located onto the appropriate area of black. A simple circular search is used for instances where the distance to the nearest appropriate black pixel is small. More complex methods that account for the recent movements of the ant are used for large jumps, since there is greater potential for locating the wrong area of black in these cases.

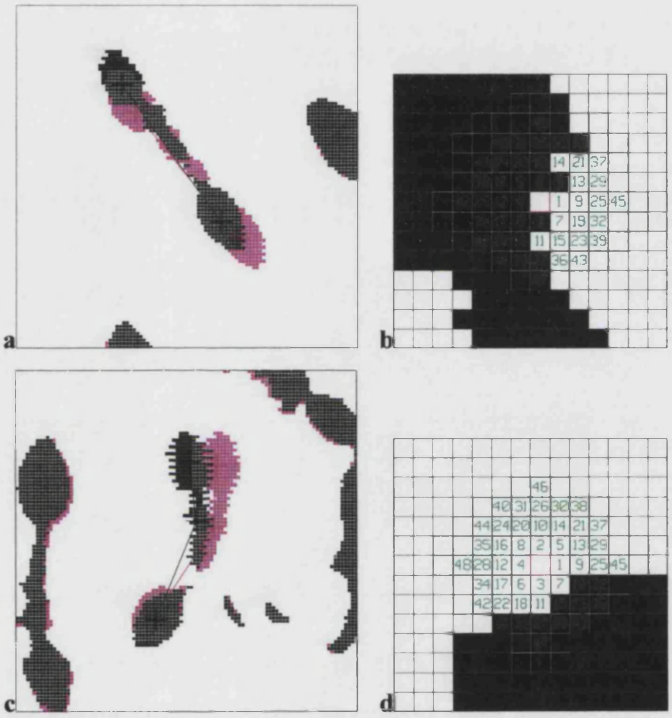


Figure 2-20 Locating lost fixes onto the nearest black pixel a and **c** show images of two fast-moving ants. The new image (black) is overlaid on the previous image (pink) and the respective crosses of the centres show that the new image does not overlap the old head fixes. **b** and **d** show a close-up of the circular search for the nearest black pixel of similar images. The position of the old head fix is outlined in pink and the sequence of the circular search is shown in green. The head was found on the 2nd pixel enquiry in **b** and on the 15th in **d.**

Figure 2-20 shows two examples of the relocation of lost fixes. The algorithm looks in increasing circles around the old centre fix until a black pixel is found. Constraints are put on accepting each black pixel - it must not be part of the area occupied by another ant and it must be within a reasonable distance from the centres of other parts of the same item. If a valid black pixel cannot be found within a 6 pixel radius of the old fix, then relax the latter constraint. If this still does not find a valid pixel then try two exceptional methods. If the ant is moving forwards quickly, then the old fix is extrapolated forwards in an attempt to catch up with the position of the ant. The second looks in an arc sideways for a turning ant. The 'C' code of the 'extrapolate' algorithm is printed in Appendix I as an example of how the worded algorithms set out in this text are implemented in 'C'.

2.5.3 Tracking ants in the nest tunnel

In each image, the TRAC program monitors ant activity in the entrance tunnel. It does this by looking at the colour of pixels at two markers in the tunnel, to pick up ants entering the nest, and discontinue tracking of ants leaving the nest. The identities and dimensions of entering ants are assigned from the identity records stored during filming, and a check is run on the identity of exiting individuals.

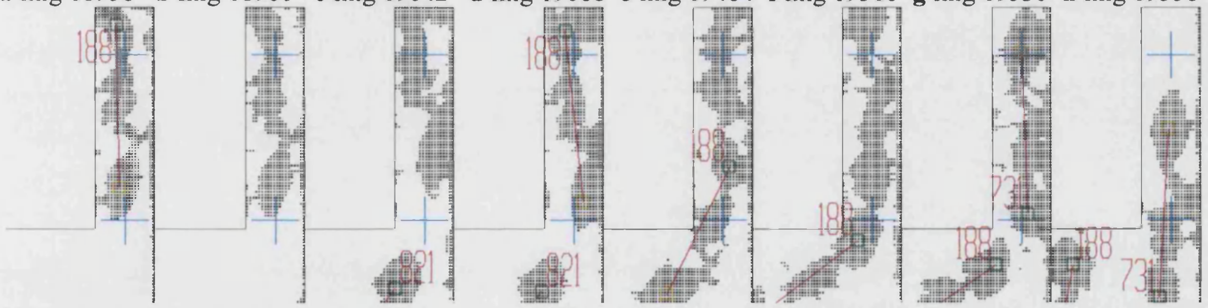

a Img 18958 **b** Img 18960 **c** Img 19042 **d** Img 19068 **e** Img 19464 **f** Img 19516 **g** Img 19530 **h** Img 19558

Figure 2-21 Tracking entering and exiting ants The sequence of images show the area around the tunnel for a period of 24 seconds of colony M, sampled irregularly, showing when tracking starts and stops for ants entering and leaving the nest respectively (See text for further explanation).

The corner of the nest which contains the entrance tunnel is magnified in Figure 2-21**a** to **h**. Image numbers are printed above each picture. The blue crosses mark the tunnel markers which are monitored for the black pixels of entering or exiting ants, the upper cross being the outer tunnel marker (OTM). Ants that are currently being tracked are numbered and the head and gaster positions are marked. Ant 188 exited at image #18960 but was picked up reversing back down the tunnel in image #19068 when both tunnel markers went black. Ant 188 continued to reverse out, making way for ant 731 which was picked up at image #19530 when it overlapped both tunnel markers.

Entering Ants

If the pixel colour of both the tunnel markers is black, then the program investigates the possibility of an incoming ant. A number of criteria need to be satisfied:

- There must be no other ant currently being tracked in the tunnel or within 2/3rds of a head width of the inner tunnel marker (ITM).
- There must be some black pixels half-way between the tunnel markers where the thorax should be.
- There must be a sizeable extent of black squarely over each marker. This is assessed by looking at the size and extent of the area around each tunnel marker.

If all these conditions are satisfied then the ant in the tunnel is picked up and tracked. In Figure 2-21**f** the incoming ant is not picked up because the head of ant 188 is too close to the ITM. In **g** this is resolved.

Once an ant has been picked up the identity list is checked for the nearest entering ant. For example when an ant entered at image #19068, the nearest entry according to the identity list shown in Figure 2-22 is '+g 188 019025' indicating that it was ant 188 entering gaster-first. If an ant of the suggested name is already being tracked the user is prompted to investigate the contradiction, usually thrown up by a small time error in the identification list.

```
-h 188 018742
+g 188 018825
+g 188 019025
+h 731 019466
-h 188 019905
```

Figure 2-22 Entries in identity file for the period shown in Figure 2-21

Exiting Ants

The program stops tracking an ant if its inner-most fix is more than 2/3rds of a head width further out than the ITM and if the ITM is clear. This ensures that it is not immediately picked up again as an entering ant. For example in Figure 2-21**b** the ITM is clear and the gaster of ant 188 is far enough away from the ITM. The identity list is also checked whenever an ant exits the nest to confirm its identity. If the ant has not yet been named and there is an entry of an ant exiting the nest within 10 seconds of the current image number, then the identity in the list entry is compared with the identity of the exiting ant. If the exiting ant has not yet been identified then it is given the name in the list.

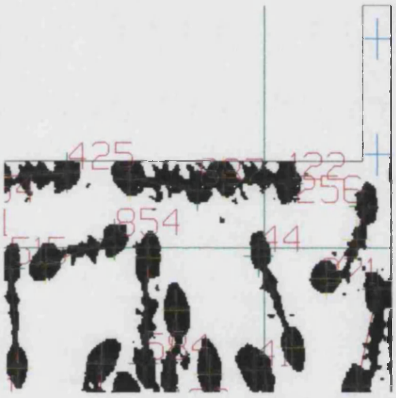


Figure 2-23 Identifying Ants Given the entry '818 18380 571 357' in the identification file, this is a portion of image number 18380 overlaid with a large green cross at x=571, y=357. The previously anonymous ant #44 is updated to ant 818.

Naming ants in the nest

Each entry in the identity list has an image number, the x- and y-co-ordinates of the head of the ant that has been identified, and the three-digit name of the ant. During tracking, when the current image number is equal to the image number of an entry in this list, the head of the nearest ant to the co-ordinates in the entry is located. This ant is then given the name in the list. If the ant has already been named, then the identities of the ant and in the entry should match - if they do not a message is posted for the user to investigate but the program continues. In this way the identities of each ant in the nest recorded by the user during filming using the FILM program are used to identify the ants that are being tracked by the TRAC program.

2.5.4 Updating the centres of each item

For each image, the centre of each bulb of each item is updated from the position of the centre of the bulb in the previous image. The old centre positions are either on black pixels in the new image, or have already been located onto the nearest valid black pixel. This section first describes the principle of the centring algorithm used in the program, and then goes on to describe the various constraints that are imposed on what areas the algorithm can use to calculate the centre. These constraints ensure that realistic proportions of ants and other items are conserved, and that areas belonging to one item are not usurped by a neighbour. These constraints are applied in slightly different ways for each of the five item types. Finally this section describes how the program deals with violations of two assumptions; when ants move more quickly than half a head-width between images, and when ants overlap.

2.5.4.1 The principle of the centring algorithm

The centre of each bulb of each item is approximated in an efficient manner by calculating the central point of four lines drawn across the bulb from edge to edge. Each successive line is drawn in a different direction, either horizontal, vertical, with a gradient of 1 and of -1, and each line includes the central point of the previous line, or, if it is the first, it includes the position of the centre of the bulb in the previous image. The centre of the fourth line is taken as the centre of the bulb for this image.

The following three figures show this sequence for the head and gasters of a worker ant. Figure 2-24 shows the position of the ant in the previous frame in pink, overlaid with the current image in black. The old centre fixes are boxed and connected with a black line. The purpose of the centring algorithm is to relocate the boxes to the centre of the new head and gaster areas.

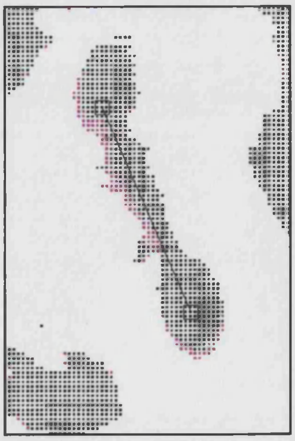


Figure 2-24 Silhouette of a moving ant

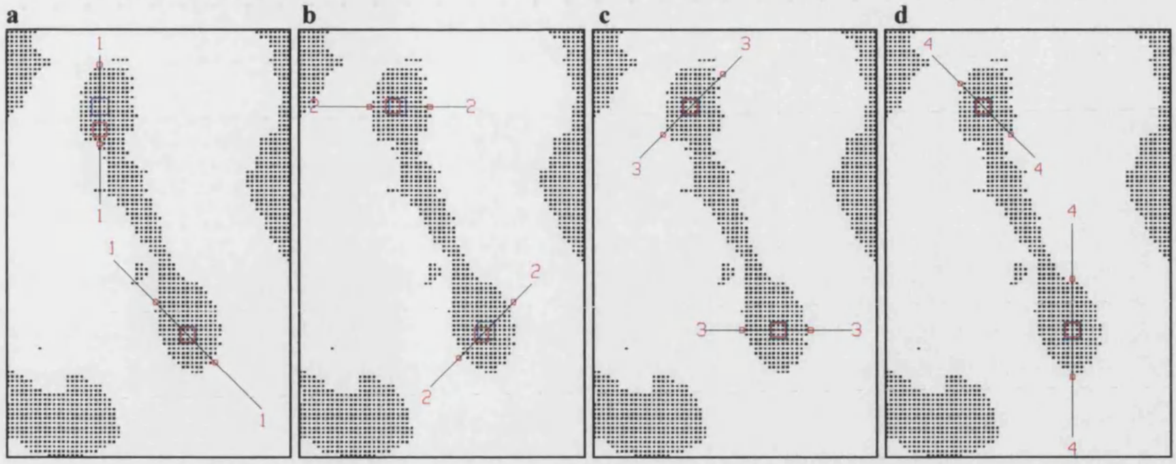


Figure 2-25 Updating the centre of the head and gaster of a straight ant (See text for explanation)

In Figure 2-25**a** the first line is drawn through the old centre position (here marked by the large red square) to both edges of the head. The first white pixels encountered are marked with small red squares. The mid-point between these two small red squares is taken as the temporary centre of the bulb, marked by the blue square. The head centre moved up and away from the edge of the head, while the gaster centre remained unchanged.

The same procedure is then applied in the remaining three directions as shown in **b**,**c** and **d**. In these, the current centre position is the centre of the previous line drawn (marked as the large red square) and the updated centre is the blue square. After **c** there is little change in the centres. Figure 2-26 summarises Figure 2-25 and is the kind of figure used hereafter. Again, the large red square marks the centre of the bulb in the previous image, while the blue square is the updated centre.

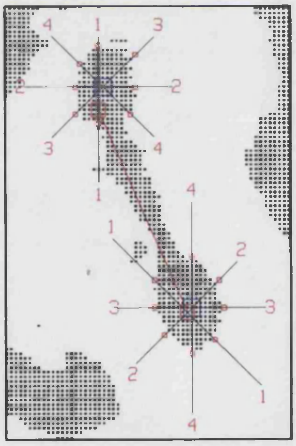


Figure 2-26 Summary of centring

Two further refinements on this basic theme are implemented in the centring algorithm:

1. For each line, the maximum distance that is searched in either direction from the current centre is restricted to the diameter of the current bulb. For example in Figure 2-27**a** the first line explored

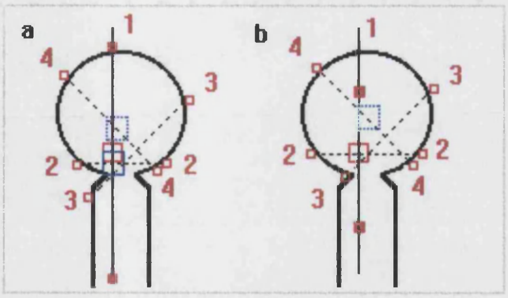


Figure 2-27 Constraints on exploratory line length for finding the centre of the head of an ant. Large red squares mark the previous centre, dotted blue square marks the updated centre after 4 exploratory lines have been drawn. The dark blue box marks the temporary centre after the first exploratory line has been drawn.

(vertically) does not extend indefinitely down the thorax. It extends a head width in either direction from the starting point.

2. The maximum allowed distance between the little red squares is restricted to the width of the current bulb. If this distance is found to be greater than the width of the current bulb (as is the case with line 1 in Figure 2-27**a**), then the distance to the furthest little red square from the current centre position is shortened, or if the red squares are found to be of equal distance from the current centre position, then there will be no movement of the centre position. This is the case for line 1 in Figure 2-27**b**, where there are no edges near the centre position in a vertical direction. Note that this improves the final position of the updated centre (dotted blue square) compared to Figure 2-27**a**.

While these restrictions on the extent of exploratory lines mean that slow-moving ants are tracked correctly, centres can escape onto the thorax or onto a touching neighbouring item. The next section imposes some more biologically reasonable constraints on the search for each centre.

2.5.4.2 Self-compatible constraints

The distance between the head and gaster of a straight ant is fairly constant. The program needs a method of making sure that the distance between the centre of the head and gaster of each ant remains within reasonable bounds. This can be encoded into the algorithm, for example for the head, by using the gaster centre as an anchor from which a minimum and maximum distance to the head can be calculated. The exploratory lines for the head are then restricted to remain within these minimum and maximum boundaries. Figure 2-28 shows these boundary distances and those describing the expected extent of the gaster from the position of the centre of the head for a straight ant.

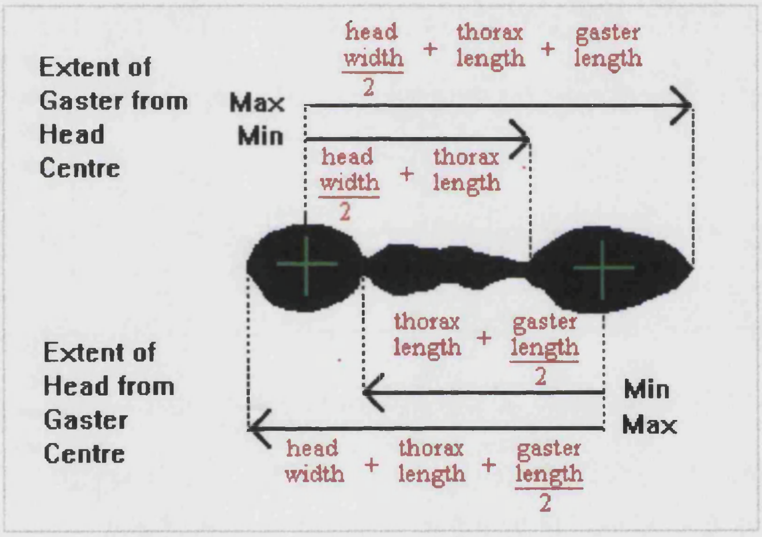


Figure 2-28 Expected dimensions of straight ants

Figure 2-29 shows how this is applied to a real ant image. The arcs, drawn in green, are boundaries beyond which exploratory lines centring the head and gaster centre cannot go. Those exploratory lines which are stopped by these self-compatible boundaries are drawn in green. For example both ends of the line drawn second (horizontally) are prevented by these boundaries from reaching the edge of the black of the bulb.

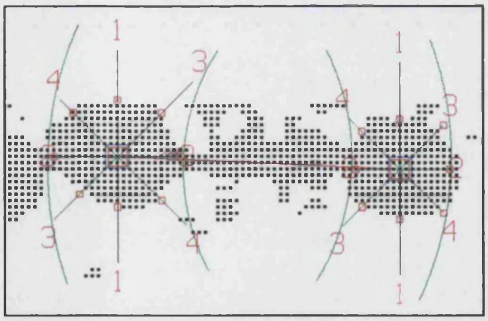


Figure 2-29 Self-compatible fields applied to a straight ant Arcs delimiting the extent of the gaster, based on the position of the head, and *vice versa,* are drawn in green.

For a bent worker or a queen the thorax fix is involved in calculating the relative extents of the head and gaster and vice versa. Figure 2-30 shows schematically how the relative distances are calculated.

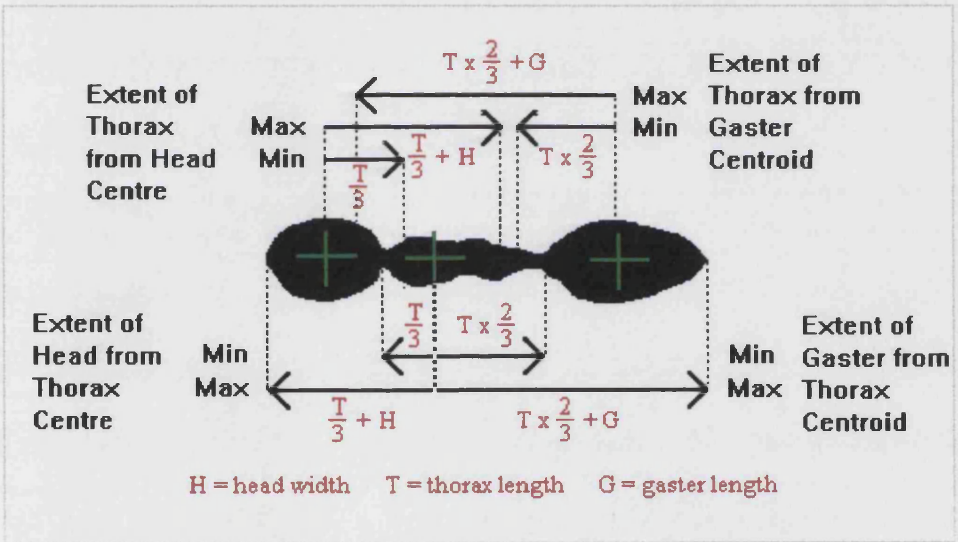


Figure 2-30 Expected dimensions of 3-bulb ants

Figure 2-31 and Figure 2-32 show how these constraints are applied for two bent worker ants and a queen respectively. Notice that the position of the thorax is constrained more by the position of the head than by the position of the gaster. This is because the head and thorax are more tightly coupled than are the thorax and gaster. The latter distance is subject to considerable variation, especially in a very bent ant, where the distance shortens as the angle of bend increases.

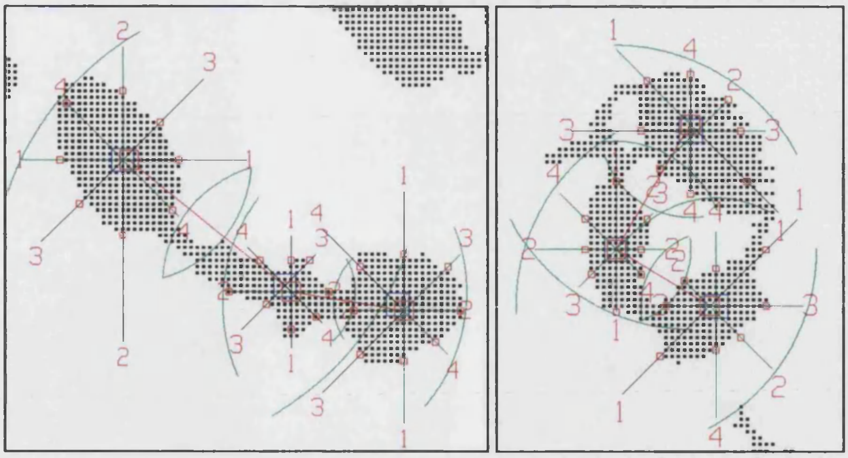
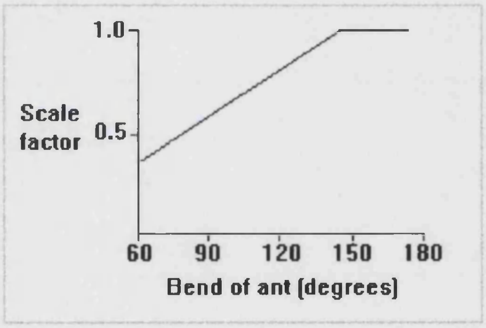


Figure 2-31 Self-compatible constraints on bent ants Arcs defining the extent of the gaster and the head are prescribed by the position of the thorax, and *vice versa.*



Graph 2-1 Relationship between gaster distance scale factor and bend of ant

Figure 2-32 Self-compatible constraints of queen

In order to allow for this, the minimum and maximum self-compatible distances between the thorax and gaster are scaled according to the bend of the ant. Graph 2-1 shows the relationship between the scaling factor and the degree of bend that was found to reflect the behaviour of the thorax-gaster distance most accurately. If this variation is not applied, the expected distances become unrealistic leading to errors as the thorax and gaster are pushed too far apart in a bent ant.

Figure 2-33 shows the self-compatible boundaries imposed on the relative positions of elliptical items in this case an ant pupa and a circular item. Naturally there are no self-compatible considerations for one-bulb circular items.

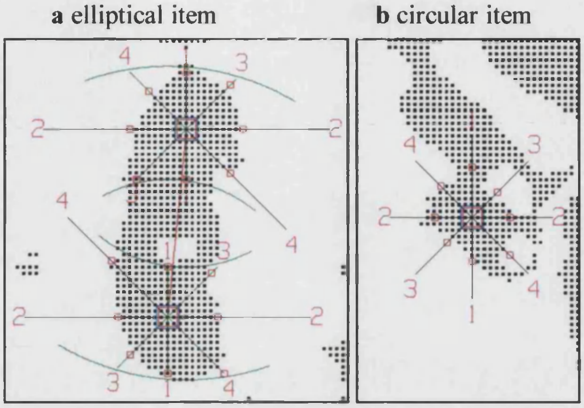


Figure 2-33 Self-compatible constraints applied to two types of brood

Self-compatible constraints ensure that bulbs within an item maintain suitable relative positions, but further constraints need to be imposed so that areas of touching neighbours are not used for centre exploration.

2.5.4.3 Ownership constraints

Explorations for updating each centre must be kept away from other items. This can be applied by surrounding each item's fixes with forbidden zones, giving the item ownership of the black pixels the zone contains. In general this means preventing neighbours exploring closer than half a bulb-width of the centre of the bulb. Figure 2-34 shows how the focal upper ant, whose centres are being updated, is prevented from extending onto the head of the ant below it. The forbidden zone is a pink circle around the lower ant's head with diameter equal to its head width. The head of an ant on the left also curtails the length of exploratory line number 2 on the gaster of the focal ant. Those lines curtailed by the occupation of pixels by neighbouring ants are drawn in pink.

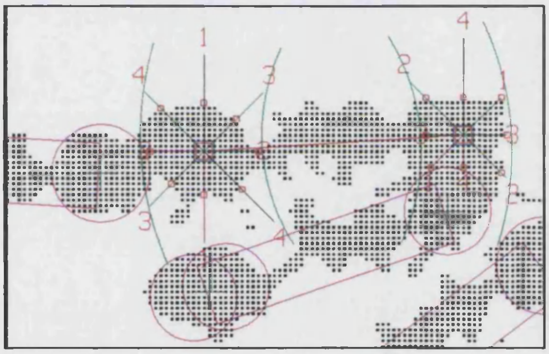


Figure 2-34 Forbidden zone around the head of a worker ants

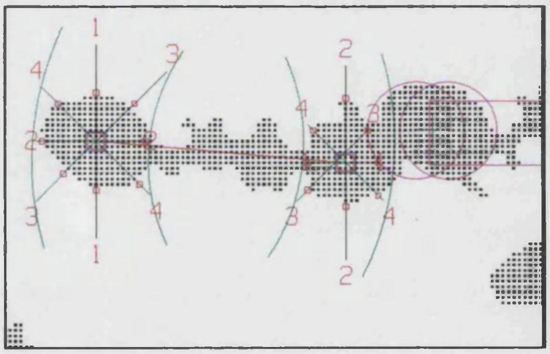


Figure 2-35 Forbidden zone around the gaster of a straight worker ant

The ownership of an oval area by the gaster is simulated by drawing two inviolate circles, each of a head width diameter, along the head-gaster line such that the maximum extent of these two circles along this line is equal to the length of the gaster. This effectively covers the area of the gaster. In Figure 2-35 the gaster of the ant on the right has curtailed the exploration for the centre of the focal ant.

Although the thorax of a straight ant is not explicitly tracked, its position is known to be between the head and gaster. The area of the thorax is protected from use by other items by a tube constructed between the head and gaster, with a width of 2/3rds of the head width of the ant. Figure 2-37 shows how the exploration for the head of the focal ant is denied access to the area of the thorax of the ant

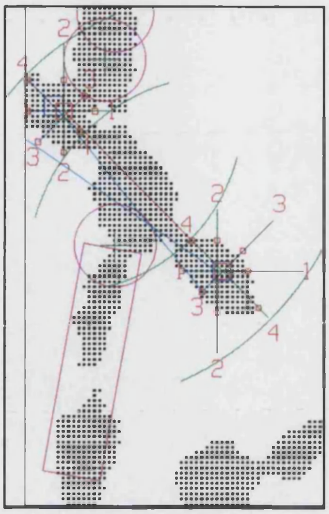


Figure 2-36 Forbidden zone of thorax of straight ant from point of view of the defender

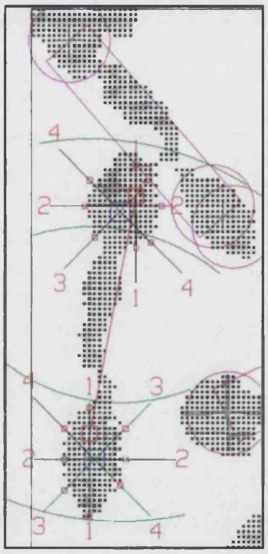


Figure 2-37 Forbidden zone of thorax of straight ant from point of view of intruder

above. Lines 1 and 3 are curtailed and are drawn in red. The algorithm that has been employed to calculate the allowed extent of a line towards the thorax tube of an alien ant is an efficient one that is not always exact to every last pixel, and the point of curtailment will depend on the angle with which the exploratory line approaches the alien thorax tube.

From the perspective of the ant whose thorax is being approached, she must not move her head or gaster fixes to positions where her thorax is overlapping the bulb of an another item. It must move as if its thorax tube described above cannot overlap with another item's owned zones. In Figure 2-36 the cyan line from the head centre towards the gaster, through the edge of the forbidden circle of the alien ant's head, is used as a boundary which lines exploring the gaster bulb are not allowed to cross. In this instance, lines 3 and 1 are curtailed by this line and are drawn in cyan. A similar line is drawn from the gaster centre towards the head, but this does not affect the exploration of the head bulb.

Figure 2-38 Forbidden zones of a bent ant

When an ant is bent, a forbidden zone is drawn around the thorax in addition to the head and gaster. A tube is now drawn between the

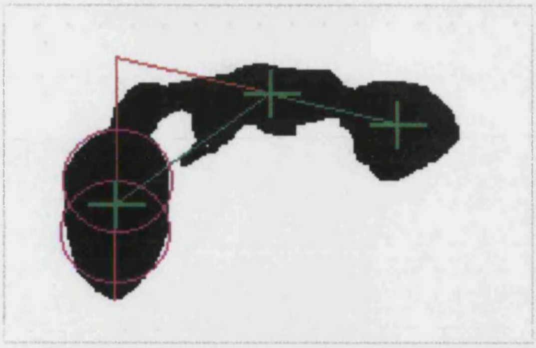
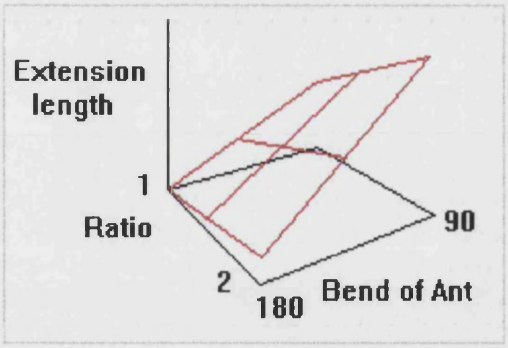


Figure 2-39 Calculation of gaster long axis using the gaster extension (See text for explanation)



Graph 2-2 Gaster orientation The qualitative relationship between extension length, bend of ant and the ratio of the length of the thorax-gaster line to the length of the head-thorax line

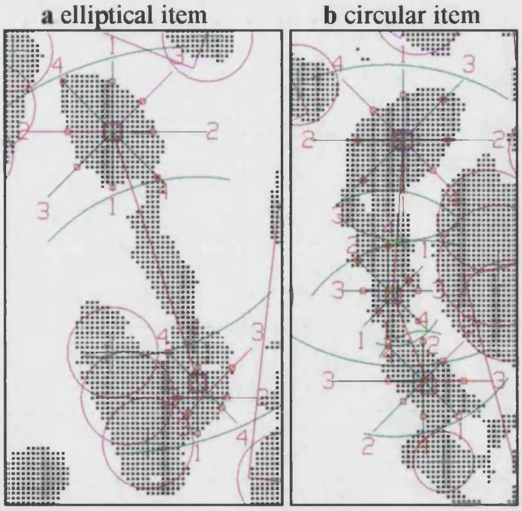
thorax and each end of the ant, although the neck tube is rarely called into play. An example of the forbidden zones of a bent ant are shown in Figure 2-38.

A further complication arises with bent ants. As the angle included between head, thorax and gaster reduces, the long axis of the gaster is no longer in line with the thorax gaster line. It always bends round more acutely (see Figure 2-40). To cater for this, the orientation of the gaster's long axis is calculated as is shown in Figure 2-39. The head-thorax line is extended beyond the thorax by a distance dependant on the degree of bend of the ant and on the ratio of the thorax-gaster length to the head-thorax length. The extension is longer for a more bent ant and for a larger ratio, as is shown in Graph 2-2. The gradient of the line between the tip of this extension and the gaster fix is then used as the long axis along which the two gaster circles are positioned. This modification has been found to be crucial in a number of instances for accurately covering the pixels of the gaster and preventing errors caused by alien fixes getting caught on the gasters of bent ants.

Figure 2-40 Forbidden zones of very bent ant The ant is on its side, with its gaster at the top of the picture. It is grooming the tip of its gaster.

The queen, shown in Figure 2-41, is treated in a similar way, but her proportions are slightly different, the thorax bulb being a full head width wide, and a supplementary larger circle is added exactly on the gaster fix because the queen's gaster is proportionately wider than a worker's.

Figure 2-41 Queen's forbidden zones


a elliptical item

b circular item

Figure 2-42 Forbidden zones of brood items

Figure 2-42**a** shows how an elliptical item is given ownership of the area covered by three circles. Two are drawn around each fix with a diameter equal to the width of the item at that end, and the third is drawn exactly mid-way between them with a diameter equal to the average of the two widths. In this case the ant that is grooming the larva has had lines 2 and 3 of its head bulb exploration curtailed by the larva's forbidden zones. Figure 2-42 shows that the forbidden zone around a one-bulb circular item is a circle with diameter equal to the width of the item.

The final constraint is that exploratory lines may not cross the edge of the nest.

2.5.4.4 The sequence of calculations

Bulbs

The order in which the centres are calculated for an item is crucial. Straight ants tend to move forward quickly, and they are more successfully tracked if the gaster centre is found before pushing the head forwards away from the gaster. The gaster is therefore calculated first for straight ants. The reverse is true for bent ants who do not tend to move quickly. It has been found that the priority for them is to locate the head and thorax first before updating the gaster fix in relation to these.

Exploration lines

The order in which the direction of exploratory lines used to calculate each centre are chosen is also important for moving items. A record of the movement of each bulb over the last five images is used to calculate the direction of movement. The line most nearly parallel to this direction of movement is chosen first. This is normally the direction in which there is most movement of the centre fix, and often gets the centre out of potentially erroneous positions.

Items

Items are updated in the same order in which they were initialised at the beginning of tracking. However the centring algorithm is applied twice for the same image on bulbs that are moving quickly. This is sometimes necessary to allow the fixes to reach the centres of their bulb for fast-moving ants where the initial position is awry.

2.5.4.5 Switching between straight and bent types

At rest and while walking, a straight line can be drawn between the head, thorax and gaster of an ant. Ants become bent during grooming, food exchange, brood care and during some manoeuvring. The position of the thorax of a bent ant needs to tracked because its position is no longer known and the distance between the head and gaster is now variable, dependent on the thorax position. The thorax of straight ants is not tracked for two reasons. Firstly, tracking two centres is more time efficient than tracking three. Secondly, the thorax centre cannot move quickly because its exploration lines are constrained from the head and from the gaster. Fast-moving bent-type ants would therefore not be faithfully tracked.

The program therefore needs to be able to switch in and out of tracking the thorax at the appropriate moment. It does this by monitoring the point where the centre of the thorax of a straight ant is expected to be. This expected thorax position (ETP) is 2/5th of the straight line distance between the head and gaster. It will be occupied by the black pixels of the thorax for a straight ant. As the ant bends, this position will become white, at which point the thorax needs to be tracked explicitly.

When the ETP becomes white the black pixels of the thorax are searched for either side of this point, perpendicular to the head-gaster line. The first black pixel encountered may not be the thorax - it may belong to a neighbouring ant, or may be part of a very small area of black, for example of a bundle of legs that become visible during grooming. Therefore each separate area of black within 2/3rd of a head width either side of the ETP is considered as being a suitable candidate for the thorax. Areas belonging to other items are rejected. The centres of the other areas are then calculated as if they were the thorax. If there is more than one such area, the thorax is chosen based on a combination of two criteria.

1. The extent of the area as measured by the total length of the exploratory lines used in finding the centre of the area.

2. The proportion of black pixels along lines between the proposed thorax centre and the head and the gaster centres.

Large areas and a high proportion of black are sought for the new centre. This part of the algorithm has to be complicated because otherwise errors occur through the wrong choice of the thorax area. Furthermore, once an area has been chosen, it is rejected if the angle between the head, thorax and gaster fixes is greater than 165°, or if the distance between the ETP and the nearest edge of the proposed thorax area is less than 3 pixels. These last two criteria ensure that ants do not switch rapidly between straight and bent types and mean that only the thoraxes of truly bent ants are tracked.

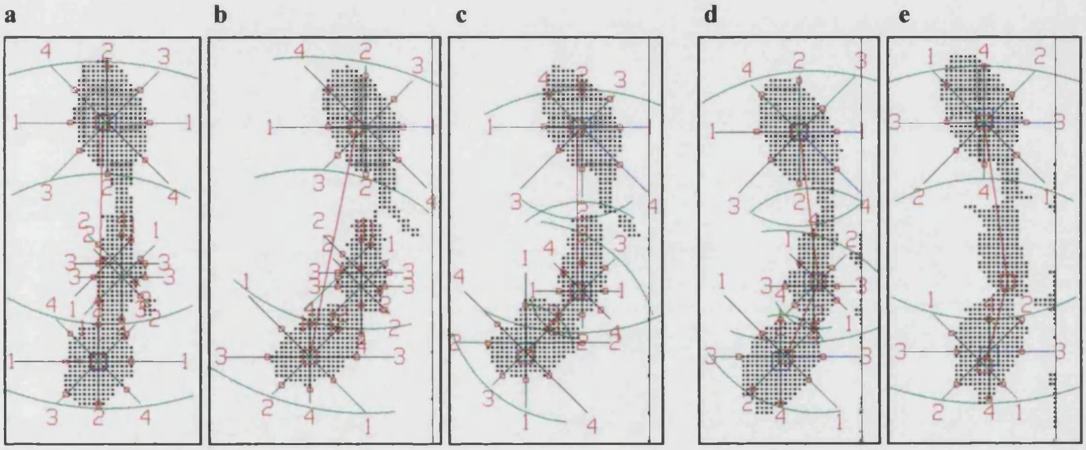


Figure 2-43 Switching into and out of bent ant type (See text for explanation)

In Figure 2-43 a series of pictures of an ant changing to bent type is shown. In **a** the ETP (not drawn) along the red line between the updated head and gaster centres (blue squares) is occupied by a black pixel. In **b** this pixel has become white as the thorax moves to the right, but the proposed thorax is rejected because the distance from the ETP to the nearest edge of the thorax area is less than 3 pixels. **c** shows the first image in which the proposed thorax is accepted. The angle included within the head-thorax-gaster is less than 165° and the thorax is sufficiently offset from the ETP.

Figure 2-43**d** and **e** show the same ant for the two images across which the ant changes back to straight mode. This occurs when the ETP becomes black again as the ant straightens up.

2.5.4.6 Exceptional modes

The assumptions on which the algorithm was developed were that the ants move in the two dimensions perpendicular to the camera i.e. that their silhouettes touch but don't overlap, and that they do not exceed a speed of half a worker head width per image. Both assumptions are violated occasionally. The first only rarely, and only because of the roof height needs to allow the larger queen freedom of movement. This means that workers can, with difficulty, climb over each other. The second violation is courted since the inter-image time interval is a major determinant of the speed of the tracking program, and the larger the interval, the faster the ants can travel between images.

Both types of violation are dealt with by classifying violating ants as being in an exceptional mode for the duration of the violation. This requires rules for entering the exceptional modes, modified centre updating rules during the modes, and rules for returning to normal tracking mode.

Overlapping ants

Ant heads occasionally overlap and ants will occasionally try to climb across each other, usually at the waist. These behaviours would clearly lead to error under the scheme of forbidden zones explained above. Two exceptional modes 'ignoring heads' and 'crossing' are therefore required. There are similarities between these two modes in that both modes involve ignoring one feature of another ant. In the first, two ants ignore each other's forbidden head zone, while in the second the climbing ant ignores the thorax tube of the ant it is climbing, which in turn drops the inviolability of its thorax. In order to change into these modes, 'stress' must be detected in the exploratory lines used to update the centres of the head and gaster of potentially overlapping ants. 'Stress' is defined as the shortfall in the expected lengths of the exploratory lines used in the centring algorithm. The expected length of each exploratory line is the width of the current bulb.

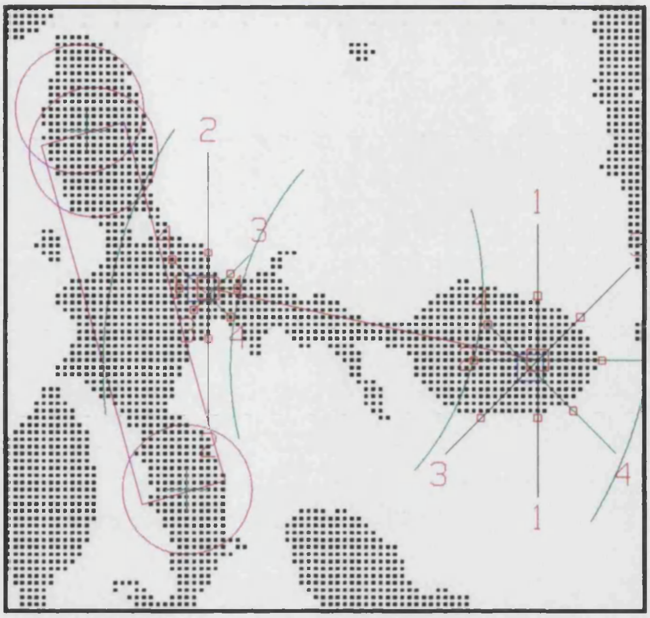


Figure 2-44 Stress in exploratory lines (See text for explanation)

Figure 2-44 shows how 'stress' is measured for a head pushing up against the thorax tube of another ant. The expected length of each exploratory line while updating the centre of a head is half a head width either side of the current centre. In the picture the forward progress of the lines is hampered by the thorax tube to the left (purple line), and by the minimum expected extent of the head from behind (green arc to the right). The thorax tube is curtailing three lines (1,3 & 4) to the left, and so the 'stress', measured in pixels, due to the thorax tube is three times half a head width minus the summed length of these three lines. On the right hand side of the head, 'stress' caused by the self-compatible arc is calculated in a similar fashion. The amount of 'stress' attributable to forbidden zones and the amount attributable to self-compatible constraints are monitored. In particular cases of extreme 'stress', exceptional overlapping modes are entered.

Both the exceptional overlapping modes have to be mutually agreed between two ants. To this end the mode is only entered if both conform to the rules of entry in the same image. However stopping the mode can be stopped by either ant, forcing the other ant to stop at the same time.

Ants 'ignoring heads'

The requirements for an ant to move into head ignoring mode are that:

1. There is another head centre within a head width of the head centre of the focal ant.

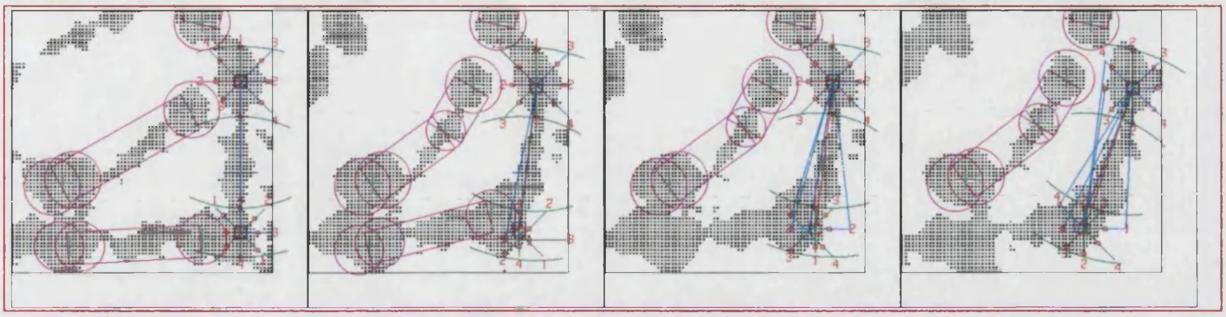
2. There is more than half a head width of stress caused by the minimum self-compatible constraint on either the head or the gaster (i.e. the ant is being compressed lengthways).

3. There is more than half a head width of stress caused by the forbidden zone of the head of the neighbouring ant.

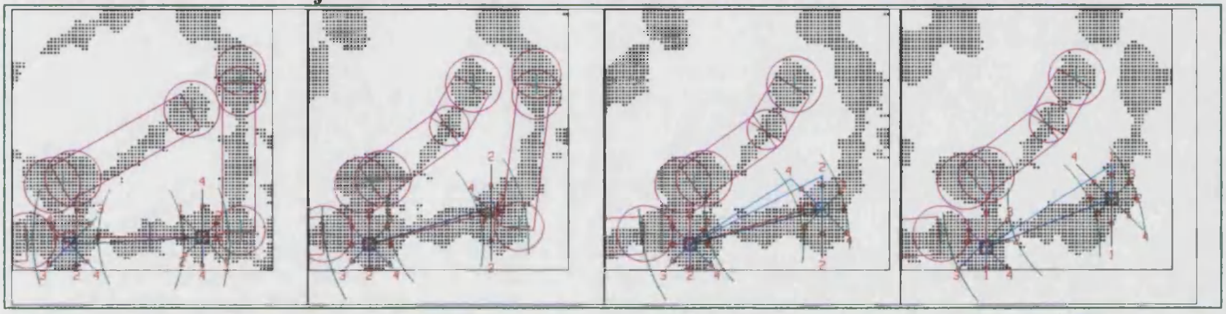
Ants stop this mode when the distance between their fixes exceeds 7/8th of a head width i.e. when they have drawn away again.

Figure 2-45 shows a sequence of images of two ants going into and coming out of head ignoring mode. In **a** and **i**, the heads of ants 244 and 818 are approaching each other, and by **b** and **j** there is quite a lot of 'stress' in the exploratory lines of each head. At image number 24614, the ants enter 'ignoring heads' mode. Note that the forbidden zones of each ant are now being ignored by the other, and the exploratory lines are free to explore the common head area (but see 'keep straight' constraint below). Through **d**, **e**, **f** and **l**, **m**, **n**, the ants remain in 'head ignoring' mode. At image number 25168, the head centres are now sufficiently far apart to stop the 'head ignoring' mode as the ants draw apart.

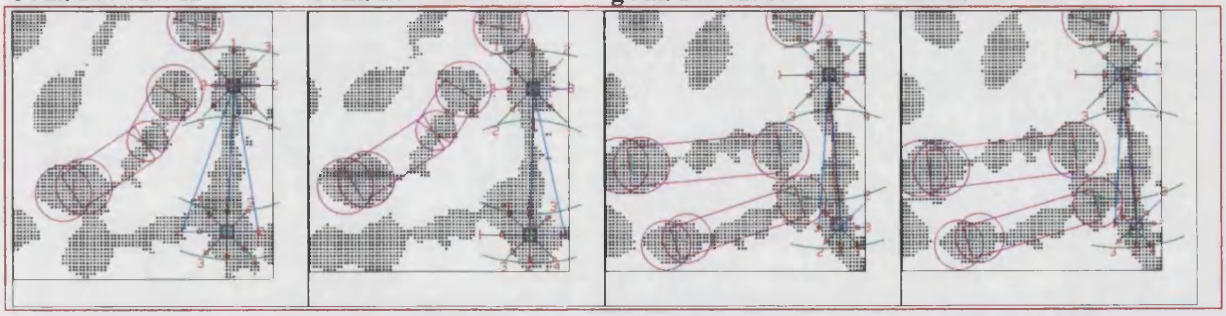
a Ant 244 #24380 **b** Ant 244 #24538 **c** Ant 244 #24614 **d** Ant 244 #24616



i Ant 818 #24380 **j** Ant 818 #24538 **k** Ant 818 #24614 **l** Ant 818 #24616



e Ant 244 #24912 **f** Ant 244 #25056 **g** Ant 244 #25168 **h** Ant 244 #25170



m Ant 818 #24912 **n** Ant 818 #25056 **o** Ant 818 #25168 **p** Ant 818 #25170

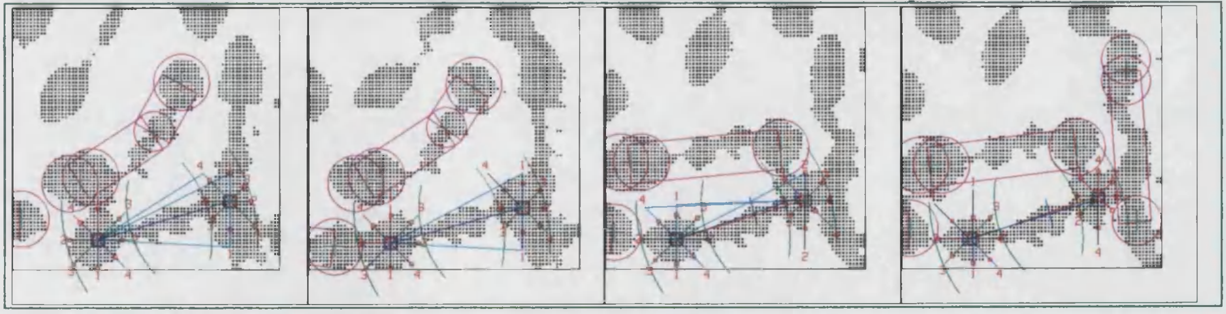
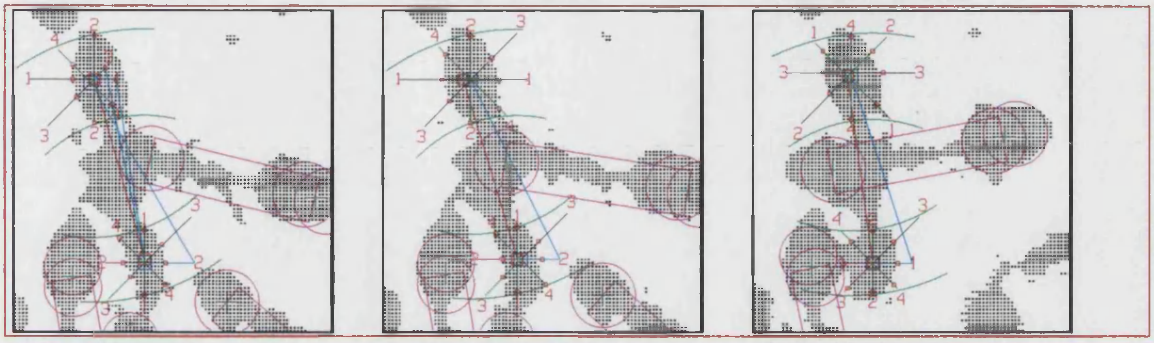


Figure 2-45 Tracking ants with overlapping heads The ant identity and image number is shown above each picture. **a-h** and **i-p** show the tracking of ants 244 and 818 respectively for the same images (See text for explanation).

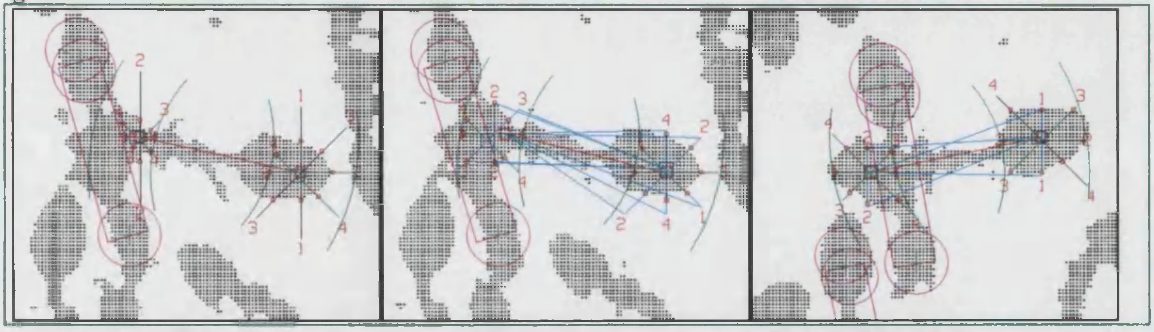
'Crossing' ants

Figure 2-46 shows ants 267 and 461 going into and out of 'crossing' mode. The rules of starting the

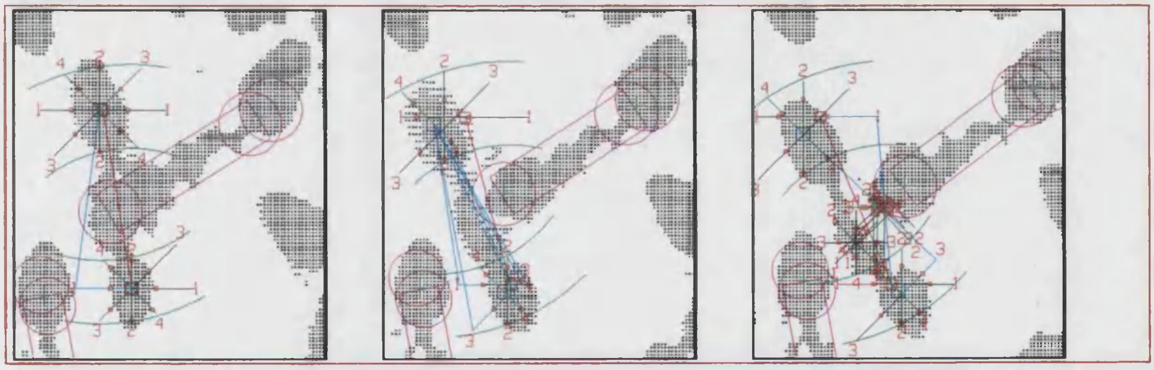
a Ant 267 #116748 **b** Ant 267 #116750 **c** Ant 267 #116770



g Ant 461 #116748 **h** Ant 461 #116750 **i** Ant 461 #116770



d Ant 267 #116868 **e** Ant 267 #116896 **f** Ant 267 #116898



j Ant 461 #116868 **k** Ant 461 #116896 **l** Ant 461 #116898

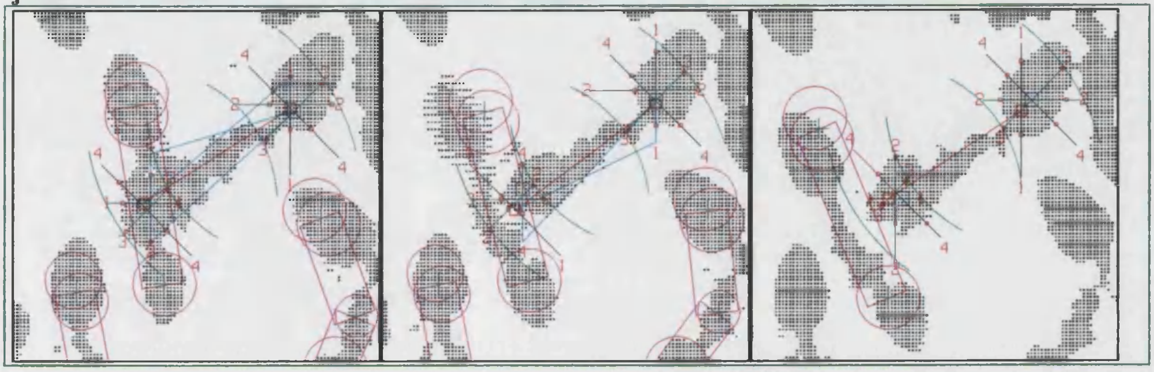


Figure 2-46 Tracking 'crossing' ants Ant identity and image number are shown above each image. **a-f** show images of ant 267 being crossed by ant 461, who is shown in identical images in pictures **g-l** (See text for explanation).

exceptional mode differ for each ant. A potential 'climber' must have similar stress criteria as in points 2 and 3 for the 'ignoring heads' ants, except that the forbidden zone in this case is the thorax tube of another ant. This other ant must have corresponding 'stress' caused by its own thorax tube. Figure 2-46g shows an ant whose head is 'stressed' ahead by a thorax tube and from behind by a self-compatible constraint. Figure 2-46**a** shows how the other ant's gaster explorations are under stress from the constraints of the position of its own thorax tube. In image number 116750 the ants commence 'crossing' mode and the thorax tube forbidden zone considerations have been dropped. 'Crossing' mode is stopped when the head-gaster lines of the two ants no longer cross and when the 'climber' has withdrawn away from the head-gaster line of the 'crossed' ant (in image number 116898).

The exact threshold 'stress' levels required to change into an exceptional mode are seemingly arbitrary. They have been established after much trial and error and are appropriate values in most but not all instances. This has been a very time-consuming part of the development of the tracking system. A significant improvement was made with the introduction of a 'keep straight' constraint.

Keeping overlapping ants straight

In the overlapping modes, where the ants share zones of black pixels, the problem of how to prevent the ignoring fixes from roving onto unwanted parts of the other ant arises. For example the head of a 'crossing' ant could slip along the thorax of the ant it is crossing, creating problems when the ants disengage.

This problem is alleviated by using the area of the thorax of each overlapping ant as a guide to the position of its head and gaster. Ants involved in exceptional modes tend as a rule to be straight, and are never very bent. During exceptional modes, exploratory lines are not permitted to explore positions that would mean that there is no area of black pixels in between the head and gaster fixes where the thorax would be expected.

This is shown schematically in Figure 2-47, where the maximum width of the head's exploratory lines are constrained to lie within lines passing from the gaster fix through the edge of the thorax. A good example of where the 'keep straight' constraint was important can be found in Figure 2-45**m**, where the head of ant 818 is prevented from going to its right and staying on the area of the head of ant 244 as the ants disengaged in pictures **n**,**o** and **p**.

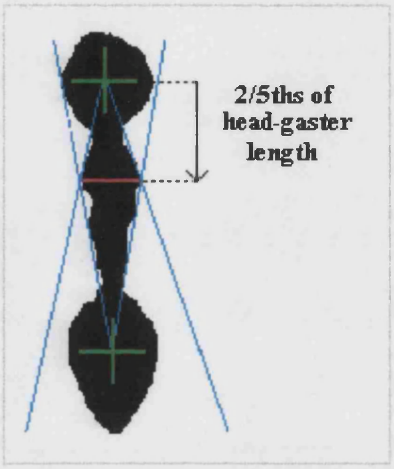


Figure 2-47 Mechanism for limiting sideways movement The blue lines are drawn from each fix towards the other end of the ant through the edges of the thorax. Exploratory lines must keep within this 'V'.

'Fast ants'

Ants quite regularly exceed the half a head width speed limit. However fast ants are quite predictable in their movements, in that they will tend to move consistently in the same direction, will slow before changing direction, and are by definition not hampered by neighbouring ants. As a result fast ants can be tracked relatively easily. Allowances are made in the centring algorithm through the 'fast ants' exceptional mode. In this mode the self-compatible constraints in the direction of movement that restrict fast progress are withdrawn.

Figure 2-48 shows the progress of an ant 122 of colony M through 15 consecutive images as it starts

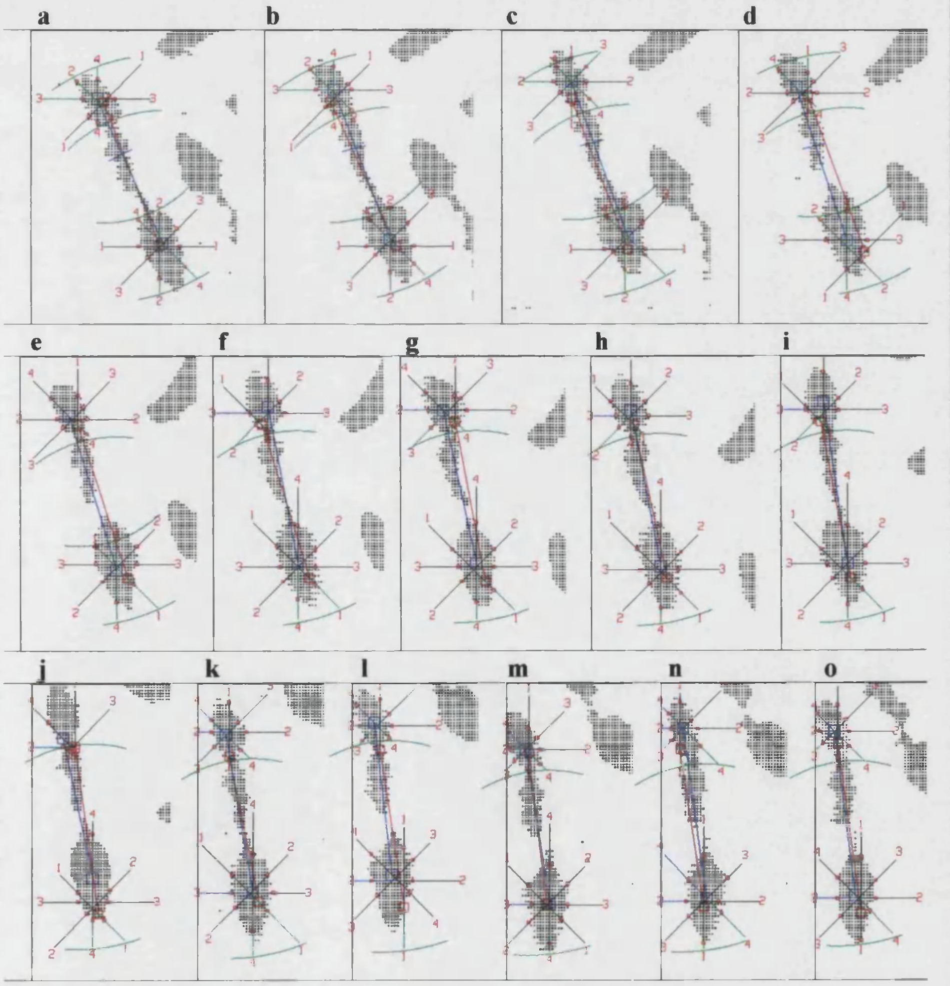


Figure 2-48 Fast moving ant Consecutive images of ant 122 of colony M are shown as it starts moving from a stationary position. The left hand wall of the nest is along the left-hand edge of each image. The speed of the ant can be gauged from the movement of the other stationary items in through the images (See text for explanation).

moving and goes into 'fast ant' mode in **e**. At this point the head has travelled more than a head width in the last five images. The maximum expected extent of the head from the position of the gaster fix constraint has been dropped. In **f** the minimum expected extent of the gaster from the head fix is also dropped as the gaster picks up speed. This allows the head and gaster fixes to move forwards more quickly. 'Fast ant' mode is stopped when the cumulative total movement in a forward direction falls below the threshold head width distance. The self-compatible constraints are then re-imposed. A similar scheme is implemented for reversing ants.

2.5.5 Storing results

After each image has been analysed the updated co-ordinates of each item are stored on disk. The path of each item is stored in separate files with its dimensions, name and the start and length of the tracking period. The co-ordinates are stored in compressed format since the fixes of 50 ants, say, for 180,000 images will soon fill most hard disks. The format of the compression is described in Appendix II.

3. Evaluation of tracking success

Extensive error-checking has been carried out on the performance and results of the tracking system. Error checks have been built into the computer programs used in this study, and a suite of checks were performed on the results of the tracking system.

3.1 Types of error

Potential errors in the ATTA can be classified into four types; coding errors, inadequate functionality, hardware error and incorrectly specified user information. These can be detected during tracking, during checks on the integrity of the results, and during analysis of the results. The majority are caught during tracking, either because the tracking comes to a halt, or because errors are caught by the program.

3.1.1 Coding errors

With a program the size of TRAC there are bound to be coding errors. However minor errors can be tolerated if they do not cause the program to fail to track items successfully, since the only requirement is that the program accurately updates and stores the centres of each item in every image. Because of the amount of data that has been processed by the program (more than 3000 ant hours, or approximately 135 million ant silhouettes) errors are most likely to have bubbled to the surface and to have caused an error in some instance. Most coding errors can be seen directly during tracking, since the behaviour of the algorithm is visually presented to the user on call for any item in the nest. This is effectively a window into the code and reveals errors to the trained eye.

Coding errors are therefore found mostly during tracking. However errors can be found retrospectively during the checks run on the result files, for example if the results have been written incorrectly, or if ants display unusual behaviour.

3.1.2 Inadequate functionality

If the tracking program cannot track items through a particular behaviour sequence using the current algorithms, then it is lacking functionality. The TRAC program will always lack functionality since it is not possible to test TRAC on all possible ant conformations that could ever arise. Indeed the current program is bound to fail under certain circumstances, for example in some complicated crossing situations and because of some small items of brood that are traversed by ants.

If an error occurs because the program is not equipped to deal with the situation, the program halts and waits for the user to modify the program or to add functionality. In very rare instances it is necessary to specify explicitly the fix co-ordinates of an item.

Small items of brood

An example of current inadequate functionality is the handling of small brood items. Brood items are pale and can be partially translucent. They therefore may not show up as complete silhouettes in the black and white images. Small items such as micro-larvae and eggs will not show up at all. This means that they cannot be tracked, and behaviours such as egg-licking cannot be detected using the ATTA. More problematic are small items that do show up, because they can be walked over and temporarily obscured by ants. As a result the tracking system is unable to track them continually, and therefore not at all. This leaves patches of untracked black pixels in the images that can snag fixes from items that pass nearby. For example on one video-tape, session 2 of colony L, a small untracked item near the entrance that was repeatedly traversed by ants caused considerable problems for a half-hour period.

3.1.3 Hardware error

The quality of the images that reach the frame-grabber from the video-recorder can vary. Occasionally images are completely unintelligible, or the inter-leaving (see next section) is not synchronised, or the image is degraded by white specks. The main source of problems has been the video-tape heads. This is not surprising given that they are very heavily used (for four days non-stop for each video-tape).

However, the system has to be able to recognise bad images and reject them. It therefore runs a series of checks on each image as it loads it into memory to ensure that the inter-leaving is satisfactory, and that the image is of the correct size - when compressed, distorted images tend to be much larger than clean images. All image quality errors are handled either in the TRANSFER program during compression, or in TRAC on loading images into memory.

Inter-leaving

Within the tracking program the inter-leaving of images can be a problem. Inter-leaved images are effectively two pages drawn on top of each other, occupying alternate lines. The two pages are redrawn asynchronously, about 1/50 of a second apart. The silhouette of a fast-moving ant in a captured image can therefore seem split, with half the lines depicting a different position from the alternate lines. In the extreme case of very fast moving ants, this shadowing effect can completely separate out two silhouettes of an ant, and the centring algorithm will be unable to track the alternately lined 'ghost' individuals. The program therefore makes a check to see how much vertical exploration has taken place for each bulb. If it is zero (i.e. alternate lines of the ant are missing) then the centring is repeated using only alternate lines of the image.

3.1.4 Incorrectly specified user information

There are two potential sources of error in user-specified information:

1. In the sizes of items specified during measurement in the first image of each video-tape.

2. In the recording of the identity list that is created during the FILM program.

A majority of the fatal errors during tracking are caused by the former, items having incorrect dimensions. In these instances the program will stop and wait for the user to resize the relevant individuals. While tracking is in progress, the user can adjust the dimensions of each item manually and a complete check on the sizes of all items in the nest can be called by the user, when all items are individually displayed and the user can confirm or alter the dimensions of each item.

The second problem is with the identity list, which controls the identity, orientation, and timing of ants entering and leaving the nest, and the identity of ants within the nest. Errors in the times, orientation, identity and position of records in the identity list can occur, especially when many ants enter in quick succession. These may cause errors during tracking if the entry cannot be found for an entering ant, or if the information contradicts current information about an ant. Some errors are not picked up by the program and checks on the result files are designed to identify these errors.

3.2 Errors detected during tracking

Only a rough guide to the type and frequency of errors during tracking is presented here. This is because a tally of all errors would be misleading since the program has evolved throughout the analysis of the tapes. Many of the errors that occurred in the early tapes have been eradicated. For example the transition from straight to bent type and the tracking of very bent ants has become very successful with few errors in the later analyses. The introduction of the 'keep straight' constraint greatly increased the success of the 'ignore head' exceptional mode. 'Fast-moving' ants very rarely caused problems in the later analyses.

During tracking, most errors occurred due to badly proportioned ants, translucent areas of brood, complicated tunnel traffic and overlapping ants, in that order. Most of these were soluble by minor alterations to the program or by altering the sizes of the items involved, but some persistent insoluble problems remained. These include some 'crossing' instances and complicated tunnel traffic (e.g. not single file).

3.2.1 Repeated tracking

Tape K1A was tracked twice; it was tracked first of all and again as the penultimate tape. During the first run, there were many errors due to badly sized ants; to brood tracking problems because no elliptical item option was then available; the then algorithm often failed for very bent ants; and there were repeated problems with entering ants being picked up erroneously. In the second run after the program had been significantly altered, there were only two errors in four hours of images, one due to a badly sized ant, and once because two ants overlapped in the entrance tunnel.

3.2.2 Example of insoluble error

The series of pictures shown in Figure 3-1 lead to an error that the tracking program was unable to solve. The problem was overcome by manually specifying the positions of the two ants at a crucial stage (at approximately image number 33860).

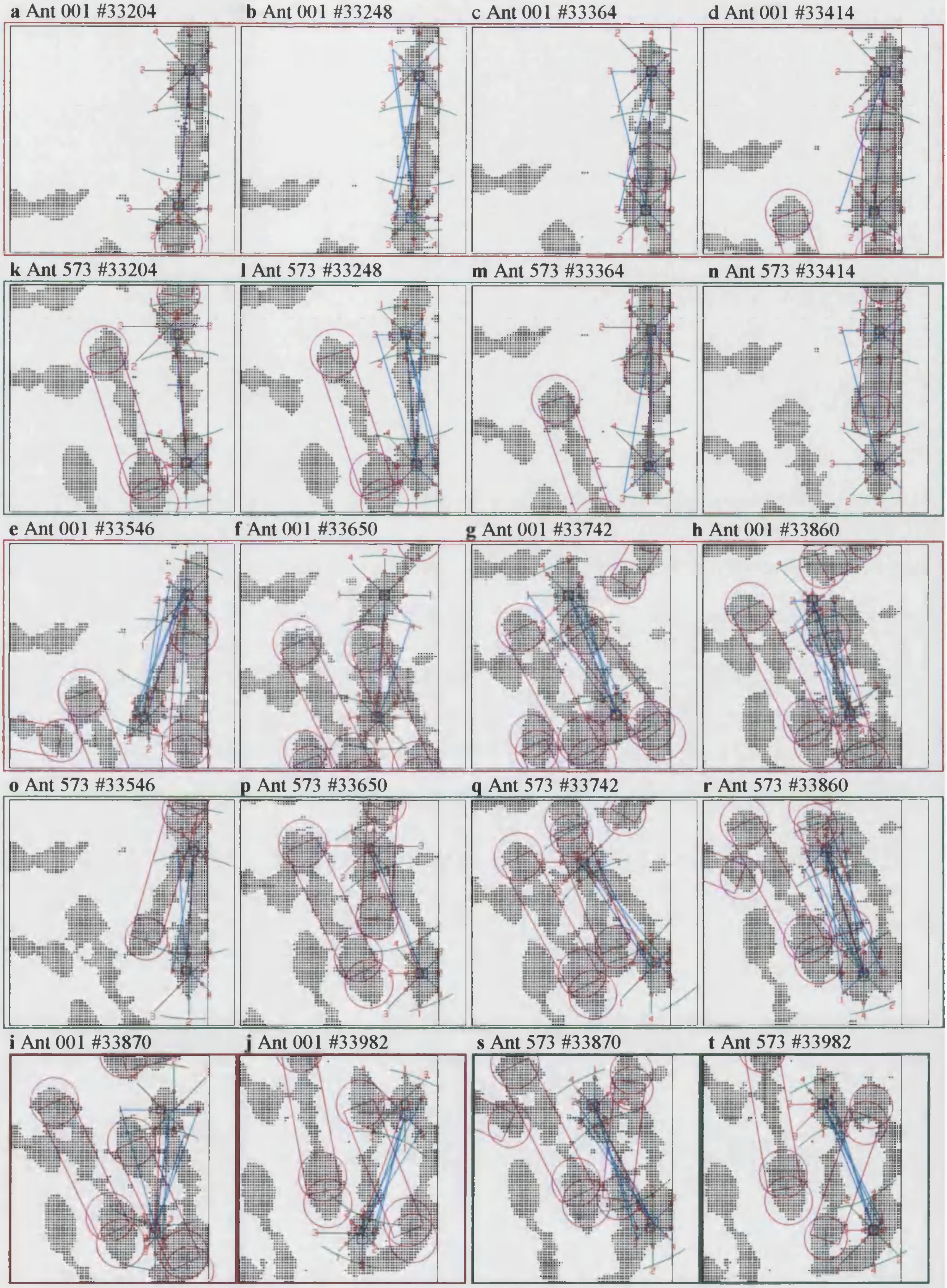
The problem arose as ant 001 (unknown identity) entered the nest and climbed over ant 573 who was lurking near the entrance. The ants went into 'head ignore' mode at image number 33248 (Figure 3-1**b** and **l**), then into 'crossing' mode at image 33364 (**c** and **m**). Ant 001 then tried to push past, and in image 33860 the head fix of ant 573 (**r**) got caught on the gaster area of ant 001, and the gaster fix of 573 moved onto the area of the head of ant 001. The problem was then compounded as the head and gasters of the two ants exchanged positions (**j** and **t**) and the program eventually halted as the head and gaster fixes were drawn unreasonably far apart.

3.3 Checks on result files

After tracking, all result files are run through a series of checking programs. These check the integrity of the results, for agreement between the entries in the identity record and the identities of ants found in the nest, and check the identity , timing and orientation of ants entering the nest.

3.3.1 Data integrity

The co-ordinates of each fix for each ant are checked to ensure that they are within the nest boundaries. For each file that finishes before the end of the four hour session, which should only happen when an ant leaves the nest, the final position of the ant is checked to ensure that it is in the exit tunnel. A rapid graphic display of the path of each ant for the four hour period is displayed and checked visually for obvious errors.



a Ant 001 #33204
b Ant 001 #33248
c Ant 001 #33364
d Ant 001 #33414

k Ant 573 #33204
l Ant 573 #33248
m Ant 573 #33364
n Ant 573 #33414

e Ant 001 #33546
f Ant 001 #33650
g Ant 001 #33742
h Ant 001 #33860

o Ant 573 #33546
p Ant 573 #33650
q Ant 573 #33742
r Ant 573 #33860

i Ant 001 #33870
j Ant 001 #33982
s Ant 573 #33870
t Ant 573 #33982

Figure 3-1 Insoluble tracking error Ant identity number and image number accompany each image: **a-j** and **k-t** follow the tracking progress of ants 001 and 573 respectively for identical images. In **a-d**, ant 001 is moving down the entrance tunnel into the nest in the top right hand corner of the image (see text for further explanation).

3.3.2 Identities of ants in the nest

Every entry in the identity list stored during filming is checked against the identities of the respective tracked paths.

3.3.3 Identities of ants that entered the nest during filming

All entering and exiting ant entries in the identity file are checked. During tracking the TRAC program picks up ants in the tunnel and then checks the list for an identity, rather than the list specifying when the program should pick up an entering ant. This is because the entries would have to be unreasonably accurate (to the nearest half a second at worst) if the list were to indicate when to pick up an ant. However, this means that entering ants that are not picked up by the program (for example if they are not in single file), are ignored even though they are in the entry list. If this happens, and there is an untracked ant in the nest, this will usually cause an error during tracking because unoccupied areas of black pixels tend to catch the fixes of other items. However it is possible that an untracked ant can survive within the nest without causing an error, providing that its silhouette rarely touches that of another item in the nest. The checks look for a result file for every list record of an ant entering the nest. Similarly, the identities of ants leaving the nest are checked, and the presence of an exiting ant for each entry is verified.

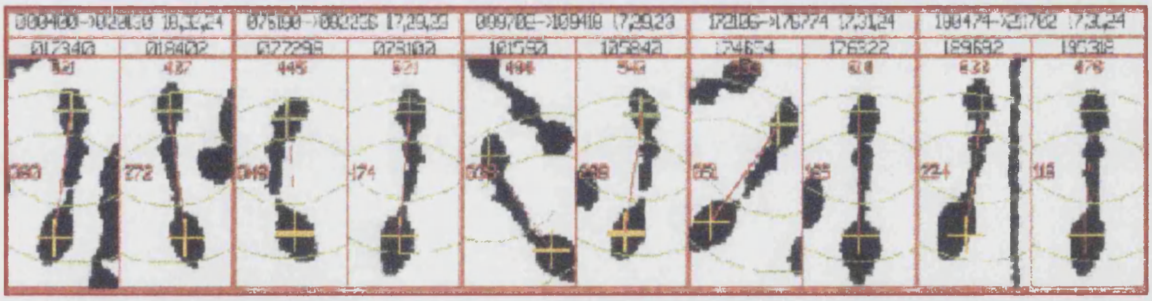


Figure 3-2 Example portraits for ant 638 of colony M in session 1 as displayed by the checking program Two portraits are shown for each in-nest period of tracked data. The start and finish image number of each in-nest period, and the dimensions of the head, thorax and gaster in pixels is given above each pair of portraits. The image number from which each individual portrait is taken is shown above each portrait. For example the first two portraits on the left show ant 638 in images 17340 and 18402 during its first period of in-nest occupation, from the start of tracking (image 400) to image 20120. Its dimensions were 18,32 and 24 respectively. The red numbers on each portrait show the co-ordinates of the centre of the ant in the nest. Ant 638 left the nest five times during the session. All silhouettes are of similar size and the green cross correctly marks the head in each picture.

The identities of all result files are also checked to see if two ants of the same name are in the nest at the same time. Two pictures of the silhouette of each in-nest period of each ant are also taken from the video-tape. The portraits of each ant are then displayed together so that the user can visually compare the relative sizes of the ant in each portrait and detect size anomalies (see Figure 3-2). This method only picks up errors between ants of significant size difference, but the orientation of each ant is also checked visually; that the head fix is tracking the head of the silhouette and *vice versa*.

3.4 Errors detection during the analysis of the result files

To date no errors have been detected in the checked result files.

Table 3-1 Errors found by the checking methods outlined in text

Session name	Two items of the same name in nest at the same time	Entering ant not picked up	Entering ant misidentified	Entering ant picked up the wrong way round	Error in initial position of entering ant	Exiting ant misidentified	Data integrity error	Coordinates out of nest area	Miscellaneous
K1	-	-	-	-	-	-	-	6[e]	-
K2	1	-	-	2	-	-	-	-	-
K3	-	-	1	-	-	-	-	-	-
K4	-	-	-	-	-	-	-	-	-
K5	-	-	-	-	-	-	-	-	-
K6	-	1	1	-	-	-	-	-	-
L1	-	-	-	-	-	3	-	-	-
L2	-	-	3	-	-	1	4	-	-
L3	-	-	3	-	-	-	-	-	-
L4	-	-	-	-	-	-	-	-	-
L5	1	-	1	1	-	-	-	-	Hidden tracking error[g]
L6	-	1	-	-	-	-	-	-	-
M1	-	-	-	-	-	1	1	-	Tracking error[h]
M2	-	1	1	-	-	2	1	-	-
M3	-	1[a]	-	-	-	-	1	-	-
M4	-	-	1	1	-	-	-	1[f]	-
M5	-	-	-	-	1[c]	-	-	-	-
M6	-	-	-	-	-	-	-	-	-
Total	2	4	11[b]	4	1	7	7[d]	7	2

Notes:

[a] result file over-written by tracking program

[b] errors mostly caused by an error in the FILM program which stored some pairs of entering ants in the wrong order

[c] ant's head initialised on gaster of previous entering ant

[d] all are errors in continuity of compressed results files caused by incorrectly terminated entries

[e] all are minor errors in the co-ordinates of exiting ants about to leave the nest, caused by poorly positioned tunnel markers

[f] error in the TRANSFER program caused images to be temporarily misaligned, in turn causing co-ordinates to go out of the nest area

[g] head fix temporarily caught on passing ant; not causing fatal error but left undetected during tracking

[h] tracking error for ant 685 caused by small untracked brood items resulted in ant head and gaster fixes being inverted

3.5 Results

Table 3-1 summarises all errors found retrospectively in the result files of the tracking program. All errors found in the suite of checking programs were corrected, either by renaming or by retracking.

4. Automated behavioural classification

The result files of the paths of all ants and brood in the nest hold much information about the positions and movements of items in the nest. They can also be pooled to give measures of colony activity and spatial use of the nest. However, the value of the results would be greatly augmented if the movements of items could be translated into behaviours. This chapter presents the first attempt at making explicit (i.e. in algorithmic terms) the definitions of distinct ant behaviours so that the computer can be programmed to detect behaviours in the sequences of ant movements.

4.1 How much behaviour can be classified automatically?

A simple behavioural classification might be whether or not an ant is moving. Movement alone, however is not a very useful measure. Defining an ant as being active, or actively engaged in any kind of behaviour other than 'resting' is more difficult, since, for example, an ant can be immobile during trophalaxis. An ant may be moving although it may not be active, for example if it is physically pushed, or while it is being groomed. The context, both in time and of the local environment are clearly essential for classifying what an ant is doing.

Precise behavioural definitions can be problematic because there are often a number of ways in which a behaviour can be done. For example, a definition (in terms of movements and context) of self-grooming would have to cover a variety of movements, from small head movements as the antennae are combed by the fore-legs, to small body movements of a bent ant as it grooms its legs, to the totally curled up position of an ant grooming the tip of its gaster. Breaking down a definition of self-grooming into the different types might be possible, but they all have some properties in common that make a general definition practicable. They all involve small repetitive movements of an ant staying in roughly the same position for a period of seconds, independent of the movement of other items. This section explores the encoding of such a loose worded description into an algorithm which can be used to classify the behaviour of ants in the nest.

4.2 Defining and detecting behaviours and cues

4.2.1 Sampling interval

The results files contain records of the position of each item every 2/25th of a second, or alternately 12 and 13 times a second. While this rate is essential for the tracking system to function, a lot of this information is redundant. The biological information therein can be sampled at a less frequent rate without substantial loss. For example, it is not essential to know exactly which fraction of a second an ant became active, if the duration of active bouts is of the order of tens of seconds or minutes. Similarly, split-second accuracy about the onset and duration of interactions such as grooming and 'trophalaxis' are not useful for the purposes of this study.

Furthermore, the behaviour of the algorithm is such that for some inactive items, a bulb centre fix will oscillate between two or more positions, depending on the starting point for the exploratory lines or the order of the exploratory lines. These movements can be ironed out by averaging the positions over a number of images.

The evolution of the algorithm during the course of the tape analysis and future changes to the program mean that ants will be tracked in slightly different ways. For example, the introduction of 'fast-forward' mode resulted in ants being tracked more smoothly between images. Averaging over images will lessen or eliminate the slight changes in tracking behaviour due to different versions of the system. Coarser sampling would also reduce the size of the result files, and a new result file format is now needed to record behavioural information for each time interval.

4.2.2 Storing behavioural information

This is collated in four steps. Firstly MOVEMENT PRIMITIVES, behaviour that can be gleaned from consecutive fixes, such as whether the item is moving, and the speed and direction of movement are recorded. Secondly, behaviour that can only be understood from a wider time context, such as whether an item is inactive or active, grooming or continuously moving in one direction is defined. These are termed BEHAVIOUR PRIMITIVES. Thirdly CUES which are available in the local environment of an ant are recorded. Lastly interactive BEHAVIOURS and TRANSITIONS can be extracted by looking at the history of the BEHAVIOUR PRIMITIVES in the context of the local CUES available to an ant, for example to identify food exchange, allogrooming, and information exchange between ants. Behaviours defined in this chapter are written in capitals to distinguish the artificial definitions from the behaviours to which they aspire.

For each individual item, all MOVEMENT PRIMITIVES, BEHAVIOURAL definitions and CUES are stored with the recorded position of each item for every sampling interval. Each of the four steps is completed on every item for a session before the next step is taken.

4.2.3 MOVEMENT PRIMITIVES

MOVEMENT PRIMITIVES are defined and stored in the transition from the raw path data produced by the TRAC program to a new more coarsely sampled behavioural data format. Increasing the sampling interval to every second was chosen as a compromise between the advantages of increasing the sampling interval listed above, and the loss of useful biological information for a large interval. The position of each item for each second is calculated as the average fix position for each second. This was chosen in preference to sampling the position every second, since the averaging will more effectively eliminate artificial movement generated by the algorithm.

Four types of MOVEMENT PRIMITIVES are defined in the transition from raw data to the averaged new format. These use the movements of the fixes of each item over the sampling interval. Their seemingly arbitrary nature is the result of trial and error to develop intuitive movement measures which are then useful for defining BEHAVIOUR PRIMITIVES.

MOVING/immobile

All 12 or 13 fixes of each second of raw data are considered to assess whether the item is showing significantly more movement than would be expected from noise in the images and noise generated from the algorithm. The current second's average fix is defined as MOVING if one or more of the following are true:

1. The maximum distance of any fix in the current second is more than three pixels away from the previous second's average fix.
2. The total cumulative movement between the 12 or 13 fixes of the current second is greater than 10 pixels.
3. The ratio of the distance between the average fix of the current second and that of the previous second to the total cumulative movement between the 12 or 13 fixes of the current second is greater than 0.2.

Speed of movement

The speed of a MOVING ant is classified as FAST if the distance between the consecutive average head fixes is greater than a gaster length, MEDIUM if the distance travelled in a second is greater than half its gaster length, SLOW if greater than a quarter of its gaster length and VERY SLOW if movement is less than this.

Direction of movement

The direction of movement for a MOVING ant is defined as FORWARD if the direction of movement over the last second is within 55° of the direction of the gaster-head vector. It is considered as BACKWARDS if the direction of movement is greater than 120° from the gaster-head vector, and the ant is considered to have moved SIDEWAYS if the direction is somewhere in between.

Bend of the ant

If the following two conditions are fulfilled then the ant is considered to be in BENT mode for the second:

1. The ant is recorded as bent for more than half the second.
2. The number of changes from straight to bent mode within the second is less than four.

4.2.4 BEHAVIOUR PRIMITIVES

MOVEMENT PRIMITIVES summarise the movements of an ant with reference to a single second or pairs of seconds. BEHAVIOUR PRIMITIVES then use these in an extended time context to describe the class of movement behaviour that an ant exhibits. It is important to note that the BEHAVIOUR PRIMITIVES defined here are an artificial classification of behaviour based on combinations of MOVEMENT PRIMITIVES. They are intended to approximate the real behaviours that an ethologist would distinguish. The next chapter will assess the success of these artificial definitions. Analyses using behaviour classified by these automated definitions must be qualified by an assessment of the success of the classifications.

Firstly, whether or not an ant is ACTIVE or INACTIVE is determined. If ACTIVE, the behaviour is described as either directional movement (WALKING), GROOM-LIKE MOVEMENT (G-LM), PAUSED or UNKNOWN. WALKING describes any FORWARD, BACKWARDS or SIDEWAYS movement that is too fast and consistently directional to be any kind of G-LM. G-LM includes self-grooming, allogrooming, feeding brood and some small repetitive movements such as licking the floor of the nest. PAUSE describes an ACTIVE ant's non-movement which, because of the context of previous and subsequent periods of ACTIVE movement are unlikely to be INACTIVE. Periods that do not fit into any of these categories are termed UNKNOWN.

ACTIVE / INACTIVE

A) Ants

Each second of the result file of each ant is searched from start to finish, and seconds are defined as ACTIVE if one or more of the following conditions are true:

1. The MOVEMENT PRIMITIVE of speed has been defined as faster than VERY SLOW.
2. If the speed is VERY SLOW and at least one of the previous five seconds has been set to ACTIVE.
3. If there is no movement in the current second, but there is some movement (VERY SLOW or faster) within five seconds on both sides of the current second.

This definition of ACTIVE seconds needs to be supplemented by a method of removing short periods defined as ACTIVE which are due to noise. A further run is therefore made through each result file looking for periods of less than 10 seconds of activity, isolated by at least 15 seconds of inactivity both sides. If the difference in position of all parts of the item between the start and finish second of the short period of activity is less than 3 pixels and no part of the item has moved more quickly than VERY SLOWLY, and if the maximum distance of any seconds within the short period of activity is within 3 pixels of the starting position, then the period of activity is changed to INACTIVE.

B) Brood

The criteria for considering an item of brood to be ACTIVE (either carried by an ant or rolled during grooming) can be more strict than the above definitions. This is because the stored positions of brood items tend to be more noisy than for ants. This is because they are not as opaque as ants and their greyscale value is closer to the threshold above which pixels are considered to be background. As a result their shapes fluctuate between images. A brood item is therefore considered ACTIVE only if its speed is SLOW or faster.

WALKING

This BEHAVIOUR PRIMITIVE defines ants as 'going somewhere', not moving on the spot. The ant must travel roughly in a straight line, or its direction of movement must be constant for a short period. All ACTIVE seconds for ants are considered as candidates for WALKING. Five types of WALKING are recognised: FAST and SLOW FORWARD, FAST and SLOW REVERSE, and SWIVEL. Ants move forwards if at all possible, and will often turn around instead of reversing. The head is by far the most mobile end of the ant, with the gaster normally taking the shortest route to follow the head. When exploring sideways, the gaster is often left in the same place while the head rotates around the anchored gaster. The SWIVEL definition attempts to identify such behaviour. The WALKING BEHAVIOUR PRIMITIVES are defined by examining the movements of an ant in a short window of three seconds. This is small enough to pick up short bursts of WALKING, but long enough so that big jerky movements during grooming are not picked up. Three measures are made of this window:

1. The average speed of the ant.
2. The direct distance travelled between the starting and finishing positions of the window.
3. The number of seconds with BEHAVIOUR PRIMITIVES defined as either FORWARDS, BACKWARDS or SIDEWAYS.

These measures are then combined as Table 4-1 shows, for the five types of WALKING.

GROOM-LIKE MOVEMENTS

This definition is designed to include a wide range of small repetitive movements on the spot, such as self-grooming, allogrooming, feeding brood solid food, nest structure licking, some liquid exchange, and some ant head to head interaction. Three definitions of G-LM are shown in Table 4-1, where in addition to the measurements made for WALKING, the ratio of the direct distance travelled to the total distance covered, the angle of rotation of the head-gaster line, and the amount of bend of the ant in the window is calculated. Four or five second windows are now considered, all seconds of which must be defined as ACTIVE and not defined as WALKING. The G-LM II and G-LM III definitions in Table 4-1 are designed to pick up BENT ants grooming themselves. Ants only bend less than 100° if they are licking the tip of their gaster. G-LM III is designed to pick this up.

PAUSE

For periods of four ACTIVE seconds, none of which have been defined as WALKING or G-LM, if the average speed of the ant over the four seconds is less than four pixels per second and the distance between the position of the ant in the first and last second is less than 3 pixels, then the ant is considered to have PAUSED for the four seconds.

UNKNOWN

ACTIVE seconds that do not fall into any of the above categories are left as unspecified. These are normally short periods of transition. between seconds that have been classified above, and are always fairly inactive.

Table 4-1 Definition of MOVEMENT PRIMITIVES

BEHAVIOUR PRIMITIVES	Window length in seconds	Average speed per second in window	Distance between position in first and last second of window	Direction of movement	Ratio of distance between position in first and last second to total distance covered	Bend of ant, or rotation of ant
Fast Forward	3	H >= GL *or* G >= GL	H >= TL *or* G >= TL	All FORWARD	-	All straight
Fast Reverse	3	H >= GL *or* G >= GL	H >= TL *or* G >= TL	All REVERSE	-	All straight
Slow Forward I	3	H >= TL/3 G >= HW/2	H >= HWx4/5	No REVERSE	-	-
Slow Reverse I	3	H >= TL/3 G >= HW/2	H >= HWx4/5	No FORWARD	-	-
Slow Forward II	3	G >= TL/3 H >= HW/2	G >= HWx4/5	No REVERSE	-	-
Slow Reverse II	3	G >= TL/3 H >= HW/2	G >= HWx4/5	No FORWARD	-	-
SWIVEL	3	H >= TL/3 G < HW/2	H >= HWx4/5	SIDEWAYS >= 1	-	-
G-LM I	5	H <= HW/3	H <= GL	2 or more changes of direction	<= 0.5	Rotation of head-gaster line < 45°
G-LM II	4	H > 3 pixels	H or G <= GL	-	<= 0.4	All BENT
G-LM III	4	-	-	-	-	Average bend < 100°
PAUSE	4	H <= 1.5 pixels	H <= 3 pixels	-	-	-

H = head G = gaster G-LM = GROOM-LIKE MOVEMENT
HW = Head Width TL = Thorax Length GL = Gaster Length

4.2.5 CUES

The purpose of logging the tactile CUES of an ant are to describe the local environment of an ant to which it can respond. This must include all objects within the antennal play of an ant, and objects that touch the body of an ant. Since the position of the antennae are not tracked, the classification of an item being within the tactile range of an ant does not specify that it touched an item, only that it could not have touched items outside this tactile range. This will clearly result in some over-estimation of the CUES available to an ant, and this will be borne in mind in future analyses.

The possible CUES that an ant can touch are other ants, brood items and the walls of the nest. CUES are also classified according to their proximity to the focal ant. They are considered to be within tactile range if within the antennal play area, and are considered to be within grooming range if close to the head of the focal ant. Hereafter, 'touch' and 'brush' are used to describe the long and short CUES respectively.

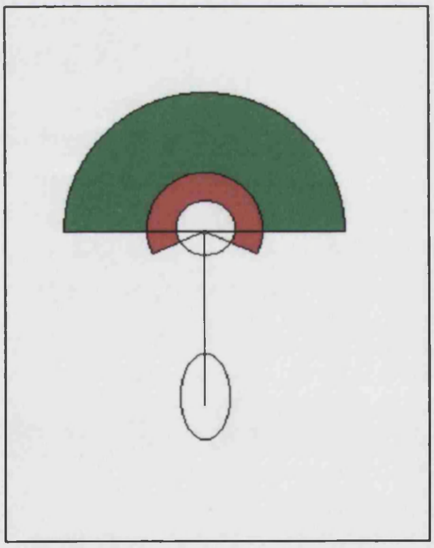


Figure 4-1 CUE detection range Green area represents antennal tactile 'touch' range, red delimits groom 'brush' range

Figure 4-1 shows the radii and arcs of the ranges of the long and short CUES. The 'brushing' arc is wider because ants can groom at right angles to their body lines whereas straight ants in general do not bend their antennae more than 90°. The orientation of the arcs are anchored by the gaster to head line.

An item must come within these arcs if it is to be regarded as a potential CUE. For example the centre of an ant's gaster must come within half a gaster length of the tactile range radius to be considered. If an ant is within 'brushing' range of an ACTIVE brood item, the ant is considered to be grooming or carrying the item, depending on the length of the carried period.

The identity of the item within tactile range is stored, be it ant or brood, or if it is nest wall, which wall is CUED. For each ant, the part of the ant closest to the head of the focal ant is stored; either head, thorax, gaster or antennae. When an ant CUE is stored, the ant being 'touched' also stores a 'touched by' CUE for the same second. So if the head of INACTIVE ant 685 is within the antennal range of ACTIVE ant 313, ant 313 stores that it 'touched' ant 685, and 685 stores that it was 'touched by' ant 313.

Adjustments are made to this general scheme for defining CUES of INACTIVE ants, ants touching each others' antennae, bent ants, ants moving sideways and reversing ants.

1. INACTIVE ants

INACTIVE ants are assumed not to use other INACTIVE items as CUES, but are assumed to be capable of 'registering' ACTIVE ants within their tactile range, and ACTIVE ants coming within tactile range of their bodies.

2. Antennal contact

Ants often alter their behaviour when their antennae come into contact with another ant's antennae. In order to pick up such CUES, the angle described by the head of the focal ant, the head and thorax of the other ant is used to scale the minimum distance required for touching. For ants head on, where the angle is near 180°, the threshold distance between the heads of the two ants must be two times the head width of the second ant on top of the antennal play radius described above.

3. Bent ants and ants moving sideways

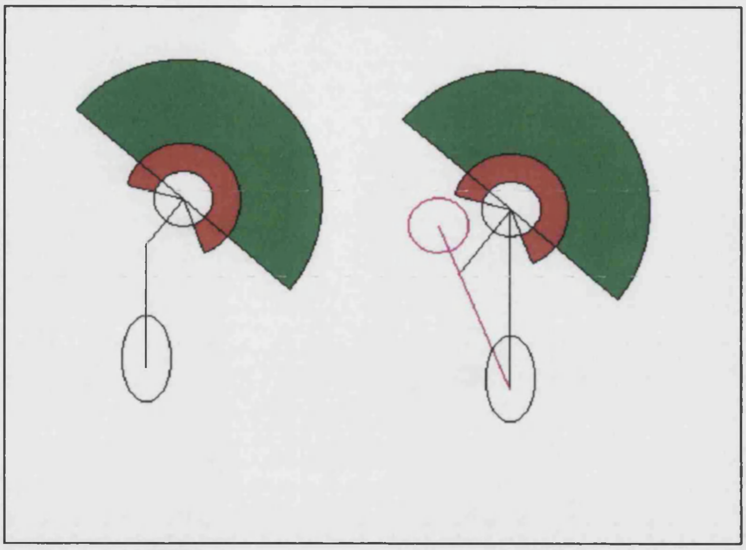


Figure 4-2 Sector orientation for bent and sideways moving ants The pink lines represent the position of the sideways moving ant in the previous second (see text for details).

Bent ants almost always bias the direction of their antennae around the bend described by their bodies. Similarly, ants moving sideways almost always bend their antennae towards the direction in which they are moving. These two common features are allowed for by changing the anchor that is used to calculate the arcs described in Figure 4-1. Whereas for a straight ant the arc is symmetrical about the head gaster line, the thorax is now used for bent ants (see Figure 4-2). For ants moving sideways, the position of the thorax in the previous second is used as the anchor (see Figure 4-2).

4. Reversing ants

In the above scheme an ant reversing into the gaster or thorax of an INACTIVE ant will not be considered, since either head must be involved for CUES to be recorded. Therefore each reversing ant is checked to locate parts of ants that are within grooming distance of its gaster. If an ant is considered to reverse into another ant, the bumped ant stores a ‘touched by’ CUE.

5. Trophalaxis

Ants involved in trophalaxis and ants feeding liquid to larvae are often immobile, and may therefore be defined as INACTIVE. Since according to the above scheme, pairs of INACTIVE ants do not regard each other as CUES, pairs of INACTIVE ants whose heads are within 'brushing' distance range of each other are stored as potential trophalaxis participants, to be confirmed in subsequent definitions.

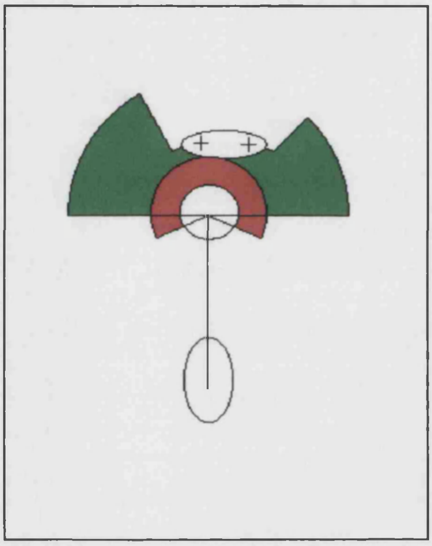


Figure 4-3 Hidden items The CUE range of an ant is curtailed by items in the foreground, in this case by a larva.

Hidden CUES

A check is run on all CUES to identify and eliminate CUES that are not physically possible because, for example, one item obscures another. All CUES to obscured items are discarded. The widths of items in the fore-ground are slightly over-estimated so that they cast a broad hidden sector behind themselves (see Figure 4-3). This reflects the inability of ants to 'touch' items in the background that do not have significant clearance from items in the foreground because their antennae are elbowed inwards.

CUE summary

Table 4-2 summarises the CUES that can be stored for the focal ant and for the 'receiver' ant or brood item.

Table 4-2 List of all CUES recorded

	Long range cues		Short range cues	
Neighbour	Stored by focal ant	Stored by neighbour	Stored by focal ant	Stored by neighbour
Ant	'Touch' Antennae	'Touched by' Ant		
	'Touch' Head	'Touched by' Ant	'Brush' Head	'Brushed' by Ant
	'Touch' Thorax	'Touched by' Ant	'Brush' Thorax	'Brushed' by Ant
	'Touch' Gaster	'Touched by' Ant	'Brush' Gaster	'Brushed' by Ant
			'Potential Troph'*	'Potential Troph'*
			None	'Reversed into' by ant
Elongated brood item	'Touch' one end of brood item	None	'Brush' one end of brood item	None
	'Touch' other end	None	'Brush' other end	None
Circular brood item	'Touch' item	None	'Brush' item	None
Any brood item			'Carry' brood item	'Carried by' Ant
Nest structure	'Touch' Wall	N/A	'Brush' Wall	N/A

* 'Potential Trophalaxis' : Both ants must be INACTIVE

4.2.6 BEHAVIOURS and TRANSITIONS

The information now collated on the movement of each ant for each second and the sequence of objects that come into and go out of tactile range can now be considered together to define the most likely occupation of an ant. Some behaviours have already been adequately defined. For example WALKING is an exclusive description that precludes any more complicated activity, though the context of CUES is important in understanding the changes in direction or pace.

Two BEHAVIOURS and one TRANSITION are described here that require both CUES and BEHAVIOUR PRIMITIVES to have been defined. Trophalaxis and the various types of grooming have yet to be defined, and contextual correlates of the ACTIVE-INACTIVE TRANSITION *and vice versa* can be extracted at this stage.

Grooming

Disentangling the various movements and CUES involved in self-grooming from allogrooming and distinguishing grooming from other small movements is a complex task. An attempt at a definition of each of these is never going to be completely successful because the ATTA does not provide information about the position of the antennae, orientation of the head (it is surprisingly mobile) and whether items are actually touching. However some progress can be made by considering a sequence of MOVEMENT PRIMITIVES and CUES defined for an ant.

It is assumed that a G-LM means that the ant is either self-grooming, allogrooming, grooming or feeding an item of brood, or licking the nest wall. The result file of each ant is considered from start to finish. On encountering a G-LM. PRIMITIVE the list of CUES for that second is searched for an item within 'brushing' range. If no such item is found, then the ant is considered to be SELF-GROOMING in that second. If an item is found, then the subsequent seconds are interrogated until the item ceases to be within 'brushing' range, or until the focal ant becomes INACTIVE. If the same item is within range for at least five seconds while the focal ant is involved in G-LM for at least 2/3rds of the time, then the focal ant is considered to be ALLOGROOMING the other item.

No attempt is made to distinguish grooming brood from feeding brood because they are difficult to differentiate algorithmically, not least because the location of the mouth of each larva is not known by the tracking system. Therefore all brood attention is grouped into the ALLOGROOM definition.

Trophalaxis

Trophalaxis is characterised by two ACTIVE ants engaging for a relatively immobile period in a close head to head formation, at the end of which both ants are generally ACTIVE, at least for a few seconds. During this period the ants normally adjust their positions at least every 20 seconds. It is rare for ants to be in such a formation during genuine inactivity because they seem to prefer space for their antennae to rest out in front of their heads.

A similar method to that used to sort out ALLO- from SELF-GROOMING is used to define 'potential trophalaxis'. For each ant, each second is consulted in sequence to find seconds where the ant is ACTIVE and comes within 'brushing' range of another ant's head. The length of the 'brushing' association is measured, up to a maximum of 120 seconds (the longest observed 'trophalaxis' event in the 18 video-tapes was 85 seconds). While the ant remains in 'brushing' range, the number of consecutive INACTIVE seconds is monitored. If this exceeds 30 seconds then the ant is not considered to be involved in 'potential trophalaxis' and is considered to be genuinely INACTIVE. If the ant remains in contact for between 10 and 120 seconds and moves occasionally during this period, but always less than SLOW speed, and is ACTIVE in at least one of the 3 seconds following separation of the two heads, then the period is defined as 'potential trophalaxis'.

Once all ants have been checked for periods of 'potential trophalaxis', a further check is then made to find periods where both ants involved have defined the same periods as 'potential trophalaxis'. Such agreed periods are confirmed as TROPHALAXIS while periods of one-sided 'potential trophalaxis' are rejected.

ACTIVE - INACTIVE TRANSITIONS

In order to investigate the spread of activity and identify the mechanisms for the colony-level activity pattern, the correlates of ants becoming ACTIVE or INACTIVE need to be identified. An ethologist observing at a nest would be able to identify events where INACTIVE ants become ACTIVE in response to physical contact with another ant. This interaction can be picked up automatically by looking for CUES just prior to an ant becoming ACTIVE.

Every transition out of INACTIVITY is considered. An ant is considered to be ACTIVATED if it has a CUE recorded in the past 3 seconds of 'touching' an ACTIVE ant, or being 'touched by' an

ACTIVE ant. The identity of this activating ant is then stored, or in case of multiple ACTIVE neighbours, the identity of the closest or most recent activator is stored. If no such CUE is found, then the ant is considered to have become activated SPONTANEOUSLY.

Every INACTIVE second is also scrutinised for CUES from or of neighbouring ACTIVE ants, in which case the INACTIVE ant is considered to be 'not activated' by such CUES. In this way, the context of ants becoming ACTIVE and those continuing to be INACTIVE is summarised.

The behavioural definitions described in this chapter have been developed in concert with a real-time visual display of the classified behaviours on the computer screen. These are overlaid on the respective video-tape images on the screen. This display, and an assessment of the success of the automated behavioural definitions are described in the next chapter.

5. Evaluation of automated behavioural classification

Each type of behavioural definition used in the previous chapter must be assessed for the accuracy with which it identifies behaviours. This means that all BEHAVIOUR PRIMITIVE, CUE and BEHAVIOUR and TRANSITION definitions need to be assessed. The assessment is performed in two ways. Firstly, the BEHAVIOURS and CUES defined in the previous chapter are displayed, overlaid on video-tape images. Secondly, behaviours collated by eye from the video-tapes are compared with the results of the automated behavioural classifications for the same periods of video-tape.

5.1 Live visual overlay

During the development of the behavioural definitions, the resulting classifications were displayed on a computer screen over live images of the nest, using a frame-grabber as before (section 2.3.6). In Figure 5-1, an example of the graphical overlay for each of the three colonies, K, L and M is shown. This display updates in time with the live video-images. In the three nest pictures of Figure 5-1, the nest entrance is in the lower right-hand corner (the image has been inverted from previous images of the nests). Each ant is portrayed as a straight or bent line with a square representing its head. Brood items are shown either as single squares (if they are circular items) or as a short line with no square (if they are elliptical items). Ants are coloured according to their automatically classified behaviour. Lines are drawn from the head of an ant to the CUES that it is alleged to touch. Long range ‘touch’ CUES are shown in orange, short range ‘brush’ CUES in purple. ACTIVE ants have black bodies and the head square colour is coded according to the behaviour of the ant (see the figure legend).

Ants classified as being ACTIVATED are shown with a blue body line (e.g. the ant near the nest entrance in the bottom picture) and the ants alleged to have ACTIVATED it are connected to it with blue lines. In this case two ants were held responsible for ACTIVATING the ant.

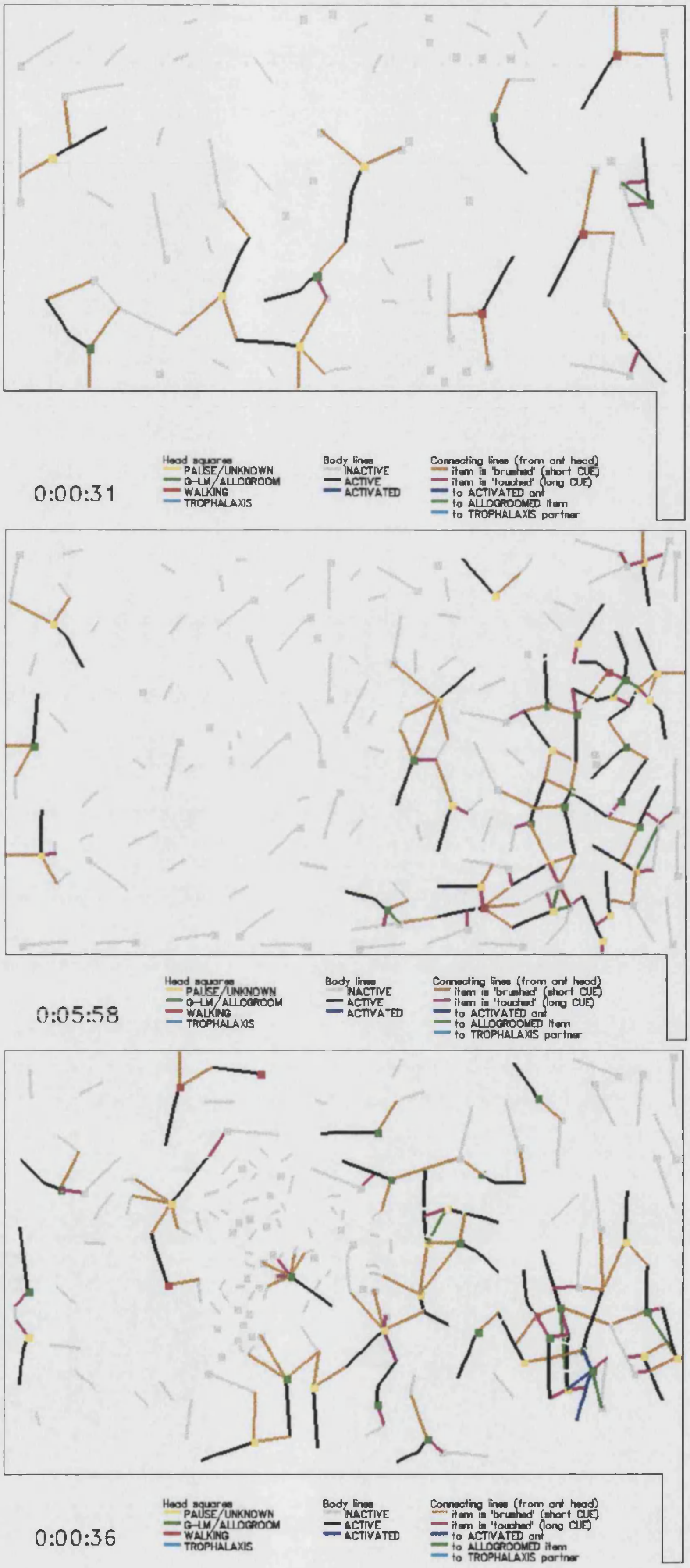


Figure 5-1 Live overlay Example overlays for sessions K1, L2 and M2 (from top to bottom) are shown. The behavioural occupation of each ant and its tactile cues are colour-coded according to the legend. The time at which the pictures have been taken is shown in the bottom left-hand corner.

This display, evolving over a simultaneous live video-image, allows the user to assess the success of the behavioural definitions, and to make the required modifications. This kind of display is also interesting in its own right because it accentuates the network of information potentially being sampled in the nest, and the spread of activity. For example, when an ant enters an inactive nest, it activates the ants in its path, which then activate their neighbours, producing a wave of activity through the nest, all connected by CUE lines.

Though assessment of the behavioural definitions using this visual method is not quantitative, it is a very useful developmental tool and qualitative check of the behavioural definitions.

5.2 Protocol for behavioural observation

Behavioural observations are made with the help of the computer because of the problems of identifying items in the video-images (as detailed below). Video-images are shown on a computer screen as above. The timings of behaviours are specified manually by choosing a focal ant (by clicking on its head) and a current behaviour type (shown in a window on screen) and then holding down the right hand mouse button for the duration of the observed behaviour. The computer records the timing of each behaviour specified for each ant. These records are then compared to the behaviours of the same period taken from the automated classifications.

In order to compare the observed behaviours of an ant with those calculated automatically from the results of the tracking system for the same ant, the identity of ants being displayed on the screen must be available to the computer. Furthermore, the identity of interacting items, such as those being groomed or those involved in trophalaxis must also be known so that the accuracy of all behavioural definitions can be assessed.

However, there is no information that the observer can use on the video-tapes to identify individuals. The images are effectively black and white so no paint marks are visible on the ants. The computer was therefore programmed to follow the time-code of the images being played on the video-recorder (as above) and to keep an updated list of the position of every ant in the nest for every image, taken from the results of the tracking program. The observer was then able to click the mouse on an interacting item, for example on the head of an ant the focal ant had just touched with its antennae, and the computer would store the time and identity of the 'touched' ant. Even though the computer has a list of ant positions updated every second, no information is displayed on screen that would bias the behavioural observations being made.

Twenty hours of behavioural observation were taken from the video-tape of session 1 of colony K. The following data were collected for each ant every second; behavioural occupation, the cues available in the ant's local environment, the reason for ants becoming active, and the identity of items encountered. Throughout this chapter the behaviours defined by the automatic technique of the previous chapter are written in capitals, while the observed behaviours are written in single quotes. Periods of behaviour defined as UNKNOWN in the previous chapter are converted to periods of PAUSE in this chapter, because an 'unknown' behavioural class is not considered. The observed 'pause' definition includes the automatically defined UNKNOWN and PAUSE.

5.2.1 Behavioural occupation

The behavioural occupation of each ant was one of 'inactive', 'walking', 'self-grooming', 'pausing', 'allogrooming' and 'trophalaxis'. These were defined as follows:

- 'Inactive': No perceptible movement.

- 'Walking': Directional movement equivalent to the intuitive notion of an ant 'going somewhere'.

- 'Self-grooming': Grooming antennae, legs or gaster. Licking the floor was also included in this class because it was often difficult to separate from self-grooming.

- 'Pause': All non-directional movement that is not grooming, often displayed in the transition between two other activities or when an ant is exploring the local environment with its antennae. This was the default activity if no other behaviour was appropriate, and included much of the low activity behaviour.

- 'Allogrooming': Grooming brood, feeding brood and grooming ants.
- 'Trophalaxis': This covered liquid food exchange and periods of mouth-to-mouth interaction between ants, even if this did not involve liquid exchange.

5.2.2 'Cues'

A 'cue' was recorded if the ant clearly touched an item (ant, brood or wall) with its antennae or if it was reversed into by another ant.

5.2.3 'Inactive'-'Active' transition

When an ant became 'active', the 'transition' was either labelled as 'activated' if another ant was apparently responsible, for example by touching the 'inactive' ant just before it became 'active', or 'spontaneous' if no such 'cue' was responsible.

5.3 Comparison of automated and observed behaviour: Results

5.3.1 Behavioural occupation

Table 5-1 compares the observed and automated interpretation of ant behaviour. Each second of video-tape that was analysed by both methods is scored in the table according to the observed and

Table 5-1 Comparison of automated and observed behavioural definitions per second

		Tracked activity						
		INACTIVE	WALKING	SELF-GROOM	PAUSE	ALLOGROOM	TRPHALAXIS	Total
Observed acitivity	Inactive	10172	1	25	27	0	0	10225
	Walking	2	373	69	252	0	0	696
	Self-groom	52	74	667	601	5	0	1399
	Pause	212	38	353	466	3	4	1076
	Allogroom	108	1	245	233	1	0	588
	Trophalaxis	119	0	33	15	5	4	176
	Total	10665	487	1392	1594	14	8	14160

automated behaviours attributed to it. For example, the left hand column of data shows those seconds defined INACTIVE, broken down into the observed behaviours for the same seconds. There was 95% congruence between seconds defined automatically INACTIVE and observed as 'inactive'. Of the remaining 5%, 2% were defined as 'pausing'.

A high proportion, 77%, of the seconds defined as WALKING were also observed 'walking'.

The total number of seconds defined automatically as ALLOGROOMING and TROPHALAXING was well short of the number observed as 'allogrooming' and 'trophalaxing'. This indicates that these two automatic definitions are too stringent. It should be noted that the definitions for TROPHALAXIS and ALLOGROOMING are known to work in other situations observed in the video overlay. The seconds in which the ants were observed to be involved in either of these two behaviours are therefore spread amongst the other behavioural types. Most of the seconds when ants were involved in 'trophalaxis' are defined automatically as INACTIVE. The 'allogrooming' seconds are split, as might be expected, between INACTIVE, SELF-GROOM and PAUSE.

The SELF-GROOM definition works 48% of the time (or 60% if 'allogroom' and 'trophalaxis' seconds are taken into account), with most seconds being confused with PAUSE. The PAUSE seconds (which, as mentioned above, includes UNKNOWN seconds) are split between 'pause', 'self-groom' and 'walking'. The lack of congruence between PAUSE and 'pause' is in part explained by the fact that 1594 seconds are defined as PAUSE whereas only 1076 seconds are observed as 'pause'.

However, the analysis of individual seconds can be misleading. Figure 5-2 shows a visual comparison of a four minute period. Twenty ants were in the nest throughout and ant 832 entered in the third minute. The upper line for each ant shows the behavioural occupations of the ants as classified by the automated definitions, the lower line the observed behaviour.

Many of the discrepancies occur during periods of very low activity. For example, ants 155, 436, 526 and 742, who do not move very much in the 4 minute period, are responsible for much of the SELF-GROOM - 'pause' and PAUSE - 'self-groom' confusion. That they are relatively inactive can be seen

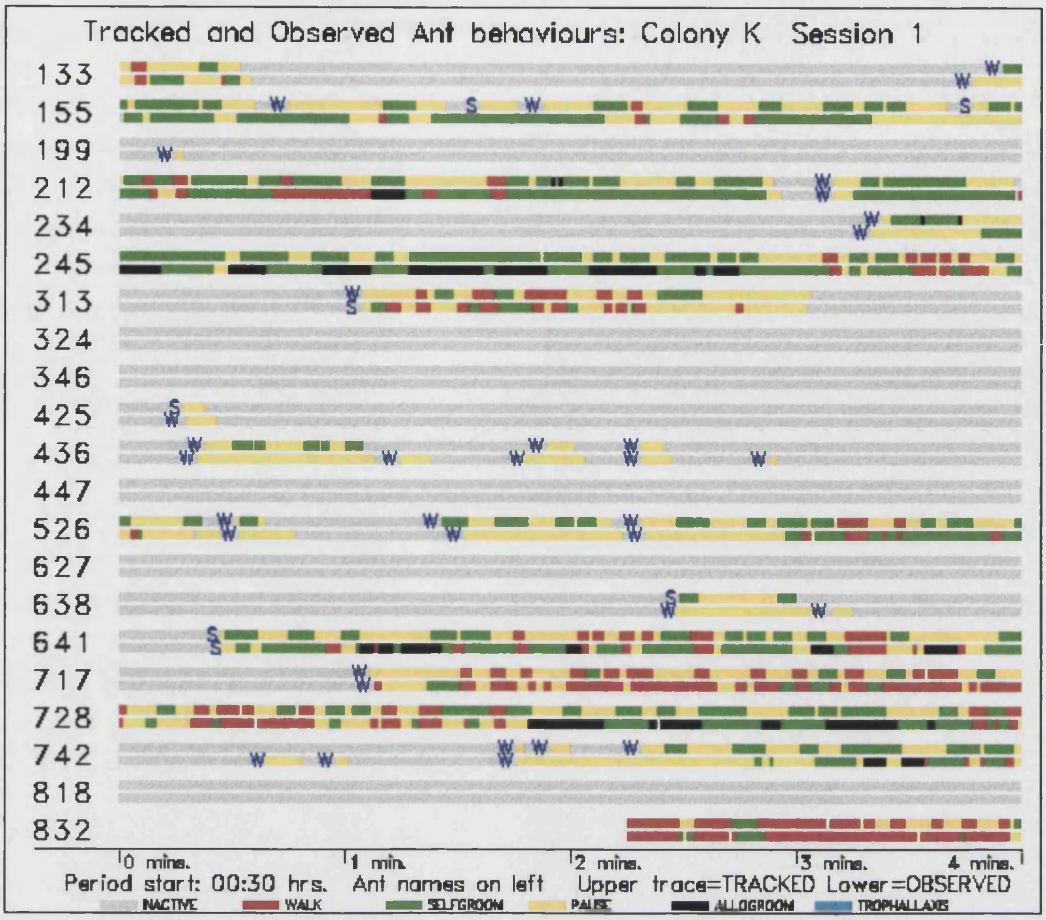


Figure 5-2 Comparison of the automated and manual behavioural classifications The blank part of the trace for ant 832 represents time spent outside the nest. 'w' indicates that the ant was considered to be activated by another ant, 's' indicates that the ant became active spontaneously.

from their frequent INACTIVE bouts. Very small 'self-grooming' movements are often misconstrued as UNKNOWN or PAUSE (e.g. ant 155). Periods of 'pausing' are often misconstrued as SELF-GROOMING by the automated definition (e.g. ant 526).

In the period for which there is both observational and automated behavioural data, there were 97 bouts of WALKING. Of these, 92 overlapped at least in part with bouts of observed 'walking'. This behavioural definition is therefore working well, although it can be seen from Figure 5-2 that the WALKING bouts tend to be shorter than the 'walking' bouts.

5.3.2 CUES

Table 5-2 Comparison of automated and observed CUE detection

		From tracked data					
		Short range CUE	Long range CUE	Not CUE	ALLOGROOM	TROPHALAXIS	Total
Observed data	Cue	439	2671	884	0	0	3994
	Not cue	736	3953				
	Allogroom	346	125				
	Trophalaxis	150	18				
	Total	1671	6767				

The automatically defined CUES are designed to over-estimate the number of contacts ants make. Table 5-2 shows that 3110 of the 3994 (78%) seconds that ants were observed to be in tactile contact with another item were also defined as being within CUE range by the automatic behavioural definitions. The fact that this is not 100% may be because the arc used by the automated behavioural definition to look for positional CUES is too narrow. However, rather than considering seconds of overlap, if the overlap of uninterrupted bouts of tactile contact between an ant and another item are compared, then a clearer picture emerges. Of the 707 bouts of CUES observed, 587 (or 83%) are picked up at least in part by the automated definition. Moreover, of the 120 bouts that are not picked up, 92 are one second bouts. These are likely to have occurred because of minor discrepancies in the timing of the observed and automated bouts.

Of further interest in Table 5-2 is the proportion of CUES defined automatically that are confirmed observationally. If the CUES that should have been ALLOGROOMING or TROPHALAXING are included in the CUE total, then 55% of automatically defined CUES are also observed - that is, the automated tracking over-estimates the number of CUE seconds approximately by a factor of two.

5.3.3 INACTIVE - ACTIVE TRANSITION

Table 5-3 shows the success of the automated definition of being ACTIVATED. Of the 24 instances when both the observed and automated behavioural data concurred that an ant became active/ACTIVE at approximately the same time (i.e. within 5 seconds of each other), 19 were correctly defined by the automated method as being ACTIVATED by another ant. Of the remaining 5 instances, 3 were defined by the automated definition as activating SPONTANEOUSLY when they were observed to have been 'activated' by another ant.

Table 5-3 Comparison of automated and observed active-'inactive' Transitions

		From tracked data						
		Activated	Spontaneous	INACTIVE	ACTIVE	ALLOGROOM	TROPHALAXIS	Total
Observed data	Activated	19	3	11	4	0	0	37
	Spontaneous	1	1	2	1	0	0	5
	Inactive	0	1					
	Active	7	3					
	Allogroom	0	8					
	Trophalaxis	2	1					
	Total	29	17					

The high ratio of 'activated' to 'spontaneous' events should be noted.

The rest of the table shows what happened when the two methods disagreed about the ant becoming active/ACTIVE at the same time. The trend in these discrepancies is shown up well in Figure 5-2. Instances of an ant observed becoming 'active' while the automated method considers the ant still INACTIVE occur (e.g. ant 742), while the reverse never occurs. On the other hand, ant 155 is observed to be continually 'active' while the automated definitions consider the ant to become periodically INACTIVE. This reflects either that the ACTIVE definition is a little conservative, or that the earlier MOVEMENT definition is too conservative.

6. Results

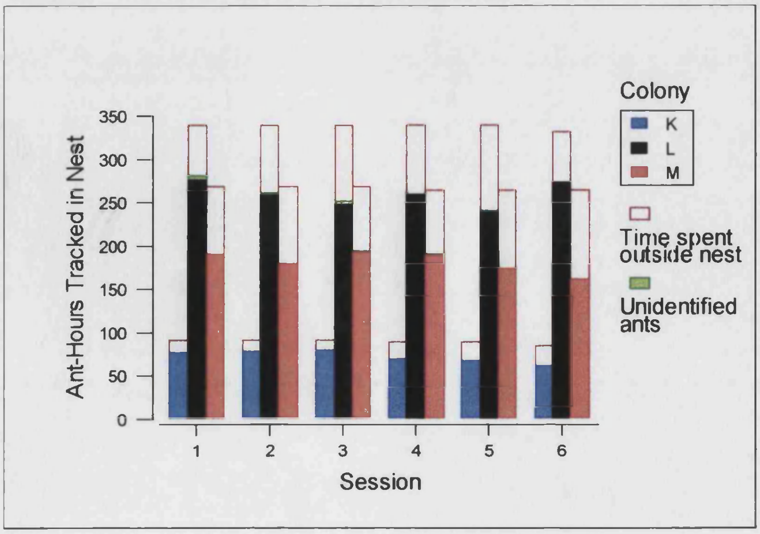
The results are presented in the following order. Firstly, the number of ant hours tracked is summarised for each session and colony. Differences between colonies and trends across sessions are explored using a simple behavioural measure; the amount of time that ants spent outside the nest. Secondly, the success of the identification scheme used in this study is appraised briefly. Thirdly, the weights and dimensions of the ants are summarised and a novel measure of the amount of reserves on board the gaster of each ant is described and calculated. This measure is then compared to the behaviour of the ants. Fourthly, the results of the automated behavioural classifications are summarised.

All statistics in the results have been calculated using Minitab for Windows v.10.2 (Minitab Inc., State College, PA).

6.1 Session Results

Graph 6-1, a summary of Appendix V, shows the number of ant hours tracked for each session of each colony. A total of 3088 ant hours were tracked, 14 of which were of unidentified ants (0.5%). Most unidentified ants were from colony L (11.2 hours) since this was the biggest colony with more ants to

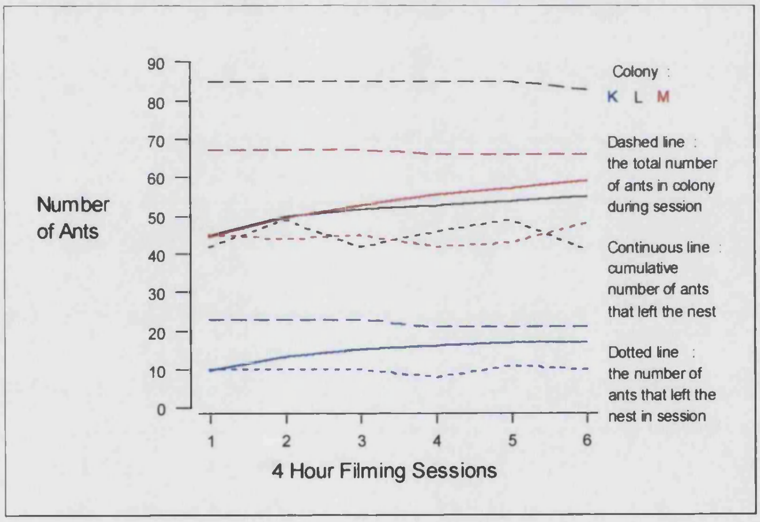
Graph 6-1 Session summary of hours tracked



identify during filming. All unidentified ants were those which either left or entered the nest during the session i.e. all ants that stayed in the nest were identified in all sessions. There is no clear trend in the number of ant hours spent outside the nest across the sessions.

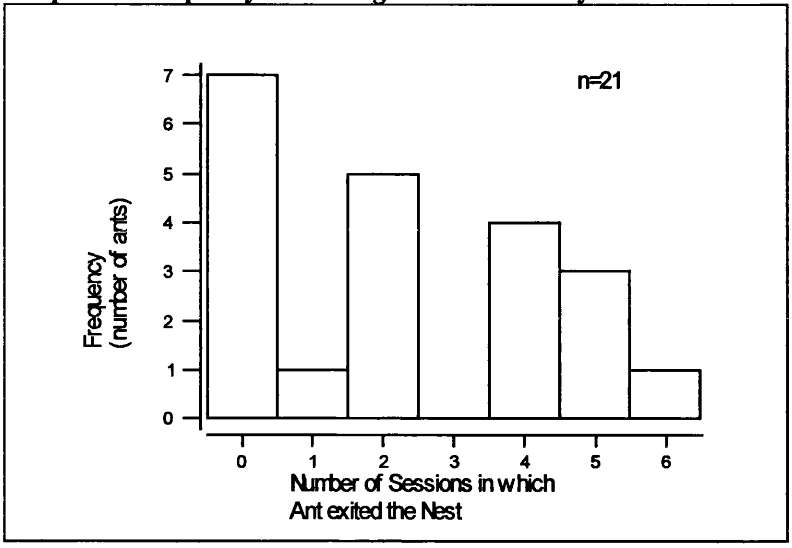
Graph 6-2 shows the average cumulative number of ants that left the nest for each single session, pair of sessions, trio of sessions etc. In colonies K and L, the number of different ants that left the nest diminishes with increasing session number. However, the cumulative number increased more strongly in colony M. 57 out of 67 ants of colony M left the nest at some point, compared to 54 out of 86 in colony L and 18 out of 22 in colony K. There is no cross-colony pattern to the number of ants that left the nest in the different sessions.

Graph 6-2 Number of Ants Leaving the Nest per Session

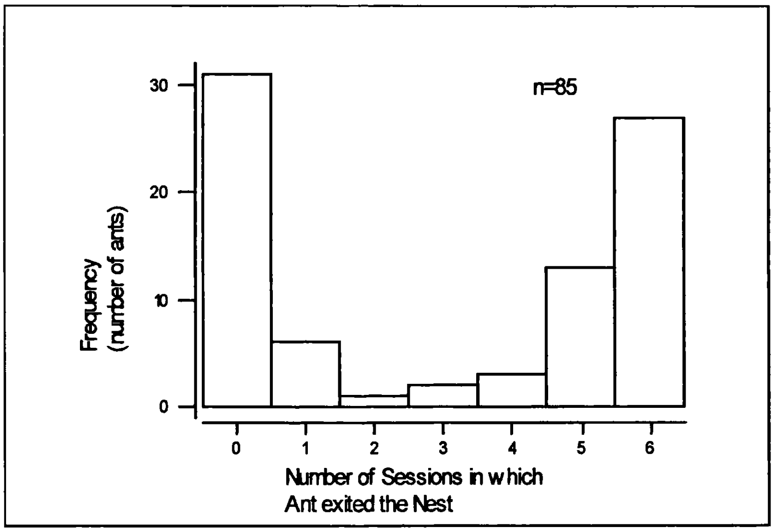


Graph 6-3 to Graph 6-5 show the frequency of the number of sessions in which ants left the nest for each colony. In colony L this is strongly bimodal, with ants either foraging in all or none of the sessions. Most ants of colony M left the nest in all sessions. There was a more even spread of frequencies in colony K.

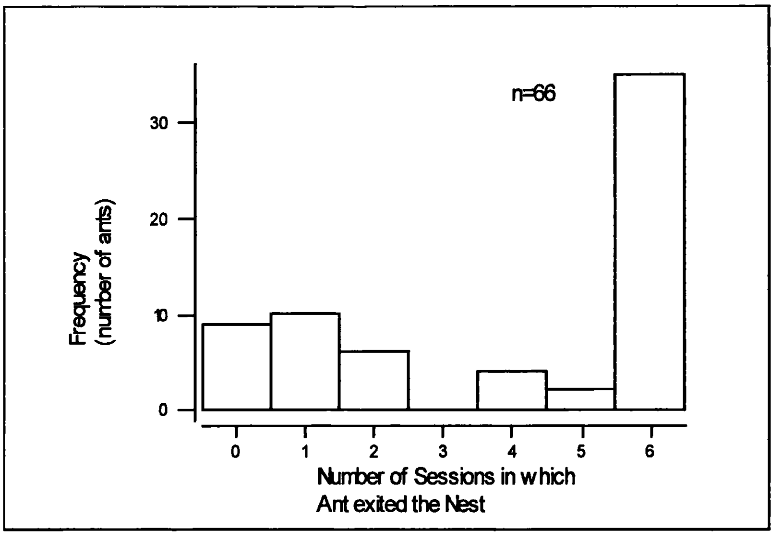
Graph 6-3 Frequency of Leaving the Nest : Colony K



Graph 6-4 Frequency of Leaving the Nest : Colony L



Graph 6-5 Frequency of Leaving the Nest : Colony M



6.2 Paint Marking

As a result of both the colour scheme used and the repainting policy, the identity of no ant was lost in the three colonies throughout their time in the laboratory. This means that all behaviour in the different video-tapes can be related, and that weights after three months in the laboratory can be compared with the weight of the same ant on collection.

The development of a marking technique to identify individuals is an integral part of the ATTA. The success of the marking during the filming period is recorded here. Of interest are any patterns in the loss of paint marks that might influence future painting strategy. The reliability of the different paint colours, of the three mark positions, and differential loss between behavioural types of individual are addressed.

The date, colony, position and colour of all paint marks lost are recorded in Appendix III. Using the information in this table a general linear model was performed with paint loss/retention as the response variable, against the treatments of colony, position and colour of mark, and behavioural type of the owner of each mark. The model was completely specified except for two interaction terms, COLONY * COLOUR * POSITION * BEH_TYPE and COLONY * COLOUR * POSITION since there was not sufficient data in the COLONY * COLOUR and the COLOUR * POSITION interaction to calculate these terms.

All three colonies K, L and M were used. Position took one of three values; thorax, left gaster and right gaster. Eight colours were used to paint the ants. The ants were divided into two behavioural types, depending on whether they spent more or less than the average proportion of time outside the nest. The response variable of paint loss was a binary qualitative measure of whether or not at least one paint mark was lost from each position, not how many were lost from each position. On releasing an ant with a newly repainted mark into her colony, the mark became the subject of much attention, and was more likely to be groomed off than older marks. Each repeated loss was therefore not considered to be an independent event.

Table 6-1 Analysis of Variance on paint mark loss

Source	DF	Seq SS	Adj SS	Adj MS	F	P
COLONY	2	8.52	5.89	2.95	25.70	0.000**
COLOUR	7	7.48	4.15	0.59	5.17	0.000**
POSITION	2	0.01	0.03	0.02	0.13	0.876
BEH_TYPE	1	1.57	1.30	1.30	11.36	0.001**
COLONY*COLOUR	14	4.06	2.54	0.18	1.58	0.081
COLONY*POSITION	4	1.30	1.57	0.39	3.42	0.009**
COLONY*BEH_TYPE	2	0.03	0.03	0.01	0.12	0.883
COLOUR*POSITION	14	0.39	0.40	0.03	0.25	0.998
COLOUR*BEH_TYPE	7	2.14	1.72	0.25	2.14	0.038*
POSITION*BEH_TYPE	2	0.24	0.20	0.10	0.86	0.422
COLONY*COLOUR*BEH_TYPE	14	0.56	0.68	0.05	0.43	0.966
COLONY*POSITION*BEH_TYPE	4	0.33	0.34	0.09	0.75	0.558
COLOUR*POSITION*BEH_TYPE	14	1.73	1.73	0.12	1.08	0.374
Error	437	50.08	50.08	0.11		
Total	524	78.45				

6.2.1 Behaviour type

There was a significant difference in the number of marks lost by the different behaviour types. Controlling for the significant colour - behaviour type interaction, ants that spent most time outside the nest lost on average 0.738 (S.D. 0.099) marks while ants that spent more time in the nest lost on average 0.328 (S.D. 0.075) marks. Ants that spent more of their time outside the nest were more than two times more likely to loose a mark.

6.2.2 Mark position

There was no difference between the propensity for marks to be lost from the three marking positions in the pooled data. However the loss from different mark positions differed between the different colonies. Table 6-2 shows that the thorax markers were least likely to fall off in colony L, but most likely to fall off in colony M, while the pooled number of marks lost were similar. Along with the

Table 6-2 Proportion of marks lost from different positions

	Colony		
Paint marker	K	L	M
Thorax	0.02	0.04	0.43
Left gaster	0.13	0.09	0.31
Right gaster	0.12	0.18	0.28
Average loss per ant	0.27	0.31	1.01

significant difference between the colonies in the number of marks lost, it is clear that there is much variation between colonies in how many and from which position marks are likely to be lost.

6.2.3 Colours

Table 6-3 The proportion of marks each paint colour lost for all ants

Colour code	1	2	3	4	5	6	7	8
Colour	White	Yellow	Orange	Red	Pink	Light blue	Dark blue	Green
Proportion of markers lost	0.11	0.09	0.11	0.11	0.49	0.21	0.25	0.04

The rate at which the different colours fell off were significantly different. Table 6-3 shows that colours pink and the light and dark blues were most likely to fall off, while green was the most reliable colour.

6.2.4 Summary

These results show that some colonies are more suitable for paint marking than others, that only some paint types are reliable, and that certain behavioural types of ant are more likely to lose their paint marks. The latter finding is important for studies where a policy of no repainting is adopted since this will bias the recognisable individuals to certain groups of the work-force. This vindicates the repainting policy used in this study.

6.3 Weights and ant dimensions

Table 6-4 shows the mean colony weights and ant dimensions for the three colonies. Colony K had the lightest and smallest workers - this is commensurate with this colony being younger than the other two. The workers of colonies L and M were similar in mean weight and size, although colony M was more variable. The queens of the three colonies were of similar skeletal size, but the queen of colony K was lighter at the end of filming. This may have been because her movement was restricted in the nest into which colony K emigrated on 20/4/95 before the last two filming sessions. Only the queen of colony K was weighed on collection. The other queens were not weighed because this queen took longer to recover from the CO_2 than any of the workers. Worker weights increased in all colonies over the tracking period, as did the variance in weight.

Table 6-4 Weights and dimensions of ants

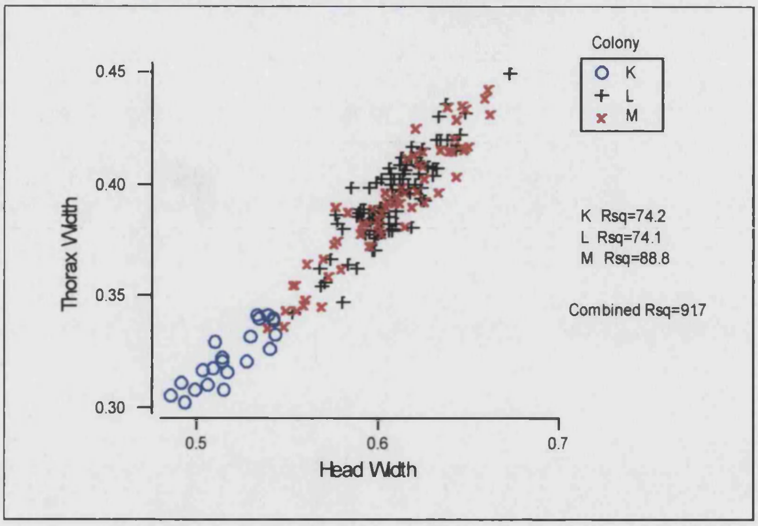
	K		L		M	
Ant Weight	22/02/95	14/05/95*	23/02/95	16/05/95*	28/02/95	15/05/95*
Mean worker weight, microg (SD)	250.9 (38.9)	259.7 (46.9)	425.7 (53.0)	461.3 (73.4)	416.2 (67.5)	451.1 (102.4)
Queen weight, microg.	1050	879	N/A	1011	N/A	1078
Ant Size†	Worker	Queen	Worker	Queen	Worker	Queen
Head width, mm. (SD)	0.52 (0.020)	0.70	0.61 (0.021)	0.699	0.60 (0.048)	0.711
Thorax width, mm. (SD)	0.32 (0.015)	0.77	0.39 (0.020)	0.7885	0.39 (0.035)	0.7735
Gaster width, mm. (SD)	0.56 (0.035)	0.94	0.70 (0.043)	0.962	0.68 (0.071)	0.9175
Gaster length, mm. (SD)	0.72 (0.075)	1.39	0.89 (0.098)	1.4655	0.85 (1.118)	1.2825

* Dates of colony freezing. Ants were weighed within 1 month of freezing.

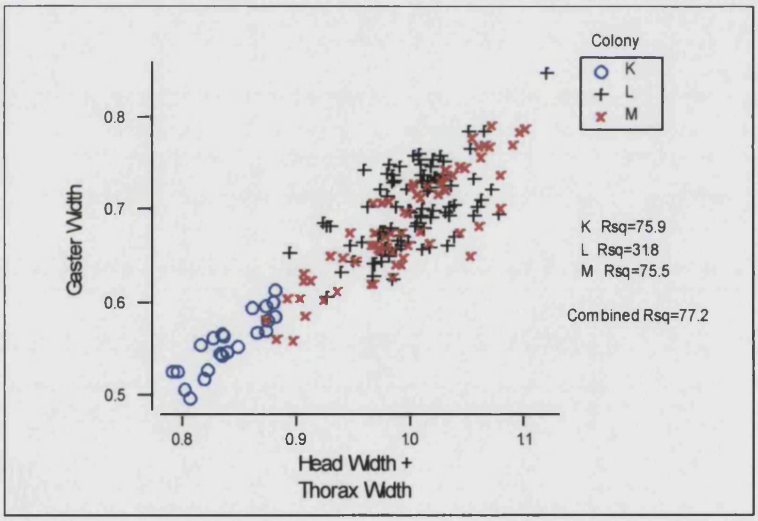
† Exoskeletal measures were taken twice, between 7/3/95 and 10/3/95, and between 5/5/95 and 10/5/95 The average value of these two measures is presented in Appendix III and summarised here.

6.3.1 The relationship between exoskeletal measurements

Graph 6-1 Relationship between Head Width and Thorax Width



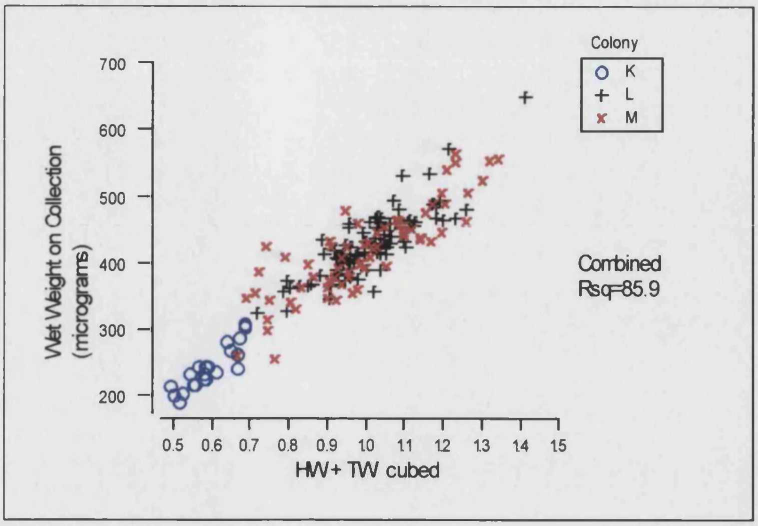
Graph 6-2 Relationship between Gaster Width and Head plus Thorax Width



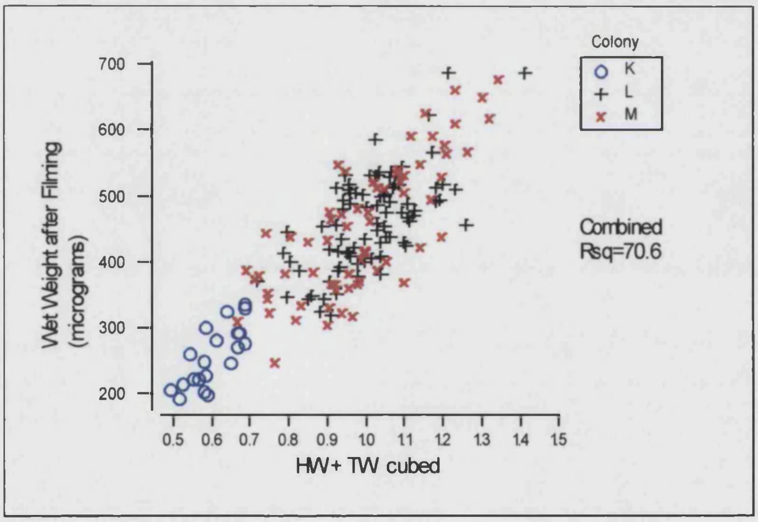
Head width and thorax width are highly correlated both within and across colonies (Graph 6-1). The two measures have been combined to give a basic measure of the exoskeletal size of an ant. This has been done to smooth the variation due to measurement error. Head width plus thorax width plotted against gaster width (Graph 6-2) shows considerably more variation than thorax width against head width. This is likely to be due to gaster width fluctuating with the size of the crop or the fat or protein reserves held in the gaster.

6.3.2 The relationship between exoskeletal measurements and weight

Graph 6-3 Plot of Wet Weight on Collection vs (Head Width+Thorax Width)^3

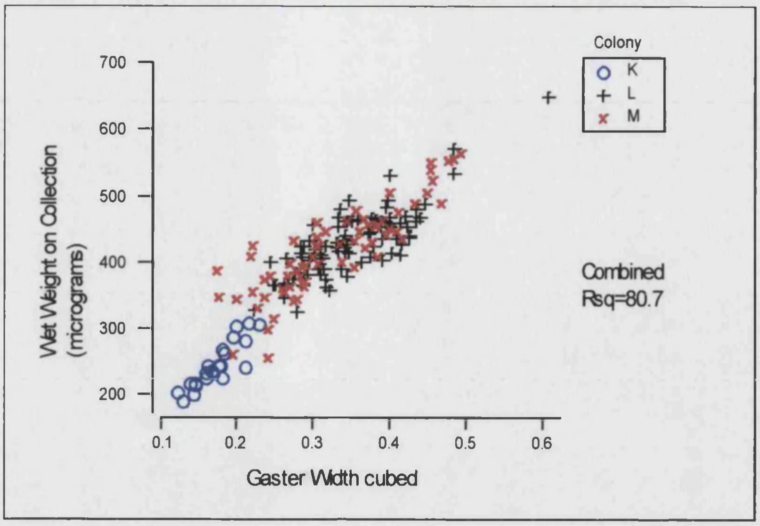


Graph 6-4 Plot of Wet Weight after Filming vs (Head Width+Thorax Width)^3

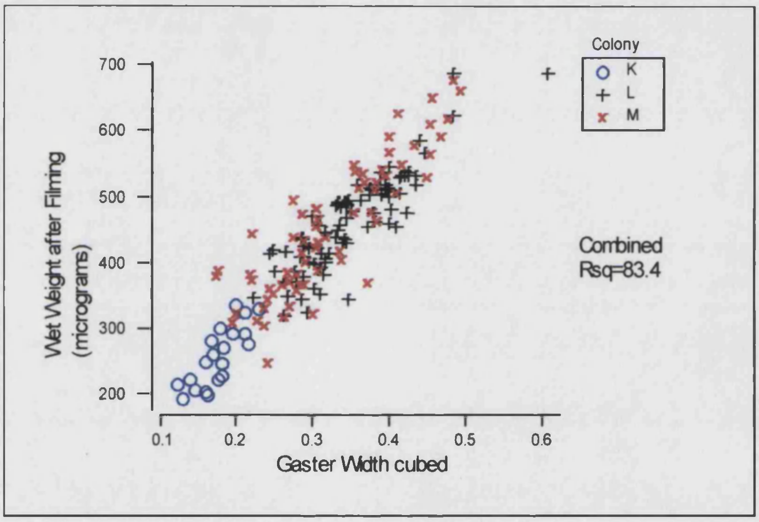


Weights are plotted against the cubed value of head width plus thorax width in order to show the linear regression of the linear exoskeletal measurements against a volumetric measure. This straightens out a slightly curved relationship. Weight on collection is strongly correlated with exoskeletal size (Graph 6-3), but this relationship becomes much more variable after 3 months (Graph 6-4), when gaster width becomes a better predictor of wet weight than head width plus thorax width (Graph 6-6). The amount of gaster reserves are therefore expressed in both gaster width and wet weight.

Graph 6-5 Plot of Wet Weight on Collection vs Gaster Width^3



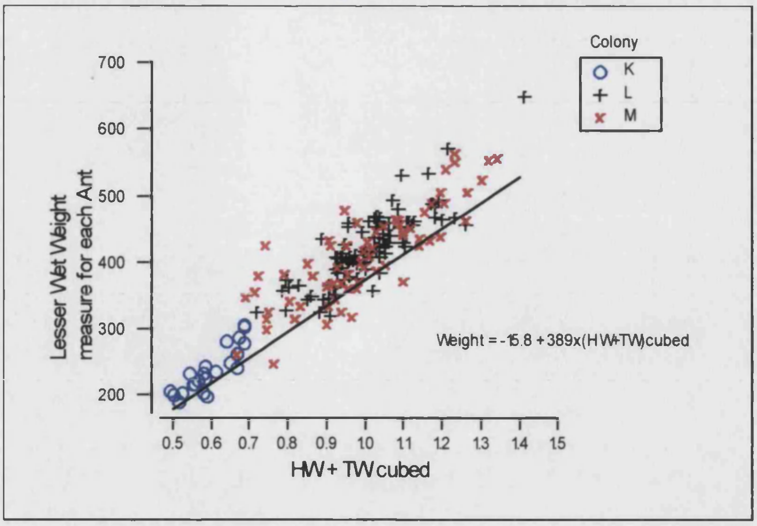
Graph 6-6 Plot of Wet Weight after Filming vs Gaster Width^3



6.3.3 A measure of reserves

Is it possible to calculate a measure of the reserves on board an ant's gaster that controls for basic exoskeletal size (measured here by head width plus thorax width)? Such a measure would be very useful to compare to the behavioural statistics of an ant. Given an ant's exoskeletal size, here measured as head width plus thorax width, it should be possible to calculate the expected gaster width of an unladen ant by looking for the smallest gaster width for a given exoskeletal size. Assuming that the unladen gaster width increases linearly with head width plus thorax width, it should then be possible to calculate the amount of reserves in the gaster width over and above this unladen width. A similar reserves measure could be calculated from the weight of an ant instead of gaster width.

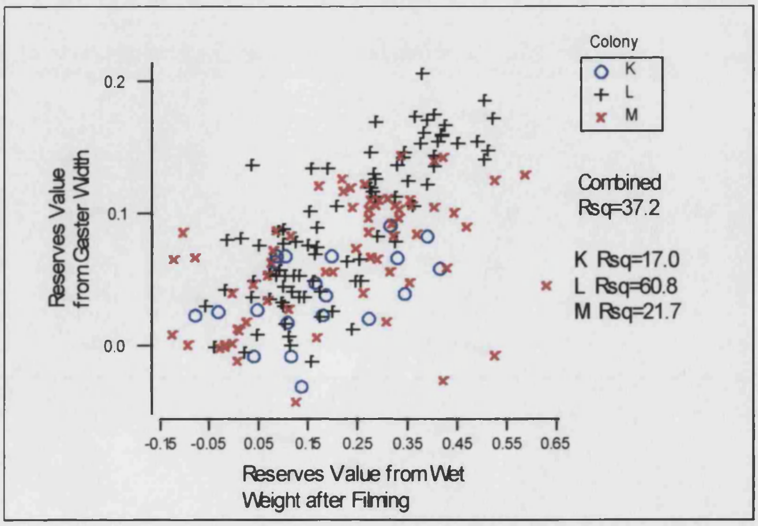
Graph 6-7 Regression of Minimum Wet Weight against (Head Width+Thorax Width)^3



Unladen weight

Graph 6-7 shows the line of minimum wet weight drawn on a plot of the lesser of either the weight on collection or weight after 3 months against ant size. This line has been calculated by dividing the data into 0.1mm ant size bins, and calculating the average value of the points that fall below the lower quartile value in each bin. The line is the linear regression through these average values. Some measurement error is expected, hence the use of the average bin value. The measure of reserves for each ant is expressed as the ratio of the weight of the ant to the unladen weight taken from this regression line.

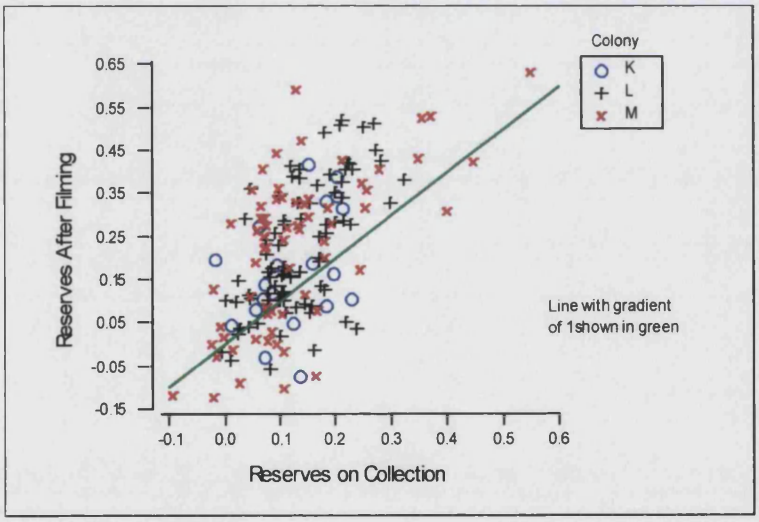
Graph 6-8 Plot of Reserves Value from Gaster Width vs Reserves Value from Wet Weight after Filming



A reserves value was calculated using weights as above and also by using gaster width. The reserves value calculated by these two methods should be similar. Graph 6-8 shows the relationship between these two measures for all three colonies. The regressions are significant for all colonies (K : $P = 0.04$, L : $P < 0.001$, M : $P < 0.001$), although the R^2 values are low for K and M. Some variance, and even outliers, might be expected since foragers must vary dramatically in weight depending on whether they have just returned with food or whether they are about to forage. It seems that there are some outliers in the bottom right-hand corner of the graph.

If there is no correlation between task and ant size, then there should be no relationship between head width plus thorax width and the reserves measures. In fact there is a weakly positive relationship between the reserves measure based on gaster width and ant size in colony M ($P=0.006$, $R^2=9.8$), otherwise the relationships are not significant.

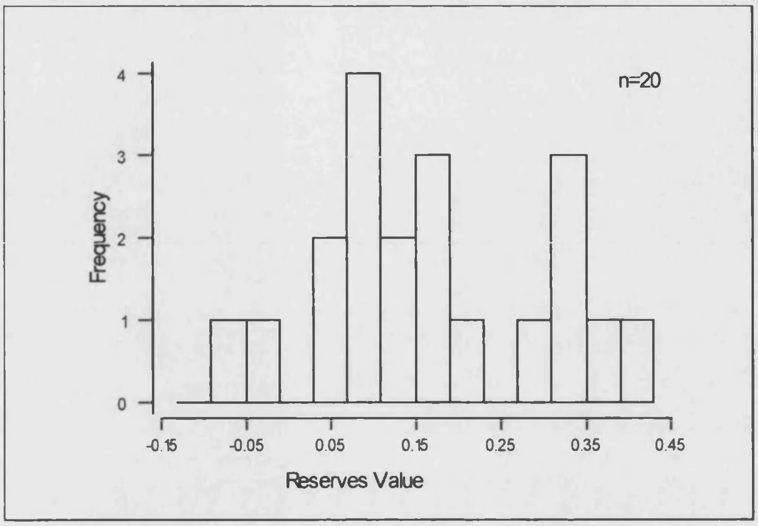
Graph 6-9 Plot of Reserves After Filming vs Reserves on Collection



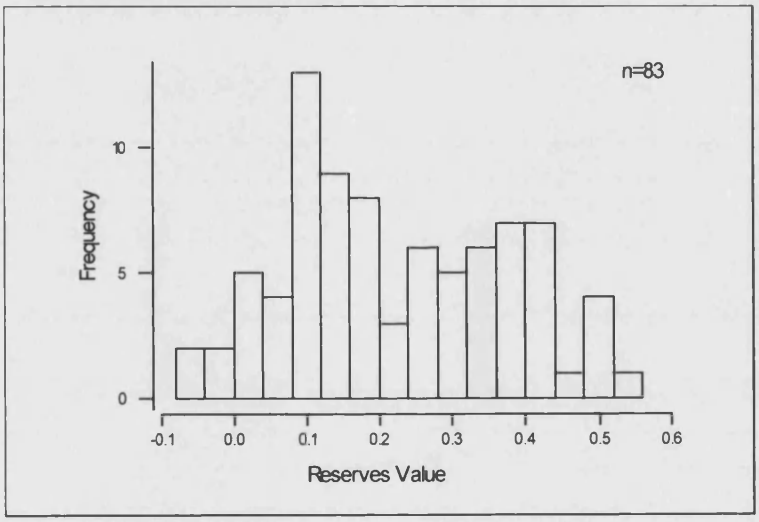
Interestingly, for all three colonies the reserves value increases for most ants after 3 months, but for some ants it decreases. This suggests that over winter reserves are spread throughout the ants in the nest, but as the season develops and foraging begins, the foragers lose weight while most ants gain weight as the following graphs show.

Histograms of reserves value taken from wet weight after three months in the laboratory are shown in Graph 6-10 to Graph 6-12. There is a suggestion that they are bimodal, or at least the distributions are

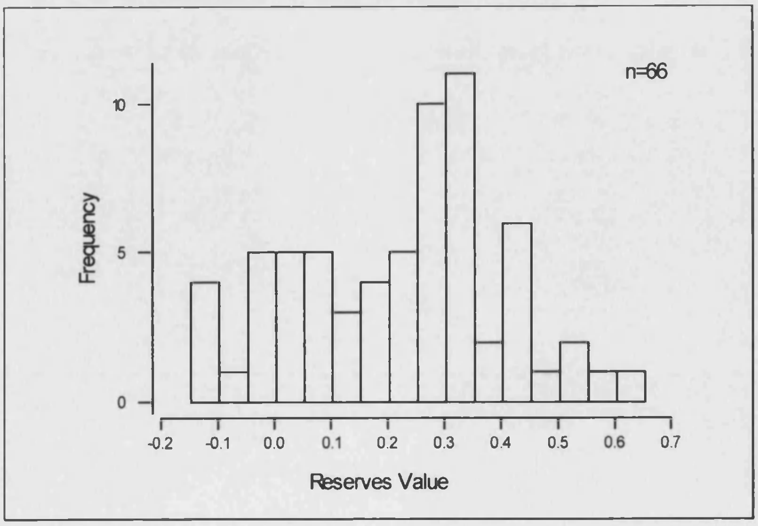
Graph 6-10 Histogram of Reserves Value : Colony K



Graph 6-11 Histogram of Reserves Value : Colony L



Graph 6-12 Histogram of Reserves Value : Colony M



more flattened than Normal distributions. This might be expected if there was a clear division between foragers and nest workers.

A behavioural measure

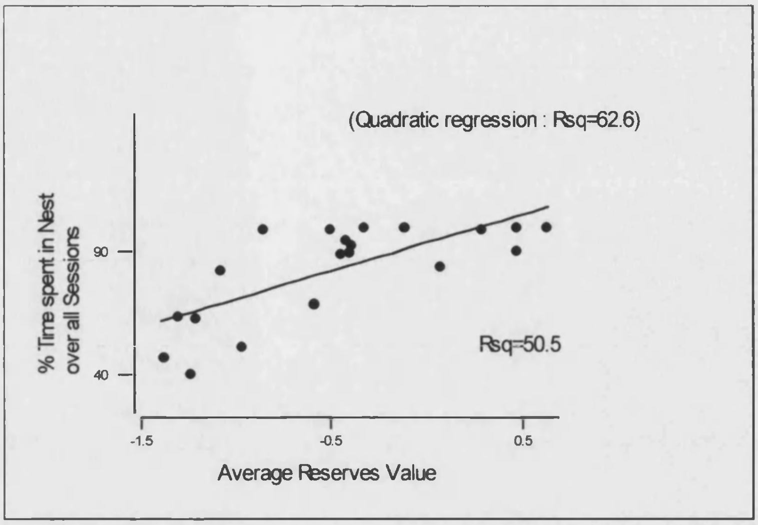
The time that each ant spent in the nest in all six filmed sessions is used as a crude behavioural measure to compare with the reserves value calculated above. Ants that spent a lot of time outside the nest are termed 'foragers', those that did not are termed 'nest workers'. The time each ant spent in the nest in each session is recorded in Appendix III.

There is no relationship between ant size and time spent outside the nest except in colony M where it is weakly positively correlated ($P=0.012$, $R^2=7.9$). This is similar to the trend seen in the relationship between reserves measure and ant size.

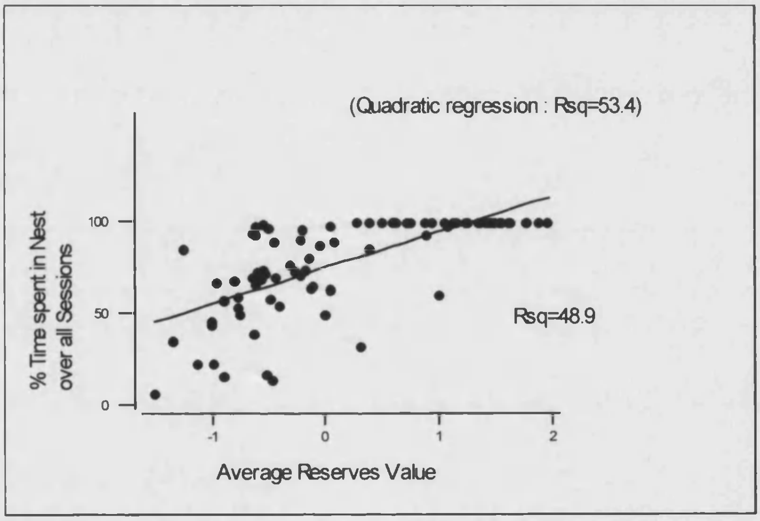
Graph 6-13 to Graph 6-15 show the relationship between the time spent in the nest against the average reserves value calculated from gaster width and that taken from weight after three months. The reserve values taken from gaster width are smaller overall than those taken from wet weight after three months (see Graph 6-8). The distributions are therefore standardised by converting the mean to zero and the standard deviations of the distributions to unity before the average reserves value for each ant is calculated. Hence in the following three graphs, the reserve values vary about a pooled mean of zero.

Both linear and quadratic regressions have been attempted. The graphs show the linear regressions, and the R^2 values for the quadratic regressions are printed on the graphs. For colonies K and L, the quadratic regressions show a better fit, while for M, the linear regression gives a higher R^2 value. All are highly significantly positively correlated ($P < 0.000$) with R^2 values ranging between 36% and 63%. Workers that spend more time in the nest have more reserves than those that leave the nest.

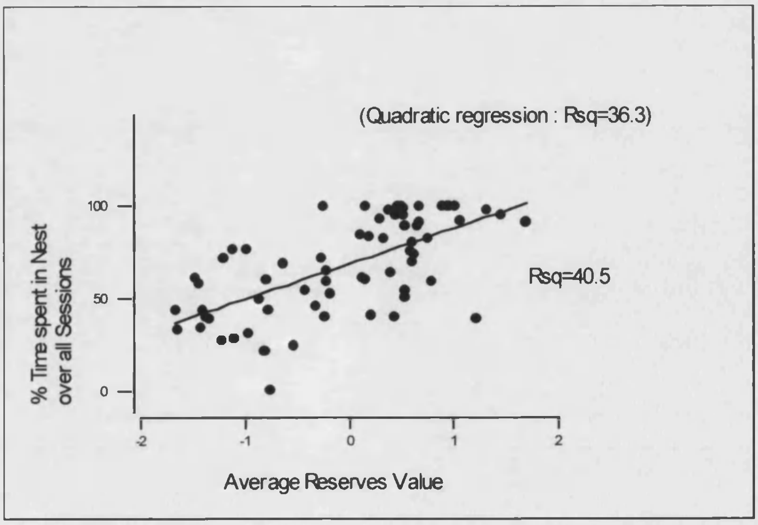
Graph 6-13 Regression of % Time spent in Nest against Average Reserves Value : Colony K



Graph 6-14 Regression of % Time spend in Nest against Average Reserves Value : Colony L



Graph 6-15 Regression of % Time spent in Nest against Average Reserves Value : Colony M



6.4 Behavioural summaries

There is much data to summarise. The summaries presented here are visual and descriptive. They are designed to give an overall impression of the 3088 ant hours of behavioural data. The data is presented in four different ways, two of which summarise the colony-level patterns of behaviour, and two of which show information about individuals. The data used in these summaries are the behavioural classifications derived from the definitions described in chapter 4, and so must be interpreted bearing the success of the definitions (presented in chapter 5) in mind. In particular it should be noted that allogrooming and trophalaxis behaviours are under-represented, and that the PAUSE and GROOM-LIKE MOVEMENT definitions do not work well for periods of low individual activity. However, as the first summary shows, the accuracy of the classification of each exact second of behaviour may not be all that important.

6.4.1 Temporal summary of individual behaviour

Figure 6-1 shows the sequence of behavioural occupation for all the ants of colony K in session 4 over the first 20 minutes of tracking. Blank interruptions to the lines occur when an ant left the nest. The behavioural occupation is colour-coded as shown in the legend of the figure. The CUES and interactions between the ants are not shown.

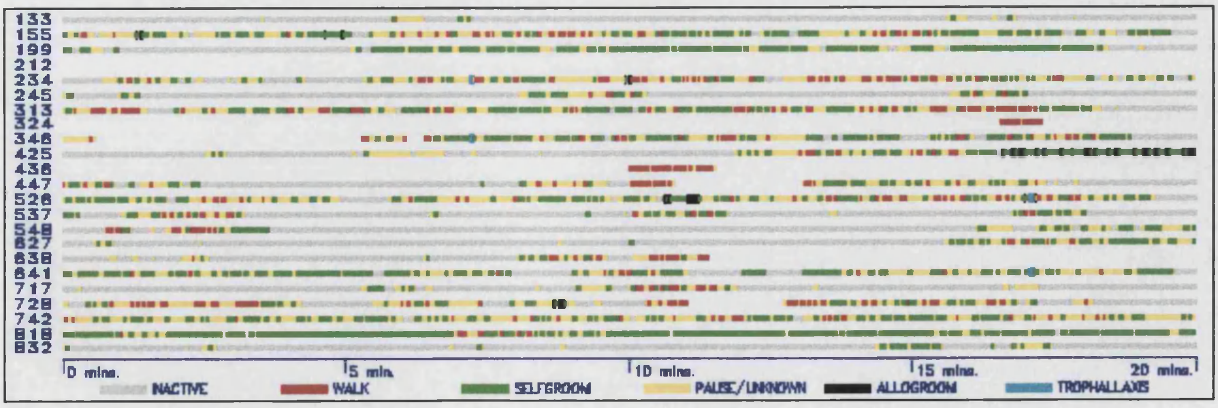


Figure 6-1 Close-up of the first 20 minutes of session K4 SELFGROOM is equivalent to GROOM-LIKE BEHAVIOUR (see text for explanation).

In chapter 5, single bouts of automatically classified behaviour were compared with single bouts from the observed data. However, it is clear from Figure 6-1 that there are longer-term 'modes' of behaviour. For example, most of the WALKING bouts (in red) tend to occur in succession, alternating for the most part with PAUSE/UNKNOWN behaviour (yellow). Similarly, periods of ALLOGROOMING are aggregated, interspersed with G-LM (or SELFGROOM) and some PAUSE/UNKNOWN. This argues that ants enter certain behavioural 'modes' for more extended periods than the bouts lengths identified in both the observational data and in the automated classifications. At least four types of 'mode' can be seen in the behavioural traces of Figure 6-1; extended periods of walking, allogrooming, self-grooming, and periods of very low activity (mostly yellow).

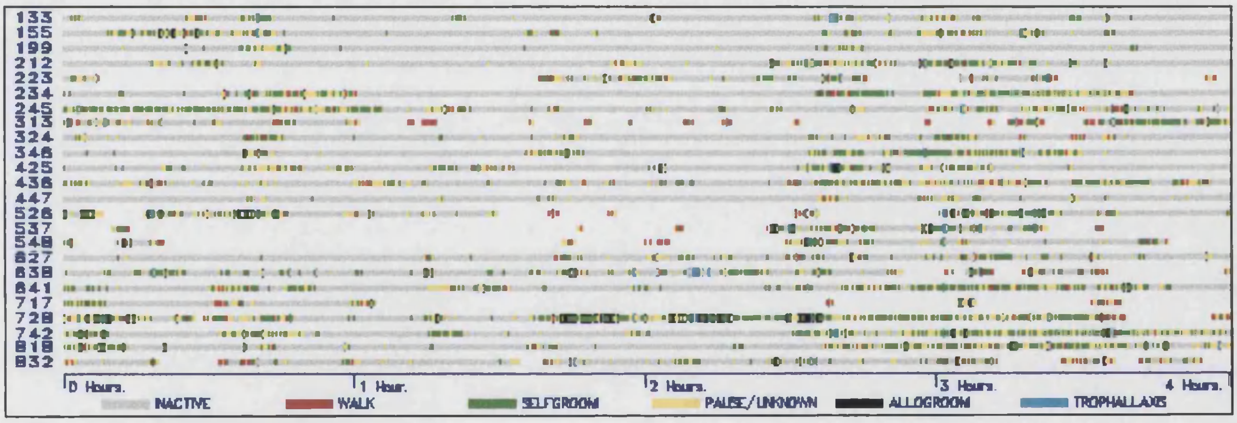


Figure 6-2 Ant behavioural histories : K1

Figure 6-2 to Figure 6-5 show the full four hours for four sessions, two from colony K and one each from colonies L and M. The other 14 sessions are shown in Appendix VI. In these figures the 'modes'

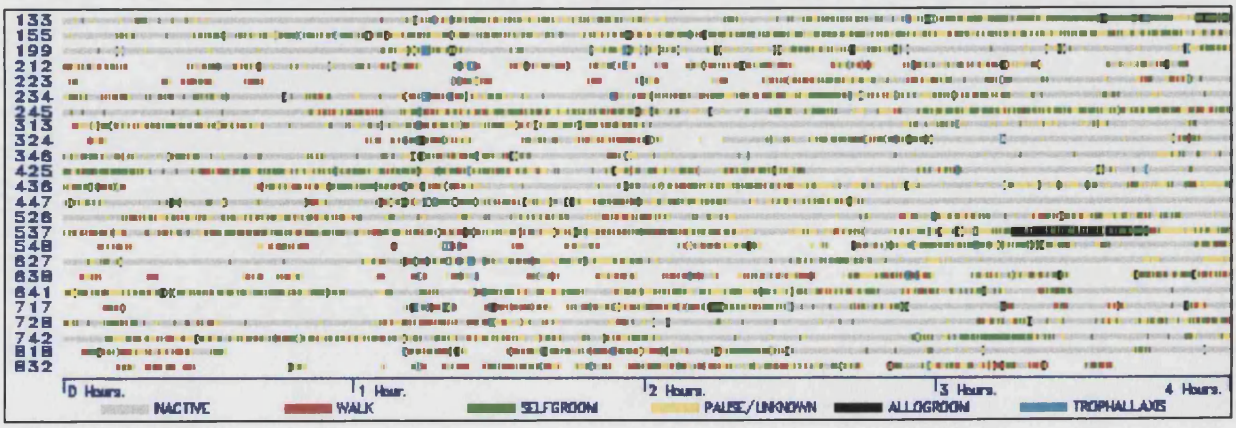


Figure 6-3 Ant behavioural histories : K3

are still clear. For example, there is little mixing of red (WALKING) with green (SELFGROOM). Individuals that leave the nest tend to do so repeatedly within a session. Individuals at the top of each figure for colonies L and M, whose number is less than 111 were unidentified ants that have been given an arbitrary number. The unidentified ants of session M3 are representative in that they normally occurred at the beginning of filming, or during periods of high activity where there was a lot of tunnel traffic.

Prior to the third session, each colony was starved of sugar and protein for 10 days. One hour into these sessions, fresh honey solution was introduced into the foraging arena. The ensuing burst of activity in the nest is clear in the figures for session K3 and M3. The dramatic increase in the amount

Figure 6-4 Ant behavioural histories : M3

of TROPHALAXIS in the half hour period following feeding suggests that the TROPHALAXIS definition is working in some instances.

The co-ordination of activity throughout the nest also becomes apparent over the four hour periods, for example in the vertical bands of activity in Figure 6-5. This is shown more clearly in the next set of figures.

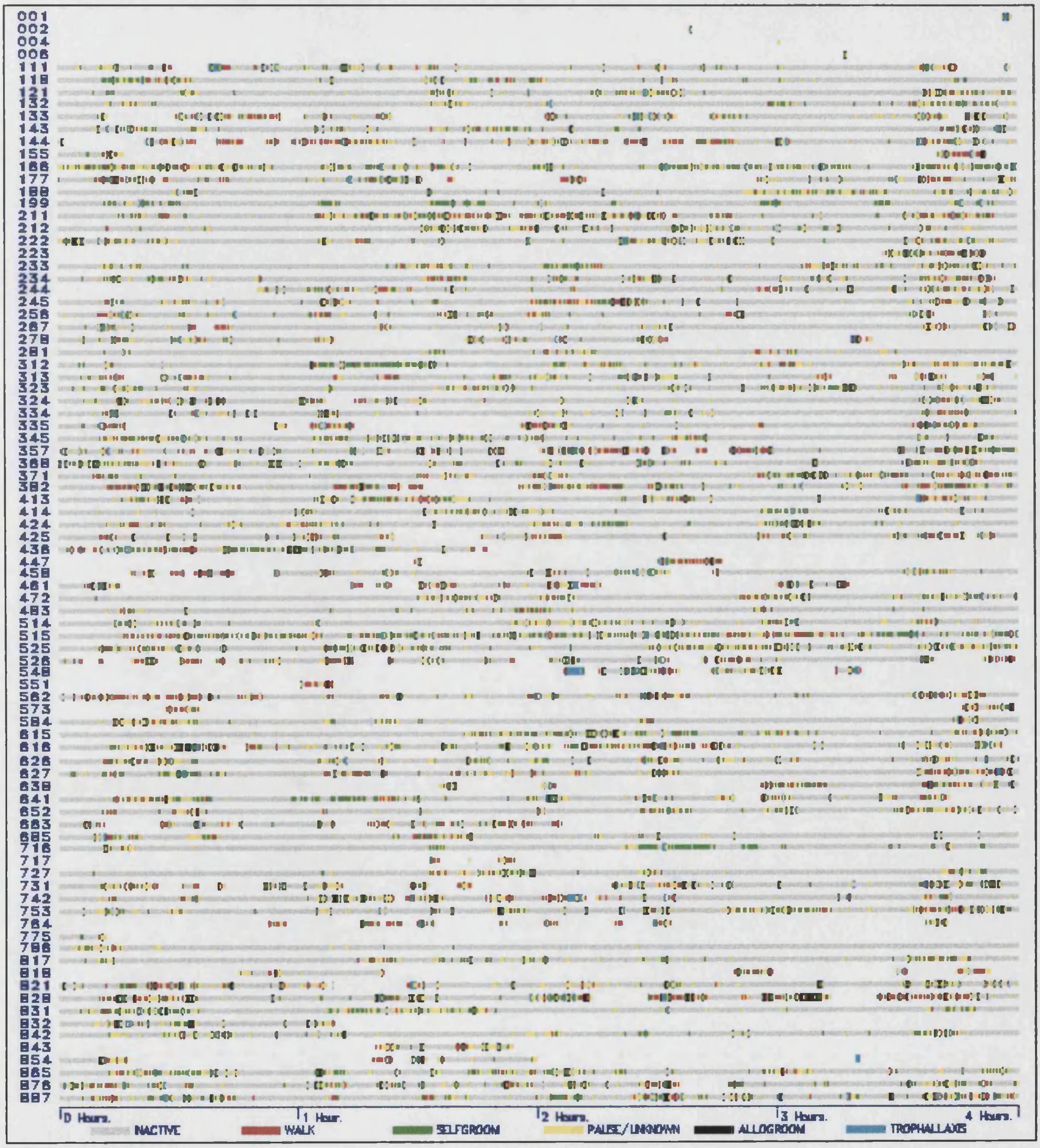


Figure 6-5 Ant behavioural histories : L4

6.4.2 Colony-level summary of behaviour

In Figure 6-6 to Figure 6-8, the number of ants in the nest occupied in the different behaviours is summarised over time. All six sessions of colonies K, L and M are shown in Figure 6-6, Figure 6-7 and Figure 6-8 respectively. The total number of ants on the y-axis represents the number of ants alive in the colony at the time of filming. The white area at the top of each graph represents the number of ants outside the nest.

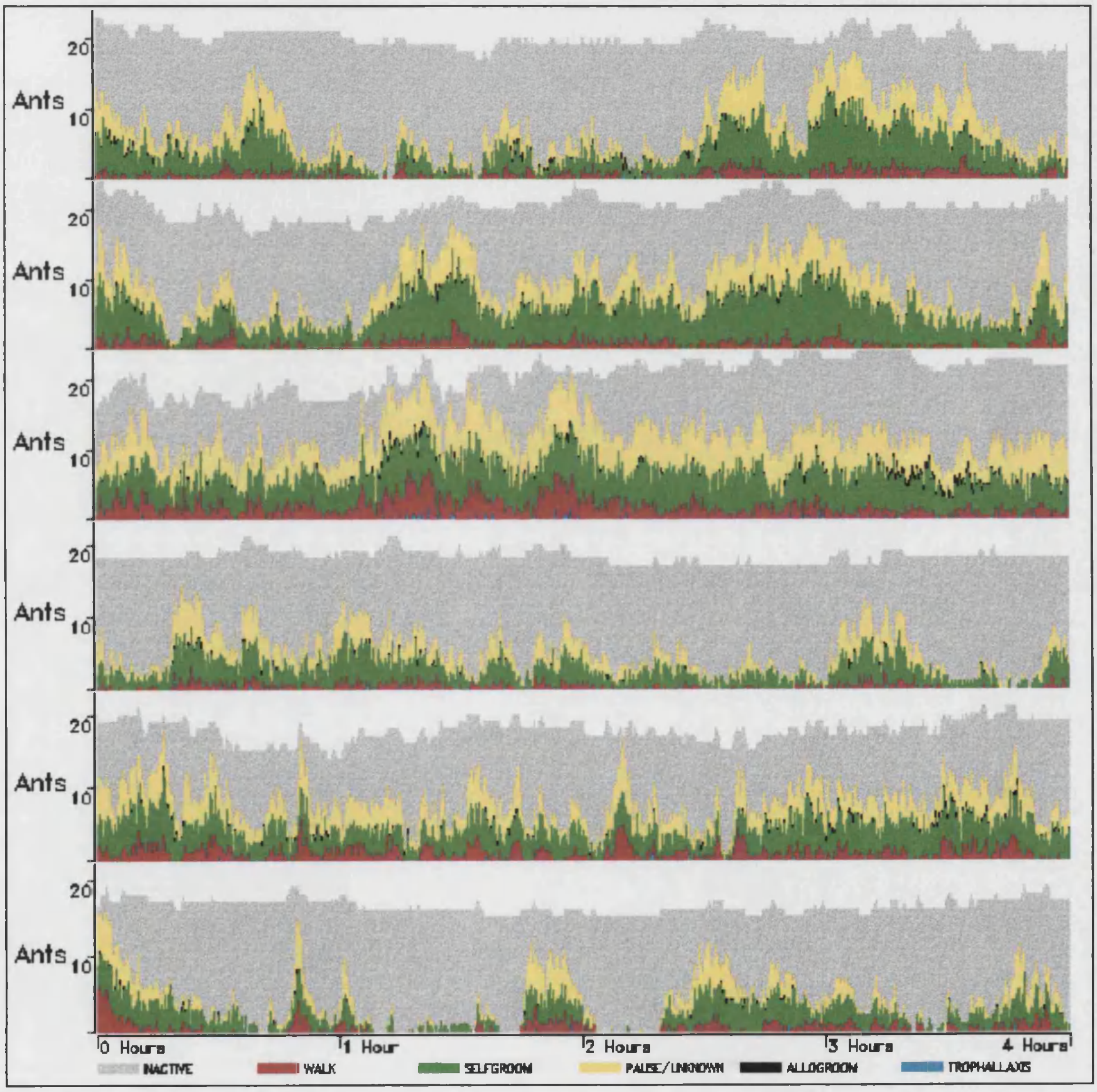


Figure 6-6 Nest behaviour summary : Colony K, sessions 1-6 (top to bottom)

Session L1 displays perhaps the clearest distinction between periods of very little activity and periods of high colony activity. However this pattern is not evinced for sessions such as M1, M2 or K5, where a relatively constant level of activity is maintained. Colony activity appears to decrease with time; colonies are less active in sessions 4-6.

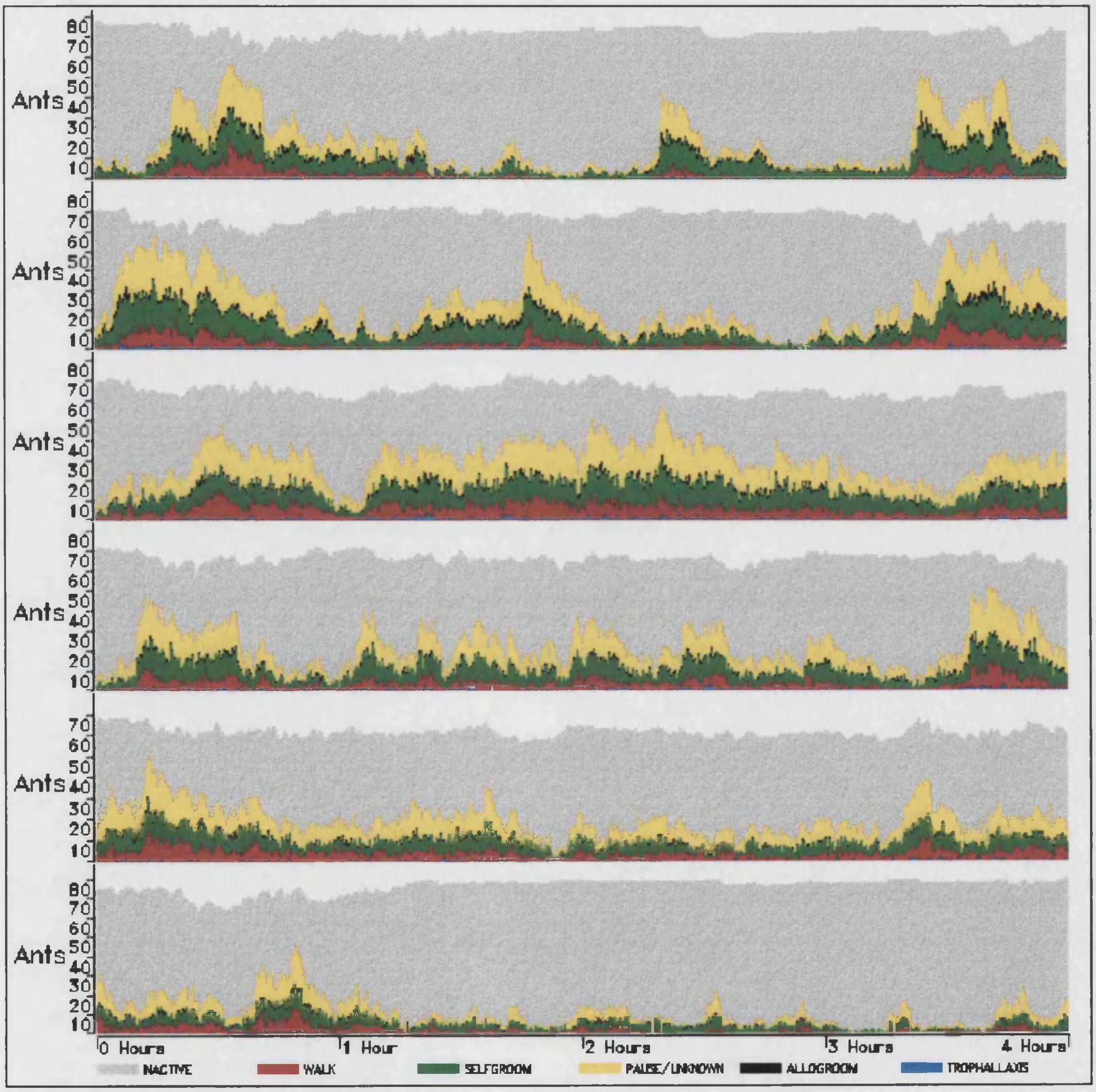


Figure 6-7 Nest behaviour summary : Colony L, sessions 1-6 (top to bottom)

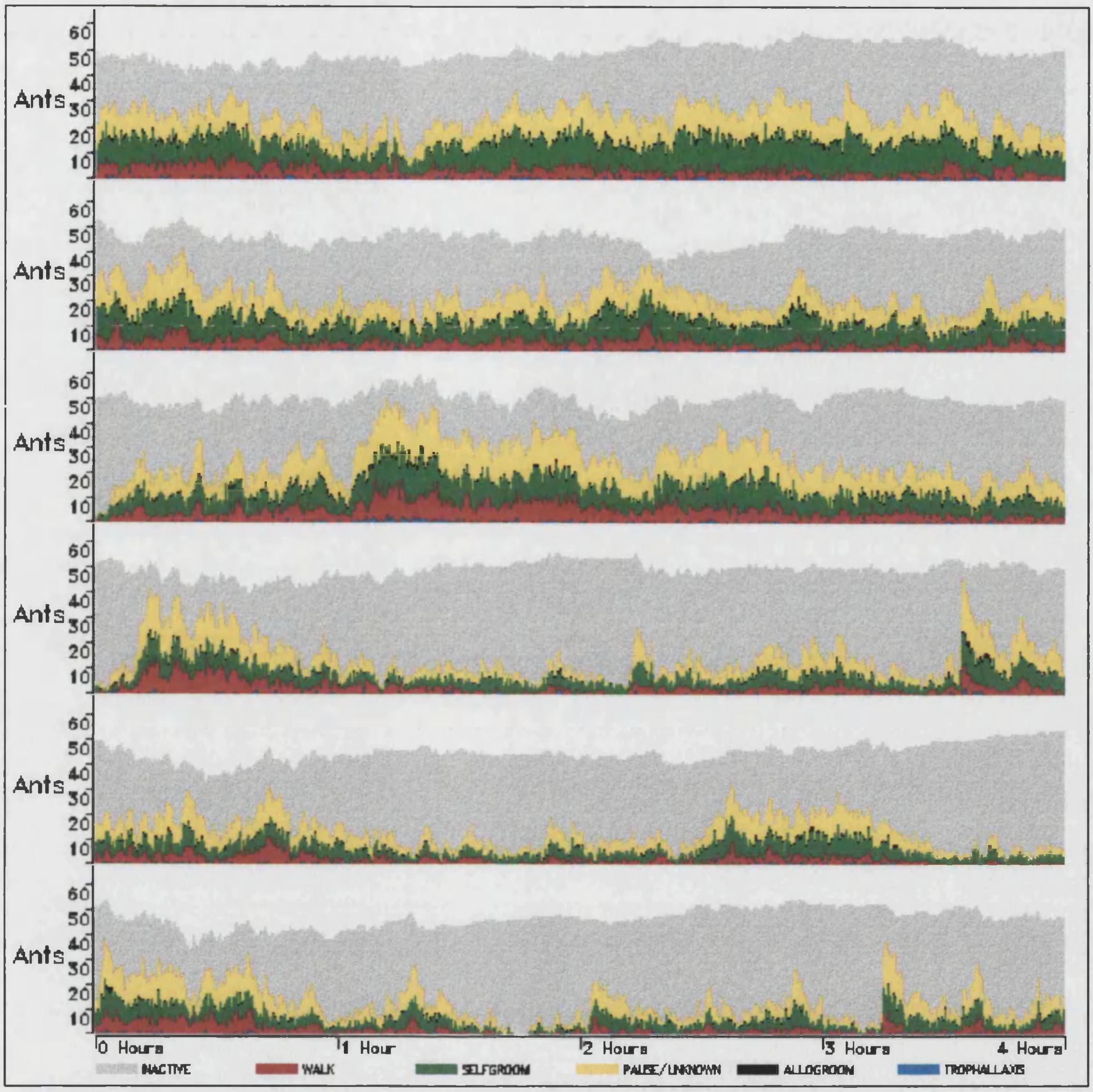


Figure 6-8 Nest behaviour summary : Colony M, sessions 1-6 (top to bottom)

There is some variation in the relative proportions of ants engaged in the different tasks for different levels of colony activity. For example, in session M1 a higher proportion of ACTIVE ants were WALKING in the first hour compared to later on in the session. However, in general the relative proportions in the figures seem to be remarkably constant.

6.4.3 Spatial summaries

The spatial location of behaviours and CUES across all sessions for each colony are summarised in Figure 6-9 to Figure 6-11. The scale for each graph is not absolute. The number of seconds in which an ant exhibited the relevant BEHAVIOUR or CUE in each grid square of the nest is tallied. The variance in the totals across the grid squares is then reduced by converting each total to its square root. Equal size bins of these square root values are calculated and coloured according to the scale at the base of the figure from white (least) to dark purple (most). Lighter graphs are those in which there is greatest variance in bin value.

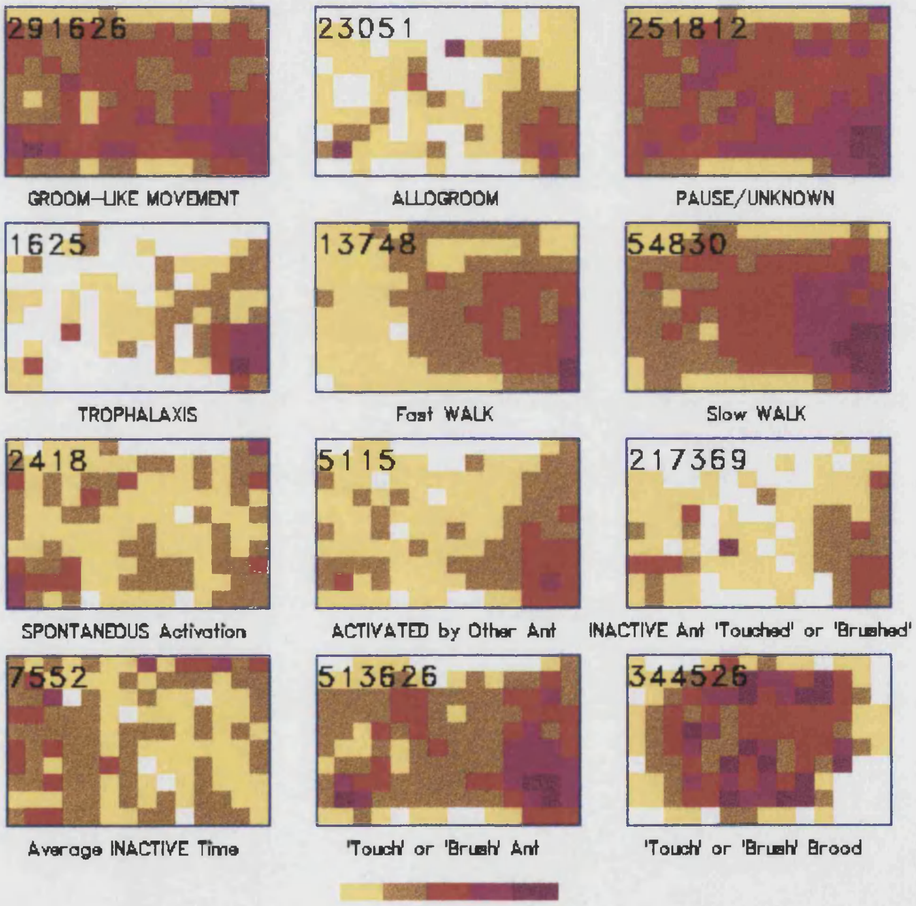


Figure 6-9 Spatial summary of behaviours : Colony K The interior of the nest is shown in each rectangle. The entrance tunnel descends off the bottom right-hand corner of the nest rectangles. The total number of ant seconds used in each figure are printed in each figure except 'Average INACTIVE Time', where the total number of bouts of INACTIVITY is printed (see text for explanation of scale).

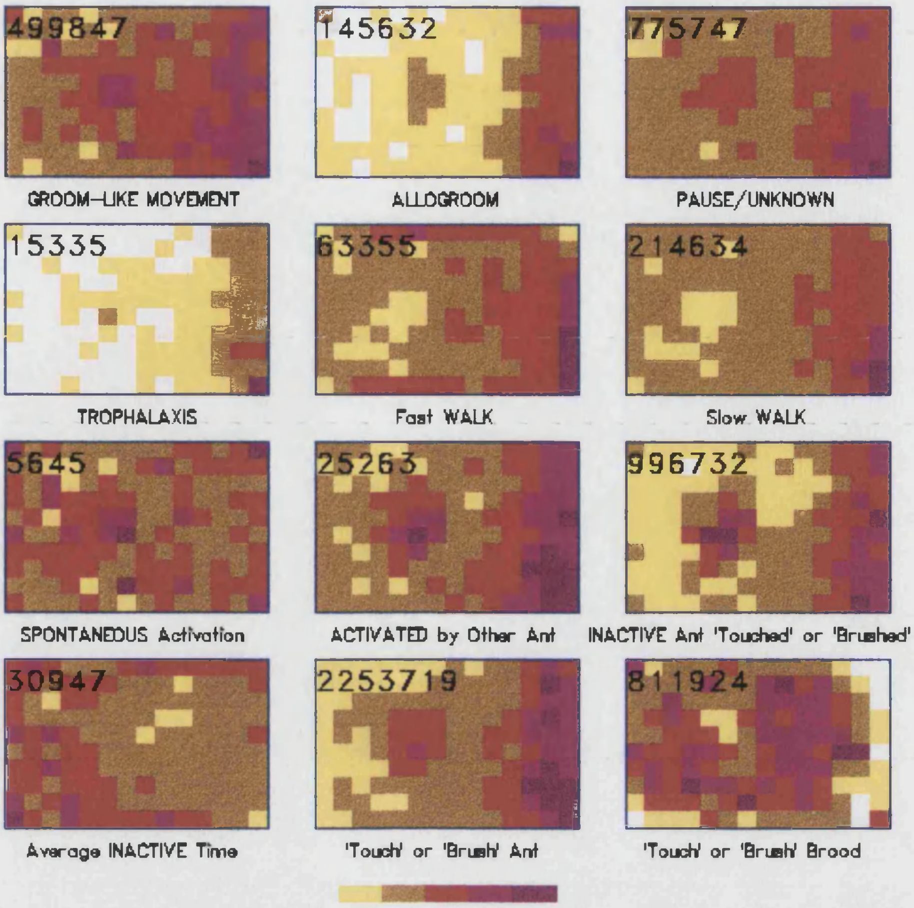


Figure 6-10 Spatial summary of behaviours : Colony L (See Figure 6-9 for explanation)

It should be noted that the nests have different structures. Colony K was considerably smaller then the other two (see Table 2-1 p.28). There was a large central pile of small brood items in colony M, causing the concentric patterns of behaviours shown in Figure 6-11. In colony L the brood items were larger and more dispersed around the nest.

Most activity occurs around or near the nest entrance in the bottom right-hand corner of each nest picture. As a result, most behaviours follow a similar spatial pattern. However there are subtle differences in the distributions of the different behaviours that are worthy of note. G-LM, PAUSE/UNKNOWN, TROPHALAXIS and ALLOGROOMING share a similar spatial pattern. They are concentrated around the nest entrance, but spread into the nest. FAST WALKING (see Table 4-1) is concentrated near the entrance and around the perimeter of the nest. SLOW WALKING is again concentrated around the entrance but more diffusely spread around the nest. Despite being a smaller colony, colony M had more FAST WALKING seconds than colony L.

While SPONTANEOUS activation seems to occur evenly throughout the nest, ACTIVATION by other ants, and ant CUES follow a very similar pattern. This pattern is the inverse of the spatial pattern of the length of average INACTIVE time.

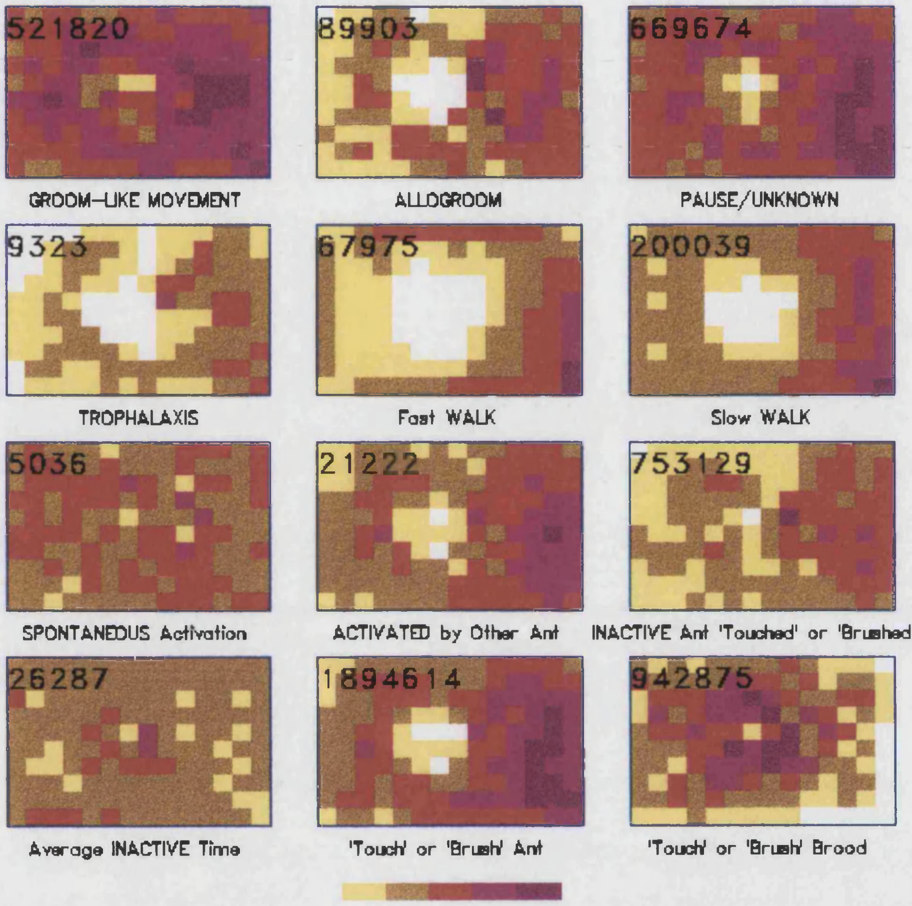


Figure 6-11 Spatial summary of behaviours : Colony M (See Figure 6-9 for explanation)

The ratio of ACTIVATED to SPONTANEOUS in colonies L and M is greater than 4:1, and greater than 2:1 in colony K.

6.4.4 Individual spatial and behavioural summaries

The spatial pattern of nest use for each individual is detailed in the following three figures. For most ants, numbered in black and blue, four maps are drawn, in two pairs side by side. The first pair (outlined and numbered in black) shows the spatial occupation of an ant before emigration, the latter pair (in blue) after emigration. If the ant died before emigration, the latter are not drawn. If the ant eclosed after emigration the former pair are not drawn.

The upper map of each pair shows the distribution of INACTIVE seconds in the nest, and the lower map shows the distribution of ACTIVE seconds. The scales of each map are absolute, in contrast to the whole nest spatial maps (Figure 6-9 to Figure 6-11), but the scales are different for pre- and post-emigration maps. For colonies K and L the colonies were emigrated between sessions 4 and 5, so there are four sessions before emigration and two after. There will therefore be approximately twice as much data in the pre-emigration maps. The scale of the post-emigration maps has been halved in order to make the maps seem similar. If this is not done, then the latter maps are mostly yellow and do not show the differences in spatial use of the nest clearly. Colony M was emigrated after session 5, so there is only one session represented in the post-emigration maps. However the scale has been kept the same as in the other two figures. The maps are drawn in the same orientation as in the spatial summaries of the previous section, with the nest entrance descending from the bottom right-hand corner of each map.

Accompanying each pair of maps is a vertical bar. This shows a time-budget of the behaviour of the ant in the maps. The height of this bar represents the number of session hours in which the ant was alive in the nest. Normally this is 4 sessions of 4 hours (=16 hours) for the pre-emigration bars in colonies K and L, and 20 hours in colony M. However, ants that died in between sessions have reduced bars. For example ant 223 of colony K (223K), escaped after session 3, so the height of the bar is reduced by 1/4 and there are no post-emigration maps. All ants in colonies L and M for which there is no pre-emigration map are callows that eclosed before the last session.

The proportion of time spent in each of the different behaviours is colour-coded according to the legend in the figures and is the same as for previous figures. Areas of white represent time spent outside the nest. For example, ant 818K spent just less than half of its time outside the nest before emigration, but most of its time outside the nest after emigration.

Unidentified ants are not shown in these figures.

Ant types

There is a clear distinction between the areas in the nest occupied by 'foragers' (ants that left the nest) of those that remained permanently in the nest. Ants that spent some time outside the nest occupied the area of the nest near the nest entrance when inside (e.g. 818K, 223L and 111M). Of those that remained in the nest, there was much variation in the pattern of spatial use. Some were evenly spread (e.g. 562L, 234L and 278M) while some seem to be specific to certain zones in the nest (e.g. 742K, 424L and 674M). Some ants that never left the nest during the filmed periods spent the majority of their time on the nest entrance side of the nest (e.g. 728K and 382L). However these are rare enough to suppose that these ants may well have left the nest at other times. The graph of the cumulative number of ants leaving the nest across sessions has not plateaued completely for colonies L and M (Graph 6-2) and suggests that there were some foragers that had not been seen leaving the nest.

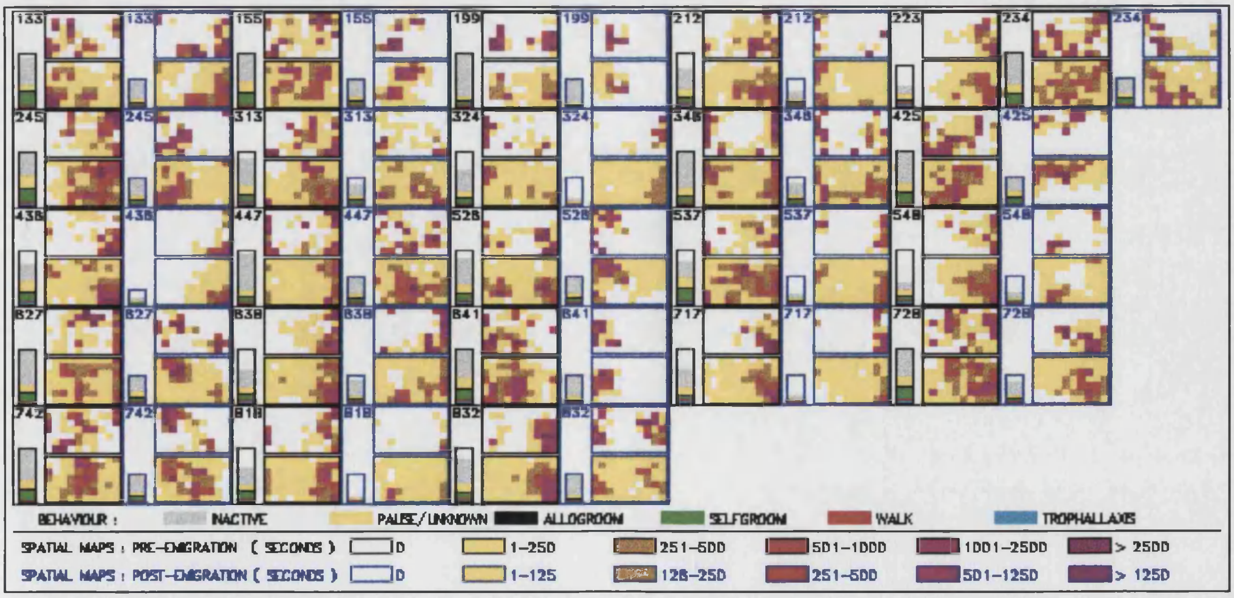


Figure 6-12 Individual spatial and behavioural summary : Colony K (See text for explanation).

Figure 6-13 Individual spatial and behavioural summaries : Colony L (See text for explanation).

The queens (all numbered 199) had limited ranges, keeping central near to or on the pile of eggs and micro-larvae. Some workers had similar ranges to the queen (e.g. 526K, 232L, 118L, 132L, 865L and 584M) while others were central but did not (e.g. 742K, 472L, 281M and 335M).

Nest coverage

Most ants covered all areas of the nest at some point during filming. Even ants that spent most of their time outside did 'tours' of the nest (around the central brood pile in colony M). However ants generally spent most of their time in a restricted area of the nest. Given the amount of time over which the tracking data was collected, the distributions are perhaps surprisingly localised. Ants of all colonies appear to have non-overlapping areas of concentrated use. Each ant occupies a different area. This implies that there is some spatial inertia that prevents ants from swapping places. This may be simply because an ant can't occupy an area if another ant is already there. Alternatively ants may recognise certain parts of the nest, through a chemical gradient or biochemical cue. This pattern is particularly striking in colony K, where the degree of overlap of areas of high activity is low.

Spatial use while ACTIVE and INACTIVE

The area in which an ant was INACTIVE was almost always very similar to the area in which it was ACTIVE.

Emigration

In all colonies the distributions before and after emigration are similar for ants that forage - they continue to use the strip of the nest near the entrance. Ants that did not leave the nest generally continued to use the interior of the nest. However, it was rare for ants to occupy the same area as before emigration. Those that did probably did so out of chance. In colony M, non-foraging workers that occupied the periphery of the large central brood pile continued to occupy the edges of the brood pile, though often in different parts of the nest.

Time budgets

Individuals were highly variable in the proportion of time that they spent ACTIVE (e.g. 447K is very much less than 245K, 188L less than 515L, and 312M less than 513M). Foragers spent a higher

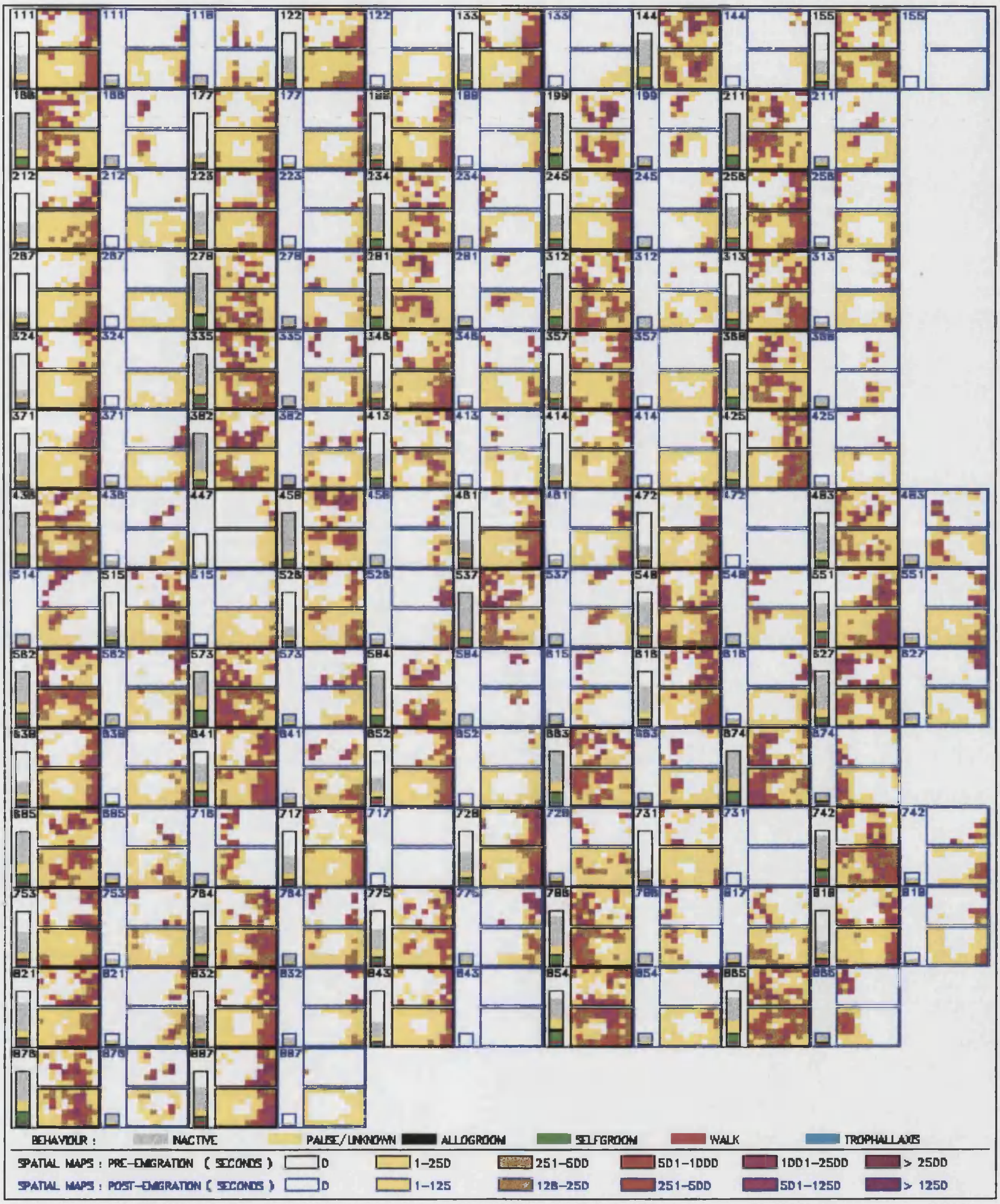


Figure 6-14 Individual spatial and behavioural summaries : Colony M (See text for explanation).

proportion of their time in the nest ACTIVE compared to those that did not leave the nest. However they tended to occupy the highly active zone around the nest entrance, and so are likely to have been activated more often than ants around the brood pile. Their time-budgets would also have be biased towards activity, since they are by definition active on entering and leaving the nest.

7. Discussion

7.1 Tracking

No published methodology is able to collect data of the quantity and quality that the ATTA produces. The quantity is a function of the automation and parallel collection of information on all the ants in a nest at once. The quality is a result of the high frequency of sampling the paths of ants. The focus on cues as an integral part of understanding ant behaviour is novel, and the attempt at automating a classification of behaviours from ant movements is unprecedented.

Other methods have been used to obtain long term information about the spatial arrangement of ants in the nest, but without the detail. Sendova-Franks and Franks (1993) recorded the positions of marked ants from slide photographs taken every half-hour, ten hours a day for five days at a time. This revealed for the first time the spatial arrangement of ants within small nests of Leptothoracine ants. Furthermore this spatial order was conserved after emigration (Sendova-Franks & Franks 1995a). This intriguing result shows that there is considerable inertia in the division of labour of these ants. The mechanism for this remains unknown, though the authors suggest that activity level might be the causal factor, with active ants foraging and inactive ones remaining in the nest. The summary results presented in this study suggest a similar pattern of spatial organisation. However there is little preliminary evidence for a two-dimensional structure that is conserved through emigration.

Intensive methods have been used to track individual ants (Fourcassié & Traniello 1995, Gordon 1995), but these attempts have had limitations that have precluded their large scale use. In the first of these studies, single ants only could be tracked, because the system could not follow ants through interactions with other ants. In the latter study, interacting foragers were tracked in a rectangular area, and an algorithm was written to keep track of ants through interactions and the coalescence of ant bodies. However, the success rate was not perfect, and the cumulative error over multiple interactions meant that knowledge of individual identity could not be assured. Both focused on ants outside the nest, where there is little complicated interaction, but the need for a wide field of view means that ants appear as little more than dots in the image.

Researchers have begun to look at the cues used by ants. For example Franks and Deneubourg (*In press*), in a study of *Leptothorax* building behaviour, assessed the building blocks that ants passed before one was picked up. This is a rare study in that it acknowledges that an understanding of the probability of an ant displaying a behaviour depends on the number and/or rate of cues it encounters.

7.1.1 Improvements to the current ATTA

A number of improvements to the existing technique that have not already been implemented are discussed in this section. Improvements to the TRAC program are required to improve the identification of ants entering the nest, to automate the measuring of item dimensions, in allowing more flexibility in the shape of non-ant items, and to the centring algorithm itself.

Ant identification

Different nest architectures might make the identification of ants entering the nest more certain, for example by ensuring that the paint marks on the back of an ant always face the observer. A Dictaphone would be useful during filming to record series of ants entering in rapid succession, but only in addition to the current automated identification procedure.

Sizes

An automatic method of checking the measurements of items during tracking is required. This has already been written for the first frame, but is not 100% successful in the case of joined silhouettes. If ants are only checked when their silhouettes are not touching another item, this should be simple to implement.

Non-ant items

Brood items come in all shapes and sizes and the current one- or two-fix items does not cater for all shapes; in particular thin but long ellipses are problematic. Introducing a middle section, as in a straight ant, would alleviate this problem. For large larvae, identifying and marking the head of the larvae as such would make the definition of the behaviour of ants feeding larvae more feasible. At the moment no differentiation is made between the head and tail end of a larva.

Centring algorithm

The centring algorithm can only allow an ant to engage in an exceptional mode with one ant, the one that is closest to it. Very rarely an ant will engage two other ants in complicated manoeuvres. For example, an ant being crossed at the waist can potentially overlap another ant's head with its own. This needs to be amended.

A more preventative approach to monitoring the development of 'stress' in fix positions would eliminate some errors, or allow them to be spotted before they have become unrecoverable. Lastly, the centring algorithm is unable to keep up with fast-moving bent ants and queens (whose thoraces are tracked) because forward movement of the head is constrained by the thorax. Different self-compatible boundary distances need to be experimented with, and perhaps the three fixes could be updated simultaneously rather than in sequence.

7.1.2 Future of the ATTA

Could an ant tracking system ever be 100% reliable?

Yes, but the system would have to be extended. There will always be some situations that the designer has not considered or seen, and hasn't allowed for. However, a hybrid system using a number of different techniques that can be called upon if the others fail may be reliable. For example, adding an automatic silhouette recognition algorithm, which takes an image and locates all the ants in the image, could be employed during troublesome periods to check the current position of the fixes. Very little alteration would be required to allow the program to run backwards in time through images. Using this, a difficult period could be approached from both sides.

However, two of the most important determinants of tracking success are the quality of the images, and the behaviour of the ants in the images. The system lends itself to colonies of monomorphic ants, since high variance in ant size means that smaller ants will be able to climb over their nest-mates.

Colour images might allow the system to identify some individuals automatically through the colour of their paint marks. However, the colour quality would have to be very good, and light reflection from the shiny gasters and paint marks is difficult to remove. Furthermore, ants in a nest can't be prevented from turning upside down, so such an approach would not be a great improvement. The use of colour might be more applicable to open situations, such as foraging, where ants are always the right way up.

Could the ATTA be applied to other areas of ant research?

The system is currently set up for the particular nest architecture used in this study. It could easily be altered to cater for different nest architectures and different situations. Leaving one or more sides of the nest open and allowing the ants to build a wall out of large grains of sand might be feasible. The building blocks would have to be opaque and large so that ants entering the field of view carrying blocks could be separated from ants entering unladen. Some work would need to be done on picking up ants entering and leaving the nest in a wide zone.

The system might be a very useful tool for studying hierarchies, since agonistic interactions are probably quite ritualised. For example, it might be straightforward to classify losers in dyads of fighting ants if they backed away or remained immobile as a sign of submissiveness.

Can the technique be applied to other animals?

It could be applied to any set-up where items moved in two dimensions and which can be filmed in such a way as to differentiate the tracking objects from their background. Obvious examples include animals and robots that move on the ground without climbing over each other, for example domestic animals such as cows, horses, deer, pigs. Rats and mice climb over each other too often. Most robots don't have the ability to climb over each other. Humans tend not to climb over each other in the normal course of public life.

A more generic approach to the number and characteristics of tracked object types would be needed so that all shapes of items can be tracked. A type would be defined by:

- the number of fixes (1+)
- the sizes or shapes of each fix, and the relative orientation of elliptical fixes
- the operators which define the relative distances between each fix of an item
- the areas of the object implicitly covered by the position of the fixes of the item
- possible changes of type if an object's shape is plastic
- the permissible movement for each type
- exceptional modes specific to certain types, although ideally these would not be necessary

General release

At the moment the ATTA is not ready for general use. It would need to be transferred completely onto a PC, the process would need to be accelerated, probably by making it capable of live tracking, and the interface needs to become much more user-friendly so that a knowledge of the inner workings is not required. Furthermore, a method of dealing with errors would be required, such as allowing the user to override the tracking system in some instances.

Hardware

Much of the hardware technology used in this project has been superseded. The bottleneck at the moment is the time it takes at least 1/3 of a second to read an image from the frame-grabber. New digital technology will allow images to be read at a fraction of this, making live tracking possible. However, using the current set-up, and adding a current top of the range Pentium chip (P200) to process images that are loaded into RAM in 1/25th of a second, it is estimated that colonies of the size used in this study could be tracked live. Further advances in hardware would allow some more complicated methods to be applied to each image to improve the success of the system.

7.1.3 Automating techniques of behavioural data capture

Continuing advances in computer technology make automated behavioural data capture a reasonable proposition for most researchers. There is a huge variety in the potential number of automated ethological techniques, so a general discussion will inevitably be incomplete. Below the arguments for and against automation that have arisen out of this study are set out.

The clear advantages of automation are repeatability, reproducibility, increases in scale and the collection of types of data that we would otherwise be unable to collect. A computer will repeat exactly the same response to identical input. This is not always true of even the most experienced ethologist. There will be variation in our attention, in our accuracy and we will be influenced by extraneous factors that bias our interpretation of behaviour, for example by projecting anthropomorphic motivation onto an animal. A computer is reliable, accurate and dispassionate.

Explicitly encoding the rules needed by a computer to interpret animal movement as distinct behaviours makes observation and experiment reproducible, since the same rules can be reused. It is also a useful exercise since it forces the encoder to be explicit about what is often implicit. This is similar to the advantages of creating word or mathematical models to force an explicit understanding of a system. As Dawkins (1983, p86) puts it, if two ethologists "could both agree on clear rules or algorithms (ideally those that a computer could use) to define each action, they would very much reduce their chance of disagreeing".

Automation can be used to collect a hugely increased amount of data, either by being able to run for longer periods, or by capturing data at a faster rate. A computer can measure many things at once, whereas we can generally only concentrate on one aspect of behaviour at a time. Computers can also collect ethological data that we are completely unable to collect, such as behaviours happening too quickly for the human eye or in alien sensory modalities, and observations that put the observer in inhospitable conditions or compromise his or her health.

However, offset against these advantages, are a number of caveats. A complicated system takes a lot of investment in time and cost to develop. A complicated system may also evolve during the course of observation as errors or potential improvements become apparent, just as a human observer is trained by experience. Automated systems may not be as robust as the human eye, so that the conditions of observation or experiment may need to be tightly controlled.

The technical limits or trade-offs in an automated system impose limits on the type of data that can be collected. For example the technique presented in this study is able to follow the gross movement of an ant, but the need to film the whole nest has meant that detail has been lost. Movements of antennae and legs that are often important to interpreting ant movement are below the level of detail of the digital images.

Hardware failures and software errors can be catastrophic to an experiment. The potential for undetected errors in software mean that rigorous checks need to be exerted over any technique. Assessment of these checks may become subjective.

Automated techniques often come under the heading of 'Objective methods'. This is surely stating the case too strongly. They are not objective - they have been set up by a subject. It is suggested that 'Repeatable' or 'Reproducible' would be more appropriate.

In conclusion there are large gains to be made in automating behavioural data capture. However, as techniques become more sophisticated, the attendant problems become more human.

7.2 Automating behavioural definitions

There is encouraging congruence between the automated and manually collected data for some behaviours (INACTIVE, ACTIVE, WALKING, waking instances, and CUE bouts) while a number of improvements in the automated definitions are needed for ALLOGROOMING and TROPHALAXIS and for periods of minimal activity. More observational data is required for the interactive behaviours such as ALLOGROOMING, TROPHALAXIS and CARRYING.

Behavioural categorisation, by observation, of a similar level of detail to that of the tracking system is not easy. It involves subjective judgement about what ants are doing, and split-second accuracy about the initiation and termination of bouts of behaviour is not possible. The observer will be biased by the recent history of each ant. It is also difficult to describe in words the intuitive and visual definitions of each behaviour. This is because it is difficult to convey the quantitative distinctions between the behaviours. For example the difference between the movements of an ant exploring slowly along a line of brood is not dissimilar to some self-grooming movements. In consequence the quantitative automated definitions have some advantages as descriptions of ant behaviour; they are not open to interpretation. The automated definitions could be used identically by other computer-based ethologists.

7.2.1 Efficiency of automation

The collection of behavioural data by observation for comparison with automatically classified behaviours is very time consuming. The video must be replayed a number of times for each ant to record the different behavioural occupations. The various cues are also often presented to an ant simultaneously but can only be recorded one at a time. Furthermore, because behaviours need to be classified for every second, prior knowledge of what is about to happen is often necessary for recording the accurate times of behaviours.

It was estimated that it took on average five times the live playing time of a section of the video-tape to record the behavioural data for one ant. Inactive ants took less, active ants much more. The repercussions of this are quite surprising. Assuming that an observer can watch a video-screen for 8 hours a day, five days a week (an impossible task), then it would take approximately 7½ years to analyse the same number of ant hours that the tracking system has tracked in this study.

7.2.2 Improvements to the behavioural definitions

More observational data is needed, especially of the rare behaviours such as allogrooming and trophalaxis. Some form of optimisation (Bunday 1984) might then be appropriate to continue the refinement of the automated behavioural definitions. This would be applicable because the building blocks of the behavioural definitions (of the MOVEMENT and BEHAVIOUR PRIMITIVES) have already been established. It is now a case of fine tuning the quantitative parameters (e.g. speed, orientation, distance) of these building blocks to obtain a better fit between the automated classifications and the observed behaviours.

The extension of the time scale of the behavioural definitions to include extended periods in different 'modes' of behaviour (as discussed in chapter 5) would be fruitful. Such 'modes' might be easier to define than the short windows currently being attempted. The 'modes' would be a useful unit for investigating the behavioural transitions of ants.

7.3 Combining non-behavioural information

Section 2.2.6 described the development of a novel method of measuring the dimensions of an ant while in the nest. In section 6.3.3 the relationship between the gaster width and the head plus thorax width was found to provide useful information about the amount of reserves (fat or sugar) in the gaster of an ant. This combination is exciting because it suggests that reserves of ants in the nest can be assessed on a regular basis without interruption. Thus changes in the amount of body reserves can be tracked, and compared to accompanying, pre-emptive or delayed changes in the behaviour of the ant. During a period of starvation, the timing of decreases in individual reserves can be compared with the timing of behavioural changes. Moreover, the colony-wide distribution of reserves can also be gauged during starvation. Do all ants lose reserves at the same rate, or does the proportion of laden ants decrease? Answers to these questions are now possible.

Gaster length was also measured at the same time as gaster width, but it was not used to develop a reserves measure because it was found that there was much variation in the angle at which gasters hung from ant bodies, and therefore that a measure of length would have been unreliable. However, gaster length almost certainly shows more variation than gaster width because the gastral tergites can extend backwards. This added variation might make up for the supposed error in the measurement. This has yet to be assessed.

It is also possible that the two measures might reflect different aspects of resource loading. The fat bodies are located under the first gastral tergite, towards the thorax, whereas the crop extends posteriorly. It may be that gaster width reflects fat storage more than liquid in the crop, and *vice versa* for gaster length.

7.4 Future directions

The data remains to be analysed quantitatively. There is therefore a huge amount of work still to be done with the current data. The priority is improving the automated definitions for TROPHALAXIS, ALLOGROOMING, and low activity behaviour. Thereafter, the investigation of contextual behaviour transition probabilities and the subsequent construction and exploration of a realistic model of nest behaviour are exciting prospects.

7.4.1 Contextual behaviour transition probabilities (CBTPs)

These are the probabilities of an ant changing behaviour given the properties of the ant, its history and the properties of the local environmental of the ant. Given enough experimental data on ants in a variety of combinations of these factors, a probability of staying in the current behaviour or changing into another behaviour can be taken straight from the data. The effect of the various influences on the transition probabilities can also be calculated.

Taking the example of the transition from active to inactive and *vice versa*, one might expect the following factors to influence the probability of an ant changing state: time in the current state, the recent history of states, the hunger level of the ant, the number of tactile cues received in the recent past, the CO_2 levels in the part of the nest, the number of inactive ants within touching distance and so on.

7.4.2 Modelling

The components of a realistic model of ant nest behaviour can be broken down as follows:

1. A description of the physical environment (e.g. nest structure, brood items and possibly chemical gradients).

2. A description of the physical properties of the agents (e.g. size, flexibility of form).

3. A description of the state of each agent. These are the intrinsic and non-permanent influences on behaviour (e.g. size, reserves, genetic predispositions, age).

4. A rule set for changing behaviour. This is the set of contextual behaviour transition probabilities of the ants.

5. The movement rules of the ants in different behaviours or 'modes'.

The ATTA can for the first time provide detailed information on components 3,4 and 5, about which many assumptions would previously have had to be made. The model can be parameterised from real ant behaviour. Such a model could then be tested using parallel experiments in the model and in real ant nests. The exploration of such a model represents one of the most interesting opportunities afforded by the novel types of data collected in this study.

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Appendix I : Example 'C' coding

An example of part of the TRAC program written in the 'C' computer language (Kernighan & Ritchie 1978) is shown below. The following function is the 'extrapolate' algorithm (p. 52). This function finds new positions for the gaster and head fixes of an ant moving forwards very quickly. The particular case that this solves is one where the fix of the gaster in the previous image is so far behind the new silhouette of the ant that the circular search algorithm (see section 2.5.2) fails to locate the edge of the new silhouette.

Starting from the old gaster fix position, the function attempts to find black pixels in the direction of the gaster to head vector of the ant in the previous image. If black pixels are found, and they are not near the fix of another item, then the gaster fix is moved onto the edge of the supposed gaster area. Since this will have brought the gaster fix towards the head, the head fix must then be pushed away from the gaster in the same direction in which the gaster fix moved.

```
#include<math.h>

//Global variables available to subroutine
int xmax, ymax;                              //Dimensions of nest, in pixels
unsigned char pixget[MAX_Y][MAX_X];          //Pixel map of nest (0=White, 1=Black)

//Macro definitions
#define WHITE 0                              //Colour of pixel map pixget[][]
#define BLACK 1                              //
#define FAIL 1                               //Error flag
#define OK 0                                 //Success flag
#define NODANGER 1                           //Return flag of Out_of_Danger() function

int extrapolate(int head_wid,int thor_len,int gast_len,     //dimensions of ant
                       int head_xy[2],int gast_xy[2])       //x and y co-ordinates
/*Local variables */
{       int newx, newy;
        float x_diff, y_diff, x_inc, y_inc, gradient, jump;
        int Out_of_Danger();                 //Prototype of function that checks if
                                             //pixel is 'owned' by another item

/* Calculate the x and y distances between head and gaster */
        x_diff = (float)(head_xy[0] - gast_xy[0]);
        y_diff = (float)(head_xy[1] - gast_xy[1]);

/* Calculate gradient of line. Guard against dividing by zero */
        if(x_diff == 0.0) x_diff=0.001;
        gradient = y_diff / x_diff;

/*x component of unit vector in direction of head is, by simple algebra,
                                      1 / square root of (gradient² + 1²) */
        x_unit_vect = 1.0 / sqrt(gradient*gradient + 1.0);

/*multiply unit vector by a quarter of a head width to get x component of line a
quarter of a head width long in the direction of the head */
        x_inc = x_unit_vect * (float)head_wid / 4.0;

/*get y component of same line */
        y_inc = x_inc * gradient;

/*Look along the gaster-head line until 2.5 head widths from gaster */
        for(jump=1.0;jump <= 10.0;jump++)
        {       newx = gast_xy[0] + (int)(jump*x_inc);
                newy = gast_xy[1] + (int)(jump*y_inc);

/*Check that new position within nest */
                if(newx >= 0 && newy >= 0 && newx < xmax && newy < ymax)
/*..that not background colour */
                        if(pixget[newy][newx]==BLACK)
/*..and not belonging to another ant
i.e. approx. more than a head width from nearest alien fix */
                                if(Out_of_Danger(newx,newy,head_wid,2)==NODANGER)
/*New gaster position found*/
                                {       gast_xy[0] = newx;
                                        gast_xy[1] = newy;

/*Now push the head position out in same direction by one ant's length*/
                                        x_inc = (float)(head_wid/2+thor_len+gast_len/2)/
                                                sqrt(gradient*gradient + 1.0);
                                        y_inc = x_inc * gradient;

                                        head_xy[0] = gast_xy[0] + x_inc;
                                        head_xy[1] = gast_xy[1] + y_inc;

                                        return OK;
                                }
        }
/*If loop completed, then no valid pixel found */
        return FAIL;
}
```

Appendix II : TRAC result file format

After every image has been analysed, the new co-ordinates for each ant and brood item in the nest are recorded. The records for each item are kept in a buffer in RAM and flushed to the hard disk when this buffer is full. The files on the hard disk are named by the type of item, the start image number of the path, and the identity number of the item. For example, 1_145224.313 is a file of the path of ant number 313 (with colour marks orange-white-orange) which entered when the time-code was 145224 (approximately 1.5 hours into the tape). The starting '1' codes for a worker ant, '0' codes for a brood item and '2' for a queen.

Each result file starts with some introductory information: duration of the period of tracking, the dimensions of the item, and the starting x and y co-ordinates in the nest. For example '360000 15 26 22 054 153 099 150' would start a file that contains the path of an ant which lasted 360000 time-code ticks which at 25 ticks a second means that the ant was tracked for 4 hours exactly. It therefore did not leave the nest at all. The ant had a 15 pixel head width, a 26 pixel thorax length and a 22 pixel gaster length and at the start of tracking the head was located at [54,153], the gaster at [99,150].

Thereafter the file contains information about changes in the x and y co-ordinates of each fix in each subsequent image, head first. So for an ant whose head, thorax and gaster were all being tracked, there will be six entries; the x and y co-ordinates of the head, thorax and gaster, in that order. The information is stored in binary format, as a series of '1's and '0's, for efficiency. Entries are written in the following format:

- If there was no change in the co-ordinate value, then the entry is 1 bit long:

  1st bit is set to '0'

- If there was a change, then the entry is 7 bits long:

  1st bit is a '1'

2nd bit is a '1' if the change in fix position was positive, '0' if negative

3rd to 7th bits represent the magnitude of the change in binary notation, from 0 - 31

For example a code of '1 0 00010' would mean a change of -2 pixels, '1 1 00101' a change of +5 pixels.

There are three exceptional types of entry:

1. The maximum magnitude of change that can be coded with five bits is 31. It is extremely rare for an ant to move more than 31 pixels in 2/25th of a second, but possible. For numbers greater than 30, 31 is written (i.e. '11111'), then the following pixel is set to '0' and the remainder is written in the next five pixels. This is repeated until there is no remainder. For example, in the unlikely event of a change of +71, '1 1 11111 0 11111 0 01001', or 31+31+9, would be stored.
2. The end of the file, or a break, is coded for by '1 1 11111 1' and rest of the current byte is ignored.
3. If an ant changes from straight to bent or *vice versa*, a signal of '1 0 11111 1' is written to register the change in the number of fixes being recorded. If the change is from two fixes to three, then rather than store the starting co-ordinates of the thorax, it is assumed that the thorax starts exactly 2/3rds of the way between the gaster and the head, and the subsequent entries for change in the thorax co-ordinates denote the deviation of the real starting co-ordinates from this default position.

Appendix III : Individual ant statistics

Table III-1 Ant statistics

Ant identity	Weight on collection (microg)	Weight on freezing (microg)	Head width) (mm)	Thorax width (mm)	Gaster width (mm)	Gaster length (mm)	% of filmed time spent in nest for each of 6 sessions						
							1	2	3	4	5	6	Av.
Colony K													
199 (Q)	1050	879	0.70	0.77	0.94	1.39	100	100	100	100	100	100	100
111	290	Died 06/03/95											
212	224	202	0.52	0.32	0.54	0.73	100	100	69	7	67	36	63
313	215	221	0.50	0.32	0.52	0.63	46	100	99	100	53	100	83
414	221	Died 04/03/95											
515	221	Died 01/03/95											
616	215	Died 01/03/95											
717	352	319	0.57	0.37	0.63	0.84	32	50	68	100	52	7	52
818	243	197	0.51	0.33	0.55	0.73	100	63	79	10	18	11	47
821	242	Died 06/03/95											
122	259	Died 07/03/95											
223	200	Esc 19/4/95	0.49	0.30	0.52	0.57	40	52	53	Esc.	Esc.	Esc.	48
324	266	247	0.54	0.33	0.57	0.73	100	100	61	1	36	9	51
425	235	282	0.53	0.32	0.55	0.76	100	100	100	100	100	100	100
526	304	330	0.54	0.34	0.61	0.76	62	81	100	100	100	100	91
627	223	228	0.51	0.32	0.57	0.65	100	100	100	100	100	100	100
728	211	205	0.49	0.31	0.53	0.65	92	100	100	100	49	100	90
731	314	Died 03/03/95											
832	301	336	0.54	0.34	0.58	0.79	83	79	56	100	99	89	84
133	241	221	0.51	0.32	0.56	0.76	100	100	100	61	100	75	89
234	240	292	0.53	0.34	0.60	0.80	100	100	100	100	100	100	100
335	297	Died 06/03/95											
436	215	Esc 19/4/95	0.52	0.31	0.53	0.67	100	63	89	Esc	Esc	Esc	84
537	187	192	0.49	0.31	0.50	0.65	27	82	100	100	63	10	64
638	230	248	0.51	0.32	0.54	0.68	82	38	49	78	67	100	69
641	243	299	0.51	0.32	0.56	0.83	100	100	100	100	100	100	100
742	280	323	0.53	0.33	0.59	0.82	100	100	100	100	100	100	100
843	253	Died 06/03/95											
144	272	Died 06/03/95											
245	261	270	0.53	0.34	0.57	0.68	100	100	100	100	100	100	100
346	307	276	0.54	0.34	0.60	0.75	100	100	100	100	75	82	93
447	284	291	0.54	0.33	0.58	0.75	100	100	100	72	99	100	95
548	202	214	0.50	0.31	0.50	0.58	40	40	52	31	57	20	40
155	231	260	0.51	0.31	0.55	0.72	100	100	100	100	100	100	100
Mean	250.9	259.7	0.520	0.324	0.559	0.718	83	85	86	79	78	73	80
St Dev	38.9	46.9	0.020	0.015	0.035	0.075							
Colony L													
199 (Q)	N/A	1011	0.70	0.79	0.96	1.47	100	100	100	100	100	100	100
111	364	342	0.58	0.36	0.66	0.71	79	84	36	90	66	81	73
212	572	688	0.65	0.42	0.79	1.22	100	100	100	100	100	100	100
313	431	400	0.60	0.40	0.67	0.77	0	59	82	83	90	89	67
414	398	493	0.59	0.39	0.71	1.01	100	100	100	100	100	100	100
515	323	369	0.55	0.34	0.65	0.91	100	100	100	100	100	100	100
616	438	490	0.62	0.41	0.70	0.89	100	69	73	92	93	99	87
717	363	418	0.60	0.37	0.63	0.78	17	74	1	3	1	0	16
818	412	396	0.60	0.40	0.67	0.94	82	80	73	24	87	90	72
821	462	479	0.63	0.42	0.71	0.87	92	93	78	91	89	91	89

Identity code: 1=White 2=Yellow 3=Orange 4=Red 5=Pink 6=Light blue 7=Dark blue 8=Green Q=Queen

Table III-1 Ant statistics (continued)

Ant identity	Weight on collection (microg)	Weight on freezing (microg)	Head width) (mm)	Thorax width (mm)	Gaster width (mm)	Gaster length (mm)	% of filmed time spent in nest for each of 6 sessions						
							1	2	3	4	5	6	Av.
122	441	Accidental death 01/03/95											
223	388	483	0.61	0.39	0.70	0.85	82	57	30	11	10	1	32
324	422	406	0.61	0.40	0.68	0.85	91	96	100	97	76	100	93
425	426	428	0.62	0.40	0.70	0.89	94	79	42	90	77	80	77
526	364	386	0.57	0.37	0.63	0.75	91	57	51	72	29	88	65
627	380	350	0.59	0.38	0.68	0.83	96	73	13	100	90	66	73
728	301	Accidental death 01/03/95											
731	401	421	0.60	0.39	0.67	0.82	20	38	69	66	44	88	54
832	414	434	0.62	0.38	0.70	0.83	100	70	76	29	0	18	49
133	482	513	0.64	0.42	0.73	0.96	86	85	100	68	52	51	74
234	429	438	0.61	0.41	0.69	0.82	77	52	30	98	92	81	72
335	452	489	0.62	0.42	0.69	0.87	99	70	27	64	71	87	70
436	381	323	0.58	0.38	0.66	0.78	96	79	36	42	74	93	70
537	409	Accidental death 01/03/95											
638	455	470	0.63	0.41	0.67	0.77	61	39	54	30	1	86	45
641	463	461	0.63	0.41	0.73	0.89	85	45	48	90	64	92	71
742	403	414	0.60	0.39	0.68	0.85	100	72	64	90	64	91	80
843	422	407	0.60	0.39	0.66	0.82	77	76	35	14	100	95	66
144	431	429	0.62	0.41	0.70	0.85	74	73	27	79	20	46	53
245	445	501	0.61	0.39	0.72	0.89	100	100	100	100	56	100	93
346	356	Esc. 1/5/95	0.61	0.40	0.68	0.89	12	0	0	0	0	Esc	2
447	481	455	0.65	0.43	0.70	0.82	0	5	4	7	10	8	6
548	408	435	0.59	0.39	0.66	0.83	80	90	27	20	63	100	63
551	466	491	0.64	0.42	0.69	0.84	49	9	17	3	6	9	16
652	405	415	0.59	0.39	0.64	0.81	100	80	100	100	100	100	97
753	460	538	0.61	0.40	0.73	0.99	40	21	100	100	0	100	60
854	375	378	0.61	0.38	0.66	0.88	77	79	48	21	22	96	57
155	493	493	0.64	0.42	0.70	0.88	91	12	96	12	41	100	59
256	389	456	0.60	0.38	0.67	0.90	82	97	47	95	87	100	85
357	466	511	0.64	0.44	0.72	0.95	0	14	27	87	8	95	38
458	464	518	0.63	0.43	0.71	0.88	66	91	44	80	43	91	69
461	461	467	0.63	0.41	0.70	0.97	8	15	16	36	3	2	13
562	382	387	0.60	0.38	0.65	0.78	98	94	100	98	96	99	98
663	387	382	0.61	0.40	0.68	0.80	85	6	46	42	34	46	43
764	399	413	0.60	0.39	0.62	0.80	8	24	81	14	0	8	23
865	460	531	0.60	0.40	0.76	0.91	100	100	100	100	100	100	100
166	438	537	0.62	0.40	0.75	1.03	100	100	100	100	86	100	98
267	417	360	0.59	0.39	0.67	0.80	94	95	38	97	10	11	58
368	415	448	0.61	0.40	0.69	0.84	100	75	100	100	100	100	96
371	361	399	0.57	0.36	0.68	0.82	100	100	100	100	100	100	100
472	376	489	0.61	0.38	0.70	0.85	100	100	100	100	100	100	100
573	412	407	0.61	0.41	0.66	0.78	10	15	96	9	0	0	22
674	372	Esc. 1/5/95	0.59	0.40	0.65	0.79	90	71	0	0	0	Esc	32
775	412	438	0.61	0.40	0.66	0.83	100	73	59	5	67	99	67
876	410	391	0.60	0.38	0.68	0.79	96	100	54	95	96	100	90
177	344	369	0.60	0.37	0.64	0.80	59	84	43	67	18	26	50
278	424	423	0.63	0.40	0.66	0.80	56	2	24	52	17	61	35
281	356	412	0.57	0.35	0.69	0.89	100	100	100	100	100	100	100
382	326	346	0.57	0.36	0.61	0.79	100	87	99	89	90	95	93
483	457	509	0.61	0.38	0.74	0.88	100	100	100	100	100	100	100
584	488	566	0.63	0.42	0.76	0.90	100	100	100	100	100	100	100

Identity code: 1=White 2=Yellow 3=Orange 4=Red 5=Pink 6=Light blue 7=Dark blue 8=Green Q=Queen

Table III-1 Ant statistics (continued)

Ant identity	Weight on collection (microg)	Weight on freezing (microg)	Head width) (mm)	Thorax width (mm)	Gaster width (mm)	Gaster length (mm)	% of filmed time spent in nest for each of 6 sessions						
							1	2	3	4	5	6	Av.
685	421	485	0.61	0.40	0.69	0.86	100	87	100	100	100	100	98
786	427	532	0.60	0.39	0.75	1.02	100	100	100	100	100	100	100
887	367	319	0.59	0.38	0.64	0.71	91	40	100	99	92	86	85
188	649	688	0.67	0.45	0.85	1.26	100	100	100	100	100	100	100
211	365	348	0.59	0.36	0.64	0.76	100	100	99	98	97	99	99
312	452	460	0.61	0.40	0.74	0.97	100	100	100	100	100	100	100
413	481	519	0.62	0.41	0.76	1.02	100	100	100	100	100	100	100
514	373	445	0.58	0.35	0.68	0.87	100	100	100	100	100	100	100
615	452	474	0.62	0.41	0.75	0.95	100	100	100	100	100	100	100
716	459	528	0.62	0.40	0.75	0.97	100	100	100	100	100	100	100
817	532	544	0.62	0.41	0.74	0.95	100	100	100	100	100	100	100
118	494	514	0.61	0.41	0.74	0.96	100	100	100	100	100	100	100
121	435	501	0.62	0.40	0.73	0.96	100	100	100	100	100	100	100
222	424	476	0.63	0.39	0.72	0.98	100	100	100	100	100	100	100
323	535	622	0.63	0.42	0.79	1.03	100	100	100	100	100	100	100
424	406	512	0.60	0.38	0.72	0.98	100	100	100	100	100	100	100
525	467	500	0.61	0.40	0.74	0.92	100	100	100	100	100	96	99
626	411	481	0.60	0.38	0.74	0.99	100	100	100	100	100	100	100
727	401	506	0.61	0.38	0.73	0.94	100	100	100	100	100	100	100
828	441	454	0.62	0.39	0.72	0.89	100	43	100	89	100	100	89
831	436	531	0.61	0.40	0.75	1.05	100	100	100	100	100	100	100
132	409	506	0.61	0.39	0.75	0.95	100	100	100	100	100	100	100
233	453	517	0.60	0.38	0.73	1.00	100	100	100	100	100	100	100
334	435	454	0.58	0.38	0.74	0.93	100	100	100	100	100	100	100
842	459	519	0.62	0.41	0.73	0.83	100	100	100	100	100	100	100
143	466	585	0.61	0.40	0.76	1.02	100	100	100	100	100	100	100
244	411	343	0.58	0.39	0.70	0.97	27	100	85	79	86	0	63
345	468	531	0.61	0.40	0.75	0.91	100	100	100	100	100	100	100
Mean	447.5	496.2	0.610	0.395	0.733	0.952	83	77	74	77	71	82	77
St Dev	57.5	75.9	0.019	0.019	0.039	0.099							

Colony M

199 (Q)	N/A	1078	0.71	0.77	0.92	1.28	100	100	100	100	100	100	100
111	459	481	0.60	0.39	0.67	0.87	57	61	43	58	77	68	60
212	357	364	0.60	0.39	0.64	0.81	4	11	83	46	80	12	39
313	377	359	0.60	0.39	0.63	0.73	85	79	25	53	60	51	59
414	408	381	0.56	0.36	0.60	0.74	26	15	46	81	48	25	40
515	344	303	0.58	0.39	0.62	0.72	37	38	26	56	25	19	33
616	378	365	0.59	0.38	0.66	0.81	92	56	53	69	92	52	69
717	343	322	0.55	0.35	0.58	0.73	28	57	53	59	69	0	44
818	353	315	0.60	0.39	0.64	0.74	14	29	85	65	29	46	44
821	394	399	0.62	0.40	0.66	0.72	95	79	60	70	78	49	72
122	384	377	0.55	0.34	0.56	0.70	64	31	71	54	54	5	46
223	342	357	0.59	0.38	0.65	0.79	87	46	100	54	40	36	61
324	397	370	0.62	0.38	0.65	0.81	12	54	41	0	50	8	27
425	423	453	0.60	0.38	0.67	0.84	79	54	69	76	56	37	62
526	431	495	0.64	0.41	0.65	0.85	7	47	20	65	36	71	41
627	488	591	0.64	0.41	0.78	0.93	100	75	79	100	100	100	92
728	378	384	0.58	0.37	0.64	0.78	50	42	95	24	95	86	65
731	384	381	0.60	0.39	0.65	0.85	14	0	24	88	5	0	22
832	390	414	0.61	0.39	0.66	0.76	63	59	63	83	12	65	58

Identity code: 1=White 2=Yellow 3=Orange 4=Red 5=Pink 6=Light blue 7=Dark blue 8=Green Q=Queen

Table III-1 Ant statistics (continued)

Ant identity	Weight on collection (microg)	Weight on freezing (microg)	Head width) (mm)	Thorax width (mm)	Gaster width (mm)	Gaster length (mm)	% of filmed time spent in nest for each of 6 sessions						
							1	2	3	4	5	6	Av.
133	352	373	0.55	0.34	0.60	0.71	66	54	75	70	38	3	51
234	503	528	0.64	0.42	0.77	1.05	100	100	100	2	100	98	83
335	422	444	0.56	0.35	0.60	0.79	100	100	100	100	100	100	100
436	460	540	0.63	0.40	0.73	0.99	96	100	100	100	100	54	92
537	564	662	0.64	0.43	0.79	1.01	100	100	100	100	100	92	99
638	464	535	0.62	0.41	0.72	0.88	20	28	87	66	12	26	40
641	254	246	0.57	0.34	0.62	0.83	84	88	53	72	87	81	77
742	295	343	0.55	0.35	0.62	0.75	100	100	55	99	100	55	85
843	372	330	0.59	0.38	0.62	0.66	54	23	44	60	26	0	35
144	428	476	0.61	0.39	0.72	0.89	100	100	100	100	36	9	74
245	456	520	0.63	0.40	0.73	0.85	96	100	62	28	11	88	64
346	456	368	0.62	0.41	0.72	0.95	83	40	36	73	10	57	50
447	462	Died 17/4/95	0.65	0.44	0.70	0.93	0	3	26	Dead	Dead	Dead	9
548	453	509	0.63	0.39	0.73	0.90	65	32	98	100	100	92	81
551	423	385	0.61	0.40	0.68	0.89	90	92	36	77	89	80	77
652	436	422	0.64	0.40	0.67	0.79	39	44	73	43	51	16	44
753	397	428	0.58	0.37	0.67	0.89	62	62	83	78	18	53	60
854	367	473	0.60	0.38	0.66	0.89	100	100	100	100	28	74	84
155	431	474	0.60	0.37	0.71	0.90	0	59	95	47	32	0	39
256	398	401	0.61	0.39	0.70	0.77	85	86	43	74	88	60	73
357	360	332	0.58	0.36	0.65	0.74	61	61	89	34	49	37	55
458	360	433	0.60	0.37	0.66	0.78	100	100	100	100	100	100	100
461	259	308	0.54	0.34	0.58	0.78	66	26	11	73	65	74	52
562	311	350	0.56	0.35	0.63	0.76	100	86	97	93	100	88	94
663	487	577	0.65	0.42	0.76	1.01	100	100	100	100	100	100	100
764	423	465	0.58	0.39	0.67	0.85	59	65	68	80	89	95	76
865	556	677	0.66	0.44	0.79	1.04	100	100	100	100	39	100	90
166	503	566	0.65	0.43	0.74	0.91	100	100	100	100	100	100	100
267	368	364	0.59	0.38	0.66	0.78	84	6	36	3	13	6	25
368	440	531	0.62	0.41	0.73	0.97	100	37	100	100	100	100	90
371	444	436	0.65	0.42	0.68	0.89	75	66	35	70	63	59	61
472	407	322	0.60	0.38	0.67	0.78	49	60	7	40	34	0	32
573	538	563	0.65	0.42	0.77	0.94	100	100	100	100	100	100	100
674	475	627	0.64	0.42	0.74	0.92	100	100	100	100	100	100	100
775	444	514	0.62	0.40	0.71	0.90	87	100	100	12	23	97	70
876	524	649	0.66	0.43	0.77	1.10	100	99	100	100	100	100	100
177	329	311	0.57	0.37	0.61	0.75	16	7	61	33	10	47	29
278	444	505	0.63	0.41	0.74	0.91	100	100	74	100	100	100	96
281	552	619	0.66	0.44	0.78	1.12	92	100	100	100	100	100	99
382	551	609	0.64	0.43	0.77	1.00	100	100	100	100	100	100	100
483	420	520	0.62	0.39	0.72	0.94	0	100	100	100	100	100	83
584	478	538	0.60	0.38	0.71	0.95	100	100	74	100	100	100	96
685	340	438	0.57	0.36	0.65	0.86	100	100	100	100	100	100	100
786	390	549	0.59	0.38	0.71	0.95	100	100	100	100	47	100	91
887	427	415	0.60	0.40	0.70	0.84	73	71	62	72	76	5	60
188	346	386	0.55	0.34	0.56	0.68	82	16	55	48	15	30	41
211	406	461	0.61	0.39	0.73	0.89	100	100	73	100	100	100	95
312	449	590	0.62	0.41	0.74	0.87	100	100	100	100	100	100	100
413	433	547	0.62	0.42	0.75	0.95	68	33	100	0	100	29	55
Mean	425.7	472.1	0.609	0.392	0.696	0.881	83	77	74	77	71	82	77
St Dev	73.2	105.0	0.033	0.029	0.057	0.104							

Identity code: 1=White 2=Yellow 3=Orange 4=Red 5=Pink 6=Light blue 7=Dark blue 8=Green Q=Queen

Appendix IV : Paint Mark Loss

Table IV-1 Paint mark loss The position of all paint marks that were lost by all ants are recorded.

Colony K

Worker identity	Missing 14/5/95	Total	
		Positions	Marks
537	T L R	3	3

Colony L

Worker identity	Repaint 05/3/95	Repaint 17/4/95	Missing 16/5/95	Total	
				Positions	Marks
212		L R		2	2
515		T		1	1
425		L R		2	2
335			L R	2	2
436		L R		2	2
144		L R		2	2
551	L R	L		2	3
155		L R		2	2
256			T	1	1
573		T		1	1
775			L R	2	2
177		L R		2	2
278		R		1	1
211			R	1	1
244			R	1	1

Colony M

Worker identity	Repaint 18/3/95	Repaint 31/3/95	Repaint 05/4/95	Repaint 17/4/95	Repaint 04/5/95	Missing 15/5/95	Total	
							Positions	Marks
212	L						1	1
515	T		T R	T R	T	T	2	7
616	T L R	T	T		T	T	3	7
717	T L R						3	3
821					T		1	1
122	T	T					1	2
425	L R				R		2	3
526		T					1	1
627					T L R		3	3
728				T	T		1	2
731	T		T				1	2
537					T		1	1
638	T	T		T	T		1	4
641	T	T			T	T	1	4
245		L R					2	2
346	L R		L		T		3	4
551	T R		T	T		T	2	5
652	L	L		L	L R		2	5
753	T	L R					3	4
854					T L R		3	4
155			L R			T	3	4
256			L				1	1
357	T L R		L		L	L	3	6
458					L		1	1
461	T						1	1
562				L	T		2	2
663						T	1	1
764	L R						2	2
865					L R		2	2
267					L R		2	2
371	L R						2	2
573					T L R		3	3
674					T	T	1	2
775					L R	L	2	3
177		T					1	1
278					T		1	1
483					T		1	1
786					T		1	1
188	T				T		1	2
413						T	1	1

Paint marks replaced or lost : T = Thorax L = Left side of 1st gastral tergite R = Right side

Appendix V : Session Summary

Table V-1 Session statistics : Ant tracking

Session name	Worker number	Number of worker hours tracked	Average % time spent in nest	Number of worker hours not identified	% of worker hours not identified	Number of entering ants
K1	23	76.2	82.8	0	0.00	69
K2	23	77.9	84.7	0.01	0.01	61
K3	23	79.0	85.9	0	0.00	167
K4	22	68.8	78.1	0	0.00	56
K5	22	67.0	76.1	0	0.00	92
K6	21	61.6	73.3	0	0	57
Subtotal	134	430.4	80.3	0.01	0.00	502
L1	85	280.7	82.6	3.99	1.40	133
L2	85	261.2	76.8	2.67	1.01	179
L3	85	251.8	74.0	3.71	1.45	207
L4	85	260.7	76.7	0.07	0.03	167
L5	85	240.8	70.8	0.77	0.32	189
L6	83	273.6	82.4	0	0	118
Sutbtotal	508	1568.7	77.2	11.21	0.71	993
M1	67	190.5	71.1	0.68	0.36	153
M2	67	179.1	66.8	0.34	0.19	189
M3	67	193.8	72.3	0.62	0.32	270
M4	66	189.9	71.9	1.18	0.62	196
M5	66	174.3	66.0	0.02	0.01	146
M6	66	161.4	61.1	0	0	154
Subtotal	399	1088.9	68.2	2.84	0.26	1108
Total	1041	3088.0	74.2	14.06	0.45	2603

Session Summary (continued)

Table V-2 Session summary : Set-up

Session name	Date	Start time	Hours on filming apparatus before filming	Black and white threshold value	Nest dimensions (pixels)	Tunnel width (pixels)	Tunnel length (pixels)	ITM position and y pixel distance to OTM
K1	21/03/95	08:02:02	23	128	609 x 415	25	102	[596,333] 59
K2	22/03/95	09:28:25	48	120	609 x 410	19	100	[598,330] 61
K3	12/04/95	08:58:29	23	128	612 x 414	22	100	[601,332] 61
K4	20/04/95	07:57:39	18	108	606 x 406	23	96	[597,324] 62
K5	24/04/95	08:01:15	19	118	672 x 428	25	87	[664,359] 57
K6	13/05/95	10:03:13	19	138	683 x 444	31	94	[669,367] 63
L1	27/03/95	09:31:21	24	150	651 x 477	20	78	[639,418] 49
L2	28/03/95	08:00:28	46	132	660 x 476	17	71	[651,416] 47
L3	16/04/95	09:01:55	34	150	649 x 474	20	77	[637,408] 52
L4	22/04/94	08:06:13	19	146	664 x 479	19	66	[657,419] 49
L5	26/04/95	10:11:41	21	148	640 x 473	21	74	[634,408] 50
L6	15/05/95	09:01:49	17	132	663 x 479	16	72	[655,412] 49
M1	24/03/95	09:19:37	24	136	650 x 474	18	82	[640,408] 53
M2	25/03/95	09:31:04	48	136	651 x 475	21	80	[640,406] 55
M3	14/04/95	07:58:41	31	152	649 x 477	22	85	[642,406] 51
M4	21/04/95	08:00:35	19	134	645 x 474	22	82	[635,405] 47
M5	25/04/95	10:08:09	20	132	656 x 478	24	81	[644,409] 49
M6	14/05/95	08:56:35	17	126	656 x 474	20	75	[646,402] 56

ITM, OTM = Inner and Outer Tunnel Markers

Appendix VI : Ant behavioural histories

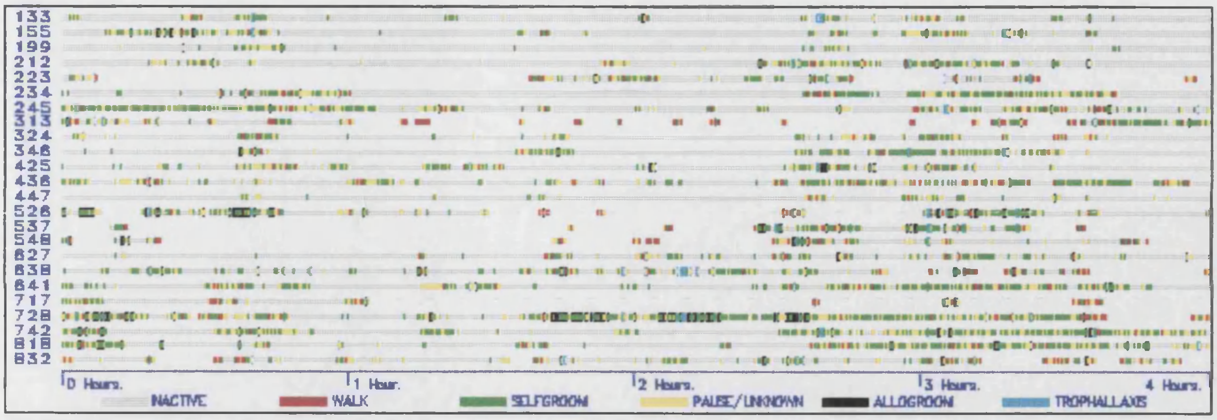

Figure VI-1 Individual histories : K1 (see p. 126 for explanation)

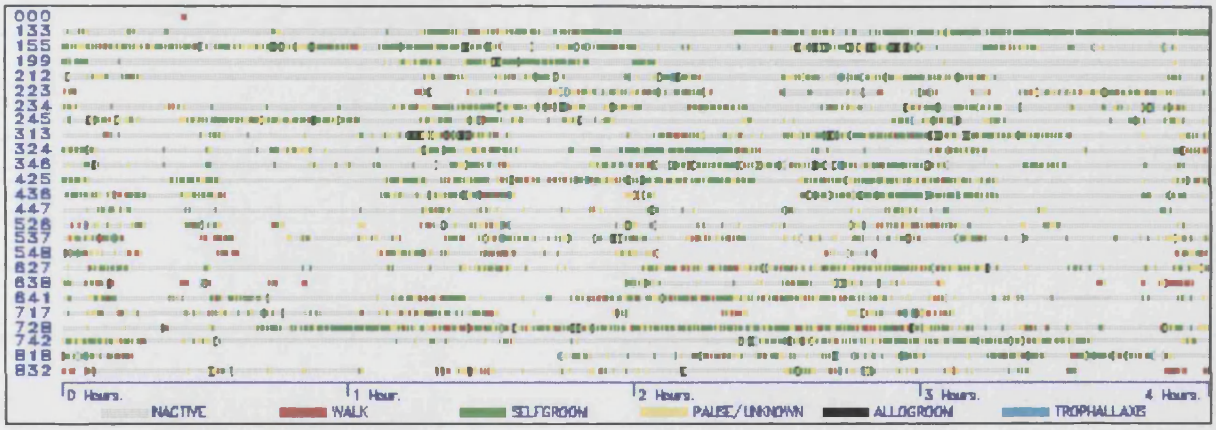

Figure VI-2 Individual histories : K2

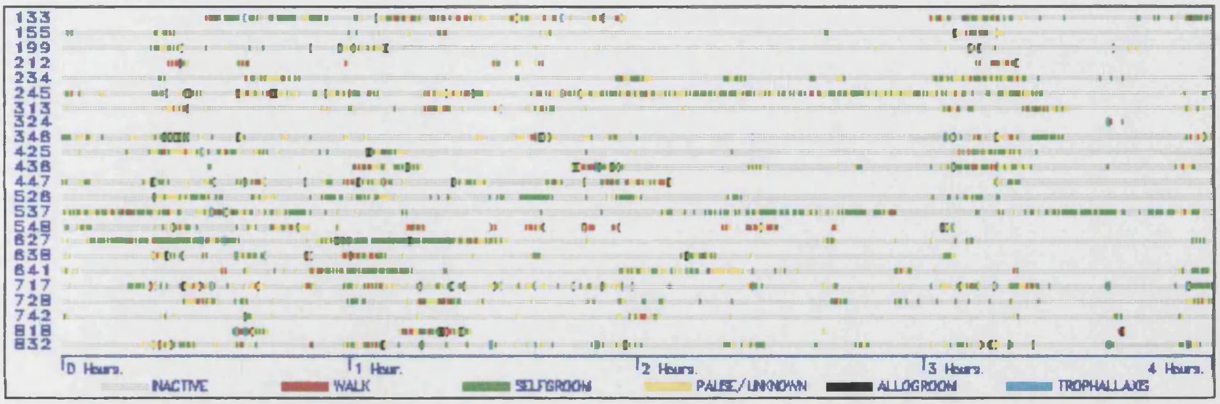

Figure VI-3 Individual histories : K4

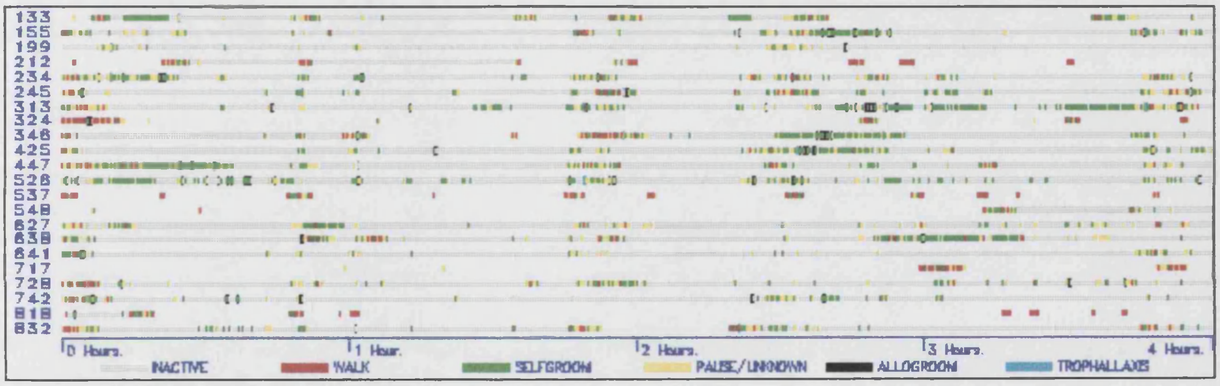

Figure VI-4 Individual histories : K6

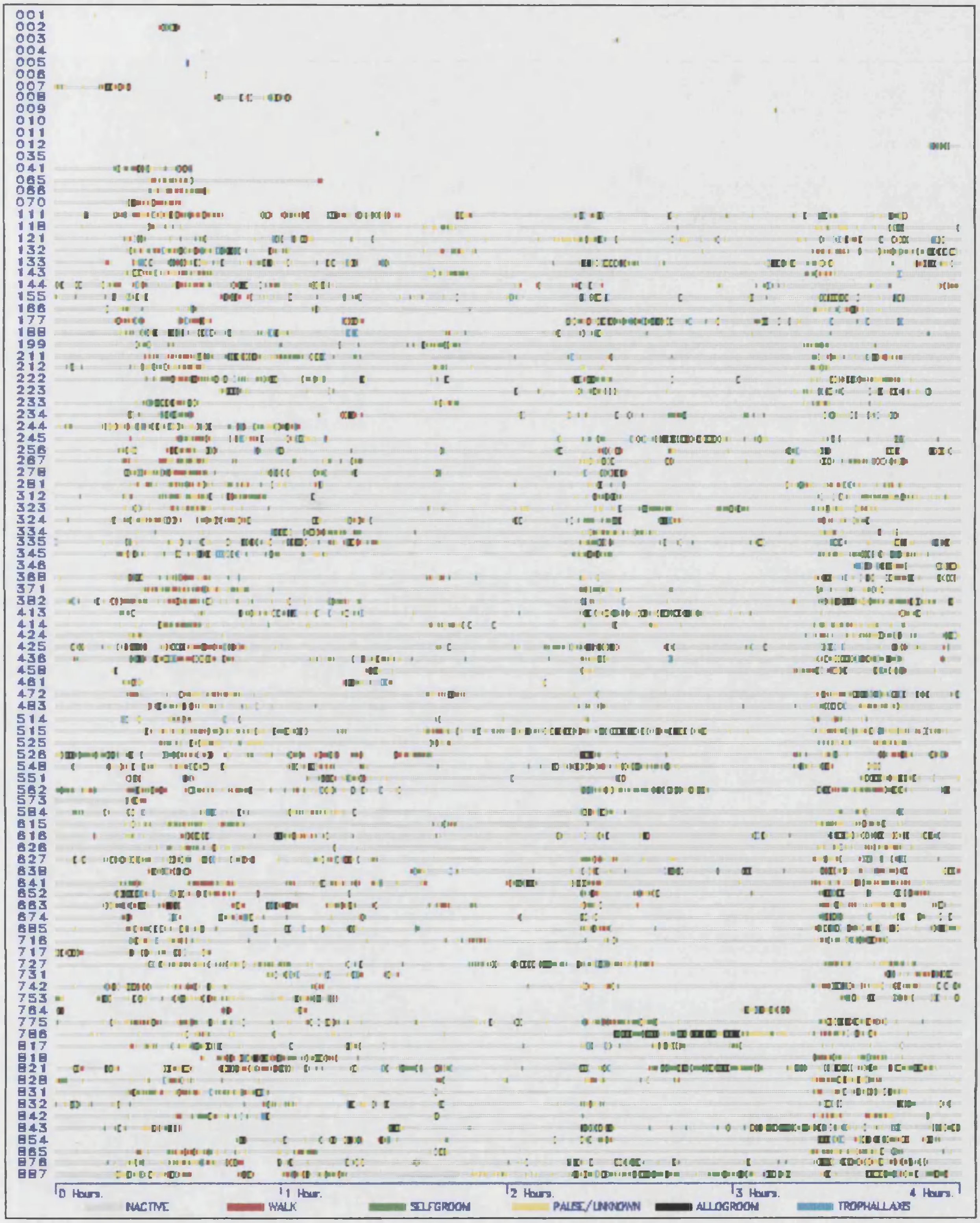

Figure VI-5 Individual histories : L1

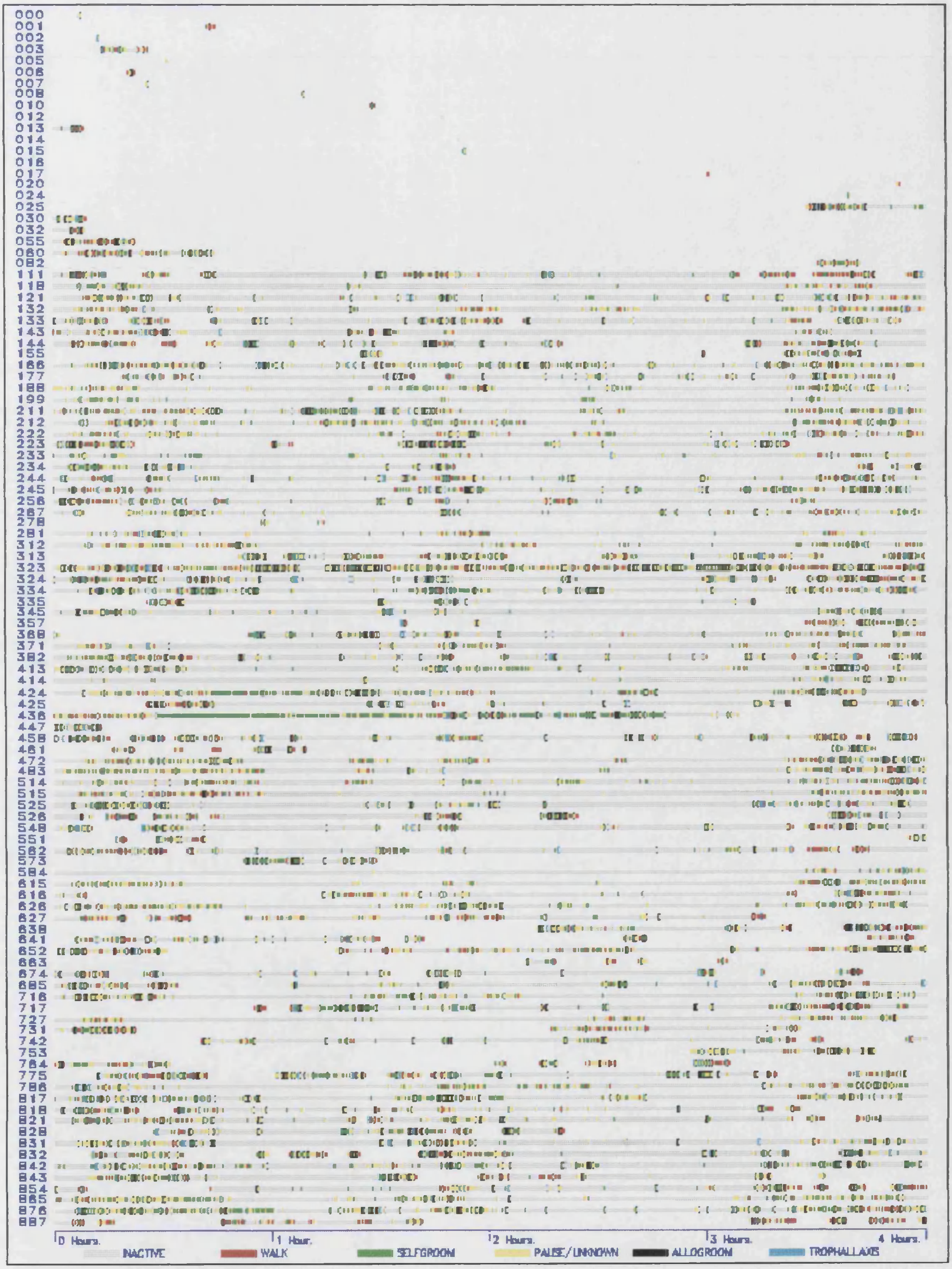

Figure VI-6 Individual histories : L2

Figure VI-7 Individual histories : L3

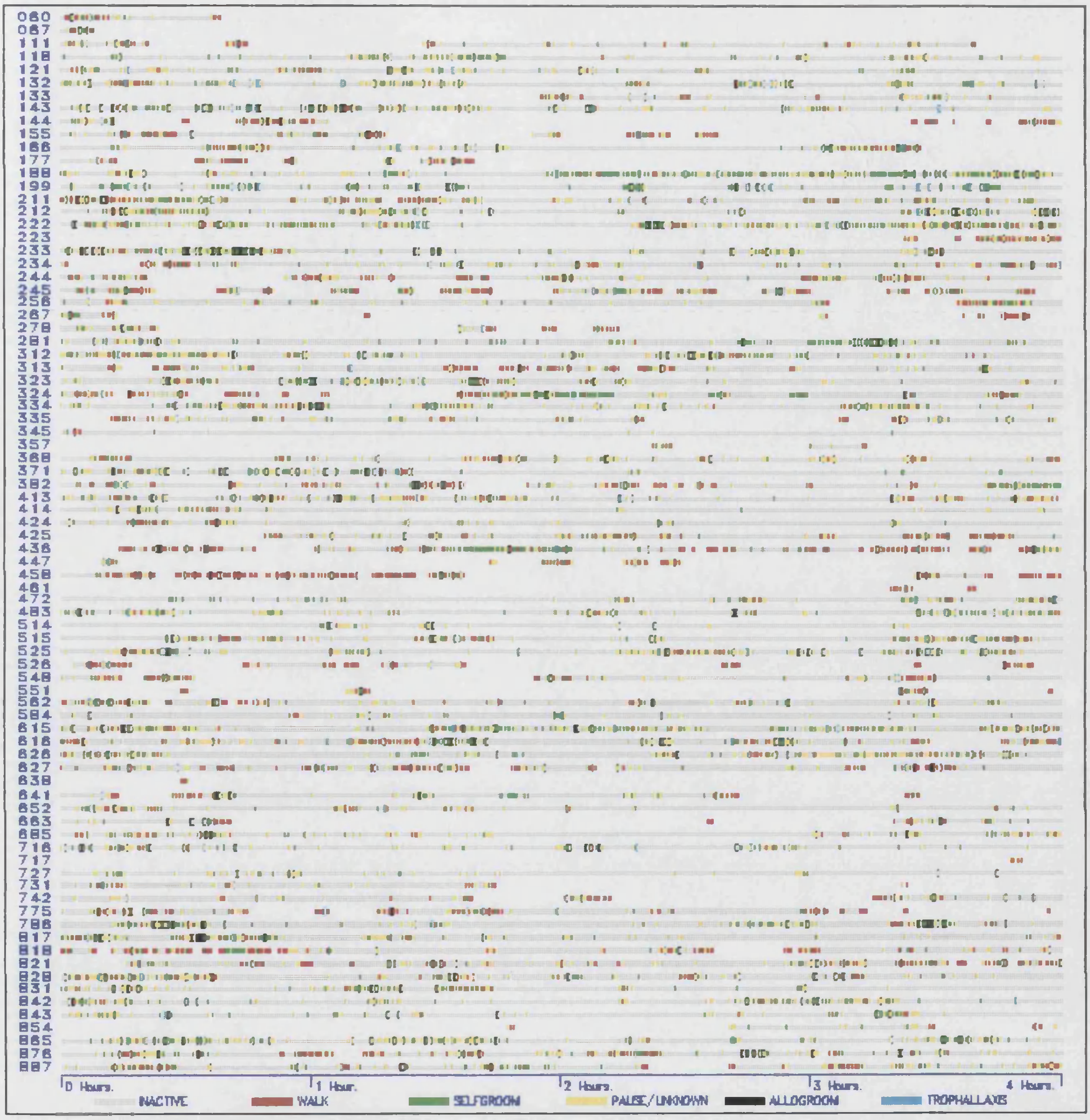

Figure VI-8 Individual histories : L5

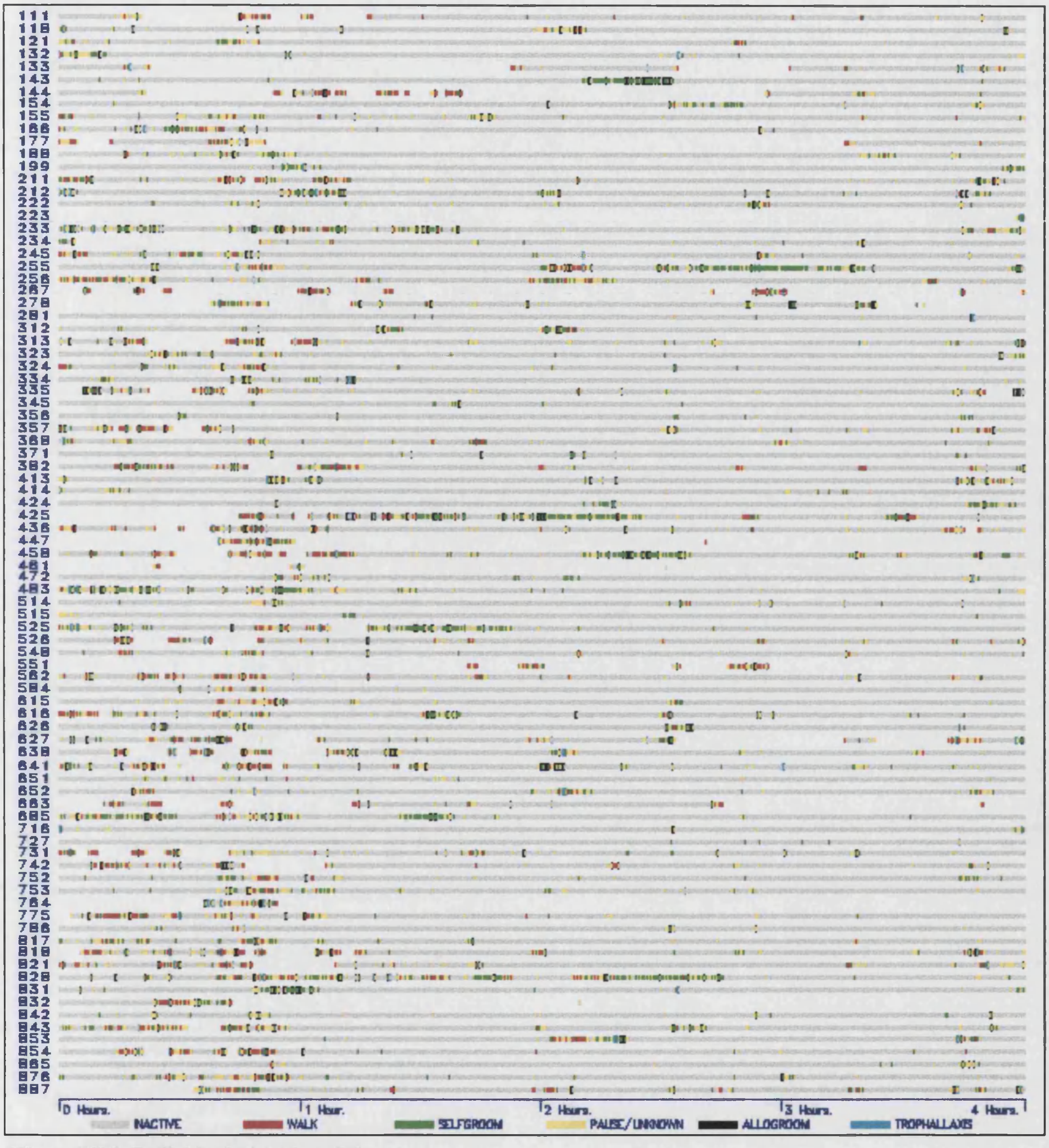

Figure VI-9 Individual histories : L6

Figure VI-10 Individual histories : M1

Figure VI-11 Individual histories : M2

Figure VI-12 Individual histories : M4

Figure VI-13 Individual histories : M5

Figure VI-14 Individual histories : M6