Automated tracking and collective behaviour in locusts and humans

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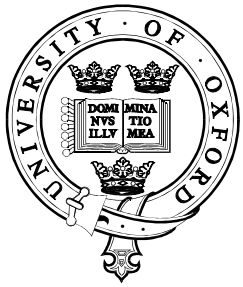

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Abstract

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The understanding of the motion of animal groups, such as birds, fish and insects, has been greatly advanced by applying principles of self-organisation – the emergence of global patterns from simple, local, interactions between individuals. The desert locust, *Schistocerca gregaria*, provides a useful model system for the experimental study of collective behaviour. During plague years, the desert locust can form aggregations extending over hundreds of km. Before developing wings, juvenile locusts form marching 'bands' which can maintain group cohesion as they migrate over large distances. In this thesis I investigate locust aggregation, group motion and individual interactions. I also apply the same principles to a study of human behaviour.

In Chapter 2, I describe the automated tracking methods that I developed and used to collect the data for the rest of the thesis. In the experiments described in Chapter 3, the relative strengths of the attraction to conspecifics and environmental heterogeneities were explored by presenting groups of locusts with two aggregation sites. I found that locusts had a preference to enter the site with the higher population. The locusts formed dynamic aggregations on the sites; no site was consistently more populated than the other, but individuals were significantly more attracted to the site with the higher current population. In Chapter 4, I consider the effect of marching experience on locust behaviour. Groups of locusts that had experience of directed marching, followed by a sudden reduction in density, behaved indistinguishably from those that had only experienced the lower density throughout, indicating a lack of hysteresis effects in collective responses to change in local population density. In Chapter 5, I investigate a locust's response to its nearest neighbour. I quantified a locust's propensity to start or stop moving according to the relative position, orientation and movement of its nearest neighbour. In Chapter 6 the techniques developed studying the locusts were applied to human groups. The response of people to different sized groups was quantified, replicating an earlier study in New York. The response was weaker in Oxford but had the same characteristics of the previous study, showing an initially linear response which saturated. The spatial distribution of gaze copying was anisotropic, tending to occur behind the group.

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Chapter 1

General Introduction

1.1 Animal aggregations and groups

Group formation is a widespread phenomenon occurring among many species of animal. There are a range of benefits individuals may gain from belonging to a group. Group membership may decrease their predation or increase their foraging success as well as provide physical benefits such as keeping warm (Krause and Ruxton, 2002). Ability to detect predators can also increase with group size (Beauchamp, 2003). Close interactions with others in a group can increase an individual's arousal level and its responsiveness to predators (Treherne and Foster, 1980). An individual can benefit from numerical dilution of risk (Wrona and Dixon, 1991, Sword et al., 2005). Predators can learn warning colouration of distasteful prey faster and more strongly when the prey are in groups (Gagliardo and Guilford, 1993). Foraging success can be enhanced when in a group by hunting together (Creel, 1995) and by using information from other individuals (Templeton and Giraldeau, 1995, Grünbaum, 1998).

In order to derive the benefits of aggregation, individuals must be able to form groups, maintain cohesion and function effectively within groups. The range of perception of an individual may be much smaller than the size of the aggregation in which it finds itself. Despite this discrepancy, large aggregations can seem to move as a single unit – for example fish schools flashing predators (Radakov, 1973) and sudden coordinated turns of flocks of starlings (Ballerini et al., 2008). Patterns can also be generated at a scale much larger than an individual, for example termite mounds (Grassé, 1959, Bonabeau et al., 1998, Korb, 2003) and the front patterns in herds of wildebeest (Gueron and Levin, 1993).

Patterns and movements of animal groups may be better understood by considering them as self-organised systems. Camazine et al. (2001) offer the following definition:

> *"Self-organization is a process in which pattern at the global level of a system emerges solely from numerous interactions among the lower-level components of the system. Moreover, the rules specifying interactions among the system's components are executed using only local information, without reference to the global pattern."*

However, the rapidly developing study of self-organized systems should not be constrained by this definition. Anderson (2002) sums up the key components of self organisation as multiple interactions involving positive and negative feedback with stochasticity creating diversity which the feedback acts on. Self-organised systems are characterised by the creation of emergent group level spatiotemporal structures or behaviour where small changes in either individuals or the environment potentially result in large global change (Anderson, 2002). In nature some global cues may be incorporated into otherwise self-organised behaviour. For example, direction preference in migrating animals may result in self-organisation "…within the context of global cues" (Couzin and Krause, 2003).

1.2 Mathematical models of group behaviour

When studying a self-organised system a reductionist approach is not sufficient. The system needs to be studied within its context (Auffray et al., 2003). Multiple interacting components can be considered together by using mathematical models and computer simulations. These models can then be used to consider hypotheses about the underlying interaction rules which combine to form a self-organised system and generate its often unintuitive emergent properties. For example, the dendritic raiding

pattern of the ant *Eciton hamatum* seem very different to the large cohesive raiding patterns of *Eciton burchelli,* however the same model of trail formation can generate both trail patterns. The difference patterns can be explained by the different distributions of prey which the ants predate (Deneubourg et al., 1989). This model was confirmed in the field by changing the distribution of the prey of *E. burchelli* which resulted in raiding patterns similar to those of *E. hamatum* (Franks et al., 1991). Ants and other social insects have provided many interesting examples of complicated group level behaviour based on simple rules (Bonabeau, 1998, Theraulaz et al., 2003). Considering the system as a whole is therefore essential in these studies.

There are three main approaches taken when modelling self-organisation. The first is discrete stochastic simulation models. For example, in his model of army ant swarming, Deneubourg et al. (1989) modelled the ants using Monte Carlo simulation on a discrete grid with each individual moving according to simple rules. Hamilton's (Hamilton, 1971) theoretical model of aggregation of a selfish herd could also be considered as such a discrete model with individuals following a simple rule to reduce their domain of danger. In these models interactions are described in terms of interactions between neighbours.

A second method of modelling self-organisation is with "Eulerian" models which use differential equations to capture the system. Examples include models of cockroach shelter-choice in which the change in the number of cockroaches in shelters is a function of the number of cockroaches in each shelter (Ame et al., 2004, Ame et al., 2006), and models of the effect of colony size on foraging efficiency among ants (Beekman et al., 2001, Sumpter and Beekman, 2003). These models are appropriate

for population-level patterns. They are based on assumptions of random diffusion and may often be solved analytically or through numerical approximation. These simplifying assumptions have the advantage that tractable and elegant models of the system are created.

The last method commonly used in modelling self-organised systems is with "Lagrangian" models. Here the equations of motion of individual units are defined along with 'social' forces due to interactions (Vicsek et al., 1995, Couzin et al., 2002). These models can incorporate detailed individual behaviour and demonstrate general principles such as transitions from disorder to order (Czirók et al., 1997, Buhl et al., 2006), lane formation (Couzin and Franks, 2003) and leadership (Couzin et al., 2005). The advantage of these models is that groups are described in terms of their natural unit of description: the individual.

1.3 The desert locust

In plague years locusts devastate crops and cost hundreds of millions of dollars to control (Enserink, 2004). A single swarm of the desert locust *Schistocerca gregaria* can contain huge numbers of individuals, extend over hundreds of km, and have a density of hundreds of millions of locusts per square km (Simpson et al., 1999). Juveniles locusts take an average 36 days to mature to winged adults (FAO, 2004). During this period gregarious locusts form large cohesive aggregations which roost together at night and form 'marching bands' that move together during the day (Ellis and Ashall, 1957). Plagues of *Schistocerca gregaria* can affect an area of about 29 million km^2 area (Symmons and Cressman, 2001).

Most of the time, however, locusts are not found in these huge groups. The desert locust exhibits an extreme density dependent phenotypic plasticity (Uvarov, 1977, Pener, 1991). At low densities the locusts take on their solitary phase; they are cryptic and actively avoid other locusts. If forced together, to feed for example, they undergo a density-dependent phase transition and become gregarious (Despland et al., 2000, Simpson et al., 2001). In the gregarious phase the locusts actively aggregate. This transition is accompanied by changes in a suite of morphological, physiological and behavioural factors (Simpson et al., 1999, Pener, 1991, Simpson and Sword, 2006). Behavioural changes have been shown to occur after only four hours of crowding (Roessingh, 1994). The large scale patterns generated by locusts provide an interesting example of a self-organised system.

Locusts are an interesting example of an animal group and ideal for the experimental study of self-organisation. They exhibit the key characteristics of a self-organised system. A locust's perception of the band or swarm is on a much smaller scale than the size of the aggregation which may extend over several 2 ha in marching bands (Ellis and Ashall, 1957) and over 20 km^2 in flying swarms (Uvarov, 1977). The behaviour of individuals is strongly affected by local interactions with their environment and conspecifics for example in phase change (Collett et al., 1998) and basking in microclimatic regions (Kennedy, 1939). Locusts are sensitive to the position of the sun (Homberg, 2004) and affected by wind (Rainey, 1951), but we can consider them to self-organise within the context of these global cues. This makes it possible to apply various mathematical models to understand their behaviour. For example, the large size of swarms makes them interesting to study with continuum approaches (Topaz and Bertozzi, 2004).

When in a band or a swarm other locusts form the majority of the environment, therefore we would expect locusts to be highly adapted to responding to their neighbours. Indeed there are differences in the visual looming detection neuron DCMD between solitary and gregarious locusts which provide gregarious locusts with an earlier and more temporally consistent response to approaching objects whereas solitary locusts have a later reaction and more variable escape response (Rogers et al., 2007). The fact that locusts are well understood physiologically combined with the relative ease of rearing large numbers in the laboratory makes them ideal for the collection of data about individuals within groups.

1.4 Tracking animals and building models

Many of the proposed models of animal groups are phenomenological, in the sense that the models are not parameterised with detailed observations. They have attempted to describe general properties of animal groups and draw general conclusions about their collective behaviour. More recently, however, the trend has been towards more quantitatively accurate models. The collection of detailed empirical evidence is required to parameterise these models. Technological advances have begun to allow the collection of huge amounts of data on groups of interacting animals, for example the simultaneous tracking in 3D of up to 2700 starling in a flock (Cavagna et al., 2008). Video tracking of cockroaches has allowed the creation of 'robot' cockroaches which can behave like and interact with live cockroaches (Halloy et al., 2007). Such detailed observations can help in the understanding of animal aggregations and the rules underpinning them which can be used to enhance models further and allow scaling from small to large groups (Parrish and Edelstein-Keshet, 1999). Our aim with

the locusts is to understand how individuals interact and build models on the basis of these interactions. To this end we need to have tools with which to analyse behaviour of individuals.

Classical behavioural observations have relied upon patient and concentrated observation, for example the continuous observation of ten locusts simultaneously (in visually screened containers) for a period of 130 h by Simpson (1981). The use of video recording can reduce the strain on an observer and is useful for capturing the interactions of several individuals simultaneously; for example studies of marching locusts in the field (Stower, 1963) and the schooling of fish (Radakov, 1973, Partridge et al., 1980). Recording the positions of multiple objects in a video sequence by hand is very labour intensive which can limit the number of individuals tracked or the length of time that the objects are tracked for.

Much of the labour involved in tracking can now be performed by computer. The advent of cheap computer power and video equipment has enabled the development of automatic tracking which is now widely available (Noldus et al., 2002, Correll et al., 2006). Whilst tracking at the group level of has become relatively advanced and completely automated, these systems still have difficulty in maintaining the identity of unmarked individuals in aggregations over long periods of time.

1.5 Aims of this thesis

This thesis seeks to address the need for data for models of locust behaviour by investigating the details of locust-to-locust interactions using computer tracking. I

investigated the degree of self-organisation exhibited by locusts in groups through exploring the following:

- the relative strengths of attraction to conspecifics and attraction to environmental cues;
- the time scale of the response to locust density when moving in a group;
- the impact of the proximity, orientation and motion of a locust's nearest neighbour on its motion.

In Chapter 2 I describe the methods that I developed to extract the information from videos of locust experiments using automatic, manual and user-assisted automatic tracking. These techniques were used to analyse the experiments detailed in this thesis as well as other studies of locusts (Buhl et al., 2006, Bazazi et al., 2008).

Locusts have been observed to form very tight basking groups in suitable microclimates in the field (Kennedy, 1939). I was interested in the degree to which the relative strengths of the attraction to conspecifics and the attraction to suitable microclimates effected the formation of these groups. Laboratory experiments had indicated that conspecific attraction only played a small role. In Chapter 3 automated tracking was used to analyse the dynamics of basking groups on two heated patches in an arena. We found that locusts were initially more likely to enter the site containing a larger number of locusts. Furthermore, throughout the experiment the locusts showed a significant preference to be in the larger group. However, in contrast to cockroach shelter selection (Ame et al., 2006) their attraction to the larger group was not sufficient to overcome the attraction to the other heated patch.

Once a band of locusts becomes aligned and begins marching, its direction tends to persist over entire days (Kennedy, 1945). Buhl et al. (2006) showed that this directional inertia could be explained as a function of group size. Groups of locusts at a density above a threshold would persistently march in the same direction. Field observation have suggested that individuals which lose visual contact with the band can continue in a straight course to catch up with the band (Ellis and Ashall, 1957). I wanted to find out what effect previous experience of high density marching had on an individual's subsequent behaviour. In Chapter 4 I showed that in the absence of navigational cues, a locust's propensity to keep marching in a sustained direction was dependent only on the instantaneous degree of crowding that it experienced, supporting Buhl et al's (2006) model. Measures of group-level behaviour were indistinguishable between groups comprised of individuals which had had experience of a high level of marching compared with those which had only an experience of marching at a low level.

The group level behaviour of interacting individuals may be explained by a range of theoretical models (Vicsek et al., 1995, Czirók et al., 1997, Couzin et al., 2002, Couzin et al., 2005). I wanted to collect empirical data of individual interactions within groups to enable the testing of such models. In Chapter 5, I performed highly detailed tracking of all individuals in groups of locusts. I found that a locust's propensity to start or stop moving was affected differently by its nearest neighbour according to the relative position, orientation and motion of that neighbour.

Considering the interactions between individual locusts enables us to better understand how they behave as a group. Humans provide another interesting example

of a group-forming species. The understanding of the movement of human crowds is essential for the design of urban spaces and provides a suitable application of the kind of empirical data collection and analysis which I have applied to locusts. In Chapter 6 we considered the principles of collective behaviour applied to people. As in chapter 3 where locusts responded experienced different sized groups on the heat patches, we were interested in how an individual's response to a static group changed with group size. We investigated the relationship between the size of a stimulus group and the proportion of passers-by who copied the behaviour of the group. This replicated a study performed in New York (Milgram et al., 1969). We fitted a threshold response to the New York data and our data from Oxford. We found a weaker response in Oxford, compared to New York, but with the same characteristic initial linear response. We also considered how the response was distributed spatially.

This thesis contributes to the understanding of group movement in locusts. It also demonstrates techniques for the analysis of locust interactions. We have developed a better understanding of the movements of juvenile locusts at a small scale. This understanding brings us closer to being able to scale up to models of large groups within the framework of self-organised systems. Such models would be useful for the control of locusts in the field by providing predictions of where bands would move, allowing the use of pesticides and other control measures to be more focused, reducing cost and environmental impact.

Chapter 2

Computer tracking of locusts and humans

2.1 Summary

Computer tracking methods for following multiple individuals were developed to measure properties of individual and groups of locust in the laboratory. In this chapter, I describe three tools, which I wrote using the Matlab computer package, for tracking multiple individuals from video. I first consider an automatic tracking tool, which requires no human intervention. This tool is able to keep track of individuals over short periods of time and therefore is suited to generating aggregate properties of group motion. This capacity allows analysis of large groups over long time periods. Next, I present a tracking tool, which enables a human observer to record the position and behaviour of objects from a video using a graphical user interface. An individual's position is interpolated between user-entered points to indicate whether or not more points need to be entered to sufficiently represent the position. This method is labour intensive but is useful for extracting detailed information in complicated scenes. Finally, I describe a tool which combines automatic and manual tracking. This augments automated tracking with human intervention when the computer loses the identity of an individual. Examples of the use of these tools in several experiments are given. These tools enabled the extraction of highly detailed observations, which formed the foundation of this thesis and two co-authored papers.

2.2 Introduction

Video recording in the study of animal behaviour is especially useful for understanding the interactions of large numbers of individuals. An observer viewing a group of individuals in real time is unable to focus on all individuals simultaneously. Even before computers were readily available, video recording helped solve this problem of observer limitation. For example, Stower (1963) filmed locusts moving in bands in the field. He quantified the behaviour of the locusts by projecting the film onto paper and tracing individuals' position frame by frame. A similar technique was pursued by Radakov (1973) in the study of fish movement. These are extremely labour intensive methods.

Automatic tracking of single free insects was achieved by Kramer (1975, 1976) by allowing an insect to walk on a locomotion compensator: a sphere which automatically moved to keep the insect on top whilst recording the 2-dimensional path that the insect would have taken. Moorhouse et al. (1978) automatically recorded activity bouts of a single locust fixed on a treadmill. The extraction of the data from videos of free moving animals has become far less labour intensive with the use of computer imaging. Computer imaging techniques have allowed automatic tracking of single individuals and small numbers of marked individuals from video; for example EthoVision (Noldus et al., 2002).

Keeping track of multiple similar and unmarked individuals in a video recording raises several problems not encountered in single-object tracking. Solutions to these problems are often application-specific and therefore require bespoke software. Automatic multi-object tracking is typically able to keep track of individuals over the

short term, which can provide a great deal of information about group level properties, for example the number of ants exploring over time (Tucker et al., 2001). Recently more generally applicable multi-object tracking software has been developed (Correll et al., 2006).

In this chapter I present the three different kinds of tracking software that I developed for use in research projects either by myself or by other members of the locust research group. This chapter aims to give a complete description of the problems and solutions required in setting up tracking experiments involving animal groups: from experimental setup through filming to image processing.

The large amounts of data generated were processed and analysed in the computer package Matlab (2004). I developed a number of Matlab functions to aid analysis. These functions are described in the Appendix and written in italics when referred to in the text below.

2.3 Experimental arena set up

In order to study the group behaviour of juvenile locusts, termed hoppers, in a homogeneous environment I designed a ring shaped experimental arena. The floor was constructed from a sheet of Formica - white laminated work surface material (Perstorp Surface Materials, Formica Ltd., Tyne & Wear, U.K.). This provided a study surface which was easily cleaned and resistant to marking. The circular wall was also made from Formica held in a round socket at the base of the arena. This ensured that the locusts in every experiment experienced the same arena conditions. The arena was lit by four fluorescent tubes which formed a tight square around the

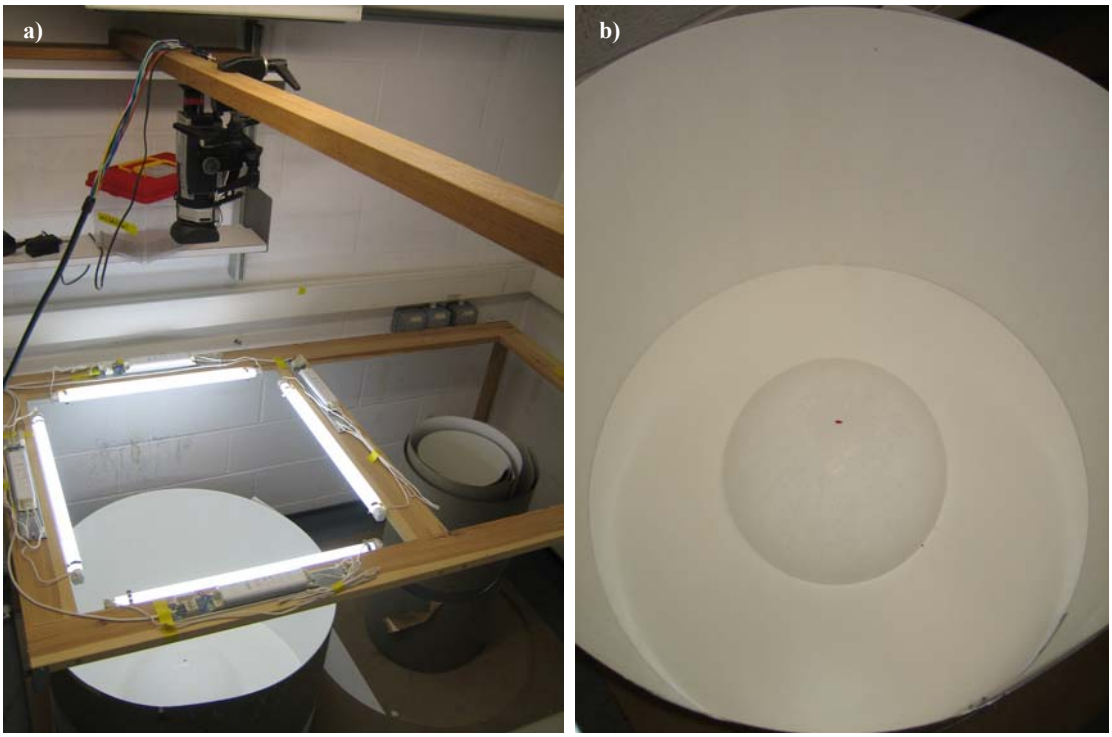


Figure 2.1 (a) The experimental arena and camera, (b) close-up of the arena.

arena and gave even lighting whilst allowing the scene to be filmed from directly above. Lighting ballasts were used to increase the inherent flicker of the lights to 15 kHz making the flicker imperceptible at the locusts' flicker fusion frequency of approximately 40–90 Hz (Miall, 1978).

A key inclusion in the arena was a central dome. This 35 cm diameter hemisphere was intended to prevent the optical flow of locusts moving in one direction on one side of the arena from interfering with the behaviour of locusts on the other side. The resulting ring-shaped arena could then be considered to have semi-periodic boundary conditions – the locusts were bounded by inner and outer walls, but able to keep moving around the circle (Figure 2.1)

The arena was filmed from directly above its centre by a standard-definition video camera (Canon XM2) using a progressive scan mode. Minimal lens distortion was

assumed as we were not interested in the corners of the image. In order to simplify the tracking, the brightness and contrast of the camera was set so that the white floor of the arena was a saturated white, reducing the detection of details such as faeces and appendages, leaving the dark bodies of the hoppers clearly distinguishable from the white background.

In preliminary trials I tried marking individuals to aid in identification, using both bright orange paper tags super-glued to the pronotum and paint spots. However, these markers could not be made sufficiently large to be distinguished on the video. Individual marking did not then seem feasible for locust tracking without a higher resolution camera or the use of multiple cameras.

2.4 Overview of Data Analysis

Quantifying behaviour was a multistage process. First, the positions of the individuals within frames had to be determined. With our laboratory set up this could be performed automatically. In more complicated environments it may be necessary for a person to manually identify objects. Automated object identification provided sufficient information for the short-term tracking of individuals which was used to extract group level properties. In order to extract individual level properties long-term individual tracking was needed. This presented several non-trivial problems which we solved by augmenting automatic tracking with human input. This allowed the identity of individuals to be maintained over extended periods. The process is summarised in Figure 2.2.

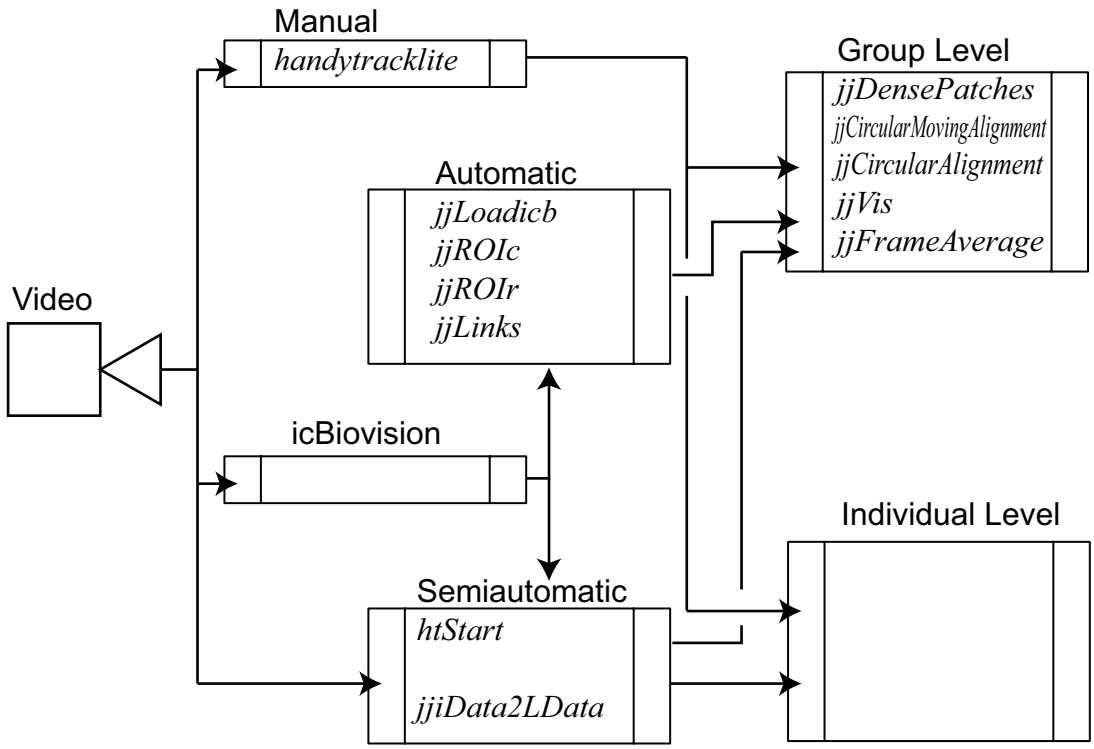


Figure 2.2 The workflow for recording and tracking multi-object experiments. The corresponding Matlab functions are in italics.

Recording the experiments

The experiments could either be recorded on to miniDV tape or directly onto the computer. Recording to miniDV tape allowed the capture of up to 90min of an experiment at 25 frames per second (fps). Recording directly to the computer allowed longer periods to be captured but required that the camera be directly connected to a computer during experiments. This latter method was preferred, especially in the laboratory. We recorded early experiments at 25 fps. This was found to be excessive, consuming large amounts of space for experiments lasting several hours. A frame rate of 5 fps was sufficient to ensure that a locust did not move more that one body length per frame unless they jumped. The locusts rarely jumped in our experiments. We used the motion jpeg codec MJPG with the minimum compression using icBiovision

developed by Dr Iain Couzin (Figure 2.3a) to minimise compression artefacts (Couzin, 2005).

Object identification in a single frame

The first step in the automated tracking of multiple objects is to identify objects in a frame of video. This was done by icBiovision. Each frame was first converted into a greyscale image. A threshold function was then applied to this image to convert it to black and white. The threshold was chosen manually to balance single object cohesion and minimise noise. It was usually set just once for an entire experiment as the light levels did not change. This resulted in the locusts, which were dark against the white arena, being represented by clusters of black pixels.

The neighbourhood around a pixel was considering when defining clusters of pixels. Two pixels are considered to be "4-connected" if they are horizontally or vertically adjacent. A pixel is in a "connected region" if it is 4-connected with another pixel already in that connected region. Thus all black pixels were grouped into connected regions.

Given a set of pixels comprising one connected region, $R = \{p_1, p_2, \ldots, p_n\}$ the following properties can be calculated (Marchand-Maillet and Sharaiha, 2000):

- the centroid (x_q, y_q) is the mean of the positions of all the pixels in R corresponding to the position of the centre of the object.

- The area is equal to the number of pixels in the connected region, that is $a = |R|$.

- The principle axis angle is the angle between the positive x-axis and the principle axis of the object. This is calculated using the discrete (k,l)-order central moment:, $\theta = \frac{1}{2}\arctan\left(\frac{2\mu_{1,1}}{(\mu_{2,0} - \mu_{0,2})}\right)$, where

  $$\mu_{k,l} = \sum_{i=1}^{n} (x_i - x_q)^k (y_i - y_q)^l$$

- The perimeter *p* can be measured simply as a sum of the number of pixels round the connected region's edge weighted according to how the pixels are connected.
- The compactness or circularity is a measure of how like a circle the object is: $c = \frac{p}{4\pi a}$. Assuming a convex object, $p = 1$ indicates a circular object and the compactness increases as the object becomes more elongated.

This information was calculated for each region in the frame and then output to a file. The information could also be overlaid on the video in icBiovision to check the tracking parameters (Figure 2.3b). This overlay was performed for every frame, generating a list of objects, their positions and their areas for an entire video sequence.

In order to determine whether or not a connected region was likely to correspond to a locust, a maximum and minimum area for a region to be valid was defined. These limits excluded the recording of irrelevant regions corresponding to excreted waste and walls. Properties of the remaining regions in a frame were calculated and recorded to a file. Each line of this output file corresponds to one object and contains

the video frame number, the object id number in that frame, position, area, principle axis angle and compactness.

This method did not perfectly detect all the locusts all of the time. There was not always a 1:1 relationship between locusts in the video and the detected objects. Two locusts sufficiently close together will be detected as a single object. A single locust may be detected as two objects. This latter error was greatly reduced by appropriately setting the brightness, contrast for the video and the threshold for conversion to black and white, but could not be completely eliminated. Finally a locust may not be detected at all. This can occur if it leaves the region of interest, by jumping on to the central dome for example, or if its area in the video becomes sufficiently small, for example if it faces vertically upwards whilst attempting to climb the wall.

Tracking from one frame to the next

Once the locations of objects have been determined in all the frames, the correspondence of objects across frames needs to be found. This correspondence is the key component required for fully-automated tracking. The output from icBiovision tells us where objects are in a frame but they are not identified with specific individuals. With a sufficiently high frame rate, objects can be linked across frames by simply considering the objects' positions. A locust is unlikely to move very far between frames, so an object at position (x,y) at time t is likely to be the same individual as an object very close to (x,y) in frame t+1. Suppose we have a set of objects O in frame t and a set of objects N in frame t+1. The Euclidian distance from every object in O to every object in N is calculated. We call the closest object in N to the position of an object o in O, the nearest neighbour of o in N and similarly for an

object n in N the nearest neighbour of object n in O is the object in O whose position is closest to the position of object n. For an object o in O to be considered to represent the same individual as n in N then n must be the nearest neighbour of o in N and o must be the nearest neighbour of n in O. If either of these conditions was not met the objects were not linked.

Two kinds of error may occur with this method. Firstly, if we assume perfect object extraction from the video, objects passing close to each other may result in misidentification. Consider an individual i_1 which moves a distance d_1 between frames at time t and t+1. If some other individual i_2 moves to a position less than d_1 away from i_1's original position in the same period, then i_2 will be identified as i_1 as long as i_2 is closer to i_1's previous position than it is to i_2's previous position. If the two individuals are moving in opposite directions they could swap identities; otherwise the identity i_2 is lost entirely. The second problem is due to objects not representing a single individual. This can result from coalescence, where two sufficiently close individuals may be detected as a single object or splitting where one individual is detected as two objects. Both of these errors will tend to introduce errors in area, speed and orientation.

Table 2.1 – An example of the difference between automatic and semi-automatic tracking. The same 30 min period from one experiment containing 5 locusts was tracked both automatically and with human intervention. The period consisted of 8983 frames, each containing 5 locusts giving a total of 44915 objects to detect. The resulting tracking information was then compared to assess the level of error in the automatic tracking.

	Automatic	Semi-automatic	% difference of automatic from semi
# split objects	327	-	-
# coalesced objects	543	-	-
Percent of objects affected	1.9%	-	-
mean activity	0.417	0.420	-0.7%
mean alignment	0.546	0.520	5.1%
mean speed	1.911	1.923	-0.6%

These errors are infrequent relative to the correctly identified individuals across two frames and so do not have a significant impact on calculating group-level statistics, which only consider successive frame links (see Table 2.1). However, the level of error is sufficient to make long term tracking of individuals fail.

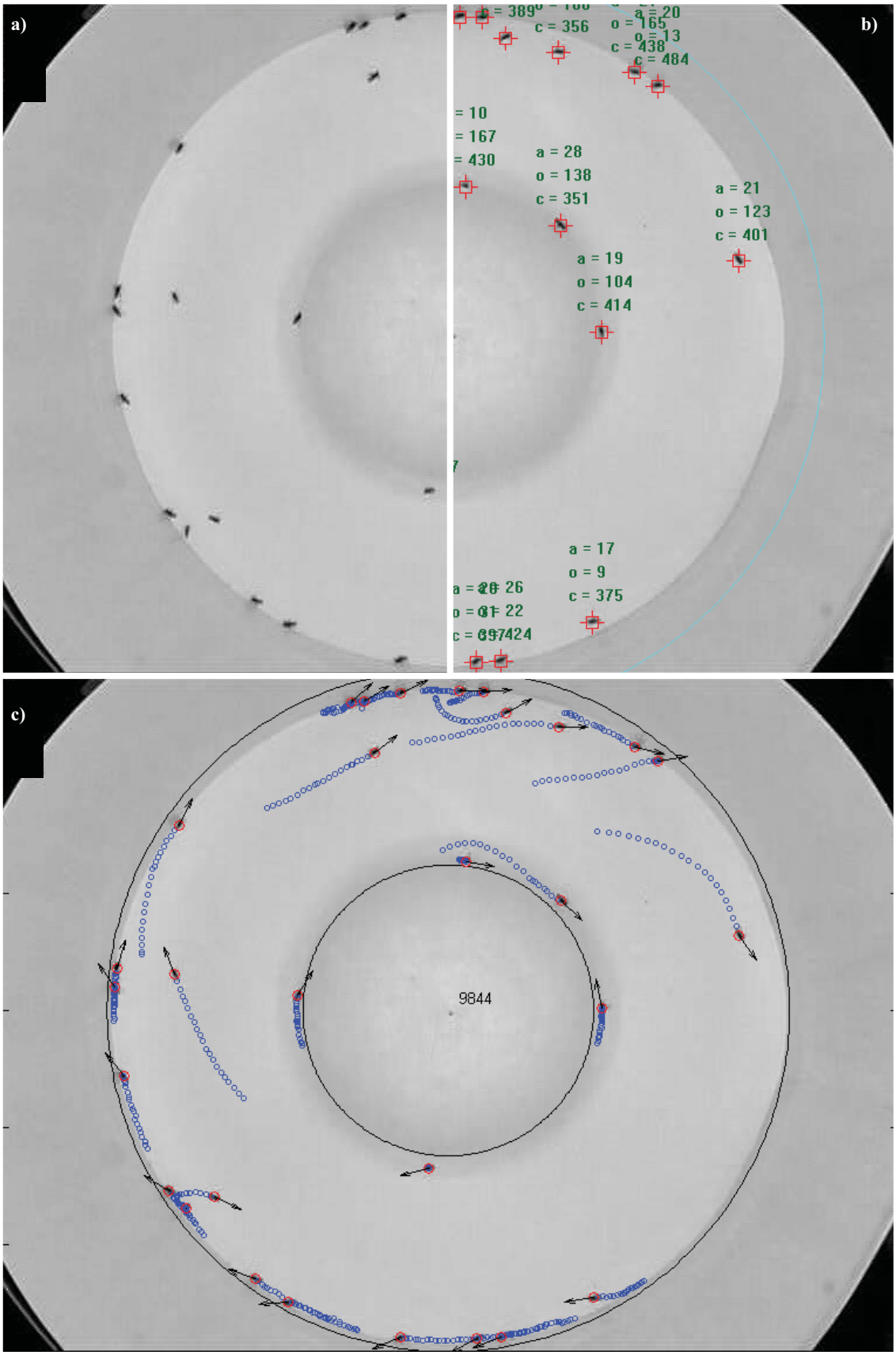


Figure 2.3 (a) An example frame capture from the video camera. (b) icBiovision identifies the position of objects in frames. (c) the objects are linked through time in Matlab.

Automatic Tracking tool

The ability to identify objects in a single video frame and then identify objects in successive frame as the same individual facilitates automatic tracking.

In order to automatically track a group of individuals, the tracked objects detected in successive frames corresponding to the same individual have to be linked. This linking can be done highly accurately between two frames, allowing the measurement of each detected individual's instantaneous speed and direction. This information allows for characterisation of behaviour at the group level at each instant. The accuracy of the group-level alignment may be improved by using additional properties extracted from the detected objects, such as the principle axis of the object. An alternative method of generating these flow properties would be to use a technique developed to analyse fluid flow - digital particle image velocimetry (DVIP). This technique generates a vector field of flow by dividing the image intio tiles and generates the average flow for each tile (Adrian, 1991). DVIP is particularly useful when the identification of an individual is difficult. However, in our set up the individuals were clearly distinguishable from the background allowing the short term tracking of individuals. The effect of the orientation of individual locusts on conspecifics was central to many of the key questions addressed in this thesis. The short term individual tracking was able to provide this information as well as providing the basis for the more detailed semi-automatic tracking described below.

Manual Tracking Tool

A manual tracking tool is useful for extracting information in complex backgrounds and recording subtle behaviours, such as where an individual is looking, or differentiating collisions from aggressive interactions. These tasks are often too

difficult to be reliably automatically detected by a computer. It can also be used for extracting features from a video, like the location of the centre of an arena for example. Manual tracking is best for the analysis of small numbers of individuals or short experiments since it is very labour intensive. Manual tracking suffers from observer bias, as the person tracking has to make decisions of how to classify what they see which may not be consistent with the classification of a different observer.

Semi-automatic tracking

Manual tracking is laborious and automatic tracking is not completely reliable. Semi-automatic tracking provides a compromise. With semi-automatic tracking the user assists the automatic tracking by resolving object identities when the computer can't. This technique reduces the amount of input required by the user and so enables tracking of more individuals and over longer periods. A human operator is able to maintain the identities of individuals, even during close encounter, and so tracks of individual locusts can be maintained over extended periods of time. Detailed tracking of all individuals within a group is a key tool in developing an understanding of the links between the individual level and group level behaviour of group living animals. An animal in a group may be reacting to an unknown number of neighbours, including zero neighbours.

2.5 Applications

Here I describe various applications to which the three types of tracking software (automatic, manual and semi-automatic) have been applied. Some of these applications form the basis for further chapters in the thesis, while others demonstrate how my code has been applied to experiments by other lab members.

Aggregation

The desert environment of the locust is highly heterogeneous, with large variations in temperature over short scales. In this environment, locusts form tight aggregations in suitable microclimates (Kennedy, 1939). We were interested in the relative strengths of the social and environmental factors which resulted in these aggregations. Hedwig Emmerig carried out experiments as part of her MSc in Biology at the Oxford University Zoology Department to investigate these factors (Emmerig, 2005). In this experiment locusts were presented with two heat patches in a homogeneous environment. Dense aggregations of locusts would form on the heated areas of the arena floor. More details of the experiments and results are found in Chapter 3. We needed to investigate the relative strengths of a locust's attraction to the heat patches and their attraction to other locusts and to see if the locusts would collectively select one of the two patches. We chose to use video tracking to obtain the number of individuals above each patch. This method minimised the disturbance of the insects during the experiment as no observer was needed in the room. Using computer tracking of the video we were able to generate a time series of the number of individuals on each patch over the entire experiment with a very fine temporal scale (25 observations per second). Thus every change in the populations on each of the heat patches was captured. Using this measure it was possible to obtain the initial growth rate on each patch, the distribution of the number of individuals on the most populous patch and the mean populations of each patch over the course of an experiment.

Ideally each locust would be individually tracked, which would have enabled direct analysis of the attraction to different sized clusters of locusts above the patches and the time each individual spent resting on a patch. It was not possible for either a

human observer or automated tracking to keep track of individuals over the course of an experiment as the locusts were unmarked and would be lost track of whenever they entered the dense aggregations. The relative sizes of the locusts and the arena meant that marking individuals was not effective. Differentiating individuals who were passing through the patch from those that were leaving after resting on a patch was similarly difficult. The most reliable measure was therefore chosen to be the number of locusts on each of the heat patches at any time.

Although it was not possible to track individuals, it was possible to use the output from icBiovision to generate a time series of the number of individuals on a heat patch. However, on the heat patches the individual locusts were very tightly packed and usually touching each other. This resulted in single objects potentially representing many locusts. The area of pixels on a patch provided a rough estimate of the number of locusts on a patch. However the area representing an individual locust varied with its orientation. Additionally, the area of coalesced locusts increased non-linearly, making the detection of the difference between higher numbers of locusts difficult. The estimate of the number of locusts on a patch was improved by considering the locusts which were not on the heat patches, given that there was a fixed population size.

The mean and standard deviation of the area of one locust was calculated from the first 3 s of the experiment, before the heat patches had heated up and while the locusts were well distributed around the arena. At this time each object was assumed to represent a single locust. Subsequently, objects whose area was within two standard deviations of the mean area were considered to represent a single locust. The number

of such objects was counted for each of the three regions being considered (each of the heat patches and the rest of the arena). The area of the remaining objects was summed in each region and the number of unaccounted locusts was divided up proportionally by the area in each region.

The accuracy of this method was tested by comparing the computed number of individuals on the spots with the observed number every 15 min for two randomly chosen experiments, one high density experiment and one low density experiment. This method was found to be 92% accurate at high densities (60 locusts) and 95% accurate at low densities (10 locusts).

Collective Motion

In the paper by Buhl et al. (2006) reproduced in the appendix, we studied the collective motion of locusts. We wanted to test the prediction from theoretical physics models that as the animal density increased in group, there would be a rapid transition from disordered to ordered motion. Experiments were carried out in the ring-shaped arena with different densities of locusts. EDA was performed and group-level statistics were generated using my automatic tracking routines. The group-level statistics where then compared with those generated by the theoretical model. Two key indices were extracted from the data: activity and alignment.

Activity

The activity was defined as the proportion of locusts moving in a frame. Buhl et al. (2006) found that immobile individuals did not influence the direction of moving locusts. Thus the effective density of the group was considered to be the active population for the comparison to the model. The size and orientation of this active

population needed to be recorded throughout the 8 h experiments and so automated tracking was used. Individuals were classified as moving or not-moving by considering their change in position between frames. Since the positions of the locusts were calculated at sub pixel accuracy, objects which were in fact stationary could appear to move due to noise in the video image. A threshold was therefore required to differentiate moving from stationary objects. The criterion for movement was that a locust changed position by more than two pixels between consecutive frames (a period of 0.2 s). This threshold was chosen by visual inspection to exclude the effects of pixel noise and also small movement due to rotations on the spot. Tracked data were overlaid on the video, which indicated whether an object was detected as moving for a given threshold. The threshold was varied until stationary locusts were correctly classified. The sensitivity of threshold choice can be seen in Figure 2.4. We found high sensitivity for a threshold of less than one, where the effect of pixel noise is strongest. In the neighbourhood of our threshold the sensitivity of activity to the threshold is approximately linear with a shallow slope.

In reducing the degree of movement of the locusts to a binary state of moving or not-moving some information was lost. We would expect that the rate of encounters, and hence the perceived active density, would increase with the speed at which locusts moved. However, since the data were averaged over an 8 h period we expect that the effect of differences in individual's speeds would not have a significant effect on the final results. It would be interesting, however, to investigate the distribution of the speeds observed in both the experiments and the model.

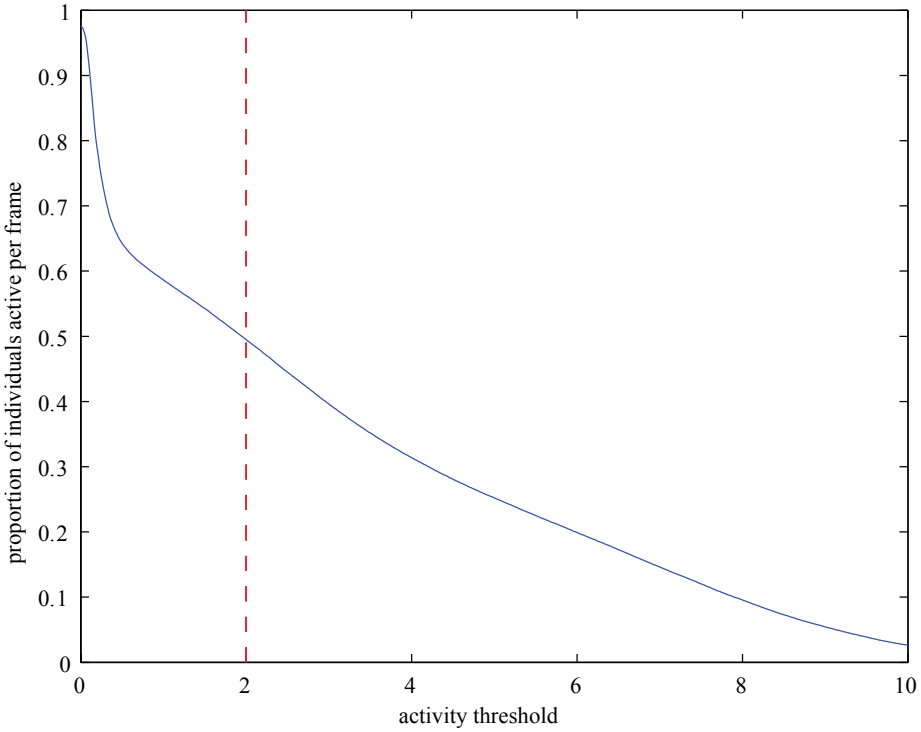


Figure 2.4 The proportion of locusts detected as being active over an experiment for different activity thresholds in pixels. The dashed line represents the threshold of 2 used in our analysis.

Alignment

We wanted to capture the degree of order within the group of locusts, corresponding to the mean velocity of all particles in the 1D SPP model. In the experiment the locusts were constrained to moving in an annulus. Therefore a locust could either move clockwise or anticlockwise around the arena. We wanted a continuous index between -1 and 1. An index with absolute value 1 represents a perfectly ordered group with all individuals moving in the same direction; the sign of the index indicates whether they were moving clockwise or anticlockwise. An index of zero should indicate a lack of ordered marching, where the locusts were either not moving or moving in different directions to each other. The index described below satisfies these conditions.

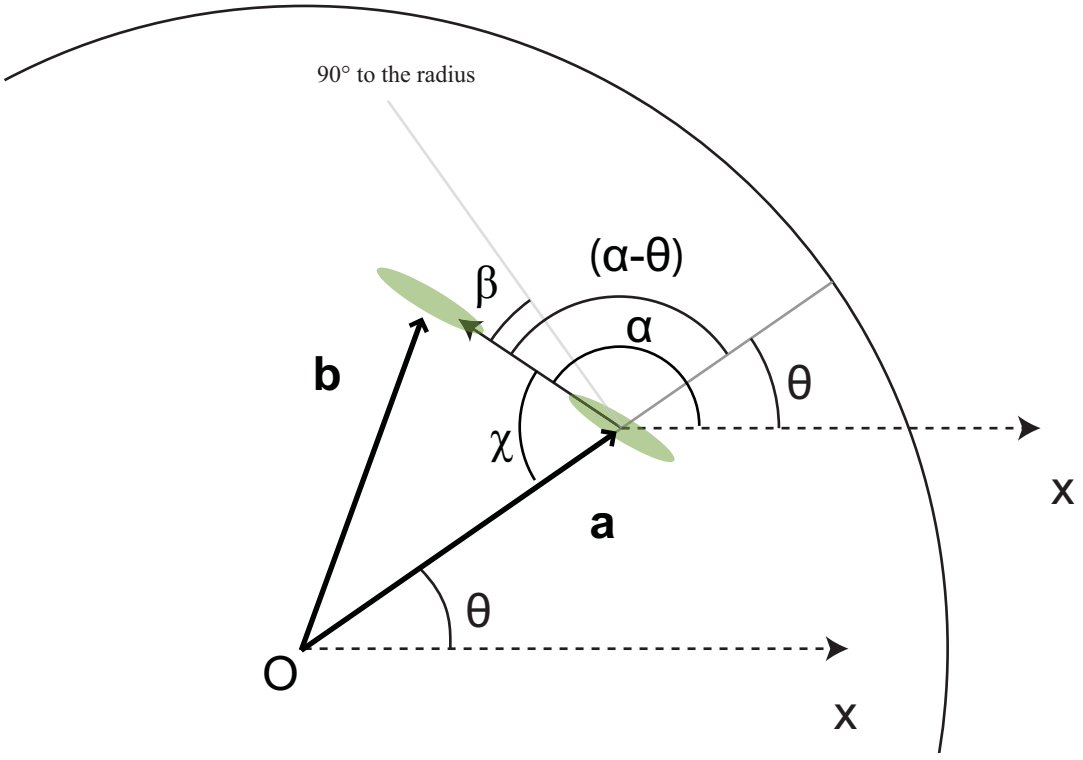


Figure 2.5 Calculating Alignment. The positions of a locust at successive time points in an arena centred at O. The position vector a is the locust location at time t and the position vector b represents the locust at time t+δt. The angles are described in the text.

When the locusts were all moving together as a polarised group they must have been taking a circular path since they were constrained by the walls of the arena. The degree to which a locust was moving on a circular path was calculated by considering the smallest angle between its position vector, relative to the centre of the arena, and its velocity vector, the difference between its position from one frame to the next (Figure 2.5).

The vector $\boldsymbol{r}_t = \begin{pmatrix} x_t \\ y_t \end{pmatrix}$ defines the locust's position at time t relative to the centre of the arena. A single locust's position at times t and $t+\delta t$ can be expressed as $\boldsymbol{a} = \boldsymbol{r}_t$ and $\boldsymbol{b} = \boldsymbol{r}_{t+\delta t}$ respectively and its the path by $\boldsymbol{b} - \boldsymbol{a}$ (Figure 2.5). We define β to be the angle that the locust's path, $\boldsymbol{b} - \boldsymbol{a}$, makes with $\boldsymbol{a}'$ – the vector perpendicular to the position vector $\boldsymbol{a}$. The vector $\boldsymbol{a}'$ is obtained by multiplying it by the 90 degree (anticlockwise) rotation matrix:

$$\mathbf{a}' = \begin{pmatrix} 0 & -1 \\ 1 & 0 \end{pmatrix} \begin{pmatrix} x_t \\ y_t \end{pmatrix}. \tag{2.1}$$

We used the dot product to calculate the smallest of the two angles between $\boldsymbol{b} - \boldsymbol{a}$ and $\boldsymbol{a}'$.

$$\cos(\beta) = \frac{(b-a) \bullet a'}{|b-a||a'|} \tag{2.2}$$

This angle was a measure of how much the locust's path deviated from the anticlockwise normal to a radius of the arena. This was transformed to be the signed angle between the radius and the direction vector as follows:

$$\chi = \beta - \frac{\pi}{2}. \tag{2.3}$$

A value of $\chi = -\frac{\pi}{2}$ indicated that a locust was facing in an anticlockwise direction, $\chi = 0$ indicated that a locust faced directly towards or away from the centre and $\chi = \frac{\pi}{2}$ when a locust faced clockwise around the arena. We call χ the relative angle. This may also be expressed as $\chi = \arcsin(\sin(-(\alpha - \theta)))$, where θ was the angle $\boldsymbol{a}$ made with the positive x-axis and α was the angle that $\boldsymbol{b} - \boldsymbol{a}$ made with the positive x-axis.

For each frame, the instantaneous alignment Φ^t was calculated as the average of the relative angles of all moving locusts in a frame, normalised to be between -1 and 1. This gave a score of how well aligned the groups was, with individuals moving in different directions cancelling each other out resulting in a low score. If the whole group is moving around in the same direction they would have a score close to 1 if they were going anticlockwise or 1 if they were moving clockwise.

This index was used as the basis for the measures of the degree of order in the experiments. These measures were the average alignment over the entire experiment, the time spend in the ordered phase (where the group alignment's absolute value was greater than 0.3), and the number of changes in direction of the entire group. The locusts tended to form aligned groups rapidly at sufficient densities and march around the arena. The time spent in these aligned states increased with density and the number of direction changes of the group decreased as density increased.

This measure would not have been appropriate if two persistent groups formed in the arena and moved in opposite directions. In this case the index would be close to zero as the index of one group would cancel out that of the other. Thus the index could indicate a lack of order when the system was in fact highly ordered.

Cannibalism

In these experiments Bazazi et al. (2008) investigated the effect of cannibalistic interactions on locust marching. It was hypothesised that locusts moved more within groups due cannibalistic interactions from behind. We wanted to determine if there was a difference in the amount of marching observed between individuals which had been desensitised to cannibalistic interactions and those which had only had a sham-operation performed on them. The role of vision was also considered by blinding locusts to the front, behind and both. The ring-shaped arena was used with the automatic tracking method above to generate the time series of activity in the different groups. The level of marching was quantified by extracting the proportion of time an individual spent moving from the activity time series. The mean speed of an individual was also compared.

No significant difference in proportion of time spent marching or speed of movement was observed between the sham-operated and desensitised locusts when there was only a single locust in the arena. However, there were significant differences in both when 15 individuals were put in the arena together. This paper is included in the appendix.

Inertia

In Chapter 4, I present an experiment investigating the role of individual experience on propensity to march persistently. I used the measure of activity described above and an improved measure of alignment to compare groups of locusts with and without highly directed marching experience.

For this experiment I extended the alignment measure to consider the orientation of both moving and stationary individuals by directly using each insect's anteroposterior axis. This extension greatly reduced the noise in the measure, allowing better precision for the small groups of 5 locusts where noise in the measure had a greater effect.

Each insects' anteroposterior (a-p) axis was extracted from the video frame using icBiovision software (Couzin, 2005). The direction in which a locust last moved was use to identify the anterior of its axis and so giving its direction vector d which can be substituted for the vector $\boldsymbol{b} - \boldsymbol{a}$ used above in the description of the alignment measure above.

The compactness of the object was used to determine whether its principle axis represented the corresponding locust's anteroposterior (a-p) axis. Compactness close to one would indicate a circular object whose principle axis orientation would be very sensitive to noise. A principle axis orientation could only be used if the object was sufficiently elongated. We set a threshold of compactness greater than 2 by inspection to determine whether the principle axis reliably represented a locust a-p axis.

The principle axis was not directed, having an orientation ρ in the range [0°,180°] so we used the movement of the locust to assign the appropriate orientation to its a-p axis of either ρ or $\rho + 180°$. We considered four cases to determine which orientation to use. In the first case, if the object had a valid principle axis, i.e. was sufficiently elongated, and was moving (as defined previously as a difference in observed position of more than 2 pixels between frames) then the orientation of the a-p axis γ was set to be the orientation of the principle axis closest to the direction of its velocity vector. In the second case, when there was a valid principle axis but the object was not moving, γ was set to the principle axis closest to the locust's a-p axis in the previous frame. If its a-p axis was not known in the previous frame γ was left undefined. The third case, when the object was moving but did not have a valid principle axis, γ was set to the direction of its velocity vector. Finally, if the object did not have a valid principle axis and it was not moving, or had undefined movement, then its a-p axis was undefined in that frame.

This method did not work during periods when a locust walked backwards. However, I observed that the locusts tended to walk backwards when avoiding other locusts and would frequently be very shortly followed by forward motion. Using the principle axis rather than the direction of motion between frames in the alignment measure greatly reduced the noise in the measure.

Individual Behaviour

To investigate the effect of the behaviour of a locust's nearest neighbour on its behaviour, I needed to track all individuals within an experiment. Additionally, to control for individual differences, the identities of each locust needed to be

maintained throughout the entire experiment. From these tracks the distance to a locust's nearest neighbour, the relative orientations of the two locusts and the movement state of each locust were extracted. These measures were broken down into discrete ranges to allow the fitting of statistical models.

Several of the inertia experiments were semi-automatically tracked using the methods described in section 0 above. The positions and identities of individuals were determined for all frames in a 30 min period in 9 experiments. This semi-automatic tracking was the basis of analysis in Chapter 5.

Gaze following

Manual tracking was used in a study replicating Milgram's (1969) gaze-copying experiments. Milgram et al (1969) investigated the relationship between the size of a group of people and the proportion of passers-by that respond to the group. Different size stimulus groups looked up at a sixth floor window from the street below for 60 s periods. A camera in the window filmed the area around the stimulus group allowing the number of passers-by that looked up or stopped to be counted.

We repeated Milgram's experiment in Oxford, this time tracking both spatial position and participant response. Manual tracking was used to record the positions of people and whether they looked up to the point at which a stimulus group of people were looking. Manual tracking was needed to track pedestrians in the complicated street environment which including many other moving objects (cyclists, push-chairs, pigeons etc.) and it was needed for the identification of passers-by's response to the stimulus group. The tracked data were transformed into real-world coordinates using

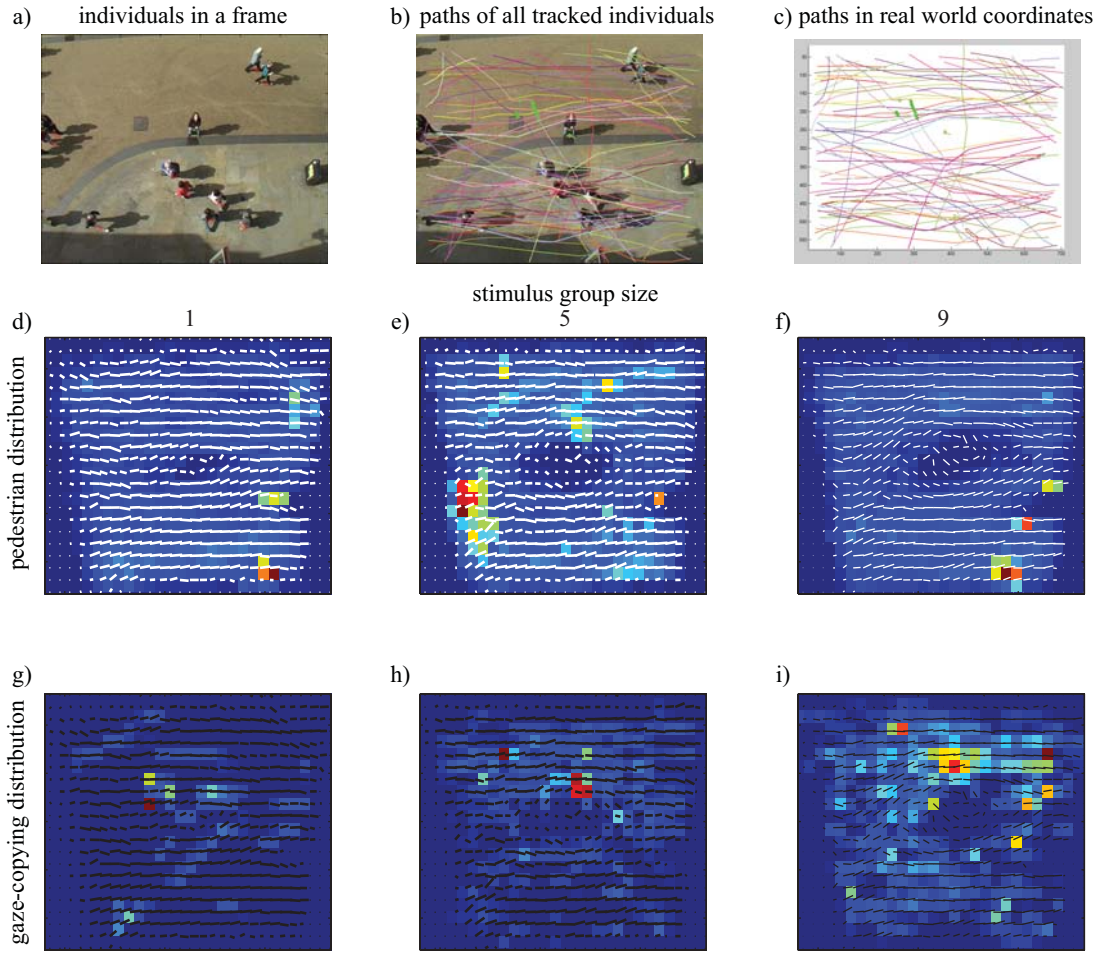


Figure 2.6 Manually tracked pedestrians. (a) Video frame in which individuals have been identified; (b) paths of all people in that trial overlaid with green circles indicating gaze-copying individuals; and (c) the same paths translated into real world coordinates. The lower panels show the spatial distribution of pedestrians and gaze-copying averaged over all replicates in 0.5m^2 cells for stimulus group sizes 1, 5 and 9. Cold colours indicate low density (with blue as the lowest) and warm colours indicate high density (with red as the highest). (d – f) show spatial distribution of the pedestrians. (g - i) show the different spatial distributions of pedestrians and where gaze-copying occurred averaged over all replicates for stimulus group sizes of 1, 5 and 9 respectively. for each treatment. The small straight lines indicate average bidirectional flow through that cell.

known positions of points on the ground and the optical parameters of the camera. Descriptive statistics of the trajectories were calculated along with the gaze-copying response to the different stimulus group sizes. Spatial visualisations of the data were also generated (Figure 2.6).

2.6 Future work

The semi-automatic tracking program is modular in design. Additions could be made to automate common tasks. For example when single locusts are detected as two objects there could be a merge objects option when the user would only need to click on a single object and it would be combined with the nearest other object. Keeping track of a how a user corrected previous actions could also speed up corrections and be very useful for dealing with a recurring problem in successive or nearby frames.

The equipment used in video tracking experiments is improving. Recording experiments using High Definition (HD) video would provide over double the resolution of the SD video we used here, going from 720 x 576 pixels to 1920 x 1080 pixels. This should allow better tracking and also allow individual marking to be more effective. With HD video close interactions would also be able to be studied more clearly.

The technical issues raised by lens distortion can now be quite easily corrected using camera calibration packages which are able to determine the properties of a given lens by analysing images of a calibration pattern. This can be used to create a mapping from points in the image to points in the real world (Heikkila and Silven, 1997) and increases the flexibility of experimental set up, as the camera does not have to be set directly above the experiment and information from multiple cameras can be integrated using real world coordinates.

As tracking technology improves, so the amount and quality of information available to an observer will increase. Making the most of these data will require new techniques. Additionally improved tracking technology provides experimentalists

with new methods to study their subjects. There is lots of exciting work to be done in developing methods and tools to analyse these data.

2.7 Appendix – Technical Details

At the end of this thesis there is a DVD containing the tracking code written for Matlab. Example code and videos are also included. This appendix contains detailed information on how the tools are used.

Automatic linking of individuals across frames

The matching of individuals across frames is performed in Matlab with the *jjLinks* function (see Box 2.1). The results of this can be visualised in Matlab by *jjVis* (Figure 2.3c).

Manual Tracking tool

I wrote a Matlab program *HandyTrackLite* to do fully manual tracking. It records the position, frame and identity of objects as the user clicks on them in a video. For a given ID, points are linearly interpolated between the user's clicks, which are overlaid in the video so that the user need only correct deviations from this path.

The tracking tool was created in Matlab 7. It should be started in a fresh instance of Matlab exclusively for the program as it clears and subsequently uses the Matlab workspace. The command *handytracklite* launches the graphical user interface for the manual tracking (Figure 2.7). Once started the filename of the video file is entered in the "AVI name" text box and then the "Initialise" button pressed and first frame of the video is displayed. If the video file is in the search path the filename alone is sufficient, otherwise the full path must be included.

Once the video is loaded it can be viewed in two different ways: either advanced in single steps or played continuously. With both methods the number of frames advanced at a time is set by the number in the "step size n" box. This can be easily

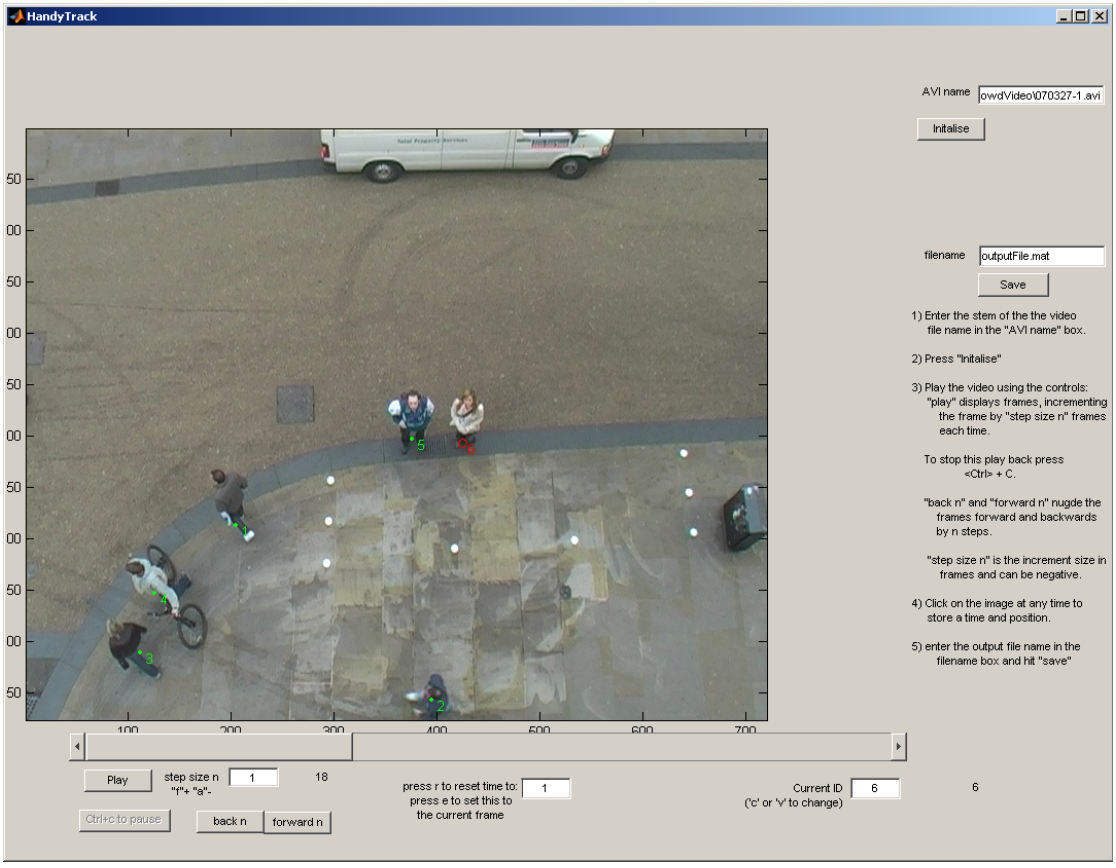


Figure 2.7 – Manual tracking with *handytracklite* applied to human tracking in a complex background.

incremented or decremented with the “f” and “a” keys respectively. The keys “s” and “d” respectively decrement and increment the currently viewed frame by the current step size. Pressing the play button starts continuous playback. In this mode pressing “g” or “r” jumps back to the frame number in the reset time box and continues playing the video. The frame number in the reset time box can be set to the current frame by pressing “e”. The video can be stopped by holding down the “ctrl” and “c” keys simultaneously.

When a user clicks on the video three pieces of information are recorded: the position of the cursor, the button that clicked and the current ID number. The current ID number is displayed in the bottom right and can be incremented and decremented with the “v” and “c” keys respectively. Previously clicked points in a frame are displayed on the video as hollow circles. The path between clicked points is linearly interpolated for the intervening frames and is represented by smaller filled circles. Left clicks are shown in red and right clicks in green and each point is labelled with its object’s ID number. The keyboard controls are summarised in Table 2.2.

Table 2.2 – The manual tracking tool's keyboard short cuts for controlling the video playback.

Key	Action
v	Increment current ID
c	Decrement the current ID
r	Start playing from restart point
e	Set the current restart point to the current point
d	Advance one step
s	Go back one step
f	Increase the number of frames advanced in a single step
a	Decrease the number of frames advanced in a single step
g	Go back to the reset point
Ctrl+c	Stop looping video

To save the tracked data, the user must enter a filename in the filename box above the "Save" button and hit "Save". The filename must have the ".mat" extension.

To resume tracking with previously saved data the user has to initialise the program as if they were starting from scratch and then run the following command:

load outputFile.mat

where *outputFile.mat* is the name of the file containing the previously saved data.

The saved file contains two Matlab cell variables called "*myFrames*" and "*myObjs*". For every video frame *f* the variable *myFrames{f}* is a list of object IDs which have been recorded in that frame. The cell *myObjs* is an array of records for each object detected containing the following information

- *myObjs{i}.start* is the first frame the object i is clicked in.
- *myObjs{i}.end* is the last frame in which the object i was clicked.
- *myObjs{i}.pos* is a matrix with one row for each frame the object is in. The x and y coordinates are stored in the first two columns and the next two columns

record the click type (left click = 0, right click = 1) and whether the point is interpolated (actually clicked = 0, interpolated = 1).

Semi-automatic tracking in Matlab

The program searches the tracking data for potential errors, assuming that there are a fixed number of objects in the arena. When potential errors are detected the user is presented with the video with the automated tracking information overlaid. The user can then graphically correct the tracking. We refer to the collection of Matlab functions which perform the semi-automatic tracking as handyTrack.

To use the semi-automatic tracking, start a new instance of Matlab and enter the command

htStart(icb, Rx, Ry, R, r, N, video1, video2, VFstart, IdxStart, IdxEnd, outputStem)

or just

htStart

The parameters are the following:

Parameter	Value
icb	The path and stem name of the automatically tracked data from icBiovision
Rx, Ry	The x and y coordinates and radius of the circular region of interest
R	The radius of the inclusive region of interest (for the arena wall)
r	The radius of the exclusive region of interest (for the central dome)
N	The number of objects in the arena
video1	The path and name of the first video of the video pair (assumes the video of the exp is split into two)
video2	The second of the video pair.
VFstart	The first frame of the video to track from
IdxStart	The index of the detected frame in the icb data file corresponding to VFstart
IdxEnd	The index of the detected frame in the icb data file to finish tracking on
outputStem	The stem name and path of the file to save the output. (generates 2

files: <stem>_0.mat and <stem>_1.mat).

For example:

*htStart('C:\icB\A060707', 382.85, 291.3,291,124, 5, 'F:\A060707_1.avi', 'F:\A060707_2.avi', 20019+10*5, 19094+10*5+1,19094+10*5+1 + 5*60*30, 'C:\A060707');*

The user is then warned that starting *handyTrack* will clear the workspace. If no parameters were passed in the command the user is prompted to enter details. If output files corresponding to the input parameters already exist the user is prompted to pick up from where they left off, otherwise it will load and process the tracking data and ask the user to initialise the tracking.

The user is presented with the first video frame with the automatically tracked data overlaid. Prompts on the figure guide the user through ensuring correctly assigning identities to the objects in the first frame.

Next, the automatic tracking data are sequentially analysed, linking objects and identities and watching for frames where there is confusion over identity. Checks are performed on the number of objects detected, the distance that an object moves between frames and for potential instances of coalesced objects. When a potential problem is found, the relevant tracking data are present to the user overlaid on the corresponding video frame. Instructions of how to fix the problem are given above the video. This typically involves using the mouse to identify erroneously tracked points or to indicate the true position of a locust. Once the frame is corrected the data are automatically saved and the kind of error which was saved is reported in a variable

fixList. The usual Matlab figure tools may be used to zoom in on the frame or resize the entire figure.

In addition to the on-screen instructions, the following additional commands can be entered into the Matlab command line:

htGoBack

This is the undo function – it undoes all the corrections to the last frame. It can be use repeatedly, going back one correct frame at time. If multiple corrections have been made in a frame, they will all be undone by one call of *htGoBack*.

htHard

Sometimes two locusts can appear to completely merge on the video. In these cases the user should run *htHard* after correcting as best they can. This will flag the frame in the variable *fixList* by multiplying the error code by 100. This list can then be inspected later and other methods used to determine which individual is which (by considering object area over time for example).

Every time a correction is made the data are saved. They are saved into two files for each experiment. They are named with the output name stem with a suffix of either "_0" or "_1", and an extension of ".mat". The first file, ending "_0.mat" contains the setting and data which do not change as the video is tracked. The file ending "_1.mat" contains the corrected tracking data. This ensures that if the computer crashes work can be recovered by starting as described above. The program *htStart* will advise the user that there are already files corresponding to the experiment and give them the

option to reload them. If the option to restart is chosen instead, all previous work on that video will be deleted.

The program displays a progress bar, which gives an indication of how far through the tracking data the user has progressed. Once an experiment has been completed, the user needs to do a final save by running the following command:

htSaveDyn()

Once the assisted track has been completed the tracking results are stored in the *idata* variable which is saved in the file ending with "_1.mat". It can be converted to the format output by *jjLink* with the command *jjiData2Ldata(idata, data)*.

2.8 Examples of using the measures in Matlab

Aggregations

The automated count of the number of individuals in dense aggregations was implemented in the code *jjDensePatches*.

Activity

The distance moved was calculated using automatic tracking by *jjLink* (see Box 2.1) and is the 8th column of the *Ldata* Matlab variable. This allowed numbers and proportions of active locusts which characterised the group to be calculated using simple Matlab commands, for example

```
sum(Ldata(:,8) > 2 & Ldata(:,12) == 1)
```

returns the number of all valid objects which have moved more that 2 pixels between frames for all tracked frames.

Alignment

The simplest method of calculating alignment considers the direction of movement of an individual as the alignment of it body. This assumes that the individual is moving forward. Stationary individuals are therefore excluded. It can be calculated in the Matlab function *jjCircularMovingAlignment*. The code to calculate the instantaneous alignment is given below where Φ^t = *InstAli(t)*.

ali = jjCircularMovingAlignment(Ldata, Rx,Ry);

InstAli = jjFrameAverage(Ldata(:,2),2. ali ./ pi, 1);*

The alignment measure based on the locust's a-p axis is implemented in the Matlab function *jjCircularAlignment*.

ali = jjCircularAlignment(Ldata, Rx,Ry);

aliByFm = jjFrameAverage(Ldata(:,2),2. ali ./ pi, 1);*

2.9 Appendix 1 – Key Data structures

icbFile - icBiovision output file

This is a text file generated when a video is tracked. The data are stored using the tab delimieted format. The first line is a header describing the column names. Every subsequent row contains the following columns:

Column #	1	2	3	4	5	6	7
Content	f	id	x	Y	a	o	c

f - video frame number (from start of first file).

id - object ID within a frame.

Box 2.1 How to load data into Matlab from icBiovision.

Loading tracking data into Matlab and linking objects over time

1) Load the data generated by icBiovision
   data = jjLoadicb(icbFiles,1);
2) Exclude objects outside of the region of interest
   myROI = jjROIc(data, [], Rx, Ry, R, include);
   myROI = jjROIc(data, myROI, Rx, Ry, r, exclude);
3) Link the objects across frames
   Ldata = jjLink(data, myROI);

x y - position of object.

a - area of object.

o - orientation of objects principle axis. (in rad)

c - compactness of object

data – raw icBiovision data imported into Matlab

This an Nx7 Matlab matrix output by the function jjLoadicb. It contains the data from one or more icbFiles, maintaining the column order described above under the icbFile data structure.

Ldata – objects linked over time

This is an Nx12 Matlab matrix generated by jjLinks. It contains details of tracked objects along with the identity of the corresponding object in the previous frame.

Col #	1	2	3	4	5	6	7	8	9	10	11	12
content	f	id	x	y	a	o	c	S	D	oid	h	roi

f, id, x, y, a, o, c as in icbFile.

S – distance moved by object between this frame and the previous one squared.

D – the angle between the positive x-axis and the direction of the motion between frames (in radians).

oid – the id of the object corresponding to this object in the previous frame.

h – the orientation of the vector from the centre of the object to its anterior (in degrees)

roi – Boolean value indicating if the object is in the region of interest as defined by jjROIc() and jjROIr().

If an object is not linked across frames S, D and oid are set to NaN ("not a number" in Matlab). Similarly, an object whose anterior can not be determined has h set to NaN.

idata – semi-automatically tracked data

This is a Nx7 Matlab containing the results of the semi-automatically tracked data, where N is the product of the number of objects tracked and the number of frames. It is similar to data, except that the 7th column contains the distance moved by object between this frame and the previous one. Additionally, the object ids in each frame correspond to individual identities.

Column #	1	2	3	4	5	6	7
content	f	id	x	y	a	o	S

f - video frame number (from start of first file).

id - object ID within a frame.

x y - position of object.

a - area of object.

o - orientation of objects principle axis.(rad)

S - distance moved by object between this frame and the previous one.

myFrames – Manually tracked frame object list.

This Fx1 cell is output from handytrackLite. For each frame this variable contains a list of individual ids which were detected in that frame and correspond to the objects in myObjs.

myObjs – Manually tracked object details

The cell myObjs is an array of records for each object detected with the manual tracking program handytrackLite. For object i it contains the following:

- myObjs{i}.start is the first frame the object i is clicked in.
- myObjs{i}.end is the last frame in which the object i was clicked.
- myObjs{i}.pos is a matrix with one row for each frame the object is in. The x and y coordiates are stored in the first two columns and the next two columns record the click type (0 = left click, 1= right click) and if the point is interpolated (actually clicked = 0, interpolated = 1).

2.10 Appendix 2 - Function reference list

jjLoadicb

jjLoadicb(filenames)

jjLoadicb(filenames, headerlines)

Loads icBiovision files, returning a matrix of their contents

data = jjLoadicb(filenames, headerLines)

filenames is either a single string containing the name of the file output from icBiovision, or a cell containing strings of several icBiovision files which will be concatenated in the same order that they are passed.

headerLines is an optional argument indicating the number of lines of text before the data starts in the data files, if omitted a value of 1 is assumed.

data = [... ; f | id | x | y | a | o | c ; ...]

f - video frame number (from start of first file).

id - object ID within a frame.

x y - position of object.

a - area of object.

o - orientation of objects principle axis.

c - compactness of object (how close to being circular is it).

jjROIr

Filter objects with a rectangular region of interest

dataROIout = jjROIr(data, dataROIin, x1, y1, x2,y2, inside)

Set up a rectangular region of (dis)interest for a data from an icBiovision file

dataROI = jjROIr(data, dataROI, x1, y1, x2,y2, inside)

data -the data from a loaded icBiovision file

dataROI - the current ROI filter data (may be [] if none exists already)

x1 and y1 - the bottom right corner of the rectangle.

x2 and y2 - the top left corner of the rectangle.

inside = 1 to include only the data inside the ROI

= 0 to include only the data outside the ROI

jjROIc

Set up circular region of (dis)interest for a data from an icBiovision file

dataROI = jjROIc(data, dataROI, x, y, R, include)

data -the data from a loaded icBiovision file

dataROI - the current ROI filter data (may be [] if none exists already)

x and y - the position of the centre of the circular ROI

R - the radius of the circular region of interest.

inside = 1 to include only the data inside the ROI

= 0 to include only the data outside the ROI

jjLink

Ldata = jjLink(data, ROI)

data – the output from jjLoadicb: the detected objects

ROI – a column vector from jjROIc or jjROIr indicating which objects to consider

The function jjLink attempts to link every detected object in *data* which is defined to be valid, that is all those with a corresponding 1 in *ROI*, with a valid object in the previous frame corresponding the same individual. If successful the speed and direction of motion of the individual is calculated. The orientation of the anteroposterior axis is also determined from a combination of the orientation of the principle axis and the direction of motion as described in the Inertia application above.

Ldata contains 12 columns

1	2	3	4	5	6	7	8	9	10	11	12
f	id	x	y	a	o	c	S	D	oldID	apa	inROI

The first 7 columns are from data:

f - frame, id – the object id in this frame, (x,y) – cords (untranslated), a - area, o - orientation,

c - compactness (1=circle)

The next five are generated in this function

S – distance moved between frames, D - direction of motion in radians,

oldID - corresponding object ID in the previous frame

apa - the estimation of the orientation of the anteroposttteria axis in degrees

inROI – logical list of whether or not the object is in the region of interest

Columns 8 through 11 are set to NaN if the object is not in the ROI or if it cannot be successfully linked to an object in the previous frame.

jjDensePatches

This command takes the total number of individuals in arena and estimates the numbers

in the spots using area approximations.

outSeries = jjDensePatches(Ldata, Ax,Ay, Bx,By, spotRad, numLocs,

areaMean, areaSD)

Ldata - output from jjLink, objects linked through time

Ax,Ay - position of patch A

Bx,By - position of patch B

spotRad - the radius of the patches

numLocs - the true number of object in the arena

areaMean and areaSD - optional: the mean and SD area of individuals in the arena. If excluded these are estimated from the first 75 frames working on the assumption that the objects are initally spread out and not in dense groups.

outSeries is a 3xF matrix with each row representing the number of locusts on patch A, B and on neither in each frame.

jjCircularMovingAlignment

ali = jjCircularMovingAlignment(Ldata, Rx, Ry)

Given the positions and orientations of individuals in Ldata generated by jjLink, this command calculates how closely each object is aligned to a circle centred at (Rx, Ry) a tracked object is based on its change in position.

For each row in Ldata the column vector ali contains a corresponding angle in radians in the range $\left[-\frac{\pi}{2}, \frac{\pi}{2}\right]$. This is the smallest angle between the radius of circle centred at (Rx, Ry) that the object is on and its velocity vector. If it is positive the locusts is facing in a clockwise direction and if it is negative the locust is facing in an anticlockwise direction. It is set to NaN if the object does not have a velocity vector (i.e. if it is stationary or could not be linked to an individual in the next frame.)

jjCircularAlignment

ali = jjCircularAlignment(Ldata, Rx, Ry)

Given the positions and orientations of individuals in Ldata generated by jjLink, this command calculates how closely each object is aligned to a circle centred at (Rx, Ry) a tracked object is based on its anteriorposterior axis.

For each row in Ldata the column vector ali contains a corresponding angle in radians in the range $\left[\frac{-\pi}{2}, \frac{\pi}{2}\right]$. This is the smallest angle between the radius of circle centred

at (Rx, Ry) that the object is on and the anteriorposterior axis. If it is positive the locusts is facing in a clockwise direction and if it is negative the locust is facing in an anticlockwise direction. It is set to NaN if it the orientation of the object's anteriorposterior axis is unknown.

jjVis – Overlay tracking data on video

jjVis(Ldata, startf, endf, step, lag, pause, filename, Rx, Ry, R, r, showObjNum)

This function plots data generated by jjLink.m

Ldata – the linked tracking data

startf – the first frame to display

endf – the last frame to display, or Inf to play until end.

step – the number of frames to advance at each step

lag – how much data from previous frames to display

pause – if set to 1 frames are shown singly with the user pressing any key to advance to the next frame, or 0 for continius playback

filename – the filename of the video file or [] to display no video

Rx, Ry – the x, y-coordinates of the centre of the arena

R – the radius of the external wall

r – the radius of the dome

jjFrameAverage – average by frame

jjFrameAverage(ID, info, valid)

This function calculates the mean of info based on frames.

ID – the Nx1 column vector of object IDs, assuming starts from 1 for each frame for all N objects detected in all frames.

info – the Nx1 column vector of values of interest corresponding to the IDs

valid – the Nx1 column vector indicating the valid columns entries with 1 are included and those equal to 0 are excluded.

It returns a column vector of the mean info for each frame, excluding NaNs and entries with a corresponding valid value of 0. Every frame is assumed to contain at least one object. A frame is defined as the rows from an ID of 1 until the row before the next ID of 1.

e.g. the average speed per frame is calculated by

jjFrameAverage(LData(:,2), LData(:,8), LData(:,12))

jjMovAve(data, window)

Calculates the moving average for the data.

jjiData2LData – convert data from handyTrack

This function converts the idata variable generated by handyTrack into the format of an Ldata variable output by jjLink.

Ldata = jjiData2LData(idata, data)

idata - output from handyTrack.

data - output from jjLoadicb or jjLink.

Ldata – result in the same format as the Ldata generated by jjLink.

The function takes the compactness of each object in idata from data or uses NaN if none is found. The direction of motion is computed from the individuals' positions over successive frames. The orientation of the anteroposterior axis is calculated similar method to that used in jjLink. It also includes a backward pass which allows the orientation of an individuals anteroposterior axis to be determined from a known orientation in a future frame if the object has a valid principle axis but is stationary.

htStart – starts semi-automatic tracking

This function initialises the semi-automatic tracking.

htStart(icb, Rx, Ry, R, r, N, video1, video2, VFstart, IdxStart, IdxEnd, outputStem)

or just

htStart

The parameters are the following:

Parameter	Value
icb	The path and stem name of the automatically tracked data from icBiovision
Rx, Ry	The x and y coordinates and radius of the circular region of interest
R	The radius of the inclusive region of interest (for the arena wall)
r	The radius of the exclusive region of interest (for the central dome)
N	The number of objects in the arena
video1	The path and name of the first video of the video pair (assumes the video of the exp is split into two)
video2	The second of the video pair.
VFstart	The first frame of the video to track from
IdxStart	The index of the detected frame in the icb data file corresponding to VFstart
IdxEnd	The index of the detected frame in the icb data file to finish tracking on
outputStem	The stem name and path of the file to save the output. (generates 2

files: <stem>_0.mat and <stem>_1.mat).

htSaveFixed – saves the fixed variables

This function is used by htStart to save the variables which are not updated after initialisation in semi-automatic tracking. It stores them in the file <stem>_0.mat, where <stem> is the name stored in the variable outputStem.

htTrack2Err – Steps through the frames

This function steps through each frames linking the previously tracked individuals with the automatically tracked objects using htLinkStep. The data are saved after each frame is successfully tracked.

htLinkStep – tries to link individuals to tracked objects

This is the key function in the semi-automatic tracking program. It allows the user to set up the initial position of all objects in the first frame and then links tracking data to these human checked points. It checks for three kinds of problems: the wrong number of objects being detected; more that one object in frame f having the same nearest neighbour of in the frame f+1; and an object moving more than the usual distance between frames.

2.11 Appendix 3 – Buhl et al (2006) From Disorder to Order in Marching Locusts

Buhl, J., Sumpter, D. J., Couzin, I. D., Hale, J. J., Despland, E., Miller, E. R. & Simpson, S. J. (2006) From disorder to order in marching locusts. Science, 312, 1402-6.

2.12 Appendix 4 – Bazazi et al (2008) Collective motion and cannibalism in locust marching bands

Bazazi, S., Buhl, J., Hale, J. J., Anstey, M. L., Sword, G. A., Simpson, S. J. & Couzin, I. D. (2008) Collective motion and cannibalism in locust marching bands. *Curr. Biol.,* 18**,** 735 - 739.

Chapter 3

Locust aggregation and response to environmental structure

3.1 Summary

A fundamental challenge to organisms living in unpredictable environments is locating appropriate habitat for resource acquisition. For many organisms, the scale of habitat heterogeneity is much larger than an individual's perceptual range. Although it is known that group-living organisms can utilise both environmental cues and the position or motion of conspecifics when attempting to find suitable habitat, the processes by which this occurs is currently poorly understood. For swarming organisms such as the desert locust (*Schistocerca gregaria*) the process by which individuals respond both to their environment, and to each other, is critical for control efforts and for understanding how such large mobile insect swarms maintain collective coordination. In this chapter, we investigate the behaviour of different-sized groups of juvenile locusts in an environment consisting of two heat patches in an otherwise homogeneous arena. For all group sizes locusts showed an initial preference for the heat patch with more locusts on it. Furthermore, for all but the largest group size, the number of locusts on the more populous of the two patches was greater than that expected if the locusts joined a patch randomly throughout the experiment. This attraction is thus implicated in a transfer of information about the location of heat patches. We further discuss these results in the context of the marching of hopper bands.

This work is based on data from experiments carried out by Hedwig Emmerig as part of her MSc (Emmerig, 2005), her experimental methods are paraphrased in section 3.3.1.

3.2 Introduction

The desert locust *Schistocerca gregaria* aggregates to form large swarms which can devastate crops and affect the livelihood of people across sub-Saharan Africa and Asia (Symmons and Cressman, 2001). Usually these insects exist at relatively harmless, low densities in the cryptic solitary phase, which tends to avoid other locusts (Despland and Simpson, 2000). When environmental conditions allow for build up of the solitarious population the density may increase. High densities affect a change in a suite of the locusts' characteristics (Uvarov, 1977) and the sight and smell of conspecifics (Simpson et al., 1999) or direct contact among insects (Simpson et al., 2001) can cause a change in behaviour with insects no longer avoiding each other and forming aggregations (Collett et al., 1998). Such aggregations occur when insects are juvenile and flightless (termed "hoppers"). "Bands" of hoppers merge as they migrate across the landscape forming groups that can cover up to 20 000 m^2 at densities from 50 to 1000 insects per m^2 (Haskell, 1992). Once matured to adulthood, flying swarms can migrate over huge distances resulting in locust invasions that can cover 20% of the Earth's land surface (FAO, 2004). Such swarms are extremely difficult to control. Understanding hopper bands thus provides a tractable problem that could prevent the initial formation of these swarms and their destructive effects (Enserink, 2004).

The desert environment is heterogeneous with large changes in temperature over very short scales due to variations in shade and shelter. Ideal microclimates may be considered a limited resource for locusts attempting to reach a sufficient temperature (Hussein, 1937, Elliot et al., 2002). This is especially true in the early morning, when insects seek basking areas as soon as they descend from roosts (Ellis and Ashall, 1957). Hoppers form very cohesive basking aggregations in the field as they pack into

available microclimates (Kennedy, 1939). Chapman (1955) observed that groups of first-instar *Locusta migratoria migratoriodes* (R. & F.) form above a heated section of a cage floor even in darkness. When presented with two warm patches the aggregation would form on the hotter of the two with a sensitivity of 1˚C. The temperature difference rather than the absolute temperature was the key factor determining which patch was chosen. Chapman (1955) suggested that these aggregations depended on the environmental heterogeneity more than conspecific interactions. However, because these experiments concentrated on the response of groups of locusts to their environment, he could not disentangle the relative importance of response to conspecifics to that of response to the environment.

To test the importance of conspecifics in aggregation, and to see how this differs in gregarious and solitarious phases Ellis (1963) looked at the behaviour of single *Schistocerca gregaria* locusts in either a homogeneous or heterogeneous environment containing decoys consisting of small groups of tethered gregarious locusts. In an evenly heated arena, gregarious locusts spent more time near the decoys than did solitary insects. Heating just the area under the decoys resulted in similar behaviour in both phases with solitary and gregarious locusts spending more time with the decoys. Collett et al (1998) went further to demonstrate that the distribution of a food resource on a small scale affected phase change as solitary individuals either 'forced' together to feed when food was clumped or able to avoid others when food was dispersed. Thus 'forced' aggregation at limited resources tends to gregarise groups of locusts. What is less clear, however, is the degree to which gregarious insects are attracted to each other and whether this attraction enables them to locate resources.

In other grouping species it has been shown that the behaviour of conspecifics plays an important role in seeking patchy resources through 'local enhancement': increased attraction to areas already occupied by others (Krause and Ruxton, 2002). Local enhancement is often mediated through positive feedback loops, whereby one individual performing a certain action is copied by others thus spreading the action through the population (Camazine et al., 2001, Sumpter, 2006). In eusocial insects, positive feedback through direct or indirect communication is a key component to a colony's collective success (Theraulaz et al., 2002). For example, *Oecophylla longinoda* ants are able to efficiently bridge gaps with a single chain emerging from many potential chains by following simple rules where the probability of entering a chain increases with the size of the chain and the probability of leaving decreases with size of the chain (Lioni et al., 2001). Positive feedback through local enhancement is not however limited to societies of highly related individuals. For example, given the choice of two shelters, groups of the cockroach collectively choose a single shelter (Ame et al., 2004, Ame et al., 2006). The mechanism for this decision is a tendency of cockroaches to spend time at a site that increases non-linearly with the number of individuals at that site.

Local enhancement may play a crucial role not only in small scale aggregation at resources but also in the formation of mass migrating bands of locust nymphs. Recent experiments on gregarious desert locusts have shown that the tendency of locusts to march in a common direction is dependent on their local density (Buhl et al., 2006). Collective motion emerges at a critical density, below which locusts move in an uncoordinated manner, and above which highly aligned dynamic groups are formed. If gregarious locusts are attracted to a resource as an increasing function of the

number of conspecifics at that resource then this process would increase local density of locusts and thus maintain gregarisation and promote marching. If the converse is true, and gregarious locusts are repelled by conspecifics, then this would reduce local density and lead to reduced marching. This second hypothesis has been shown to hold for solitarious locusts, which are repelled by conspecifics. In this chapter we show that the first of these alternatives hypotheses holds, and investigate locust aggregation dynamics at two heated patches.

3.3 Methods

3.3.1 Experimental Setup

The study was carried out using flightless third-instar desert locust (*Schistocerca gregaria*) nymphs. The insects were reared as in Roessingh et al. (1993). Locusts collected the evening before were used in experiments starting at 0900 h and 1200 h. Experiments which started at 1500 h used locusts collected that morning. Experimental insects were kept in the culture room in smaller bins with food *ad-libitum* until they were required. Once used in an experiment, a locust was returned to the culture to continue development to adulthood, but kept separate from locusts used in subsequent experiments. Handling and presence in the arena did not seem to have any harmful effect on the nymphs.

The trials were performed in an 85 cm diameter circular arena with two heated patches in the floor inspired by Ellis' set-up (Ellis, 1963). The arena's floor and 50 cm high walls where made of white plastic laminate (Perstorp Surface Materials, Formica Ltd., Tyne & Wear, U.K.). To prevent the insects climbing the walls they were coated with Fluon (Fluoropolymer dispersion; Whitford Plastics Ltd., Runcorn, U.K.). The arena was placed on a table which had two diametrically opposed 5 cm diameter holes

cut in it 1 cm from the edge; we call these the heat patches and label them ‘A’ and ‘B’. A 15 W lamp under each hole produced two neatly delimited, but invisible, heated patches on the otherwise homogeneous arena floor. The arena was lit by two parallel 55 W compact florescent tubes (“daylight plus”) placed 42 cm above the wall at the sides distant from the patches. The other lights in the room were off during the experiments so as to avoid any influence of external visual stimuli. A digital video camera (Canon XM2) was mounted 1.50 m above the centre of the arena to film the experiments.

Each treatment consisted of 1, 10, 20, 30, 40 or 60 locusts in the arena and was replicated eight times. The experiments were initially randomised but subject to changes due to availability of suitable insects. The 1 and the 30 treatments were exceptions, being carried out after all the other experiments. Replicates for all densities were equally distributed among starting times to balance a potential influence of a diurnal cycle. The locusts were introduced into the centre of the barren arena with no food or perch, to habituate for 60 minutes after which the lamps under the heat patches were turned on and the next 90 minutes were filmed. The ambient temperature on the arena floor was 20-29 ^{o}C. The local temperature at the heat patches was above this range stabilising at an average 38 ^{o}C after five minutes. The normal activity temperature range for locusts is 24 – 36 ^{o}C (Hussein, 1937). The difference in temperature between the centre of the heat patch and a point 3.5cm from the centre of the heat patch was 7 ^{o}C compared with a difference of only 3.5 ^{o}C over the 35.5 cm between 3.5 cm from the centre of the heat patch to the centre of the arena. After every experiment the arena was cleaned with water and alcohol (75%) to minimise the influence of any chemical deposits. The surface temperature was controlled and the

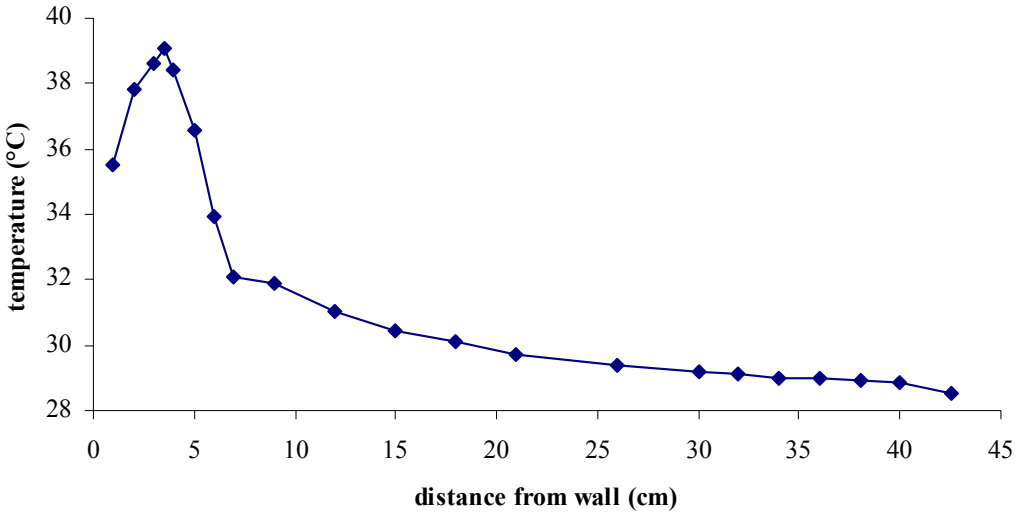


Figure 3.1 – An example of the temperature profile across one half of the arena passing through the heat patch.

position of the lamps swapped so that any potential temperature gradient would not persist over repeated experiments.

3.3.2 Data Analysis

3.3.3 Patch populations: obtaining numbers on the heated patches

We define a locust to be on the heat patch if it is within 4.1cm of the centre of a heat lamp and the *patch population* is the total number of locusts on one patch. We define the *available population* to be the number of locusts on neither heat patch. The *arena population* is the sum of patch populations and the available population. Two processes were applied to the video footage in order to identify the patch population of each heated patch, A and B, at any time. Initially, by identifying clusters of dark pixels from the white background the positions and size of all objects in the arena were determined using automated tracking software (Couzin, 2005). Further processing was used to differentiate single locusts from tight clusters of locusts. Tight clusters presented a significant problem at the heated patches where groups of locusts were identified as only one or two objects by the tracking software. The uncertainty in

the number of individuals represented by these large objects was resolved by counting the correctly identified single locusts away from the heated patches and dividing the remaining locusts between the two heated patches proportionally by area. Since the locusts were not observed to rest on top of each other, this provided an accurate measure of the patch population. The accuracy of this method was tested by comparing the computed patch populations with the observed every 15 minutes for two randomly chosen experiments, one high density experiment and a low density experiment. This method was found to be 92% accurate at high densities (60 locusts) and 95% accurate at low densities (10 locusts). The resulting data was then smoothed using a 12 frame moving average and rounded to the nearest integer to reduce noise in the data. This resulted in three time series for each experiment: the patch population at A at time t: A_t; the patch population at B at time t: B_t; and the available population at time t: C_t.

3.3.4 Initial growth rate

The initial growth rate of the patches was measured in two ways. First, to test whether the patch containing the most locusts was most attractive to conspecifics, the proportion of locusts entering the more populous of the two patches was recorded for the first ten entries. Locusts entering a patch when there were equal patch population at A and B were excluded from this analysis. This test provided a density-independent way of assessing whether the growth of population at the patches was biased to that with the highest local population. In order to test whether there was any consistent bias for patch A or patch B that could account for bias in the entries, we also recorded the proportion of entries to each of these patches.

The growth rate at the patches was also measured in a second way, by using the time series of patch population. The time series A_t, B_t and C_t of the two patch populations at A and B and the available population respectively, were smoothed further with a one minute moving average to give A'_t, B'_t and C'_t. The difference equations (3.1), (3.2) and (3.3) were fitted to smoothed time series, using least-squares linear regression, to obtain r_A and r_B where r_A is the initial growth rate at patch A per available individual in the arena per frame and similarly r_B for patch B.

$$A'_{t+1} - A'_t = r_A C'_t \tag{3.1}$$

$$B'_{t+1} - B'_t = r_B C'_t \tag{3.2}$$

$$C'_t = N - A'_t - B'_t \tag{3.3}$$

In order to capture the initial growth we only fitted to data from the start of the experiment, when the heat lamps were turned on, until one of the patch populations exceeded two thirds of its mean patch population for that experiment (rounded up to the nearest integer). During this period we assumed that there were no departures of resting individuals and that the effect of individuals passing through was minimal. The initial growth rate at A, r_A is the slope of the straight line through the origin was fitted to $A'_{t+1} - A'_t$ against C_t. Similarly r_B was obtained using B'_t.

3.3.5 The mean patch populations

The patch populations were seen to reach a dynamic equilibrium after an initial growth period of less than 25 minutes. We thus calculated the mean patch populations during the 60 minute quasi-stable period between 25 and 85 minutes. A two-tailed binomial test was used to decide if the number of times that A was more popular than B differed from that predicted by a random patch preference. This was calculated for each treatment.

3.3.6 The distribution of individuals on the most populous patch

The number of individuals on each heat patch was sampled every 60 s during the 60 minute quasi-stable period. These data were assumed to be independent and aggregated within treatments. We termed the patch with the greater population at any instant the *winning patch*. The distribution of the number of individuals on the winning patch was obtained for each total number of locusts on both patches.

The expected distribution under the null hypothesis—that locusts already on a patch would have no effect—was constructed as follows. Firstly the expected probability mass function (the discrete probability distribution function) of the number of individuals a on patch A is given by the binomial distribution with $p = 0.5$, see equation (3.4). This variable also defines the number of individuals on patch B as we are assuming that the total number of locusts on both patches is equal to T.

$$\mathrm{P}(A=a) = \binom{T}{a} p^{w} (1-p)^{T-w} = \binom{T}{a} \left(\frac{1}{2}\right)^{T} \tag{3.4}$$

If $a \geq T - a$ then A is the winning patch with population $w = a$, otherwise B is the winning patch with population $w = T - a$. By definition, the number on the winning patch $w \geq T / 2$, so the probability of observing $w < T/2$ is zero. For $w > T / 2$, the probability of observing w is the union of the probability of observing $w = a$ or $w = T - a$ (see equation (3.5)). Finally, if $T / 2$ is an integer, the probability of observing $w = T / 2$ is simply the probability of observing $w = a$. Thus the expected probability of the number of individuals on the winning patch is given by equation (3.6).

$$\begin{aligned} P(A=w) + P(A=T\text{-}w) &= \binom{T}{w}\left(\frac{1}{2}\right)^T + \binom{T}{T-w}\left(\frac{1}{2}\right)^T \cdots \\ &= \binom{T}{w}\left(\frac{1}{2}\right)^T + \binom{T}{w}\left(\frac{1}{2}\right)^T = 2\binom{T}{w}\left(\frac{1}{2}\right)^T \end{aligned} \tag{3.5}$$

$$P(W = w) = \begin{cases} 0 & w < \frac{T}{2} \\ \binom{T}{w}\left(\frac{1}{2}\right)^T & w = \frac{T}{2} \\ 2\binom{T}{w}\left(\frac{1}{2}\right)^T & w > \frac{T}{2} \end{cases} \tag{3.6}$$

The observed and expected distributions were compared using a G-Test for each of the five treatments (Sokal and Rohlf, 1994). The expected frequency was obtained by multiplying the proportion expected by the total number of observations for a given T. Note that T = 1 was excluded from the analysis since the expected and observed are necessarily identical. For each test the data were combined in two stages. For every total number of individuals on both patches, T, we had both an observed and an expected distribution. The number of different T observed increased with the number of individuals in the arena. Thus for larger population sizes the number of observations for a given T was smaller. Therefore the distributions for each T were combined to form 9 ranges of T within each treatment. For each T the number of observations was counted. The data for the T with the smallest number of observations was combined with the data from its smallest neighbour, thus representing a range of T. This process was repeated until there were 9 T-ranges left: T_1, T_2, …, T_9.

To meet the requirements of the G-Test, classes within the each of the T-ranges with an expected frequency of less than 5 were combined with their smallest adjacent class. Each of the 9 T-ranges T_i then contained a_{T_i} classes of observed frequencies $f_1^{T_i}, f_2^{T_i}, ..., f_{a_{T_i}}^{T_i}$ and expected frequencies $\hat{f}_1^{T_i}, \hat{f}_2^{T_i}, ..., \hat{f}_{a_{T_i}}^{T_i}$. The G-Test statistic G was calculated from these classes using equation (3.7) and tested against a χ^2–distribution with $\sum_{i=1}^{9}(a_{T_i} - 1)$ degrees of freedom (Sokal and Rohlf, 1994). For classes with $f_a^{T_i} = 0$, $f_a^{T_i} \log\left(\frac{f_a^{T_i}}{\hat{f}_a^{T_i}}\right)$ was defined to be zero.

$$G = 2\sum_{i=1}^{9}\sum_{a=1}^{a_{T_i}} f_a^{T_i} \log\left(\frac{f_a^{T_i}}{\hat{f}_a^{T_i}}\right) \tag{3.7}$$

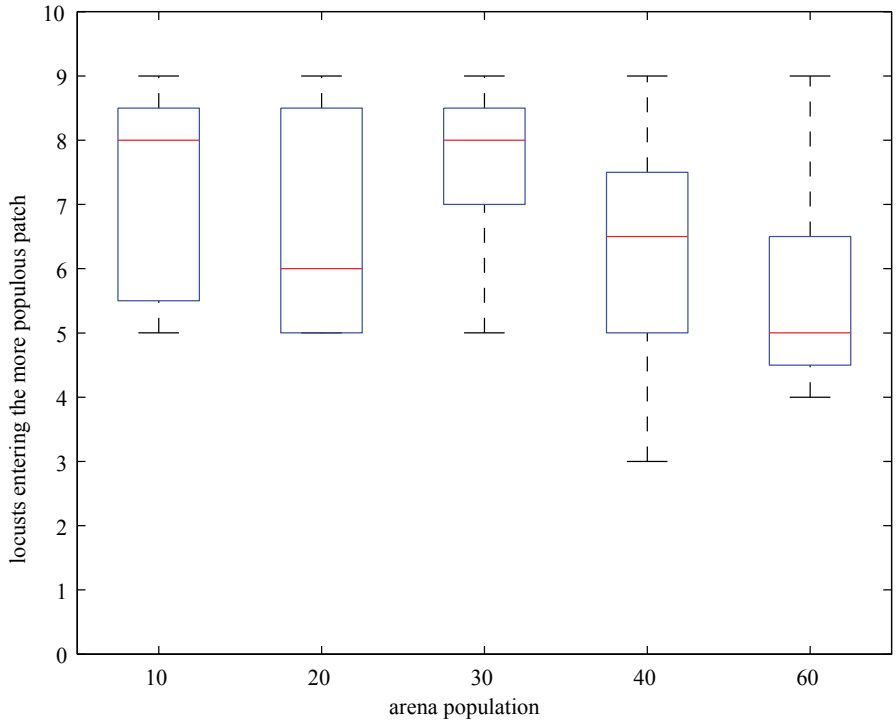


Figure 3.2 – How many of the first 10 locusts enter the more populous patch. All treatments except the 60 treatment show that the locusts enter the larger heat patch significantly more frequently than would be expected by chance (Binomial test $p \leq 0.017$). There is no significant difference was detected between treatments (Kruskal Wallis $\chi^2 = 6.21$, d.f. = 4 and $p = 0.184$).

3.4 Results

3.4.1 Initial growth rate

When the heat lamps were turned on, a locust was more likely to enter the patch with a larger population at all but the highest treatment sizes. For every treatment apart from 60, the majority of the first ten locusts entering a patch entered the patch with more individuals, see Figure 3.2. The number of locusts entering the patch with the larger population in the 10, 20, 30 and 40 treatments differ significantly (Binomial test $p \leq 0.017$, N=80) from the number expected by the null hypothesis that the locusts are randomly choosing a patch regardless of its relative size. However, the number entering the patch with the larger population do not differ significantly between treatments (Kruskal Wallis $\chi^2 = 6.21$, d.f. = 4 and $p = 0.184$).

The time it took for all 10 locusts to encounter the heat patches decreased as treatment size increased. This bias to entering the most populated patch was not caused by an intrinsic bias to patch A or B: the majority of the first ten locusts entered patch A in

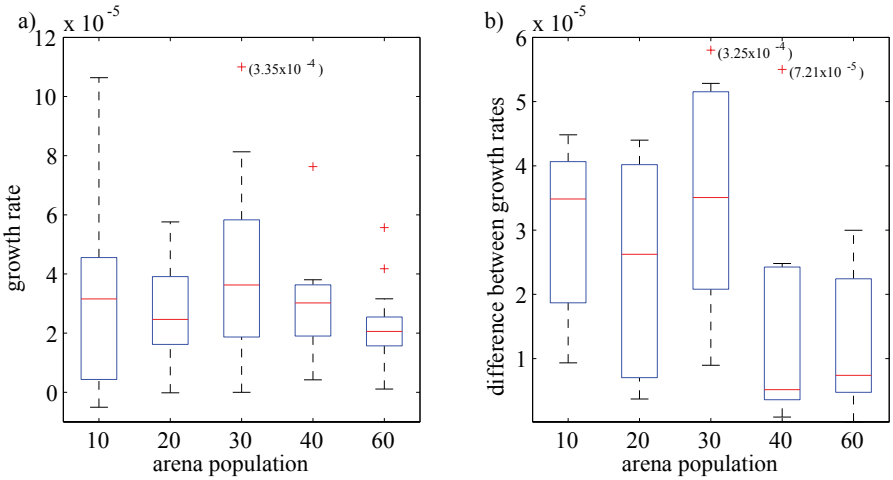


Figure 3.3 - Initial growth rates: (a) The initial growth rates r_A and r_B combined for all experiments in each treatment. There was no significant difference between these individual growth rates between treatments (Kruskal-Wallis H test at A:$\chi^2 = 3.542$, 4 d.f., $p = 0.469$; at B χ^2 = 3.542, 4 d.f., $p = 0.512$) (b) The absolute difference between the initial growth rates on each heated patch by treatment. There is a significant difference between the treatments here (Kruskal-Wallis H test $\chi^2 = 10.917$, 4 d.f., $p = 0.028$). There is similar growth at both heat patches in the 40 and 60 treatments whereas in the 10, 20 and 30 treatments we see higher growth at one patch than at the other. The text next to a '+' indicates the growth rate for that outlier which is not plotted on the same scale.

25 out of 47 experiments (Binomial test $p = 0.280$, N = 47).

Growth rates per available individual were constant between treatments (Kruskal-Wallis H test at A: $\chi^2 = 3.542$, 4 d.f., $p = 0.469$; at B: $\chi^2 = 3.542$, 4 d.f., $p = 0.512$), see Figure 3.3a. Since there were more available individuals in the higher treatments, this finding implies more rapid growth on the patches for higher arena populations than for lower arena populations. However, the difference between the growth rates of the patches within the same experiment is statistically different between the treatments (Kruskal-Wallis H test $\chi^2 = 10.917$, 4 d.f., $p = 0.028$). There is a greater difference for the 10, 20 and 30 treatments than for 40 or 60 treatments (Figure 3.3b). In the 40 and 60 treatments the rapid growth reduced the chance of there being significant differences in the patch populations for positive feedback to act on. Again there was no significant bias towards heat patch A or B, with a higher growth rate occurring for patch A in 23 out of 40 trials (Binomial test $p = 0.1341$, N = 40).

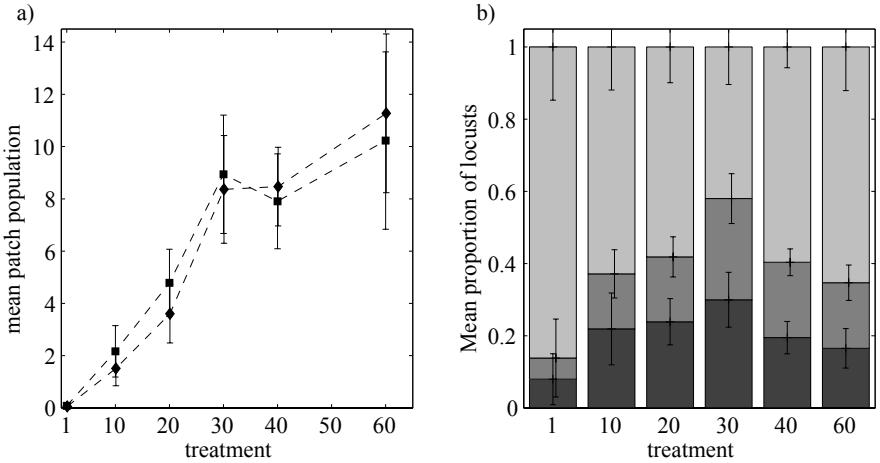


Figure 3.4 - Distribution of locusts between patches by treatment. a) The mean patch population at A(■) and B(♦) during the quasi-stable period by treatment. b) The proportion of time spent by all individuals on the patches A (■) and B (■) and in other parts of the arena C (■) by treatment during the quasi-stable period. This data suggests that increasing the density increases the group's sensitivity to the heat patches until the higher density passes 30 locusts in the arena.

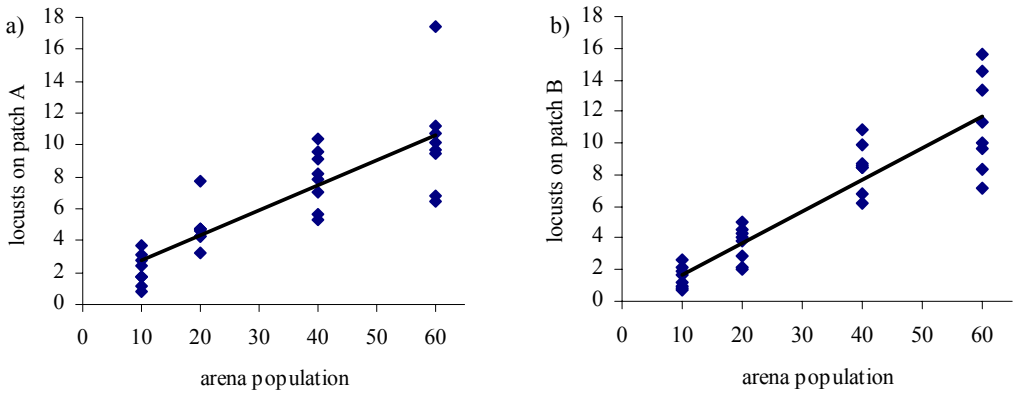


Figure 3.5 - Distribution of locusts on (a) patch A and (b) patch B excluding the 1 and 30 treatments. The 1 and 30 experiments where carried out after all the other experiments were completed. Excluding these treatments suggests a different story. We find that the mean patch population data fits a straight line with $R^2 = 0.69$ and $R^2 = 0.82$ on patches A and B respectively.

3.4.2 The mean patch population

Figure 3.4 shows the number and the proportion of individuals on the heat patches during the 25 minute to 85 minute period (also see Table 3.2 in appendix). There was no bias in the time spent on patch A or B, with neither patch consistently having more than the other across all experiments (Two-tailed binomial test $p = 0.318$). The patch population increases with treatment density reaching a carrying capacity of approximately 10 locusts per patch. The proportion on the heat patches increases with treatment up to the 30 treatment and then decreases again. The proportion on the patch for the 1 treatment is significantly different than all other treatments (Mann-Whitney U test $p \leq 0.007$) as is the 30 treatment (Mann-Whitney U test $p < 0.003$). The 10, 20, 40 and 60 treatments did not significantly differ from each other (Mann-Whitney U test $p > 0.130$).

If we exclude the 1 and 30 treatment experiments, which were carried out after all the other experiments, we see that there is strong evidence of a linear relationship between the mean number of locusts on a patch and the arena population ($R^2 = 0.69$ for patch A and $R^2 = 0.82$ for patch B). See Figure 3.5.

3.4.3 The distribution of individuals on the most populous patch

The plots in Figure 3.6 show the observed and expected distributions of the populations on the winning patch as well as their difference. It suggests that there are more individuals on the winning heat patch than we would expect from the random distribution for the 10, 20 and 30 treatments, with the 60 treatment seeming to show little deviation. This indication is confirmed by the G-Tests for each treatment (Table 3.2). All treatments except for the 60 treatment differ significantly from the null hypothesis that the locusts join a patch at random.

Table 3.1 The results of the G – Test comparing the distribution of the number of locusts on the winning heat patch to that expected with no social interaction.

Treatment	10	20	30	40	60
G	60.303	96.103	59.786	51.974	40.838
df	8	20	30	31	35
p	4.07×10^{10}	6.23×10^{12}	0.0009773	0.010537	0.22932

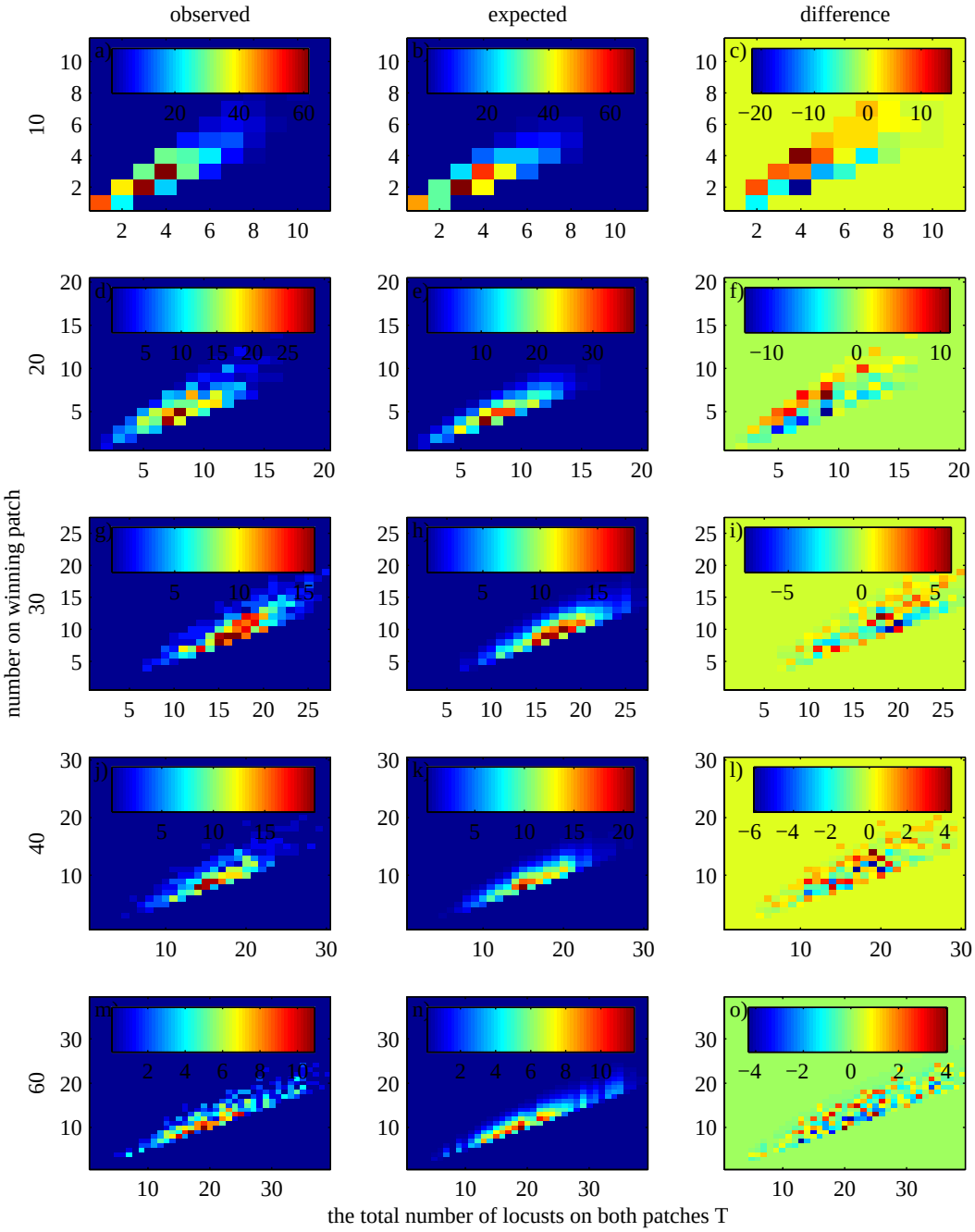


Figure 3.6 – The distributions of the observed (a, d, g, j, m) and expected (b, e, h, k, n) frequency of the number of locusts on the more populous patch given the number of individuals *T* on both patches. The number expected subtracted from the number observed is plotted in the right column (c, i, l, o).

3.5 Discussion

As in earlier experiments on *Locusta* (Chapman, 1955), our experiments show that gregarious *Schistocerca gregaria* form aggregations on heat patches. Unlike earlier experiments, we have now shown that the attraction to the patch was significantly enhanced by the presence of conspecifics. Attraction to conspecifics was seen immediately after the heat patches were turned on, with the most populated hot spot being the hot spot which was most likely to be joined by more locusts. We cannot be sure if this effect resulted from groups forming off the heat patches and then moving on to them or if the groups formed on the patch. These findings are analogous to studies of ants (Dussutour et al., 2005), cockroaches (Ame et al., 2004, Ame et al., 2006), other insects (Prokopy and Roitberg, 2001) and birds (Drent and Swierstra, 1977, Collins and Sumpter, 2007). Throughout the animal kingdom, aggregation is due to the combination of environmental stimulus and interactions with conspecifics.

The desert environment of locusts' is heterogeneous and warm basking areas are a valuable resource. Considering all experiments, our results show that attraction to conspecifics significantly increases a locust's ability to locate heat spots and as locust density increases so to does information transfer (Krause and Ruxton, 2002). A locust alone in the arena spends a relatively small amount of time on the patches, with some locusts failing completely to find them. Locusts were more likely to join a patch with a higher population. Thus presence of others seems to increases sensitivity to environmental heterogeneity. Our experiments provide evidence for the hypothesis, often stated but seldom tested, that individuals living in loosely associated groups benefit from information transfer (Krause and Ruxton, 2002, Danchin et al., 2004, Dall et al., 2005, Sumpter, 2006). Increasing density corresponds to increased

information exchange as more insects find the heat patches and spend more time there. However this conclusion is dependent on inclusion of the 1 and 30 treatments. The 30 treatment experiments were carried out immediately after the other experiments by the same person using the same protocol. The data from these experiments introduce the deviation from the linear trend in Figure 3.4 which casts doubt on their validity.

The locusts did not form static groups and did not collectively choose to bask on one of the two heat patches. This is in contrast to experiments on cockroach shelter choice (Ame et al., 2004, Ame et al., 2006). In those experiments, when the number of cockroaches exceeded the carrying capacity of either of a pair of identical shelters, the cockroaches distributed themselves evenly between the shelters. When both of the shelters were large enough to accommodate all the cockroaches – that is, when the carrying capacity of a single shelter was greater or equal to the population size – the cockroaches collectively selected a single shelter to roost under. In the experiments presented here, the carrying capacity of a heat patch was at least 24 locusts. Thus we would have expected one patch to dominate at the lower density experiments (10 and 20 locusts) and an equal split at the higher densities (30, 40, 60). We may not have observed this phenomenon because of the high activity level of the locusts during the experiments. It would have been interesting to see how groups formed at natural basking times in the early morning and evening.

Despite the locusts not selectively choosing a single patch we do see that there is evidence of individuals not choosing a patch at random. Figure 3.6 shows a higher than expected population on the more populous patch in the low density treatments,

suggesting that the more populous patch is either more attractive to passing locusts or that locusts are less likely to leave the larger patch. Note that the more populous patch would change throughout an experiment with there being no consistent bias to one physical patch or the other. The increased attraction of a larger patch is supported by the observations of the initial growth of the heat patches in figure 3.2. As the treatment size increases the patches become saturated and so the distribution of the number of individuals on the winning heat patch approaches that of the random distribution.

Precisely quantifying staying and leaving time as a function of the patch population presented several technical challenges, which we partially solved here using computer automated analysis. We generated a time series of number of individuals on a patch, but were unable to track the identity of every individual. Changes in the patch populations were observed to be due to a locust moving onto the patch and stopping; passing through a patch without stopping; or returning to a patch after a short absence. Furthermore, groups could form off the patches initially and then move simultaneously onto or through them. These details cannot be extracted from the time series of patch populations. Ideally quiescent basking individuals on the heat patches would be individually tracked, providing insight into the attractiveness of basking locusts compared with marching locusts. Such technical limitations could be overcome by, for example, marking the locusts or recording higher resolution video at the patches using auxiliary cameras.

Despite these limitations, we have begun to disentangle the mechanisms by which aggregation occurs. We have shown that the probability that a locust arrives at a patch

increases with the patch population. This is very suggestive of local enhancement, where their attraction to other locusts can help them local suitable microclimates. However, Figure 3.5 suggests that the locusts spend a fixed proportion of their time on a patch, on average, which was not dependent on the number of individuals on the patch.

While showing a tendency to aggregate, the groups forming on the patches are highly dynamic, with individuals joining and leaving frequently. The tendency to aggregate may then lead to local increases in density, which in turn promotes marching. Theoretical models have shown that if individuals are both attracted to and align with each other, then they can form extremely large mobile groups that move in a common direction (Gregoire and Chate, 2004). Previously, we have shown that at a critical density locusts form highly aligned moving groups (Buhl et al., 2006). In the next chapter I follow on from (Buhl et al., 2006) to consider the impact that an individual locust's experience of marching has on alignment and activity. The combination of aggregation and alignment could be the key to how locusts negotiate their complex heterogeneous environment, and prove essential in our understanding, and possible control, of marching locust bands.

3.6 Appendix

3.6.1 Mean numbers on hot spots

There is no bias towards one hot spot or the other over the first 12 minutes or the 60 minute period 25 minutes into the experiment. However a significant bias was detected towards A in first 25 minutes of the experiment and over the entire 90 minutes, see Table 3.2.

Table 3.2 - The number of repetitions of each treatment in which the mean number of individuals on patch A exceeds the mean number on patch B for different time periods. There were 8 repetitions for each treatment. A * indicates significance in a two tailed binomial test.

Treatment	0- 12 min	0- 25 min	25-85 min	0-90min
10	6	7*	5	6
20	6	6	6	6
30	4	5	5	5
40	5	5	3	5
60	4	7*	2	4
All	25	30*	21	26*

Chapter 4

Marching experience and gregarious inertia in locusts

4.1 Summary

Nymphs of the desert locust *Schistocerca gregaria* form large marching bands which often move in the same direction over a number of days. This ‘gregarious inertia’ only occurs when locusts reach a sufficiently high density. Here we investigate the role of individual experience on gregarious inertia. We compare the activity, alignment and association of locust nymphs subjected first to high densities then to a reduced density to those in a control group kept at the reduced density. Our results indicate that experience of a particular density has little or no influence on an individual locust's propensity to march in the same direction. Rather, we find a robust relationship between the instantaneous activity of a group of locusts and the degree to which they align with one another. We compare linear and non-linear fits to this relationship between activity and alignment, and discuss its implications for understanding collective motion of animal groups.

4.2 Introduction

The collective motion of animal groups, such as birds, fish and insects has attracted a great deal of theoretical and experimental interest (Vicsek et al., 1995, Parrish and Edelstein-Keshet, 1999, Camazine et al., 2001, Couzin et al., 2005). Locusts are an excellent model system for investigating collective motion because their physiology has been studied extensively (Uvarov, 1977) and because they produce large scale collective patterns. Locusts are also of ecological and economic importance: the desert locust *Schistocerca gregaria* has a devastating effect during plague years (Enserink, 2004). The defining feature of locust physiology is a density dependant phenotypic plasticity (Uvarov, 1977). At low population densities locusts occur in the 'solitarious' state, in which they behave cryptically and actively avoid other locusts. When resources become depleted, by drought for example, these solitary locusts are forced together when they feed (Collett et al., 1998). This close proximity causes a change in behaviour, physiology, colour and shape, as the locusts enter the 'gregarious' state (Despland et al., 2000). Gregarious locusts are characterised by greater activity, attraction to conspecifics and a suite of morphological, physiological and molecular changes (Pener, 1991, Simpson et al., 1999, Simpson et al., 2005, Simpson and Sword, 2006).

Before developing wings, behaviourally gregarious juvenile locusts form marching bands: large aggregations which move collectively in the same direction (Uvarov, 1977). Initially, small marching groups form after roosting at night. These small bands subsequently merge and Kennedy (1945) observed that when two bands met, the smaller band joined and aligned with the larger band. Similarly in flying swarms 'gregarious re-alignment' was observed, where small streams of aligned flying locusts

reoriented to align with larger streams they met (Kennedy, 1951). Once a band was underway – moving and aligned in one direction – it would maintain its direction for entire days (Kennedy, 1945). By maintaining its direction the band could avoid previously exploited, and potentially exhausted resources (Dingle, 1996). Kennedy (1945) hypothesised that this directional inertia was due to interactions with neighbouring locusts which would force a dissident locust moving in a different direction to the band to realign itself with the band and so maintained the majority direction. To test this idea, Kennedy used a mirror to alter the apparent location of the sun, exploiting the locusts' use of the sun in navigation (Homberg, 2004). Kennedy (1945) reported that in a thin stream of locusts the majority of individuals stopped and reversed direction when the sun was shone on them from the opposite side, however, in dense bands, locusts caught in the reflected rays were unable to turn due to locusts arriving behind them. Kennedy termed this persistent maintenance of direction due to interactions with conspecifics 'gregarious inertia'.

Buhl et al. (2006) formalised this argument for gregarious inertia by applying a simple self-propelled particles (SPP) model proposed by (Vicsek et al., 1995, Czirók et al., 1997) to locust interactions. In the model, particles are more likely to move in the direction of their neighbours due to mutual tendency to align with near neighbours. This model in itself was sufficient to explain the increasing degree of directional inertia as active group size increased in a ring-shaped arena (Buhl et al., 2006). Directionality was quantified by an alignment index, which captured the average direction of the movement of all locusts in a ring shaped arena, whereas the active group size was the mean number of moving individuals in the arena.

Having commenced marching as part of a band, as a result of local interactions between individuals, the questions arises as to whether the propensity of an individual to continue marching in the same direction increases over time, independently of the immediate presence of conspecifics: an effect we will term 'individual inertia'. There was no such term in the model of Buhl et al. (2006), with agents having no memory of time spent marching in a given direction, but the possibility remains that individual inertia plays a role in collective behaviour. Indeed, there are reasons to suggest that such a mechanism would be of benefit to individuals, buffering against stochastic changes in local density that otherwise would disrupt aligned motion and increase the likelihood that gregarious locusts become separated and begin to solitarise behaviourally. Partial behavioural solitarisation occurs rapidly when gregarious locusts are removed from a group (Roessingh, 1994) and would cause locusts to stop marching and be repelled by others. Locusts which solitarise prematurely, and consequently actively remove themselves from the group, are likely to be at increased risk of predation, as shown in another band-forming orthopteran, the Mormon cricket (Sword et al., 2005). Field observations have noted that band density is lower at the rear of the band (Ellis and Ashall, 1957), and that whereas locusts at the rear can lose visual contact with the band, they still continue on a straight course. Buhl et al. (2006) reported that individuals marched faster and hence further, at higher densities. This response has also been observed in the field, where gregarious locusts in smaller bands tend to march less than gregarious locusts in larger bands (Ellis and Ashall, 1957).

The diagnostic test that individual inertia plays a role in collective movement is to determine the extent to which time spent marching in a group influences the resilience

of group alignment to a sudden reduction in density to below the threshold for stimulating collective aligned movement. If individual inertia is apparent, then reducing the density that an individual experiences should not immediately reduce its alignment if it has spent time marching previously. Conversely, if alignment is entirely due to gregarious inertia, with no contribution of individual inertia, we would expect a reduction in density to lead to a rapid reduction in alignment. Indeed, theoretical work on SPP models suggests that for every group density there is a unique stable steady state for the mean alignment of the group and that alignment should undergo a rapid switch, or non-linear phase transition, from a low alignment to a high alignment at some critical point (Vicsek et al., 1995, Czirók et al., 1997, Czirok et al., 1999, Gregoire et al., 2003). Thus changes in density should lead relatively quickly to changes in alignment and hence changes in the degree of directional inertia. Here, by comparing locusts with experience of marching under different densities, we test whether individual inertia is a mechanism for maintaining directional inertia in a group.

When considering control measures, more general questions arise about the factors which enhance or inhibit directional inertia and group alignment (Enserink, 2004). For example, how does the propensity to march change throughout the daily cycle? And how does it change with respect to the local locust density? We also investigate these factors by comparing the marching of locusts at different times in their daily cycle at constant temperature.

4.3 Methods

4.3.1 Subjects

All experiments were carried out on second instar crowd-reared Desert locust *Schistocerca gregaria* nymphs. For details of how these insects were reared see (Roessingh et al., 1993). Insects aged 24 to 48h after ecdysis to the second instar were selected to control for the effect of the variation in both time spend marching and marching speeds with age during an instar (Ellis, 1951). It has also been observed that marching bands often consist of locusts of the same instar (Ellis and Ashall, 1957).

4.3.2 Experiments

To compare the behaviour of locusts which had experienced different degrees of marching we considered two groups. Gregarious locusts were pre-conditioned for one hour in an arena at either a high density, where there was strong marching, or low density, where they marched much less. The number of locusts in the high density experiments was then dropped to that of the low density experiments. This resulted in groups of locusts that had marched different amounts but spent the same time in the arena. The behaviour of the two groups was then compared.

To simulate relatively low and high densities, respectively, either 5 or 30 locusts were placed into a ring shaped arena and filmed from above. After an hour the populations were set to 5 and filmed for a further two hours. We called the experiments with an initial population of 30 the high density experiments and those with an initial population of 5 locusts the low density experiments. We employed the 80 cm diameter circular arena with a 35 cm diameter central dome as used by Buhl et al. (2006). The locusts were placed in the arena in darkness and the experiment started when the lights were turned on. After approximately one hour (m±sd: 61.9±4.1 min) the lights were turned off for 110±36 seconds. We call this period the blackout and it

allowed us to manipulate locust density with minimal disturbance to the insects in the band. During the blackout, locusts in low density experiments were left undisturbed but in high density experiments 25 locusts were removed by the light of a red hand lamp (locusts have very poor perception of the red end of the light spectrum, (Vishnevskaya and Shura-Bura, 1990)). The locusts froze when the lights went out and were unresponsive under the red light conditions, not exhibiting the escape response observed when approached under normal lighting. Some individuals would creep in the darkness but the slow nature of their motion meant that density could be altered without visibly disturbing insects (also see Appendix 4.6.2). We remove the blackout period from the time series which follow.

Experiments were run in both the morning and the afternoon. Morning experiments (AM) were started between 0900 and 1000 (lights-on during the LD 12:12 photoperiod being 0800). Locusts had been provided with food *ad-libitum* the previous day, but not on the morning of the experiment. It should be noted that feeding and activity are greatly reduced during the dark phase (Simpson, 1982). Afternoon experiments (PM) where started after 1200 with locusts provided with fresh food for at least three hours before the experiment began. Of the 14 AM experiments 6 were at the low density and 8 at the high density. Of the 23 PM experiments 11 were at the low density and 12 at the high density (also see Appendix 4.6.1).

4.3.3 Behavioural measures

The behaviour of the locusts was quantified by three measures: alignment, activity and association. These measurements were made using computer tracking software.

Alignment

An *instantaneous alignment* index of the population was calculated from the orientations of all the individuals in each frame. Each insects' anteroposterior axis was extracted from the video frame using icBiovision software (Couzin, 2005). The direction in which a locust last moved was use to identify the anterior of its axis and so giving its direction vector ***a***. This was compared to the unit vector, ***b*** - the vector perpendicular to the line from the centre of the arena to the locust, pointing in an anticlockwise direction. The smallest angle, θ, between ***a*** and ***b*** was given by:

$$\theta = \cos^{-1}\left(\frac{a \cdot b}{|a||b|}\right). \tag{4.1}$$

This angle was transformed to be a continuous index χ in the range -1 to 1 by

$$\chi = \frac{2\theta}{\pi} - 1 \tag{4.2}$$

A value of χ = -1 indicated a locust facing in an anticlockwise direction, χ = 0 when a locust faced directly towards or away from the centre and χ = 1 when a locust faced clockwise around the arena. We define χ_i^t as the alignment index for locust *i* at time *t* min. The *instantaneous alignment* Φ_0^t is the average alignment index for all *n* locusts in a frame at time *t* min and is given by:

$$\Phi_0^t = \frac{1}{n}\sum_{i=1}^{n} \chi_i^t \tag{4.3}$$

.

We also calculated the *s*-min averaged alignment Φ_s^t:

$$\Phi_s^t = \frac{1}{300s} \sum_{\tau=300t}^{300(t+s)-1} \Phi_0^{\tau/300} \tag{4.4}$$

Note that there were 300 frames in one min. We also used the absolute of this when combining data from different experiments. We defined the *absolute alignment* to be $\left|\Phi_s^t\right|$=abs(Φ_s^t). It should be noted that a low value of $\left|\Phi_s^t\right|$ may be obtained due to low alignment during the entire *s* min period or due to shorter periods of clockwise and anticlockwise alignment cancelling each other out. In other words $\left|\Phi_s^t\right|$ is sensitive to the time scale *s* considered.

To summarise the time course of alignment across experiments the 10-min absolute alignment, $\left|\Phi_{10}^t\right|$ was considered at 10 min intervals. To determine if the group maintained its direction before and after the blackout the sign of the product $\Phi_{-10}^{t_d} \cdot \Phi_{10}^{t_d}$ was considered, where t_d was the time of the blackout. A positive value indicated that the group maintained their direction across the blackout and a negative value indicated a change in direction.

Activity

The proportion of moving locusts was recorded for each frame. We defined the instantaneous activity at time *t*-min as

$$\Delta_0^t = \frac{m}{n}, \tag{4.5}$$

where n was the total number of locust in the frame at time t and m was the number of locusts which moved more than approximately 3 mm (corresponding to 2 pixels on the video) by the next frame. This threshold prevented apparent movement due to pixel noise in the video being recorded as actual activity (icBiovision uses sub-pixel accuracy, (Parker, 1993)). We also defined the s-min averaged activity Δ_s^t as

$$\Delta_s^t = \frac{1}{300s} \sum_{\tau=300t}^{300(t+s)-1} \Delta_0^{1/300} . \tag{4.6}$$

To assess the effect of the blackout period and in the case of high density experiments the removal of locusts, we measured the time until the locusts became active once the lights were turned back on. It was observed the when the lights were turned on some locusts would jump but then the entire group would remain quiescent for a period. Therefore for each experiment the time until the first locust moved was recorded, ignoring activity in the 2 s period immediately after the lights went on in which all jumps were made.

Association

We measured the association index Ψ^t for the experiments as a proxy to the degree of gregariousness of the locusts. For every locust, i, the number of other locusts, N_i^t, within one body length was counted for a frame at time t min. The average was then taken for all individuals, n_t, in the experiment.

$$\Psi^t = \frac{1}{n_t} \sum_{i=1}^{n_t} N_i^t \tag{4.7}$$

This was calculated every five minutes during the hour after t_d, the time of the blackout, and summed to give the cumulative association index $\Psi_{60}^{t_d}$.

$$\Psi_{60}^{t_d} = \sum_{\tau=1}^{12} \Psi^{t_d+5\tau} \quad . \tag{4.8}$$

An isolated-reared locusts' probability of being classified as solitarious, its P(sol), decreases linearly with increasing association index (Bouaichi, 1996). The change in P(sol) due to crowding, ΔP(sol)^{C}, may be written as:

$$\Delta \mathrm{P(sol)}^{C} = -a\,\Psi_{240}^{t} - b \tag{4.9}$$

Where $a = 6.75\text{x}10^{-3}$, $b = 0.145$ and Ψ_{240}^{t} is the cumulative association index over 240 min (Bouaichi, 1996). We assume that Ψ_{240}^{t} is equivalent to four times Ψ_{60}^{t}.

The probability of a crowd reared locust being classified as solitary, P(sol), increased from 0.02 to 0.2 after the first hour of isolation (Simpson et al., 1999). Thus the change in P(sol) due to isolation per hour is given by:

$$\Delta \mathrm{P(sol)}^{I} = 0.2 - 0.02 = 0.18 \tag{4.10}$$

If we assume these effects are linear and additive we can calculate the change in P(sol) for the low density locusts over the first hour as

$$\begin{aligned} \Delta \mathrm{P(sol)} &= \Delta \mathrm{P(sol)}^{I} + \Delta \mathrm{P(sol)}^{C} \\ &= (0.2 - 0.02) - (a\,\Psi_{240}^{t} + b) \\ &= 0.18 - 4a\,\Psi_{60}^{t} - b \\ &= -2.7\text{x}10^{-2}\ \Psi_{60}^{t} + 3.5\ \text{x}10^{-2} \end{aligned} \tag{4.11}$$

Relationship between activity and alignment

Both linear and nonlinear models were fitted for absolute alignment against activity for the 10 min after time t_d. Two functions were considered, the linear function $\left|\Phi_{10}^{t_d}\right| = \mathrm{m}\,\Delta_{10}^{t_d} + c$ and the non linear function $\left|\Phi_{10}^{t_d}\right| = \begin{cases} \left(\Delta_{10}^{t_d} - h\right)^r & : \Delta_{10}^{t_d} > h \\ 0 & : \Delta_{10}^{t_d} < h \end{cases}$. This relationship is that which would be predicted if there was some form of phase transition in the locusts collective behaviour (Vicsek et al., 1995, Czirok et al., 1999). The parameter h is the critical point at which alignment becomes non-random and r is the exponent determining how alignment increases after the critical point. Both functions were fitted using a nonlinear least-squares data fitting by the Gauss-Newton method with the data weighted by the reciprocal of the standard deviation of the alignment during the 10 min. Specifically, the standard deviation in the alignment was:

$$\frac{1}{3000}\sum_{\tau=300t_d}^{300(t_d+10)-1}\left(\Phi_0^{\tau/300} - \Phi_{10}^{t_d}\right)^2. \qquad (4.12)$$

The sum of the residuals squared from this weighted model was used to assess the comparative fit of the linear and non-linear models.

Statistical tests were performed in SPSS (2005) using alpha = 0.05.

4.4 Results

Figure 4.1 shows three typical time series for each of both high and low densities for PM experiments. At the start of the experiments the locusts were unaligned. In the high density experiments they tended to become rapidly aligned and maintained persistent marching in the same direction (i.e. directional inertia) for the rest of the

first hour. The low density populations took longer to become aligned and some remained mostly unaligned in the first hour. During the subsequent two hours the remaining locusts tended to become more aligned and more active. This figure gives an overall impression of how activity and alignment change through time and varied among experiments. We used our indices of alignment, activity and association to quantify these changes over all experiments.

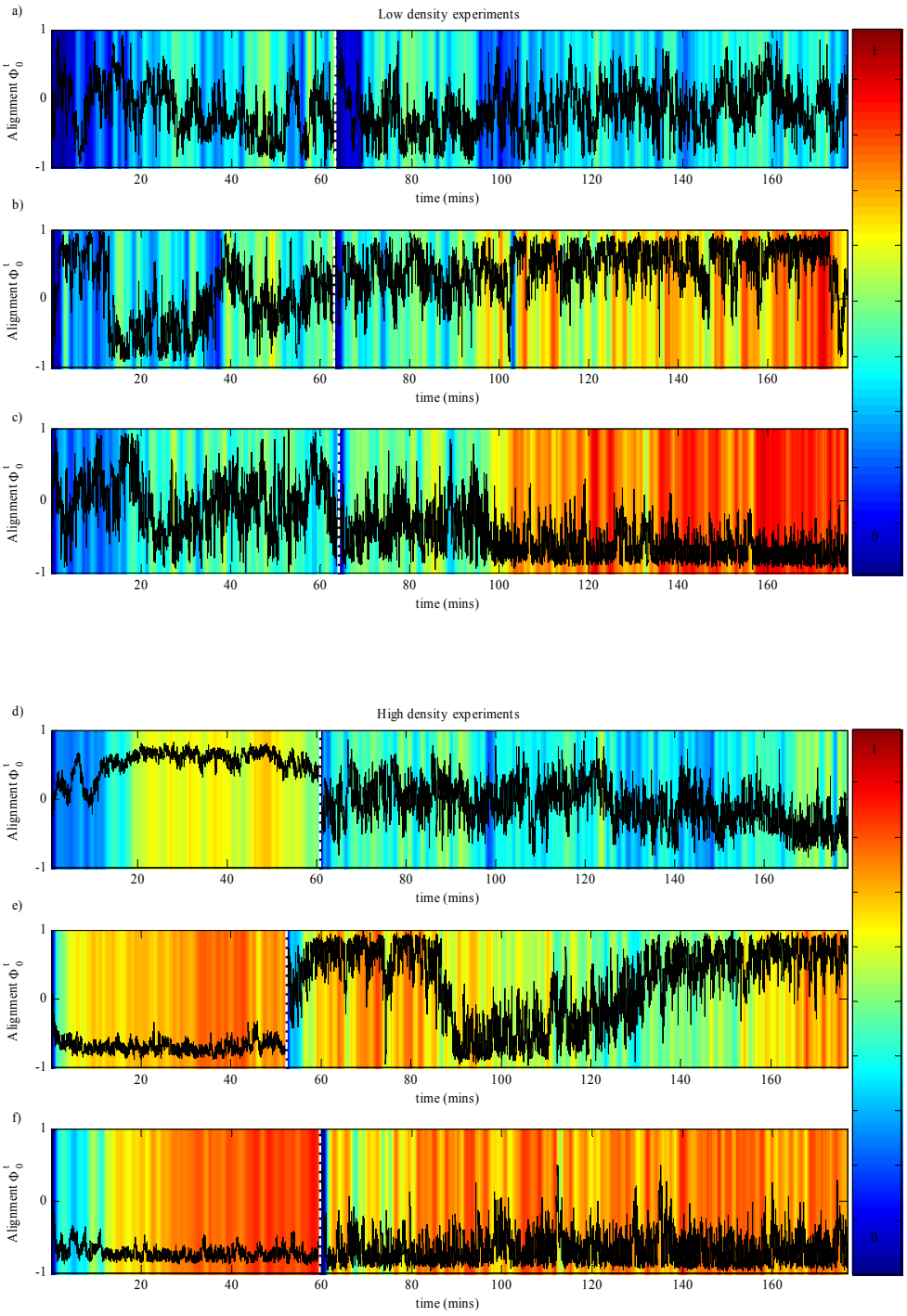


Figure 4.1 Time series showing alignment and activity of (a-c) low density PM experiments with an initial population of 5 locusts and (d-f) high density PM experiments with an initial population of 30 locusts. The solid black line in each panel shows the instantaneous alignment Φ_0^t, a measure of how well individuals are aligned with each other, over the three hours. The background colour is the one min moving average of instantaneous activity Δ_0^t, the proportion of individuals which are moving in a given frame at time *t* (blue being lowest activity levels and red the highest). The time of the blackout t_d, when the population was reduced to 5 locusts in the high density experiments, is indicated by white dashed line but the blackout itself is not included in the time series.

Figure 4.2 shows the change in absolute alignment averaged over 10 min intervals. In PM experiments the alignment was significantly different between groups of 5 and 30 locusts within 10 min of the start of the experiment. In the AM experiments it took 40 min for this difference to arise. During the 10 min period before the blackout, the absolute alignment in the high density experiments was more than double that of the low density experiments in both AM and PM experiments.

After the blackout the alignment in the high density experiments rapidly dropped to

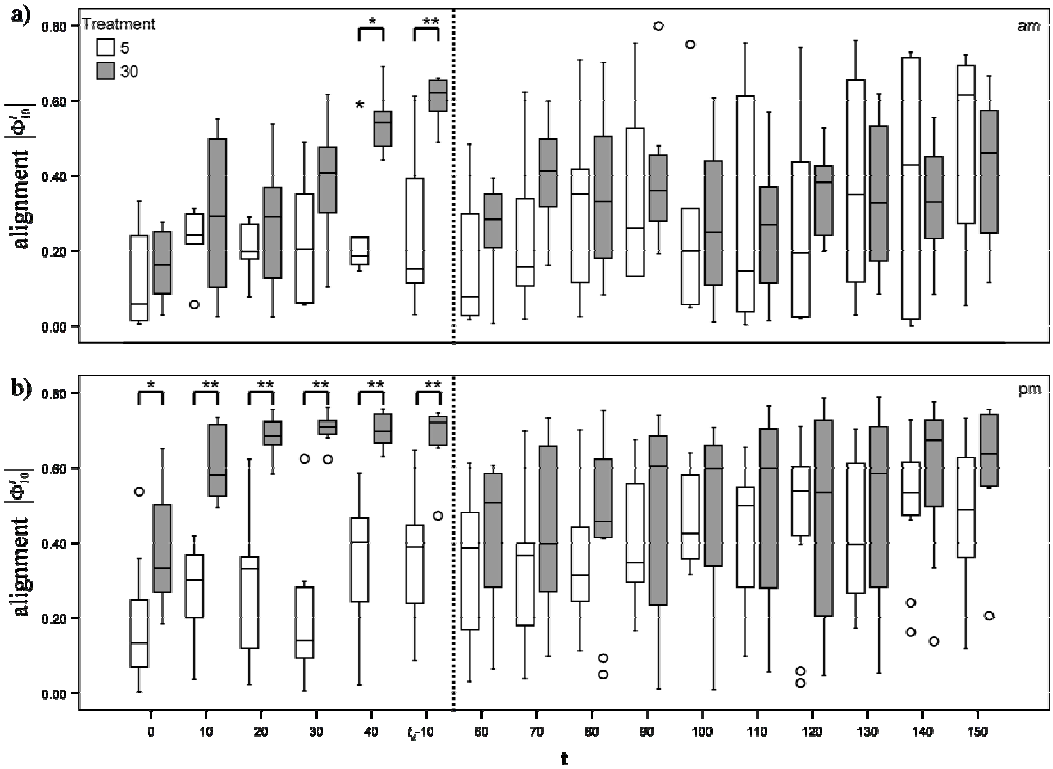


Figure 4.2 Box plots of the average absolute alignment $\left|\Phi_{10}^{t}\right|$ in (a) AM and (b) PM experiments. Each box summarises the absolute alignment during a 10 min period starting at time *t*, with low density experiments represented by hollow boxes and high density experiments by grey boxes. Brackets indicate significant differences between the treatments, with * indicating $p < 0.05$ and ** indicating $p < 0.005$ in Mann-Whitney tests. The dotted line separates the periods before and after the blackout, when the population was reduced to 5 locusts in the high density experiments. Since there is variation in the blackout t_d, the final box before the blackout is $\left|\Phi_{-10}^{t_d}\right|$ referring to the 10 min interval immediately before the blackout.

the same level as in the low density experiments. The locusts were slightly, but not significantly, more aligned for the high density experiments in the 10-min period after the blackout (after insect removal) compared with the low density experiments (Mann-Whitney AM: $U = 15$, $p = 0.524$; PM: $U = 50$, $p = 0.808$). There was no significant difference in absolute alignment between high and low density experiments in any period after the blackout. In high density experiments the absolute alignment was significantly lower in the 10 min after the blackout in both the morning and afternoon experiments (Wilcoxon Signed Ranks test AM: $Z = -2.521$, $p = 0.012$; PM: $Z = -3.059$, $p = 0.002$). There was no significant change in alignment in the low density experiments across the same period (Wilcoxon Signed Ranks test AM: $Z = -1.753$, $p = 0.080$; PM: $Z = -1.007$, $p = 0.314$).

The locusts in the low density experiments tended to maintain their direction across the blackout but this was not observed in the high density experiments. We specified here that the group maintained direction if the sign of the ten-min average alignment was the same before and after the blackout, that is $\Phi_{-10}^{t_d} \cdot \Phi_{10}^{t_d} > 0$, where t_d was the time of the blackout. In the low density experiments there was a significant maintenance of direction with 15 of 17 experiments showing no change in direction (two-tailed binomial test $p = 0.0065$). There was no significant maintenance of direction in the high density experiments, with 8 of 20 experiments showing no change in direction (two-tailed binomial test $p = 0.5034$). Also see Appendix 4.6.3.

Figure 4.3 shows how the 10-min averaged activity changes over all the experiments. During the first 60 min, activity increased like alignment. In the 10 min preceding the blackout there was a large difference in activity between the 5 and 30 experiments.

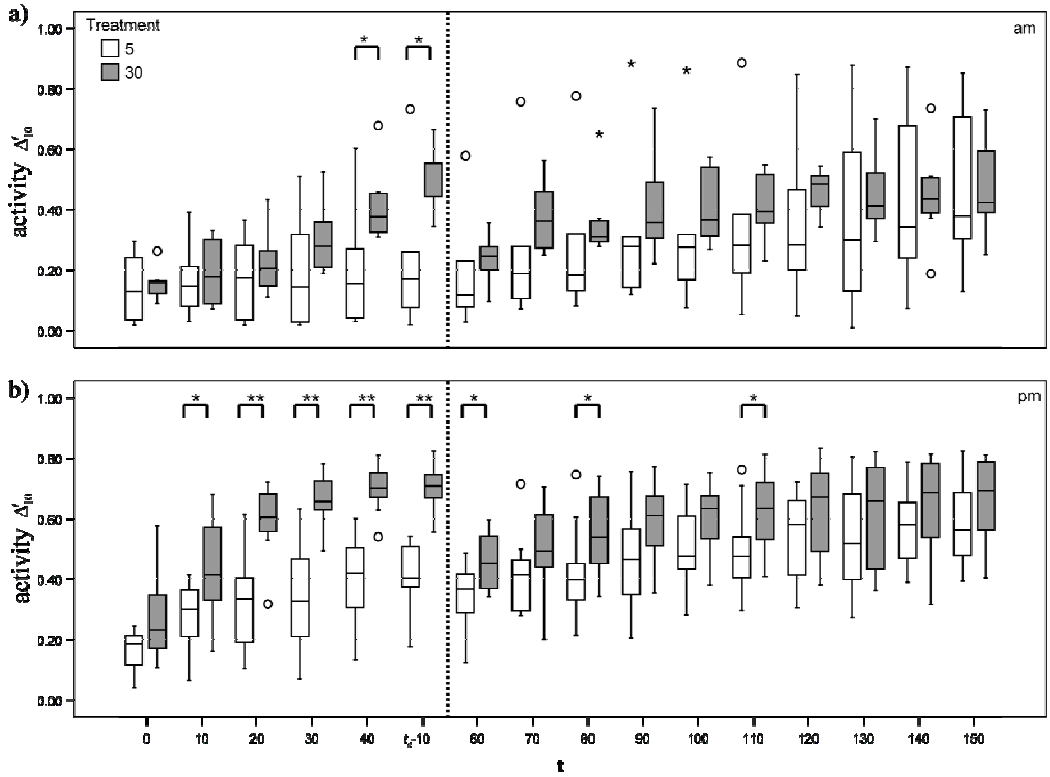


Figure 4.3 Box plots of the average proportion of active locusts Δ^t_{10} in (a) AM experiments and (b) PM experiments. Each box summarises a 10 min interval starting time t, with low density experiments represented by hollow boxes and high density experiments by grey boxes. Brackets indicate significant differences between the treatments, with * indicating $p < 0.05$ and ** indicating $p < 0.005$ in Mann-Whitney tests. The dotted line separates the periods before and after the blackout, when the population was reduced to 5 locusts in the high density experiments. Since there is variation in the time of the blackout t_d, the final box before the blackout is $\Delta^{t_d}_{-10}$ representing the time 10 min before the blackout.

This difference was significant after the first 10 min in the PM experiments (Mann-Whitney test $p < 0.05$ for intervals after 10min and $p < 0.005$ for intervals after 20 min). For AM experiments the increase in activity was slower. The activity in high density AM experiments increased more slowly than the corresponding experimental condition in the afternoon. Furthermore the activity in the low density AM experiments was constant during this period. The difference between activity in AM experiments was only significant in the last two 10 min intervals before the blackout (Mann-Whitney $p < 0.05$).

The effect of initial density on the activity during the 10 min immediately after the blackout using ANOVA indicated that there was a significant effect of whether the experiment was an AM or a PM experiment ($F_{1,33} = 22.418$, $p < 0.001$) and a significant effect of initial density ($F_{1,33} = 5.083$, $p = 0.031$). The proportion active was transformed using $\arcsin\left(\sqrt{\Delta_{10}^{t_d}}\right)$ to meet the assumptions of the ANOVA.

After the blackout the activity in the high density experiment fell, approaching the levels in the low density experiments. The activity was higher on average in the high density experiments. This difference was significant in some intervals in the PM experiments; the 10-min periods 0, 20 and 50 min after the blackout (Mann-Whitney $p < 0.05$). However, there was no significant difference in the time it took for the locusts to become active between the experiments in either morning or afternoon experiments (Mann-Whitney AM: $U = 19$, $p = 0.573$; PM: $U = 63$, $p = 0.880$).

The cumulative association index, $\Psi_{60}^{t_d}$, showed no significant difference between high and low density experiments within either the AM or PM groups for the hour after the blackout (Mann-Whitney Test AM: $U = 22$ $p = 0.794$; PM $U = 44$, $p = 0.171$). In all cases the locusts experienced enough contact with others to result in a negative change in P(sol) the probability of being classified as solitarious (see Table 4.1).

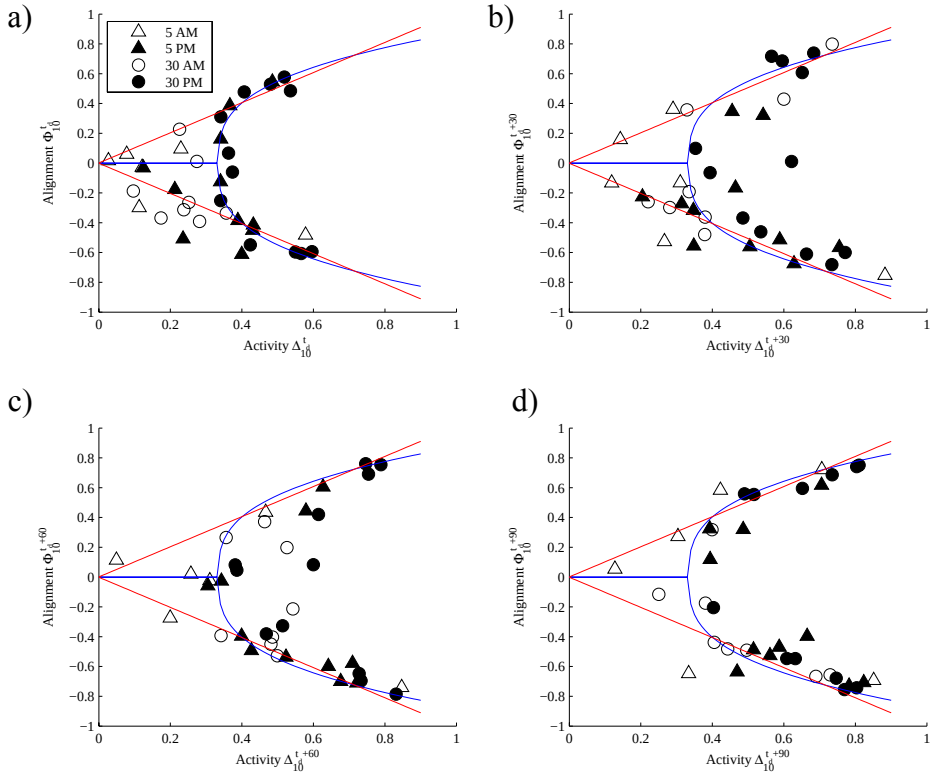


Figure 4.4 The 10 min averaged activity Δ_{10}^{t} plotted against the 10 min averaged alignment Φ_{10}^{t} (a) immediately after the blackout, when the population was reduced to 5 locusts in the high density experiments, and (b) 30, (c) 60 and (d) 90 min after the blackout. See methods for calculation of both linear and non-linear fits. For the linear fit *m*≈1.01, *c*≈ 0.001, sum of squares of the residuals = 2.75^2. For the nonlinear fit *h*≈0.334, *r*≈0.334, sum of squares of the residuals ≈ 3.78^2. The same curves are plotted in all panels.

Figure 4.4 shows the relationship between activity and alignment across all of the treatments immediately after the blackout, as well as 30, 60 and 90 min after the blackout. A linear relationship between activity and alignment gave a better fit than

Table 4.1: The mean and standard deviations of $\Psi_{60}^{t_d}$, the cumulative association index over the hour after the blackout for all the experiments by treatment. The predicted change in P(sol) based on the mean $\Psi_{60}^{t_d}$ is also given.

	AM		PM	
	5	30	5	30
Mean $\Psi_{60}^{t_d}$	1.67	1.95	2.25	1.53
Std $\Psi_{60}^{t_d}$	1.09	1.14	0.293	1.01
ΔP(sol)	-0.01	-0.0177	-0.0259	-0.0064

the non-linear model. However, the magnitude of the residuals of the linear fit was large below the critical point and small above the critical point, as represented by *h* in Figure 4.4. Activity below the critical point *h* was associated with high variance in alignment, while above the critical point absolute alignment has lower variance. In Figure 4.4 the fit of the relationship between activity and alignment was made directly after the blackout. This relationship continued to hold later in the experiment, even though the locusts became more active through time. This relationship also appeared to be independent of the time of day at which the experiment was started and the density treatment.

4.5 Discussion

The individual inertia hypothesis – that individuals' propensity to march in the same direction increases with experience of marching – is not supported by our results. We found that locusts which had marched strongly for one hour and then suddenly experienced a reduction in density, changed their behaviour within 10 min to that of individuals that had been at the low density for the preceding hour. The gregarious inertia hypothesis – that interactions with local neighbours forces individuals to move in the direction of the majority – appears to provide the primary explanation of directional inertia.

The locusts' alignment rapidly changed when the density was reduced to the levels in the low density control. Immediately after the blackout there was a significant difference in activity between the density treatments, but in the period between 10 and 20 min this difference became non-significant. In both AM and PM treatments any difference in alignment and activity between the low and high density treatments was

small compared to the difference after the change in density. Taken together these results suggests that the biggest influence on an individual's propensity for directed marching is the instantaneous density of active conspecifics that individuals experience, rather than the previous experience of marching.

Our findings do not contradict the use of navigational cues by individuals in maintaining direction after disturbance as anecdotally reported by Kennedy (1945) and Uvarov (1977) or after losing visual contact with the band (Ellis and Ashall, 1957), since such cues are largely absent in our experiments. However we emphasise that the strength of individual inertia due to these cues would, in a group context, not have to be particularly strong in order for directional inertia to be maintained.

Whilst locusts in both the AM and PM low density experiments tended to maintain their direction over the blackout, this was not observed in the high density experiment. In the field, individuals who march out of the edge of the band tend to turn around to return to the band (Ellis and Ashall, 1957). A response to rapidly reduced density may be to wander such that the probability of encountering the band is increased. Perhaps the locusts in the high density experiments do not maintain direction over the blackout due to such a response – it should be noted however that no general reversal of direction was detected either. Within the arena the only cues a locust has of the direction it is going in is the curvature of the wall and the orientation and motion of other individuals around it. The sudden vanishing of 25 locusts could be disorientating – despite the process of removing them not being a disturbance in itself. On average we would expect that the remaining five locusts, after the removal, would move in the average direction of the group prior to removal. Unfortunately, however, we are

unable to be sure of the direction of movement of the remaining individuals immediately prior to the blackout since they could not be identified individually. To understand better how individual interactions lead to alignment we need to make longer term tracking experiments where we look at how individuals respond to nearest neighbours (Partridge et al., 1980).

There was no evidence that the locusts in the low density treatment became behaviourally solitarised, since the density treatments did not have significantly different values for their aggregation index. The time before the locusts first moved after the lights went on was also the same across density treatments, suggesting no change in the tendency of individual locusts to become active. There was, however, evidence of a self-excitatory effect on activity in the period before the blackout. The proportion of locusts active was significantly higher for the high density treatments. This was surprising, since only a weak relationship of this type was observed in our earlier experimental work (Buhl et al., 2006). This observation is however consistent with the hypothesis that locusts are activated by cannibalistic interactions as in Mormon crickets (Simpson et al., 2006).

The activity and alignment in the AM experiments was lower than that observed in the PM experiments. The major difference between locusts in these treatments is that the locusts in the AM experiments were mostly roosting in the dark during the hours before the start of the experiment, which results in a partial behavioural solitarisation (Roessingh, 1994) and hence a reduced marching tendency. In contrast, the locusts in the PM experiments were active, although not marching, for at least three hours before the entering the arena, as part of their normal diurnal feeding and activity

period. Ellis (1951) found that activity reached higher levels in locusts which had marched more since they last fed.

In order to analyse the time series of activity and alignment over the course of the experiment the data was broken down into 10 min chunks (see Figure 4.2 and Figure 4.3). There is the risk that by averaging over 10 min periods and then aggregating these periods across experiments, the data may be misrepresented. Within an experiment short-term periodicity is smoothed out when averaged over 10 mins. Longer-term periodicity may also be overlooked if it is out of phase in different experiments when the 10 min chunks are combined. In the case of alignment, highly aligned marching which flipped direction during the ten min interval would get a low score due to the cancellation of the clockwise and anticlockwise components. The 10 min period was chosen to minimise this problem. However, the box plots of alignment of 5 locusts show a large range across experiments. This wide spread could be due to true differences between experiments or oscillation in the underlying time series being out of phase between experiments. Preliminary EDA supported the former. Despite these issues consideration of the 10 min periods before and after the black out still provided clear evidence of the alignment of the high-density treatment rapidly reducing to similar levels as the low-density treatment after the black out. It would be useful to perform further, more detailed analysis of these time series.

While there are large differences in the activity and alignment - between AM and PM treatments, at different times during experiments and even between experiments of the same treatment – there was a consistent relationship between activity and alignment (Figure 4.4). The proportion of active locusts is a strong predictor of alignment and

directional inertia: a relationship which holds independently of all the other factors at work during the experiment. This observation has important consequences for locust control. Control measures which reduce the activity of a marching band will not only slow the marching of the group but also reduce their directional inertia. Less active locusts will fail to align and start to disperse.

In this paper we have improved the accuracy of our measure of alignment. In our previous work we measured only the direction of moving locusts (Buhl et al., 2006). Here we have also calculated the direction of stationary locusts. This has allowed us to make more solid quantitative predictions about the relationship between activity and alignment. The best fit using least squares to this relationship was linear. However, there remains support for a non-linear relationship similar to that predicted by the model in Buhl et al. (2006). There is high variance in the alignment observed below the critical activity level *h*, after which we see a bifurcation in the alignment and a drop in the variance. The change in variance with activity thus produces residuals inconsistent with a linear model, but more consistent with a change in phase. Here we have measured an exponent close to ⅓ for increase in alignment after the bifurcation, but given the difficulty in making non-linear fits to data such as this we should give an approximate range of this exponent between ¼ and ½. In this experiment we had aimed, by using a younger instar of locust than Buhl et al. (2006), effectively to reduce the density of the locusts. It is clear, however, that if we are to quantify the exact nature of the relationship between activity and alignment a larger arena or field experiment is needed.

Differences in collective patterns can result from changes in the behaviour exhibited by individuals. They can also arise from the intrinsic properties of the system where patterns at the collective level emerge as an outcome of many interactions at a very local scale. Changes in the density of individuals or the environment can result in spectacular changes at the collective level without implying any change in the behaviour of the individuals (Pratt and Sumpter, 2006, Detrain and Deneubourg, 2006). A major contribution of theories of self-organisation has been to demonstrate that the latter type of explanation applies in a wide variety of situations (Camazine et al., 2001, Sumpter, 2006, Beekman et al., 2001, Franks et al., 1991). At the same time, much of the research on locusts has concentrated on understanding the physiological and behavioural mechanisms which change within an individual as a function of density (Simpson et al., 1999, Bouaichi, 1996, Collett et al., 1998, Roessingh et al., 1993). In the context of the formation and maintenance of marching bands we see a combination of both individual and collective effects. Experience of high densities leads to gregarisation of solitary locusts and the formation of bands, but once gregarised the group alignment is maintained by the emergence of a collective inertia. Changes in the environment and density have effects that span many biological scales: from the movement of large swarms down to the neuronal pathways governing individual behaviour. It is understanding the link between these different scales which allows us to understand how complex systems, such as locust swarms, function.

4.6 Appendicies

4.6.1 Start time and activity before blackout

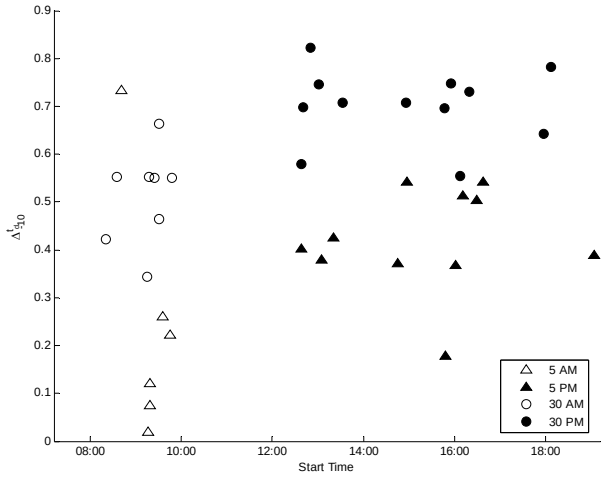


Figure 4.5 - Activity 10 min before the blackout $\Delta_{-10}^{t_d}$ plotted against the time at which the experiment started. Low density experiments are indicated by triangles and high density experiments by circles. Unfilled shapes indicate experiments in the AM group and filled shapes indicate experiments in the PM group.

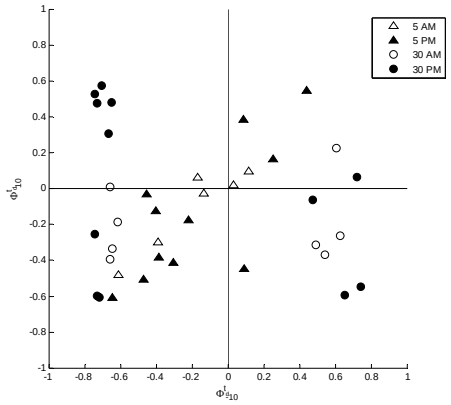
There was an unanticipated effect of time of day on activity. Figure 4.5 suggests a difference in activity between the first experiments of the day and subsequent experiments. Activity 10 min before the blackout $\Delta_{-10}^{t_d}$ is significantly different between AM and PM experiments (Kruskal-Wallis Test $\chi 2 = 4.535$, df = 1, p =0.033). The activity $\Delta_{-10}^{t_d}$ is also significantly different between the four groups defined by initial density treatment and AM or PM (Kruskal-Wallis Test $\chi 2 = 23.497$, df = 3, $p <$ 0.001).

4.6.2 Effect of the blackout

In the high density experiments, individual locusts were more active than those in the low density experiments. At the start of the experiments locusts in the high density treatment become active more quickly than those in the low density treatment once the lights were turned on (Mann-Whitney AM: U= 7, p =0.014; PM: U=26.5, p = 0.049). After the second blackout 60 min into the experiment, when the number of

locusts was set to 5 in both treatments, there was no significant difference in the time it took for the locusts to become active between the treatments in either morning or afternoon experiments (Mann-Whitney AM: U=19, p = 0.573; PM: U=63, p = 0.880).

4.6.3 Direction before and after blackout



Chapter 5

The effects of neighbours on locust activity

5.1 Summary

The effect of a locust's nearest neighbour on its propensity to move was investigated by individually tracking locusts over an extended period. The effects of proximity, the relative orientation of the locusts and the movement state of the nearest neighbour were considered. Groups of five locusts were tracked over a period of 30 min. Each locust was identified in every frame and its identity maintained over the full 30 min by user-assisted computer tracking. The data was analysed using logistic regression. I found that neighbours within 5 cm of a locust had the biggest effect. Having a stationary neighbour tended to make a moving locust stop. There was also evidence of the relative orientations and positions of the locusts being significant. Locusts approached from behind were more likely to keep moving. When a locusts and its nearest neighbour faced each other they were more likely to stop.

5.2 Introduction

There have been a number of studies of behavioural interactions within individual locusts in isolation. Simpson and Ludlow considered the time series' of bouts between feeding of individual *Locusta migratoria* with respect to a range of factors using survival analysis (Simpson and Ludlow, 1986). Moorhouse et al. (1990) placed single *L. migratoria* on a treadmill to study changes in their tendency to stop marching in response to stimuli using similar techniques. Macdonald and Raubenheimer (1995) also looked at the time series of moving and stationary bouts of individual locusts in isolation by applying hidden Markov models using the same species.

There have also been studies of groups of locusts which considered interactions between individuals. Buhl et al. (2006) demonstrated that predictions of non-linear transitions in self propelled particle models, where individuals used only local information and simple rules, were observed in groups of the desert locust *Schistocerca gregaria*. Collett et al. (1998) investigated the effect of habitat structure on phase change with groups of locusts *S. gregaria*. Group level properties have been considered earlier here in chapters 3 and 4 looking at response to habitat heterogeneity and experience of marching respectively.

There have been fewer studies which considered aspects of individual locust behaviour within groups. Stower (1963) filmed locusts in the field in Kenya and laboriously tracked individuals moving within a marching band. Ellis (1953a, 1953b) studied individual locusts' responses to groups of tethered locusts and in response to being in visual contact with marching locusts. Buhl et al. (2006) considered the interaction radius of individuals. Despland and Simpson (2006) found that having an

active locust within 5 cm stimulated activity in stationary locusts. Bazazi et al. (2008) have considered the propensity of locusts to start moving following physical contact.

In studies of animal behaviour in general, little progress has been made in understanding how animals respond to the presence and movement of conspecifics at an individual level. Theoretical models predict that the tuning of individual interaction rules can result different emergent patterns at a group level when many agents interact locally (Vicsek et al., 1995, Czirók et al., 1997, Couzin et al., 2002, Couzin et al., 2005). Empirical studies of the interactions of individuals within groups are therefore needed to test these models. The most detailed studies have been made in fish. Behaviour of schools of fish was considered by Partridge and Pitcher et al (1980) who identified how fish positioned themselves relative to each other when schooling in 3 dimensions. Tein et al. (2004) investigated individual fish's decision rules by considering their acceleration with respect to their nearest neighbour. Jeanson et al. (2005) analysed cockroach nymphs' response to small aggregations in a circular arena by estimating transition rates between movement states as a function of aggregation sizes.

In this chapter, I employ detailed individual level tracking of small groups of *S. gregaria* to investigate the effects of a locust's nearest neighbour on its movement behaviour. I consider three properties of the nearest neighbour to a focal locust: their proximity; whether they are moving or not; and their orientation and position relative to a focal locust.

5.3 Methods

5.3.1 Experiments

I looked in detail at the behaviour of individual locusts from the experiments reported in chapter 4. In these experiments five second-instar *Schistocerca gregaria* locusts had spent 60 min in a ring-shaped arena, before the light was turned off for a short period (mean±SD = 97±33 s) , and then turned on again. I reanalysed the video for the 30 min after the light was turned on again for nine of the experiments. These were experiments which had had an initial population of five locusts and had been started between 1200 h and 1300 h.

5.3.2 Data collection

The locusts were filmed at a rate of five frames per second. The positions of individuals were extracted from this video using semiautomatic tracking techniques described in the chapter 2. This intervolved searching through automatically tracked data to locate likely errors, for example when the number of detected locust in the arena changed or when an individual moved an unusually long distance. An operator would then be presented with the video with the edited tracking data overlaid. Sometimes when two individuals completely coalesced on the video frame for several frames even an experienced operator was unable to determine appropriate identities when they separated. In these cases the identities were arbitrarily assigned and the time noted. Next, the area of each individual was plotted over time. Although the area of an individual varied in the short term, it tended to characterise an individual locust over longer time periods. This characteristic area was used to determine the identity of an individual after an arbitrary assignment when two locusts' identities may have been swapped. The tracking software also extracted the orientation of the principle axis of each detected object (Couzin, 2005). This principle axis orientation was combined with the most recent direction of motion of an individual to determine the

orientation of each locust's anterior-posterior axis. This method would sometimes fail on the rare occasions that locusts walked backwards or when their principle axes were not valid. An object's principle axis was not valid if it was not recorded due to the object not being automatically detected or if an objects shape on the video was insufficiently elongated to define a principle axis. Finally, the data for the anterior-posterior axis orientations of all individuals were completed by manually adding orientations for individuals with invalid principle axes.

After this tracking, the position and orientation of each of the five locusts in an experiment was known in every frame unambiguously for the entire 30 min period. As described in chapter 4, the locusts were inactive for a period after the lights were turned back on; I therefore excluded the first 3 min of behaviour from our analysis.

5.3.3 Descriptive statistics

The first analysis was to calculate descriptive statistics for each locust over the entire period. I calculated the mean and standard deviations for their moving speed, moving bout length, stationary bout length, and the proportion of time spent moving.

Defining movement bouts

Determining when an object is stationary or moving is complicated because the tracked position of individuals is affected by pixel noise. A locust which is actually stationary will tend not to have a fixed tracked position. I therefore use a threshold, with movement below this threshold level considered to be due to pixel noise. In order to choose this threshold, the starts and ends of the longest periods of low activity, where the locust was observed not to move, were manually found for each locust in

two experiments. The mean and standard deviation of the differences in position observed between successive frames was calculated. I then calculated the mean of these means and the mean of the standard deviations to get an overall mean and standard deviation of observed displacement of stationary locusts between frames. The mean displacement of an object representing a stationary locust was 0.181 ± 0.13 px (m±SD) in experiment 1 and 0.198 ± 0.12 px in experiment 9. This gave us a threshold of 0.54 px per frame, based on the mean plus three standard deviations. I call this value the *movement threshold*. If an individual's observed position changed by more than this it was defined to be moving.

In order to further validate our choice of movement threshold I investigated how moving speed, moving and stationary bout lengths and the proportion of time spent moving depend on this threshold. I calculated each of these measures for a range of thresholds for each experiment. This is plotted in Figure 5.1 with the calculated moving threshold plotted as the vertical dashed line. Figure 5.1 shows that the choice of moving threshold had a very strong effect when it was less than approximately 0.5 px. The effect was smaller until 2 px, above which it had a strong effect on the mean stationary bout length and the perceived activity level. The choice of moving threshold $h = 0.54$ extracted from the video thus seems to be appropriate. There appear to be differences in the descriptive statistics between experiments, however they are affected by the movement threshold in the same ways.

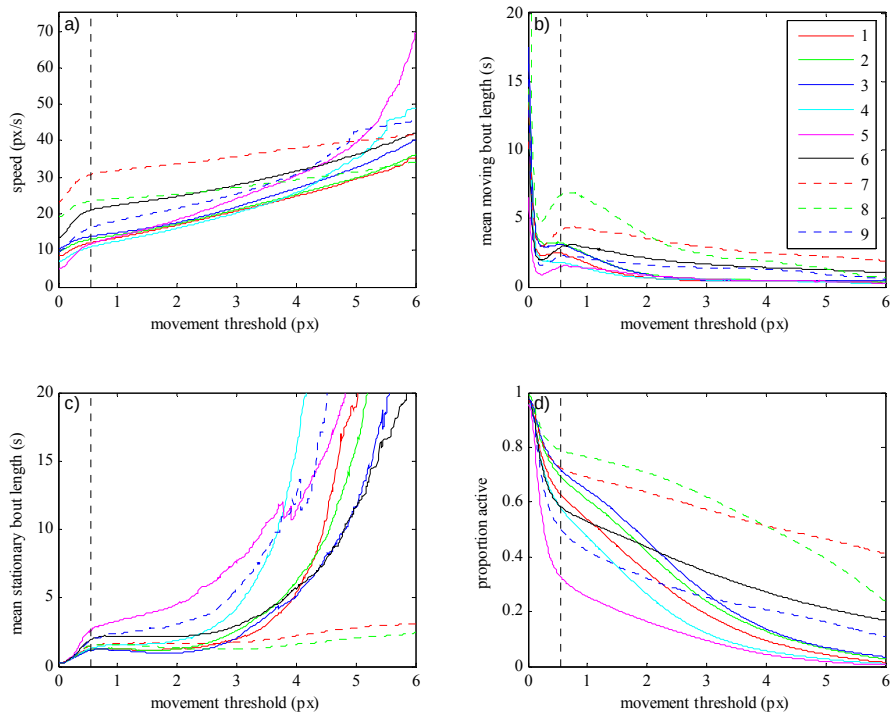


Figure 5.1 – The effect of the movement threshold on the descriptive statistics for each experiment (labelled 1 to 9). The mean speed, moving bout length, stationary bout length and activity for different thresholds. The vertical black dotted line indicated the threshold value determined from the mean+3*sd = 0.54 of observed displacement between observed positions of stationary individuals.

Given the movement threshold, I defined the movement state of the locust over the course of the 30 min as a sequence $C_t : \mathrm{t} = 1,2,...,9000$, where $C_i = 0$ if the locust was not moving in frame i, and $C_i = 1$ if it was moving. The above criterion for the moving threshold defined instantaneous motion. When considering the locusts' behaviour I wished to differentiate between small shuffling behaviour and sustained walking. I wanted to have a minimum behavioural bout duration so that short bouts of one behaviour within the other were ignored. To do this I considered two different methods: a moving average and an iterative method.

Initially I tried to smooth the activity data by applying a threshold to a moving average. At frame t I considered $\frac{1}{2k+1}\sum_{i=t-k}^{t+k} C_i > 0.5$, that is whether the locust was moving in the majority of frames in the range t-k to t+k. This reduced the number of rapid switches to and from a state. However, in certain circumstances rapid transitions could still occur. In order to enforce a minimum bout length I performed the following iterated procedure. I removed all bouts of length 1 by repeatedly applying the moving average threshold with $k = 1$, until it did not alter the activity series – when all bouts of length 1 had been removed. Next this was applied with $k = 2$, and so on up to $k = m - 1$, where m was the minimum bout length. I call this time series $\overline{C}_i^m$.

5.3.4 Moving and Stationary Bouts

I can extract bouts of activity and inactivity simply from the time series $\overline{C}_i^m$. I define a bout as the period started when a locust enters one state until it changes to a different state, the states in this case being active and inactive. The length of a bout is the duration of this period. I define $\{x_i^s\}$ to be the sequence of bouts for a state s, where s=0 refers to a stationary bout and s=1 a moving bout.

I investigated the distribution of the activity bouts under the assumption that the behaviour of each locust in the arena was independent of the others and that they were memoryless. This allowed us to model the bouts as a continuous time Markov chain. In a Markov chain, the probability of moving from one state to the next is independent of the history of previous states and so the bouts follow an exponential distribution. I tested to see if the bout lengths followed an exponential distribution using a Kolmogorov-Smirnov test. Since I imposed a minimum bout length on $\overline{C}_i^m$, I would

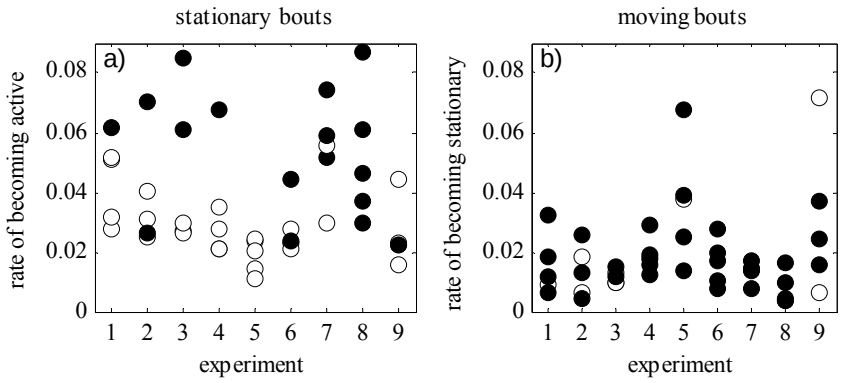


Figure 5.2 – The termination rates for stationary and moving bouts of each locust in each experiment. Filled circles indicate bout series where I can not reject the hypothesis that they are exponentially distributed. The moving threshold was set at 0.54px with a minimum bout length of 1 s. These rates are based on the transformed bouts giving the termination rate after the 1 s minimum has elapsed. Note that some point overlap.

expect a displaced exponential distribution, I therefore transformed the data by subtracting *m* the minimum bout size from each bout and removing all bouts of length zero (Haccou and Meelis, 1992). In each of the experiments the moving and stationary bouts lengths were tested to see if they followed an exponential distribution. The number of experiments in which the majority of locusts activity bouts were rejected as following an exponential distribution were counted for a range of minimum bout sizes.

The rate parameter λ for exponentially distributed bouts was estimated using the maximum likelihood method in Matlab for the chosen movement threshold and a 1 s minimum bout length (5 frames). These are shown in Figure 5.2.

5.3.5 Effect of neighbours

Changing states between moving and stationary

To test the effect of other locusts on an individual's propensity to start or stop moving I considered the distance between individuals when they change state. For a locust L I define N_{ab} to be the number of times that a locust goes from state *a* to state *b* in

consecutive frames (note that *a* can equal *b*). Assuming that the locusts are changing state independently of each other and with constant probability, I can estimate the state transition probability P_{ab} of changing state from *a* to *b* as the maximum likelihood estimator of the termination rate of exponentially distributed bouts follows (Haccou and Meelis, 1992):

$$P_{ab} = P(C_t = b \mid C_{t-1} = a) = \frac{N_{ab}}{N_{aa} + N_{ab}}. \qquad (5.1)$$

This can be extended to consider the distance to the nearest neighbour. The nearest neighbour of a locust was defined to the closest other locust not occluded by the central dome in the arena (it is therefore possible for a locust not to have a nearest neighbour if all others in the group are occluded). In order to get a reasonable number of observations I considered the effect of the distance to a nearest neighbour by breaking it up into ranges. I used six ranges bounded at 0 cm, 2.5 cm, 5 cm, 10 cm, 20 cm, 30 cm and 72.5 cm (with locusts averaging 1.8 cm in length). These were chosen following preliminary investigations into other ranges considering the maximum interaction distance observed in Bulh et al. (2006) and with 72.5 cm being the maximum distance two locusts can be apart without the dome occluding the line of sight between them. I defined $NN_t(r)$ to be 1 if the distance between the focal locust and its nearest visible neighbour was less than *r* at time *t*, otherwise it was set to 0. I defined $N_{ab}(r)$ to be the number of times that a locust goes from state *a* to state *b* between frames *t* and *t+1* and when $NN_t(r) = 0$; that is the number of frames in which a state transition from *a* to *b* is observed when the nearest neighbour is at least a distance of *r* from the focal locust. I defined $NR_t(r_1, r_2) =$ NOT($NN_t(r_1)$) AND $NN_t(r_2)$; that is $NR_t(r_1, r_2)$=1 if the nearest neighbour is in the range $[r_1, r_2)$, and $NR_t(r_1, r_2)$=0 if the nearest neighbour is outside that range. I defined $N_{ab}(r_1, r_2)$ to be the

number of times that a locust goes from state *a* to state *b* between frames *t* and *t*+1 and when $NR_t(r_1, r_2) = 1$; that is the number of frames in which a state transition from *a* to *b* is observed when the nearest neighbour is in the range $[r_1, r_2)$.

An activity bout is censored if its start or end is undefined. When considering the length of bout when a locust's nearest neighbour was within a certain range and state it can only be considered to be uncensored if the bout both started and finished while the neighbour was in that range. However, assuming that the censoring is random the maximum likelihood estimator for the transition rate is simply the number of uncensored bouts divided by the combined lengths of the censored and uncensored bouts (Haccou and Meelis, 1992). Thus I can define transition probabilities given that a locust had a nearest neighbour in range $[r_1, r_2)$ as follows

$$P_{ab}(r_1, r_2) = P\left(C_t = b \mid C_{t-1} = a, NR_{t-1}(r_1, r_2) = 1\right) = \frac{N_{ab}(r_1, r_2)}{N_{aa}(r_1, r_2) + N_{ab}(r_1, r_2)} \qquad (5.2)$$

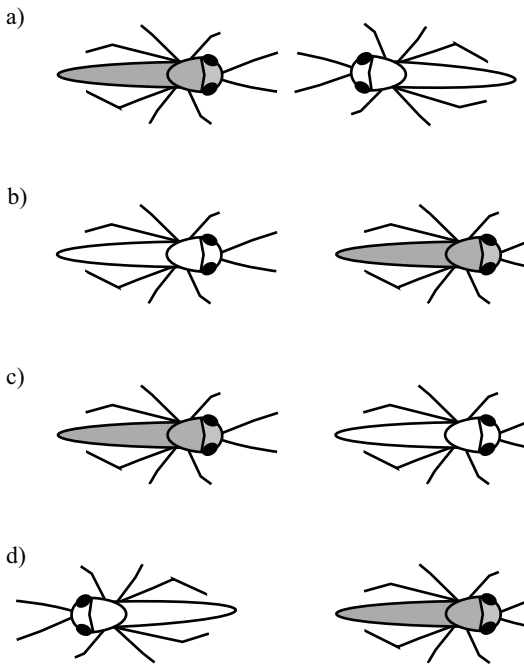
The effect of having the nearest neighbour beyond that range was defined by:

$$P_{ab}(r) = P\left(C_t = b \mid C_{t-1} = a, NR_{t-1}(r) = 1\right) = \frac{N_{ab}(r)}{N_{aa}(r) + N_{ab}(r)} \qquad (5.3)$$

The effect of having the nearest neighbour in a range was compared across experiments using a Wilcoxon's signed-rank test was then applied to $P_{ab}(r_1, r_2) - P_{ab}(r_2)$ for each range. This indicated whether or not there was an effect on transition rates due to neighbours.

Transitions depending on the movement and orientation of the nearest neighbour

I broke down the behaviour further by considering the state of a locust's nearest neighbours. I consider three pieces of information about neighbours: their activity, their orientation and whether they were ahead or behind the focal locust. A locust L_1 is defined to be facing towards a locust L_2 if the dot product of the direction vector of L_1 and the vector from L_1 to L_2 is greater than or equal to 0; that is if the angle between these vectors is less than or equal to 90°. In order to succinctly describe the relative orientations of a locust and its nearest neighbour I will use the following terms illustrated in Figure 5.3: *facing*, *ahead*, *behind* and *opposed*. The combinations of relative orientations and moving states of both locusts give sixteen possible 'states'.



	neighbour 'state'	neighbour orientation	relative position of neighbour
a)	facing	towards	front
b)	ahead	towards	rear
c)	behind	away	front
d)	opposed	away	rear

Figure 5.3 – The naming convention for the relative orientations of a locust and its nearest neighbour. The focal locust represented by the grey and its nearest neighbour white. The terms describing the relative orientations are defined as follows (a) *facing:* the focal locust is facing its nearest neighbour and its nearest neighbour is facing it; (b) *ahead*: the focal locust is ahead of its nearest neighbour, which is facing the focal locust; (c) *behind*: the focal locust is behind its nearest neighbour and facing towards it with the nearest neighbour facing away; (d) *opposed*: the two locusts are both facing away from each other.

The transition rates with the nearest neighbour in a state *S* were then calculated as:

$$P_{abnS}(r) = P\left(C_t = b \mid C_{t-1} = a, NN_{t-1}(r) = n, S_{t-1} = S\right) = \frac{N_{abnS}}{N_{aanS} + N_{abnS}}, \qquad (5.4)$$

where N_{abnS} is the number of transitions from the focal locusts movement state a to state b, given that they have a nearest neighbour in state *S* if n = 1 or given that they have a nearest neighbour which is not in state *S*.

For each range I calculated the difference between the probability that a locust became active given its nearest neighbour was out of the range and the probability that a locust became active given that it had its nearest neighbour within the range. I went on to consider not just the existence of the nearest neighbour in a range but also its relative orientation and movement state. Wilcoxon's signed-rank tests were performed on these differences to indicate effects of having a neighbour at a certain range and in a given 'state'. I used a significance level of $\alpha = 0.05$ in all tests. These tests give an indication of the importance of the different factors. However, as the data is divided up into different factors more test are performed and so the risk of Type I errors increases.

Logistic regression model

As an alternative approach, which avoids problems of multiple testing, deals with interactions, and is relatively robust to the distribution of errors in the data, I conducted an additional analysis using logistic regression models to assess the interactions of the different nearest neighbour traits. I considered the effect of five categorical dependent variables on whether an individual became active (and similarly

for when it became stationary). The factors encoded the locust's unique idenitity (ID), and the 'state' of its nearest neighbour described by three binary variables which determined whether or not the nearest neighbour was: to the front of the focal locust (P); oriented towards the focal locust (O); and whether or not it was moving (M). The distance to the nearest neighbour was also included as categorical variable encoding the same 6 ranges used above (D). For each combination of these factors I recorded the number of times that a stationary individual remained stationary between successive frames and the number of times that it became active between frames. I also recorded the number of time that a moving individual kept moving between frames for each combination and the number of time that it stopped. Any combinations of factors for which either no change in behaviour (from active to inactive or visa vera) or no persistence in behaviour was observed between frames was removed from the dataset. Data was obtained for one randomly selected individual in each of the 9 experiments. The model was chosen by using the forward stepwise NOMREG procedure in SPSS (2005). All main effect terms were available (ID, D, M, O and P) as well as all 2-, 3-, and 4-way interactions between D, M, O and P.

The parameters of the logistic regression model may be interpreted using risk odds ratios. A risk odds ratio shows how the likelihood of an outcome, for example of a stationary individual becoming active, changes due to differences in the predictors in the model. The risk odds ratio for predictor vector $\boldsymbol{X}_{1i}$ compared to predictor vector $\boldsymbol{X}_{0i}$ in a model with parameters β_i is given by:

$$ROR_{X_1,X_0} = \exp\left[\sum_i \beta_i\left(X_{1i} - X_{0i}\right)\right] \tag{5.5}$$

Testing for satisfaction of Markov property

Table 5.1 shows that the distribution of bout lengths is sensitive to the minimum bout length. Given this there is still a difference between the distributions of the moving and the stationary bout lengths. The moving bout lengths appear to be more likely to be exponentially distributed than stationary bout lengths. With a minimum bout length of 1 s, the moving bouts are longer on average than the stationary bouts. I also fitted the Weibull distribution to the bouts which had been broken down by the different states of the nearest neighbour. This distribution has two parameters and is able to capture increasing and decreasing probabilities of bout termination with bout length. When the Weibull shape parameter is equal to 1 the distribution is identical to the exponential distribution, implying that bout termination does not depend on bout length. I counted the number of locusts where the 95% confidence interval for the shape parameter contained 1 (Table 5.2). I found this to be true in 72% (n = 432) of cases.

5.3.6 Sampling

I have considered only one randomly selected locust from each experiment in order to avoid effects of non-independent observations. When two locusts were reciprocal nearest neighbours they experienced very similar conditions: their relative orientations

Table 5.1 – The number of experiments in which the hypothesis that the bouts are distributed exponentially can be rejected for the majority of the locusts. This was tested on activity data smoothed to different degrees to achieve different minimum bout sizes. For each locust in each experiment the distribution of moving and stationary bouts were each tested against an exponential distribution with mean obtained from the data with a Kolmogorov-Smirnov test. I counted the number of the nine experiments in which at least three (out of five) locusts' moving or stationary bouts did not follow an exponential distribution.

Min Bout (fms)	1	2	3	4	5	6	7	8	9	10
Moving	9	5	3	3	1	0	0	0	0	0
Stationary	9	9	9	8	7	6	4	3	2	2

were either identical (both *facing* or both *opposed*) or paired (one *ahead* and one *behind*); the distance to their nearest neighbour was identical; and all the other factors which I ignored were similar – positions in the arena, locations of other neighbours, the time spent marching and their experience prior to entering the arena.

5.4 Results

5.4.1 Transition rates

A stationary locust whose nearest neighbour was within 2.5cm of it was more likely to become active than if its neighbour were further away (Wilcoxon's signed-rank test p = 0.0117, n = 9). Figure 5.4a shows the difference between the probabilities of an individual changing state from stationary to moving, given that their nearest neighbour was in a given range and the probability that an individual changes state

Table 5.2 - Investigation of fitting bouts to Weibull distributed data. A Weibull distribution was fitted to the (a) stationary and (b) moving bouts of to each of the single, randomly chosen, locusts from each experiment. These were calculated by Matlab's maximum likelihood estimator considering right censoring. A Weibull distribution with shape parameter 1 is identical to the exponential distribution. Here, for each combination of states, I counted the number of locusts whose bouts fitted a Weibull distribution with a shape parameter 95% confidence interval containing 1. The numbers in brackets indicate the number of locusts which could have the fitting performed on them in that state. Summary percentages are given for each distance and each relative orientation.

a)	**becoming active**						
proximity (cm)	**0 - 2.5**	**2.5 - 5**	**5 - 10**	**10 - 20**	**20 - 30**	**30 - 72.5**	
facing	5 (9)	3 (9)	6 (9)	6 (9)	7 (9)	7 (9)	**63%**
ahead	3 (9)	3 (9)	4 (9)	6 (9)	7 (9)	7 (9)	**56%**
behind	5 (9)	6 (9)	8 (9)	9 (9)	9 (9)	7 (9)	**81%**
opposed	6 (9)	7 (9)	8 (9)	8 (9)	9 (9)	9 (9)	**87%**
	53%	**53%**	**72%**	**81%**	**89%**	**83%**	**72%**

b)	**becoming inactive**						
proximity (cm)	**0 - 2.5**	**2.5 - 5**	**5 - 10**	**10 - 20**	**20 - 30**	**30 - 72.5**	
facing	6 (9)	4 (9)	7 (9)	5 (9)	4 (9)	5 (9)	**57%**
ahead	6 (9)	9 (9)	9 (9)	7 (9)	9 (9)	6 (9)	**85%**
behind	6 (9)	7 (9)	8 (9)	7 (9)	9 (9)	9 (9)	**85%**
opposed	2 (9)	6 (9)	6 (9)	7 (9)	5 (9)	6 (9)	**59%**
	56%	**72%**	**83%**	**72%**	**75%**	**72%**	**72%**

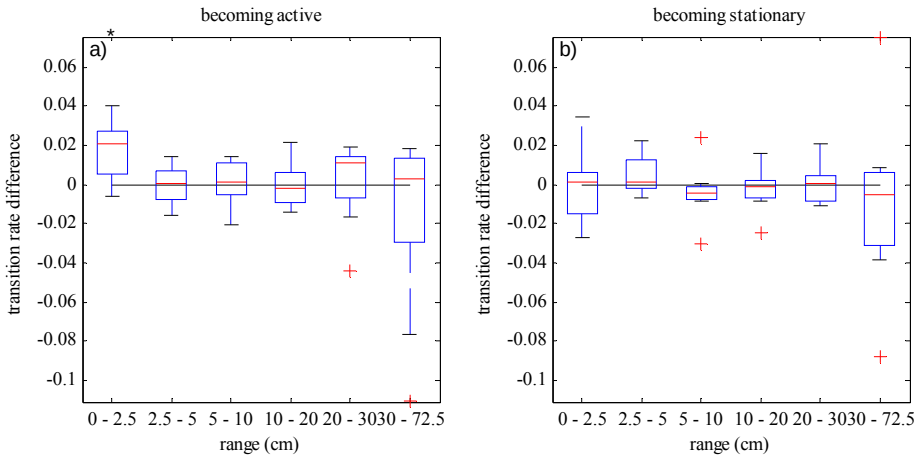


Figure 5.4 – The difference in probability of an individual changing state when its nearest neighbour is in the stated range and when its nearest neighbour is beyond the stated range. This is based on one randomly chosen individual from each experiment to ensure independence. Wilcoxon's signed-rank tests were performed on each with * denoting $p < 0.05$.

given that their nearest neighbour was beyond that range. I tested for differences in the other ranges and found no other significant deviations. When not considering the activity state of their neighbours, there is no significant effect of the presence or absence of neighbours on the rate at which active locusts become stationary (Figure 5.4b).

The activity state of nearby neighbours had a significant effect on an individual's propensity to change state. Figure 5.5a, c show that a stationary locust was significantly more likely to become active if it had a moving neighbour, but only if that neighbour was within 2.5 cm of it. There was no significant difference if its nearest neighbour was stationary (Wilcoxon's signed-rank test $p = 0.0039$ and $p = 0.496$ respectively, $n = 9$). A moving locust was significantly more likely to stop if there was a stationary neighbour within 2.5 cm of it (Wilcoxon's signed-rank test $p = 0.0273$, $n = 9$). This pattern appears to be reversed when the neighbour was moving, with the probability of becoming stationary decreasing for nearer neighbours. This last observation was not however statistically significant.

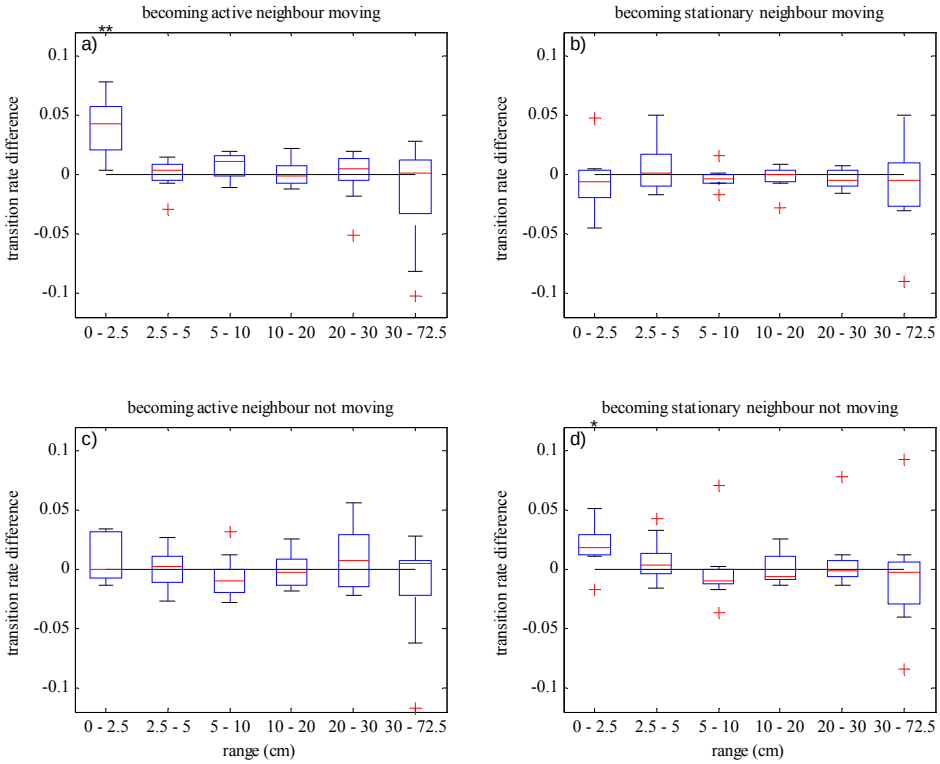


Figure 5.5 The difference in rated when the movement state of the locusts are considered. Wilcoxon's signed-rank tests were performed on each with * and ** denoting $p < 0.05$ and $p < 0.005$ respectively.

The relative orientation of a locust's nearest neighbour had a significant effect in certain ranges. In Figure 5.6 the transition rates were broken down by the four relative orientations of the individuals: *facing*, *ahead*, *behind* and *opposed* as defined in Figure 5.3. When a locust was *facing* its nearest neighbour there was a significant effect when the neighbour was in the 2.5 – 5 cm range on both stationary locusts rate of becoming active and active locusts rate of becoming stationary (Wilcoxon's signed-rank test $p = 0.0273$ and $p = 0.0195$ respectively, $n = 9$). Individuals were less likely to become active and more likely stop (Figure 5.6a & b).

-Figure 5.6c shows that when a locust was *ahead* of its nearest neighbour it was significantly more likely to become active and significantly less likely to stop when

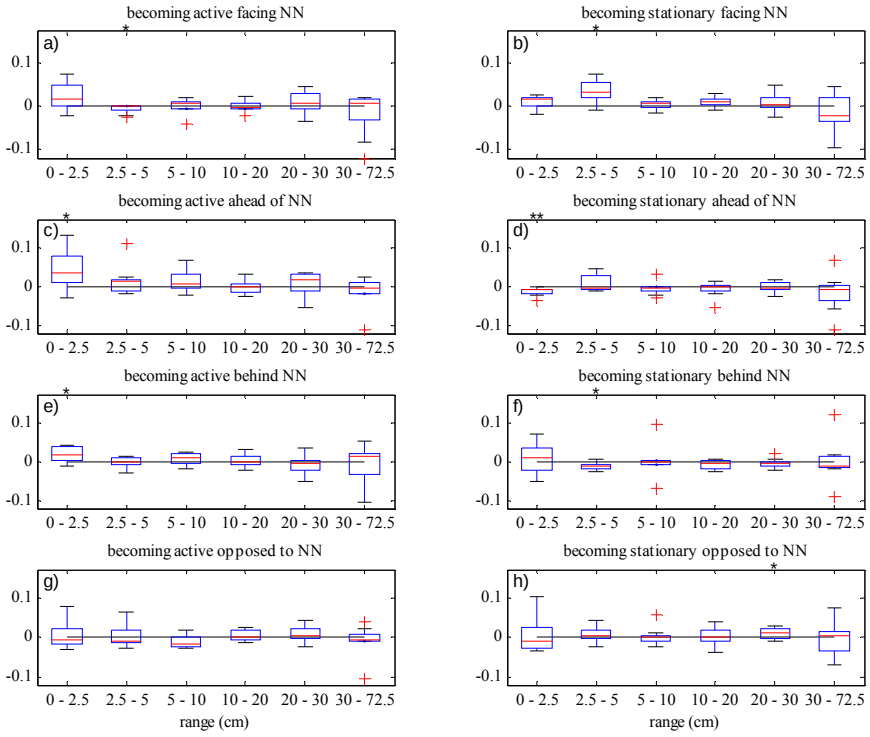


Figure 5.6 – transition rates by orientation of the focal locusts and its nearest neighbour. Wilcoxon's signed-ranks test were performed on each range with * and ** denoting $p < 0.05$ and $p < 0.005$ respectively.

the neighbour was in the 0 – 2.5 cm range (Wilcoxon's signed-rank test p =0.0273 and p = 0.0039 respectively, n = 9). When a locust was *behind* its nearest neighbour it was significantly more likely to become active with a neighbour in the 0 – 2.5 cm range and significantly less likely to stop in the 2.5 – 5 cm range (Figure 5.6e & f; Wilcoxon's signed-rank test p = 0.0273 and p = 0.0117 respectively, n = 9).

5.4.2 Logistic regression model

For becoming active, the best fit logistic regression model to the data from a single locust in each experiment generated by the SPSS NOMREG procedure is presented in Table 5.3. Of the 25834 cases observed, 3082 (11.9%) were excluded due to censoring. This generated the following model:

$$\text{logit}\,(\text{P(becoming active)}) = \alpha + \beta_1\,\text{ID} + \beta_2\,\text{D} + \beta_3\,\text{P} + \beta_4\,\text{O} + \beta_5\,\text{P}\times\text{O} \qquad (5.6)$$

Table 5.3 The logistic regression model of becoming active. Based on data from one individual in each experiment. The log likelihood ratio test of an effect compares the final model with a reduced model which excludes that effect, small significance indicates that the effect contributes to the model. Redundant parameters are set to zero and not shown.

Model: $\eta = \alpha + \sum_i \beta_i X_i$, with P(becoming active) = $e^{\eta} / (1 + e^{\eta})$

Variables in equation	Coefficient β_i	Std. Error	Wald	Significance of Wald test[§]	Significance of change in log likelihood ratio
Constant	-3.602	0.175	421.492	< 0.0005	†
Individual					< 0.0005
5	0.756	0.174	18.933		
9	0.685	0.217	9.946	0.002	
11	0.143	0.175	0.668	0.414	
18	-0.197	0.167	1.383	0.240	
25	-0.431	0.158	7.430	0.006	
30	-0.015	0.165	0.008	0.928	
33	0.337	0.166	4.103	0.043	
40	0.197	0.190	1.073	0.300	
41	0[‡]	.	.	.	
Distance					<0.0005
0 – 2.5	0.800	0.149	28.866	< 0.0005	
2.5 – 5	0.326	0.161	4.119	0.042	
5 – 10	-0.102	0.153	0.443	0.506	
10 – 20	-0.003	0.132	0.000	0.984	
20 – 30	0.130	0.144	0.814	0.367	
30 – 72.5	0[‡]	.	.	.	
Orientation: away	0.091	0.119	0.581	0.446	†
Position: rear	0.016	0.119	0.017	0.896	†
Position × Orientation					< 0.0005
rear × away	-0.617	0.170	13.091	0.000	

† This reduced model is equivalent to the final model because omitting the effect does not increase the degrees of freedom. ‡ This parameter is set to zero as it is redundant. § All effects tested had 1 d.f.

where α is the constant, ID is the locust identity, D is the six level categorical distance to the nearest neighbour, O and P indicate whether or not the nearest neighbour is oriented towards the focal locust and in front of the focal locust respectively. This model fits the data (Deviance = 253.5, d.f. = 257, p = 0.550) and shows that there is a significant effect of distance and significant interactions between P and O (see Table 5.3).

There was significant variation between individual locusts. All else being equal, the most reactive locust was 3.28 times more likely to become active than the least reactive locust. The Wald test indicates that the effect of distance become non-

significant beyond 5 cm (Table 5.3). A locust with a nearest neighbour within 2.5 cm or within 2.5 – 5 cm is respectively 2.46 or 1.53 times more likely to become active than a locust with a neighbour in the 5 – 10 cm range.

There is a highly significant interaction between the effects position and orientation. We describe the combinations of position and orientation using the neighbour 'states' in Figure 5.3: *facing, ahead, behind* and *opposed*. A locust was most likely to become active if it was *behind* its nearest neighbour and least likely to become active if it was *opposed* to it nearest neighbour ($ROR_{behind,\ opposed} = 1.82$). A locust which was *ahead* of or *facing* its nearest neighbour had a very similar likelihood of becoming active ($ROR_{ahead,\ facing} = 1.02$) with both being approximately 1.7 times more likely to become active than if they were *opposed* to their nearest neighbour. There was not a significant effect of whether the nearest neighbour was moving.

In fitting the becoming inactive data to the model 9733 of 42413 cases were censored (22.9%) and so excluded from the analysis. The model was fitted using the same method and factors as used in the model of becoming active, generating the following model (Deviance 267.6, d.f. = 256, $p = 0.297$):

$$\text{logit (P(becoming inactive))} = \alpha + \beta_1 \text{ ID} + \beta_2 \text{ D} + \beta_3 \text{ M} \qquad (5.7)$$

Its parameters and their significance are given in Table 5.4. As with the becoming-active model there was a strong influence of the individual locust, with the maximum risk odds ratio being 5.56 between locusts 9 and 25. In contrast to the becoming-active model, whether or not the nearest neighbour was moving was significant, with stationary nearest neighbour making a locust 1.5 times more likely to stop than moving ones.

Table 5.4 The logistic regression model of becoming stationary. Based on data from one individual in each experiment. The log likelihood ratio test of an effect compares the final model with a reduced model which excludes that effect, small significance indicates that the effect contributes to the model. Redundant parameters are set to zero and not shown.

Model: $\eta = \alpha + \sum_i \beta_i X_i$, with P(becoming stationary) = $e^{\eta} / (1 + e^{\eta})$

Variable in equation	Coefficient β_i	Std. Error	Wald	Significance of Wald test§	Significance of change in log likelihood ratio
Constant	-3.670	0.163	507.283	<0.000	†
Individual					< 0.0005
5	-0.735	0.170	18.590	<0.000	
9	-0.977	0.214	20.793	<0.000	
11	-0.532	0.174	9.397	0.002	
18	-0.198	0.167	1.408	0.235	
25	0.739	0.162	20.774	<0.000	
30	-0.095	0.165	0.335	0.563	
33	-0.279	0.170	2.689	0.101	
40	-0.617	0.192	10.360	0.001	
41	0‡	.			
Distance					0.002
0 – 2.5	0.040	0.153	0.067	0.795	
2.5 – 5	0.338	0.147	5.291	0.021	
5 – 10	-0.090	0.147	0.379	0.538	
10 – 20	-0.295	0.133	4.955	0.026	
20 – 30	-0.073	0.140	0.274	0.601	
30 – 72.5	0‡				
Movement: stationary	.386	0.092	17.741	<0.000	< 0.0005

† This reduced model is equivalent to the final model because omitting the effect does not increase the degrees of freedom. ‡ This parameter is set to zero as it is redundant. § All effects tested had 1 d.f.

The effect of distance was also significant but with neighbours in the 2.5 – 5 cm range having the biggest impact. A locust with a nearest neighbour in that range was 1.3 times more likely to stop than if it had a nearest neighbour in the 0 – 2.5 cm range, and 1.5 times more likely to stop than if it had it nearest neighbour in the 5 – 10 cm range. Position and orientation of the nearest neighbour were not present in the model, suggesting that they do not significantly influence a locust's likelihood of stopping.

I present the data from all 45 locusts from the 9 experiments in the appendix. The data was pooled to consider the average transition rates for all individuals. This summarised the interactions for all the factors which I considered (Figure 5.7). This was not corrected for between individual differences, but it illustrates the general effects.

5.5 Discussion

I was interested in how a locust was influenced by its neighbours whilst moving in a group. I hypothesised that the distance to an individual's nearest neighbours, the relative directions that the locusts faced and whether they were moving would influence a locust's propensity to start or stop moving. I used two tools to measure the effect of the neighbours on individuals. The logistic regression model of a locust's propensity to become active showed that the distance to, relative orientation and position of a nearest neighbour were significant factors. The logistic regression model of a locust becoming stationary showed that the distance to and whether or not the nearest neighbour was moving were the significant factors. The nonparametric analysis of the state transitions supported the logistic regression in the most part, but

differed by indicating a significant effect of the interaction of 'state' and distance not observed in the corresponding logistic models, this reduces our confidence in the significance of these higher level interactions.

While the assumption that transitions were Markovian was not completely demonstrated by the data, fitting the data to a Weibull distribution indicated that the distribution of movement and stationary bout lengths was not significantly different to an exponential model in the majority of cases (72%) (Table 5.2). Calculating the significance differences of the transition rates using Wilcoxon signed-rank test allowed analysis free from distributional assumptions. However, results regarding the interactions were harder to interpret using this method and prone to type I errors due to the multiple tests run. Fitting logistic regression models facilitated a much clearer interpretation of the interactions, but is limited by the assumption of independent error terms as we would expect error to be correlated with the individual. I dealt with these issues by controlling for individual locusts and assuming Markovian transitions. Therefore the logistic regression provides the more reliable analysis.

I found that a locust's propensity to switch between walking and stationary behaviour was strongly influenced by very local interactions with conspecifics. Only locusts within 5 cm (2.78 body lengths) appeared to have a significant effect on increasing a locust propensity to change state. This is consistent with the distance found by Despland and Simpson (2006). It is somewhat lower than the 13.9 cm (~6 body lengths) estimated by Buhl et al. (2006) of the range at which alignment was affected. This may be explained by the difference in the size and visual acuity between the 2nd instar locusts in these experiments and the 3rd instar locusts used by Buhl et al. (2006).

The significant reduction in propensity to become stationary when an individual had a nearest neighbour in the 10 – 20 cm range suggest that individuals are nevertheless able to respond to individuals at longer distances.

The inclusion of the relative orientations and positions as well as distance in the transition probabilities suggest that a locust is attracted to the abdomen of conspecifics and repelled by the head (Figure 5.6c, d, e and f). The logistic regression analysis showed that greatest stimulation to start moving was when a locust was facing the abdomen of its nearest neighbour. Bazazi et al. (2008) have shown that locusts are more likely to become active through tactile contact with their abdomen and has shown that the visual detection of other locusts approaching from behind strongly stimulates marching. Our results from direct testing of the transition probabilities support this. Individuals which were 2.5 – 5 cm behind their nearest neighbour were less likely to stop and more likely to start if the nearest neighbour was up to 2.5 cm from it (Figure 5.6e, f). A locust *ahead* of its neighbour, on the other hand, seemed to be repelled when the neighbour got too close, being more likely to keep moving or start moving when the nearest neighbour is at a close range. When face to face, a locusts' motion tended to be inhibited at a range of 2.5 – 5 cm. The interaction between position, orientation and distance was not detected in the logistic model. This may have been due to insufficient sampling

There did seem to be an effect of whether or not a nearest neighbour was moving. The analysis of the transition rates indicated that stationary nearest neighbours had a significant effect on moving locusts and moving locusts had a significant effect on stationary locusts at the closest range when no other interaction were considered

(Figure 5.5). The logistic regression supported the effect of movement being significant for locusts becoming stationary after controlling for the other interactions. This would be expected since gregarious locusts are attracted to each other and have been shown to settle close to tethered locusts in the lab (Ellis, 1953b). In field observations very slow moving locusts moving in bands have been observed to accelerate, following a short delay, after being overtaken by another locust (Stower, 1963).

In this analysis I only considered whether a locust's neighbour was moving or not. Locusts in the experiments moved at different speeds and one might hypothesize that faster-moving locusts would have a bigger impact than slower-moving ones. It would be interesting then to break down the speed of the nearest neighbour into ranges, as was done with the distance to the nearest neighbour. Speed ranges would however introduce more states and so spread out the data more thinly. This shortcoming would require the individual tracking of more experiments to generate sufficient data.

When all 45 locusts rather than 9 focal locusts were considered I found good agreement with our findings from 9 locusts (see appendix). There was strong support for a 'push from behind' (Simpson et al., 2006, Bazazi et al., 2008). The detection of a difference in reaction distance to moving and stationary locusts which are facing a locust is also interesting. It should be noted that this data suffers from the independence issues noted in the methods.

There is strong evidence in the literature that a locust's instantaneous probability of changing state (from stationary to moving and visa versa) increases with the time

since its last change (Simpson and Ludlow, 1986, Moorhouse et al., 1990). However, this may be less true for gregarious individuals. Studies of the visual looming detection neuron DCMD in locusts have demonstrated that there are differences in the sensory-motor pathways of gregarious and solitary locusts (Matheson et al., 2004, Rogers et al., 2007). The signalling of object approach by DCMD habituates more strongly in soliary than in gregarious locust (Matheson et al., 2004). This results in solitary locusts having a later acting and more variable response to approaching objects and gregarious individuals having an earlier and more temporally consistent response.

In the analysis presented here bouts were assumed to start from when the state describing the locust's relationship with its nearest neighbour last changed, rather than from when it was last in a different movement state. The time since an individual last changed state could be included as an additional covariate. This could then be used to assess the degree of habituation to the stimuli from a locust's near neighbours. Survival analysis seems to be the appropriate tool for considering these data and the interactions of the covariates (Cox and Oakes, 1984).

Here I have demonstrated a method for quantifying interactions between locusts. This is a first step towards developing an executable behavioural model of individual interacting locusts (Tucker et al., 2001). As more information becomes available to behavioural researchers through the use of video tracking methods which take advantage of this data need to be developed further.

5.6 Appendix

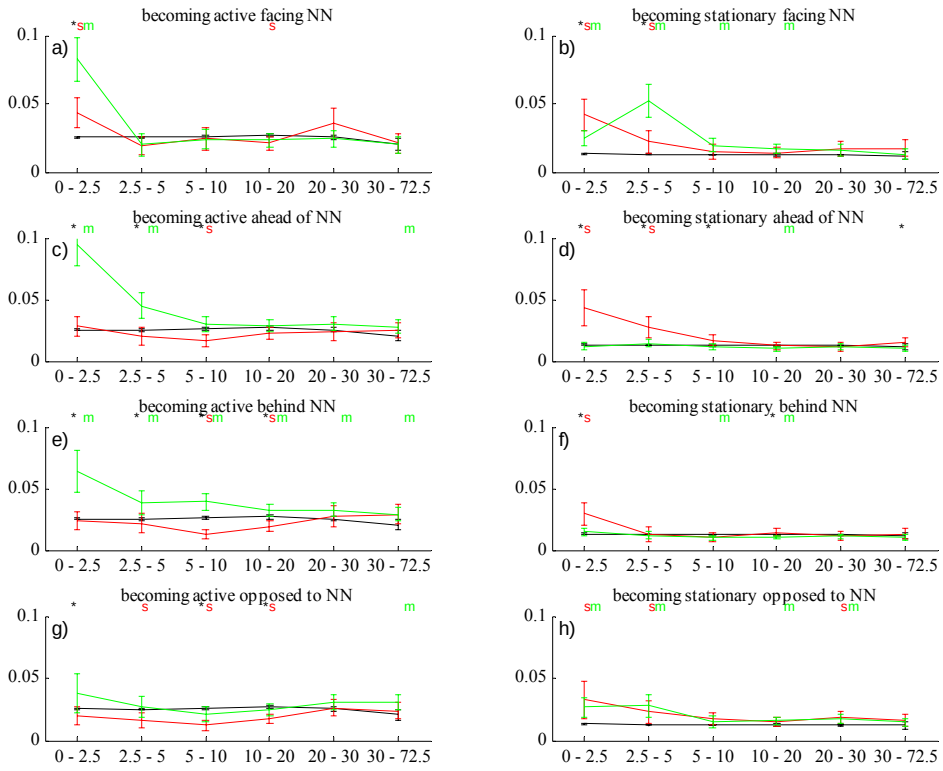


Figure 5.7 – Pooled data results with 95 % confidence intervals (assuming exponentially distributed bouts). The black line denotes the rate when the nearest neighbour is beyond the range. The red line represents the case when the NN is stationary and the green line corresponds to time when the NN is moving. For each range a "*" indicated that zero fell outside the confidence interval of the difference between the rate given a moving or stationary NN. A red "s" indicates that zero falls outside of the 95% confidence interval of the difference between the rate given a stationary NN vs having no NN, and a green "m" indicates that zero falls outside of the 95% confidence interval of the difference between the rate given a moving NN vs having no NN.

Chapter 6

The drawing power of crowds revisited

6.1 Summary

In this chapter the response to different sized groups of conspecifics was studied in humans. We replicated in Oxford, UK, a study of gaze-copying in people carried out in New York, USA in 1968. A stimulus group looked up at a building for 60 s and the response of passers-by was captured on video. We investigated the relationship between the size of the stimulus group and the proportion of passers-by responding to the group. Our study supported the earlier work, finding that the proportion of passers-by who copied the stimulus group by looking up at the building increased with stimulus group size. We quantified the responses of both the original study and ours by fitting a threshold response curve. People in Oxford were less responsive than those in New York. However we did find a similar initial linear response to group size in both cities. This response level is below that necessary to result in spontaneous crystallisation of a crowd – where stopping behaviour spreads rapidly through a crowd. We also considered the spatial distributions of the gaze-copying and found that people were more likely to look up when they were behind the stimulus group.

6.2 Introduction

On two winter afternoons in 1968 Stanley Milgram and two colleagues performed a simple, yet revealing experiment on social behaviour of humans. On a busy New York street they placed a small stimulus crowd of individuals each of which looked up at a window of a nearby building. They then observed the proportion of passers-by who looked up at the window as they walked past the crowd. The results were clear. The larger the crowd the larger the proportion of passers-by who would stop and/or look up (Milgram et al., 1969). These experiments reveal the importance of social interactions even in the most every day setting.

It is the inter-individual interactions which are key to many of the different grouping behaviours observed in group-living animals (Couzin and Krause, 2003). This principle is true for people as well as locusts. The gaze-copying observed by Milgram et. al (1969) is suggestive of local enhancement seen in many social and grouping animals (Krause and Ruxton, 2002). In chapter 3 I considered the response of locusts passing-by different sized groups of conspecifics. I found that, as with the New York pedestrians, individuals were more likely to join a larger group. In chapter 5 I started to investigate the individual level interactions of the locusts and the effect relative orientations and motion had on an individual's likelihood to start or stop moving. Here we study this phenomenon in people at the individual level using the techniques I developed for investigating collective behaviour in locusts.

In the 40 years since Milgram's experiment, there has been a great deal of experimental and theoretical research in to the social behaviour of humans and other animals. Individuals adopting the behaviour they see performed by others is now

accepted as one of the principles both of collective animal behaviour (Deneubourg and Goss, 1989, Krause and Ruxton, 2002, Sumpter, 2006) and of sociology (Schelling, 1978, Ehrlich and Levin, 2005, Hedstrom, 2005). Depending on the form by which behaviour is transferred from one individual to another various consequences can be seen at the level of the group. For example, Granovetter (1978) describes a model where individuals decide whether to engage in some form of action (such as a rioting, use of contraception or voting for a particular party) when a threshold of others have already engage in the action. He showed that groups with similar average preferences may generate very different collective behaviour, depending upon the order in which they make the decision. In general, a threshold like response, where above a certain group size an action becomes much more probable to be undertaken, generates strong dependency on initial conditions while linear responses, where response is proportional to stimulus group size, does not (Nicolis and Deneubourg, 1999, Sumpter and Pratt, 2008).

These more recent empirical and theoretical studies raise a number of interesting questions about Milgram's original study. Firstly, what is the functional form of the relationship between group size and proportion looking up? Although Milgram's original data seems to reveal a threshold-like relationship in the proportion of individuals looking up, the threshold at which half the passers-by look up is very close to one individual. This makes it difficult to determine empirically whether the response is a steep threshold or simply a linear increase near to 1 which later tails off. Establishing this functional form is important if we want to infer that gaze copying will lead to a 'tipping point' (Gladwell, 2001) or 'crystallisation' (Ball, 2004) of

copying behaviour, where groups of individuals looking up will spontaneously emerge. Such crystallisation can only occur for threshold-like responses.

A second question is about the reason individuals look up (Bond, 2005). One hypothesis here is that individuals socially conform to the behaviour of others (Milgram, 1963, Asch, 1955). If a passer-by sees a fellow pedestrian looking up she or he feels they need to conform with this behaviour and looks up as well. A second hypothesis is that the passers-by feel they can gain some information from looking up. It would be unusual for a crowd to gather without there being something worthwhile to look at and it is then beneficial for the passer-by to check what they are looking at. To some degree this question can be investigated by looking at the spatial positioning of individuals when looking up: are passers-by more or less likely to look up when they know they are in the field of view of the stimulus group?

In this chapter, as well as celebrating the 40th anniversary of Milgram's experiment by repeating it in a new setting, we try to answer these two questions.

6.3 Methods

6.3.1 Observational setup

We replicated the experiment on gaze-copying performed by Milgram (Milgram et al., 1969) described above. All experiments were conducted between 21 March 2007 and 10 April 2007 on Cornmarket Street, Oxford, UK. Crowds were filmed from above using a CanonXM2 miniDV camcorder atop a roof of one of the buildings on the street (Figure 6.1). For simplicity we also used this placed camera as our 'stimulus'.

As will be clear from our results, there was a very low spontaneous probability of individuals locating the camera used for filming in this experiment.

A group of confederates (our stimulus crowd) entered Cornmarket from a hidden location in a small side-street and in the middle of our observation area stopped and looked up towards the camera. This gaze was maintained for 60 seconds. At the end of this period the group was signalled to disperse. After the area was cleared we waited 3 minutes before another stimulus group entered. We found that due to the relatively high rate of flow on this street that this was a sufficient time between experiments. We conducted trials with groups of 1, 2, 3, 4, 5, 7, 9, 12 and 15 individuals chosen in random order. Between 6 and 14 replicate experiments were conducted for each group size.

Figure 6.1 (a) View of Cornmarket Street, Oxford from our vantage point. (b) View of the camera from the perspective of pedestrians at street level. The experimenter was not observable during trials.

6.3.2 Data analysis

Tracking

We tracked all 3, 917 pedestrians who entered a 10 m x 8 m region during our trials by estimating for each frame the point on the ground directly below the body (usually in-between the feet). We also recorded, for every frame, whether or not individuals were looking in the same direction as the stimulus group. This was done manually using point-and-click software we developed. Although a very time-consuming procedure it is the only method that ensures the accurate detection of gaze-copying. All data was anonymous, individuals being given sequential numerical identities based upon when they entered the scene (Figure 6.2).

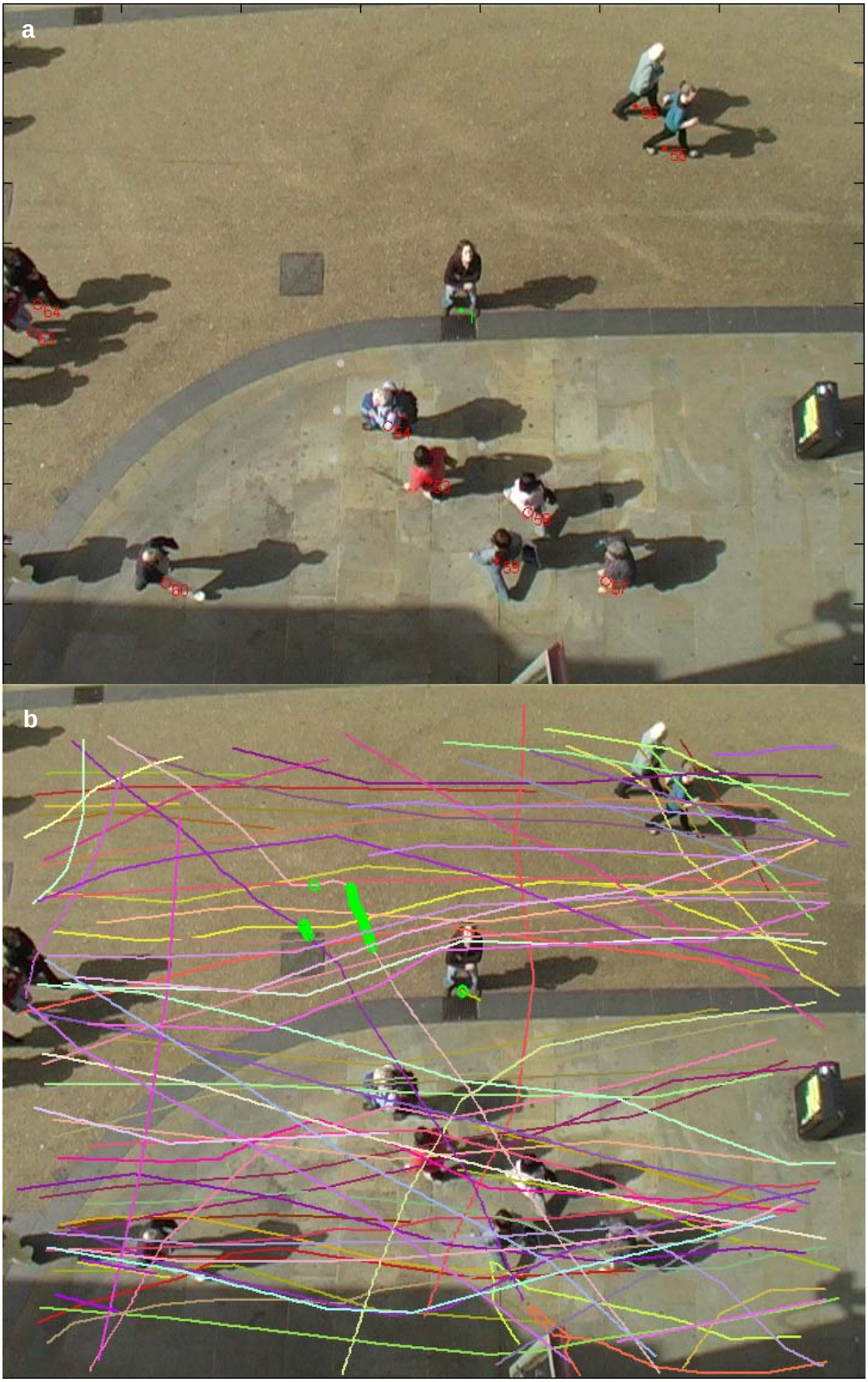


Figure 6.2 (a) Individuals are each assigned unique, but anonymised, identities. (b) A 1 min sequence of tracks showing the motions of pedestrians as coloured lines projected onto the ground beneath their feet and sections of trajectories where the individual was estimated to have copied the stimulus gaze shown in bold green. Note a stimulus individual is in the centre of the frame.

Camera angle and lens distortion

Due to the fact that the camera is not directly above the crowd (Figure 6.1b) and that the lens has inherent curvature the tracked positions are distorted. We therefore needed to determine the internal camera geometric and optical characteristics in order to remap pixels in the image to their corresponding real world positions. We used the four step method of Heikkila and Silven (1997) which involves replacing the physical parameters with non-physical implicit parameters that are used to interpolate between known 'tie-points' in the scene. To do this we take into consideration the focal length, skew and distortions to correct our geometry and recalibrate trajectories such that they appear as if we filmed from directly above the crowd. An overview of this technique and links to useful technical papers on this topic can be found online (Bouguet, 2007).

Behavioural Analysis

Calibrated trajectories were analysed in Matlab using our own custom-written code. We calculated individual the passers-by' position, speed, the tortuosity of their path and when, where and for how long they copied the gaze of the stimulus group. We categorised an individual as stopped if its speed fell below $0.2ms^{-1}$. We also discretised the region of filming to allow us to analyse the spatial relationship between crowd density, flow patterns and where individuals tended to copy the gaze direction of the stimulus crowd, or of each other.

Response fitting

In order to quantify the response of passers-by' to the stimulus group we fitted the function

$$P(N) = m\frac{N^k}{T^k + N^k}, \tag{6.1}$$

where $P(N)$ is the observed frequency of looking up and N is the stimulus group size. The fitted parameters, T, m and k characterise the type of response: m is the maximum proportion of individuals that will look up, T is the threshold group size at which $m/2$ individuals will look up and k determines the shape of the functional response. The larger the value of k the more like a threshold switch the response becomes (Sumpter, 2006, Sumpter and Pratt, 2008). The fit of this curve was made using the non-linear fit function in Matlab. We fitted this function both to our own data and retrospectively to the data of Milgram.

6.4 Results

6.4.1 Response as a function of stimulus group size

Passing pedestrians did copy the gaze direction of the stimulus group (Figure 6.3a). As group size increased so too did the tendency to copy gaze direction, although this tendency saturated for larger groups. Fitting equation (6.1) to the data gave m=0.63, T=6.4 and k=1.42. Contrasted with a similar retrospective fit to Milgram's data (m=0.91, T=1.2 and k=1.05) we see that the propensity for individuals to look up is much lower in our observations. However, the k parameter is close to 1 in both cases, indicating a similar functional response in both studies.

Individuals that copied the gaze of the stimulus crowd looked in the stimulus direction between 1 and 6 times (Figure 6.4a) and there was no significant effect on the time spent looking and whether or not an individual had looked previously (Figure 6.4b).

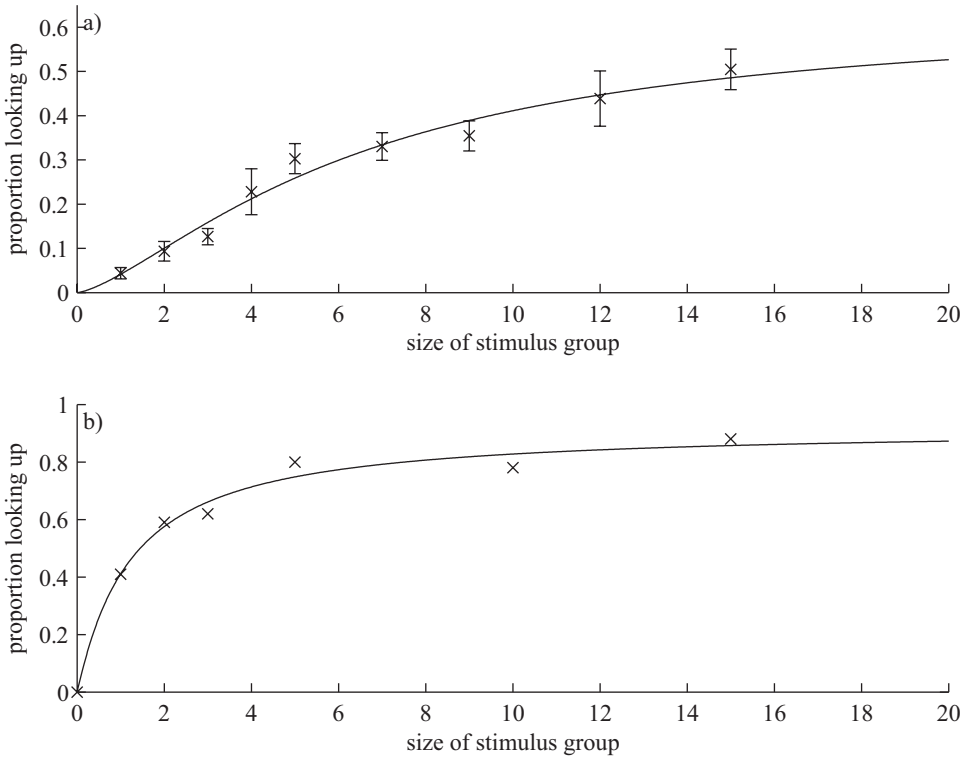


Figure 6.3 The relationship between the probability that passers-by will copy the gaze of the stimulus group as a function of stimulus group size fitted to equation (6.1): (a) from the Oxford experiments, mean ± SE, *m*=0.63, *T*=6.4 and *k*=1.42; (b) data from Milgram et al (1969), *m*=0.91, *T*=1.2 and *k*=1.05.

That is gaze duration seems not to depend on whether an individual has previously looked or not and remains relatively constant throughout trials.

6.4.2 Speed and stopping

As in previous studies of pedestrian speeds (Henderson, 1971, NYC Department of City Planning, 2006) we found a relatively widely spread distribution of natural speeds which follows a Gaussian distribution with mean of 1.228 ms^{-1}, and standard deviation 0.295 ms^{-1}. This is in close accordance with previous comparable data from New York City where the mean speed of pedestrians was calculated as 1.281 ms^{-1} with standard deviation 0.216 ms^{-1} (NYC Department of City Planning, 2006).

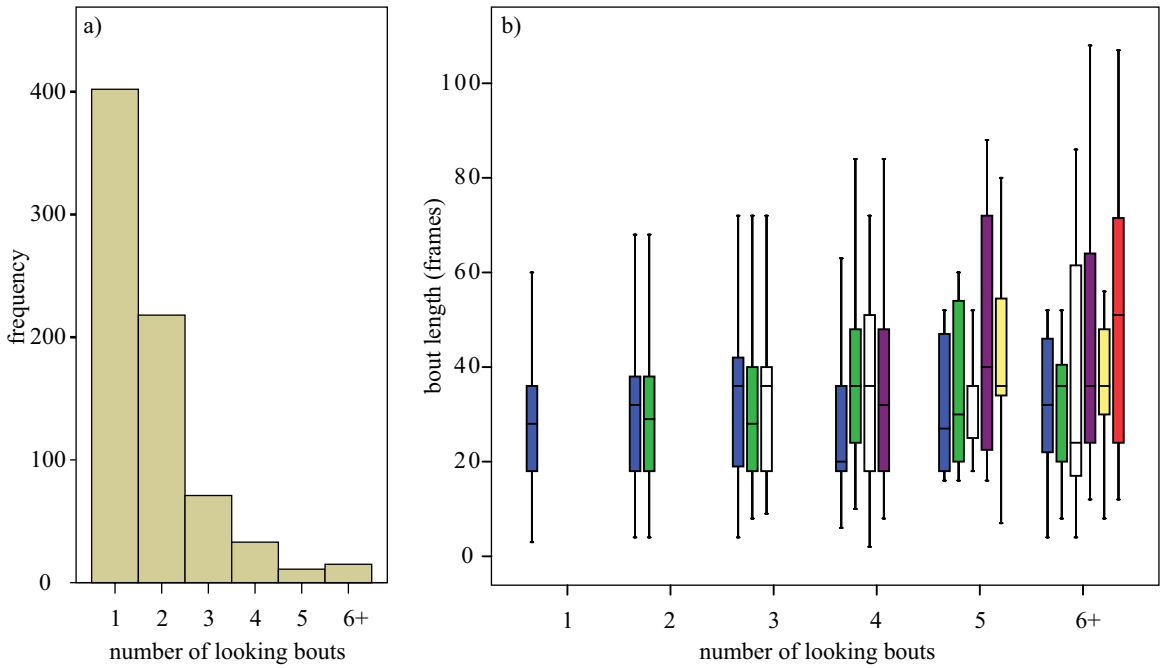


Figure 6.4 Number and length of looking bouts for those individuals which looked up. (a) Frequency distribution of the number of looking bouts and (b) the boxplots for the duration of the looking bouts broken down by number.

In order to investigate the relationship between pedestrian speed and gaze-copying we compared the trajectories of individuals that did, and did not look up, when they walked through the scene. We found two strongly statistically significant, but weak, relationships between speed and gaze copying behaviour. Firstly we found that individuals that did copy gaze tended to walk more slowly than individuals which did not look up (t-test $t=7.845$, $df = 2770$, $p < 0.0005$). Furthermore individuals that did perform gaze copying moved slightly more slowly when looking up, as opposed to when they were not looking up, shown in Figure 6.6 (paired sample t-test, $df = 732$, $t = 7.687$, $p < 0.0005$). However, individuals exhibited only a very low propensity to stop when gaze-copying and, when they did so, the duration of the stop tended to be very short (Figure 6.6).

6.4.3 Spatial aspects of gaze-copying

The direction of gaze of a group seemed to have little influence on the density of individuals moving in front or behind the stimulus group, with roughly equal numbers of individuals going behind or in front of the group independent of group size (Figure 6.7a,c,e). Individuals approaching the group tend to veer off in the direction which takes them round it with the least effort. In other words, there does not appear to be a

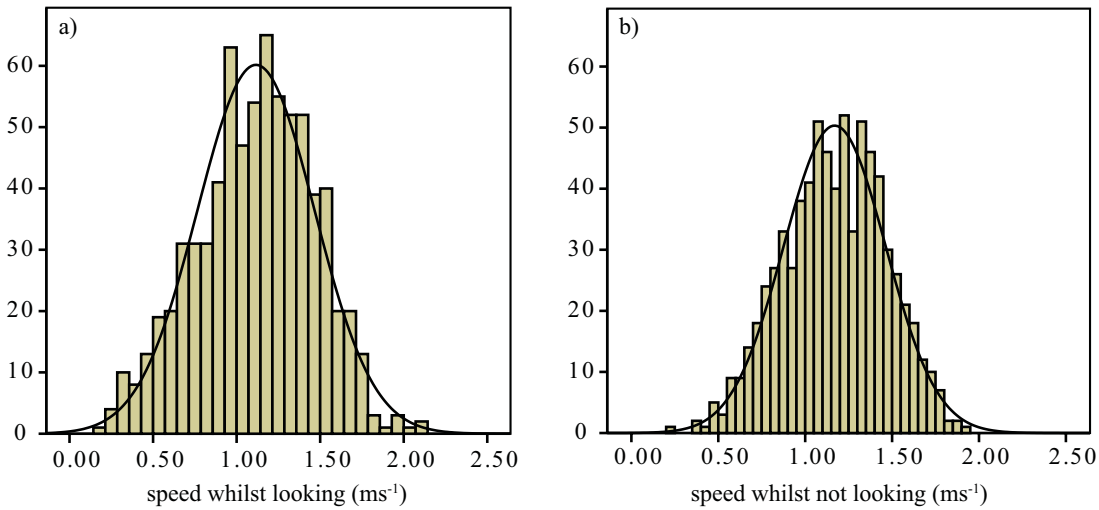


Figure 6.6 Speed distributions of pedestrians which did look for (a) trajectory segments whilst they were gaze-copying (mean = 1.1142, standard deviation = 0.350, N = 733) and (b) trajectory segments whilst they were not gaze-copying (given that they did look up at some point during the study) (mean = 1.1749, standard deviation = 0.291, N = 733).

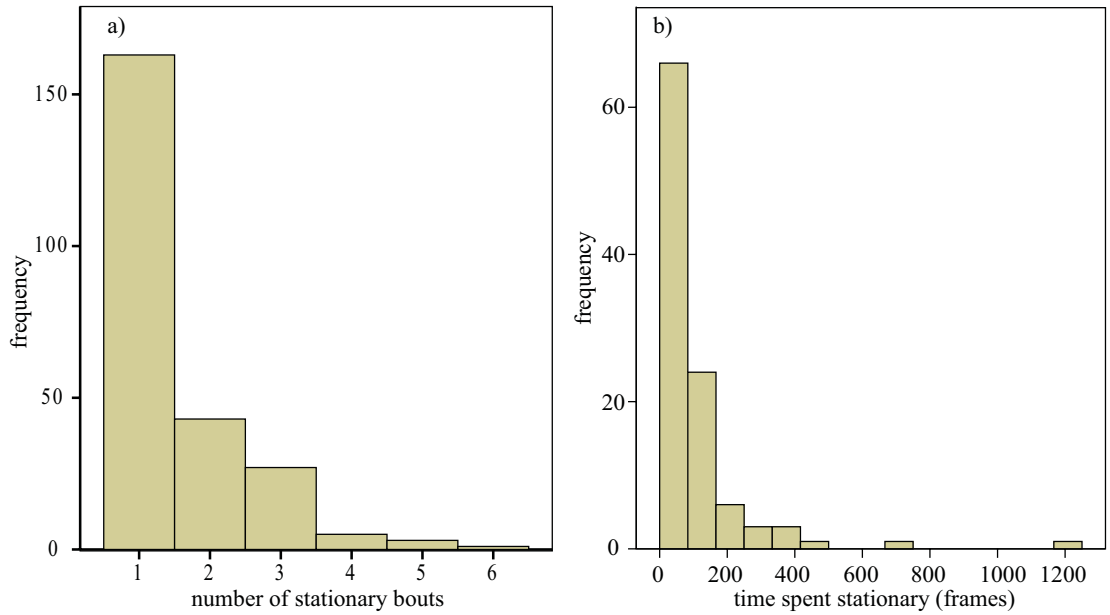


Figure 6.6 The frequency distribution of (a) the number of stationary bouts and (b) time spent stationary during looking bouts. Individuals who did not spend any time stationary are excluded.

bias to go in front or behind the group. Gaze does not however spread evenly in all directions. Individuals 'behind' the stimulus group tended to have a higher propensity to copy gaze than did individuals in front of (and thus 'within' the gaze of) the group (Figure 6.7b,d,f).

6.5 Discussion

We confirm the experimental results of Milgram et al. (1969). Individuals influence the gaze of those around themselves. Quantitatively our results differ from Milgram et al. in terms of the strength of response. Our stimulus group in Oxford attracted much less attention than the New York group, with a threshold response at 6 rather than 1 individual and a maximum probability of responding of 63% rather than 91%. While it might be interesting to speculate on cultural differences in the relative interest of the dwellers of these two cities have in the behaviour of others, the weaker response in our experiments could equally well be due to subtle differences in the composition of the stimulus group, time of day, and so on. Cornmarket Street in Oxford has many visual stimuli - with shop fronts being prevalent. This was not so in the New York study site. The competition for gaze was therefore higher in Oxford.

While threshold and maximum response probability changed between experiments, the crucial parameter k, which determines the steepness of the response, did not. In both cases k was slightly larger than 1, indicating an initially linear response to group size. Unlike Milgram's original experiments, the threshold in our experiments is sufficiently far from one person in order that we can be confident in the estimate of this parameter. Taking the two experiments together indicates a robust linear response to group size.

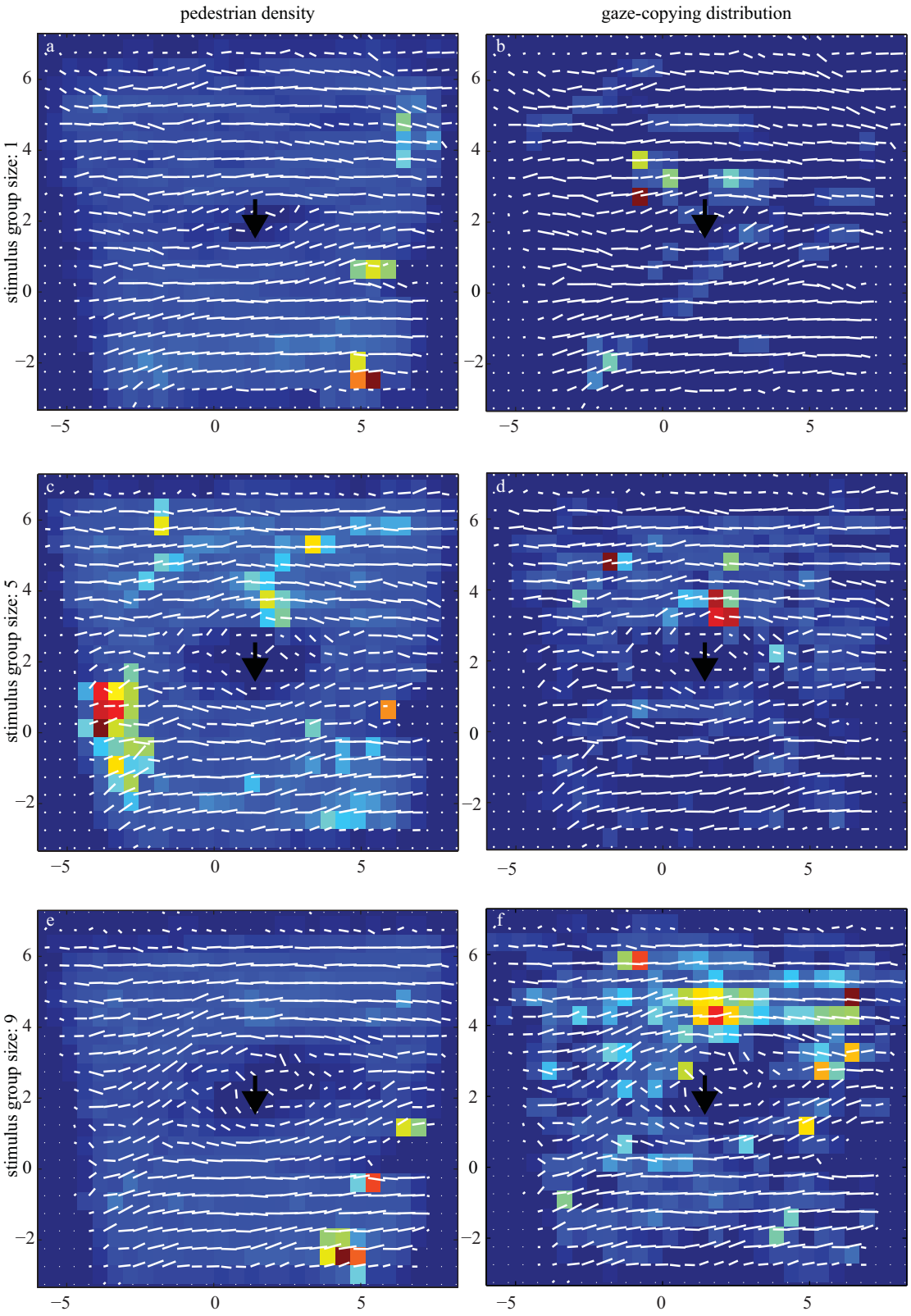


Figure 6.7 The spatial distribution of pedestrians' locations (a, c and e) and spatial distribution of the gaze-copying behaviour (b, d and f). Blues indicate low density and reds high density and the units are meters. The black arrow indicates the direction of the stimulus gaze. The white lines represent the averaged direction of pedestrian flow modulo 180° in each 50 cm^2 box. Data presented are the mean for all replicates for each of three group sizes 1, 5 and 9.

The linear response has important consequences for how we view group size dependent behaviour. Previous interpretations of Milgram's results have suggested that crowds may 'crystallise': such that stopping behaviour will, above some threshold rapidly spread through a group of individuals (Woloszyn et al., 2007, Tarnow, 1996). Such crystallisation will not however occur for k=1. We can see this by developing a mean-field model of individuals looking up. Let N be the number of individuals looking up at a particular time, R be the number of passers-by per minute and $1/l$ be the average length of a period of looking up. The rate of change of the number of individuals looking up is then

$$\frac{dN}{dt} = Rm\frac{N^k}{T^k + N^k} - lN \tag{6.2}$$

where m, T and k are as defined in equation (6.1). For k=1, solving $\frac{dN}{dt} = 0$ reveals a single non-zero stable steady state for these equations at $N = Rm/l - T$. Thus, provided the stimulus is strong enough, there will be a steady self-sustaining flow of onlookers. This is in contrast to the case where k>>1 where there exist multiple stable steady states to equation (6.2) corresponding to 'crystallized' and 'non-crystallized' groups of onlookers. While our measured value of k is slightly greater than 1, when random fluctuations are taken in to account, it is not sufficiently large to generate significant crystallization.

Animals, such as ants and fish, which use density based responses to make collective decisions often have values of k of around 3 or higher (Pratt and Sumpter, 2006, Ward et al., 2008). Interestingly, the response functions that have been measured in apes are more similar to those seen in our experiments, with k≈1 (Meunier et al., 2007). This could however simply reflect the relative importance of accuracy in the decisions

tested within the different experimental setups (Bond, 2005). Experiments to determine responses in fish and ants often involve decisions which have direct consequences for their safety. Theoretical studies have shown that high values of *k* produce more accurate decisions, while low values of *k* produce faster decisions (Sumpter and Pratt, 2008). Determining whether something is going on over a high street shop is probably not very important to the average passer-by and is thus reflected in a weak response to a stimulus group.

Unlike Milgram et al (1969), we have characterised the movement and positions of passers-by which look up relative to the stimulus group. A surprising result here is that gaze-copying occurs more readily to the sides and behind stimulus individuals than in front of them. This would suggest that copying is not due to social pressure or conformity (Asch, 1955) or some form of obedience (Milgram, 1963). Instead, individuals appear to use gaze-copying in order to gain information about their environment. These results support the hypothesis that in situations where individuals are not directly confronted by the peer stimulus group their response is more linear than threshold-like (Bond, 2005).

Chapter 7

General Discussion

7.1 Summary of results

This thesis contributes to the understanding of group movement in locusts. It also demonstrates techniques for the analysis of locust interactions, which can be extended further in future. A fuller understanding of groups from the individual level is needed for the formation of valid models of the behaviour of locusts at larger scales. Such large scale models could be of great benefit to locust-control efforts for helping in the prediction of the motion of marching bands. However, there is still a great deal of work required before this capability is achieved. The understanding gained though the study of locusts within a self-organisation framework also benefits the study of other group living animals.

In chapter 2 the tools required for the computer tracking of individuals were presented. Such techniques had not been successfully applied to the detailed study of groups of locusts before. The automatic tracking tool with no human intervention allowed for the collection of data from experiments containing large numbers of individuals over long time periods. This tool was able to keep track of individuals over short periods of time and was suitable for capturing group level properties such as activity and alignment. This automatic tracking enabled the collection of data for a study into the effect of group size on the degree of order in marching (Buhl et al., 2006) and the influence of cannibalistic interactions in locust marching (Bazazi et al., 2008) as well as obtaining the data used in chapters 3 and 4.

For tracking in more complicated environments I developed a fully manual tracking system which enabled an observer to track the position and other information about an object from video using a graphical user interface. This method is very labour

intensive but is useful for obtaining highly accurate data. It was used in chapter 6 to study the movement of humans in complex backgrounds. These previous two tracking tools were combined to include human intervention when automatic tracking was likely to make an error. Tracks could then be maintained even when locusts were very close and the automatic tracking failed. This correction of misidentifications facilitated the tracking of individual locusts over extended time periods enabling the collection of data on close interactions between individuals where we would expect the strongest interactions to occur. This data then formed the basis of chapter 5.

At the collective level, I have looked at both aggregation and collective motion of locusts. Chapter 3 focuses on aggregation. I showed that locusts, given a choice between two identical heat patches had a preference for entering the patch with the greater number of other locusts already on it. The number of individuals on the more populous of the two heat patches was significantly different from the number expected if there were no social influence. However, unlike similar studies on cockroach shelter choice (Ame et al., 2006), which inspired my work, the locusts did not collectively select a single shelter even when the group size was smaller than the carrying capacity of a single patch. The locusts instead formed dynamic groups above each patch with individuals frequently entered and left. However, single gregarious locusts in the arena spent a much smaller proportion of their time on a heat patch, with some locusts not finding either patch over the course of the entire experiment. This latter observation strengthens the hypothesis of some transfer of information about the locations of the heat patches when individuals were in a group. Taken together the results of this chapter indicate that density dependent aggregation is not as important in locust interactions as was first supposed.

Collective motion was however an important component of locust behaviour. Many models of collective motion in groups assume that individuals can be considered as memoryless particles (Vicsek et al., 1995, Couzin and Krause, 2003). In the joint paper with Buhl et al. (2006) and chapter 4 I considered the collective movement of the locusts. In chapter 4 I found that locusts did indeed seem to respond to their immediate group density, rather than their previous density. Locusts which had experienced marching in a high density crowd were indistinguishable from locusts which had only experience of a low density once they placed in groups of the same low density. Furthermore I found that there was a robust relationship between the level of activity in the group and the degree to which the group aligned. This supported earlier work by Buhl et al. (2006) to a certain extent although also brought up some important differences.

Chapter 5 changed focus from the collective to the individual. I tracked groups of 5 individuals at 5 frames per second for 30 min using-assisted automatic tracking. This ensured that individual identities were maintained over this period. The proximity, relative orientation and the movement state of a locust's nearest neighbour were considered as factors influencing its probability of moving. I found that neighbours within 5 cm of a locust had the greatest effect. This agreed with previous studies (Despland and Simpson, 2006). Stationary neighbours tended to make a moving locust more likely to stop and similarly, having moving neighbours tended to make a stationary locust more likely to start moving. We also observed differences in a locust's reaction depending on whether the neighbour was facing towards or away from it.

In chapter 6 the response of individuals to different sized aggregations of people was investigated. We replicated a study of gaze-copying in people carried out in New York, USA in 1968 (Milgram et al., 1969). A stimulus group looked up at a building for 60 s and response of passersby was captured on video. We investigated the relationship between the size of the stimulus group and the proportion of passersby responding to the group. Our study supported the earlier work, finding that the proportion of passersby who copied the stimulus group by looking up at the building increased with stimulus group size. We quantified the responses of both the original study and ours by fitting a threshold response curve. People in Oxford were less responsive than those in New York. However we did find a similar initial linear response to group size in both cities. We also considered the spatial distributions of the gaze-copying and found that people were more likely to look up when they were behind the stimulus group.

7.2 Future Work

7.2.1 Using the collected data

In this thesis I have collected and analysed a great deal of information about the movement of juvenile locusts at both the individual and group level. The data collected from the individually tracked locusts in chapter 5 suggests that the alignment of individuals may be due to a push from behind, an hypothesis which is supported by recent work (Simpson et al., 2006, Bazazi et al., 2008).

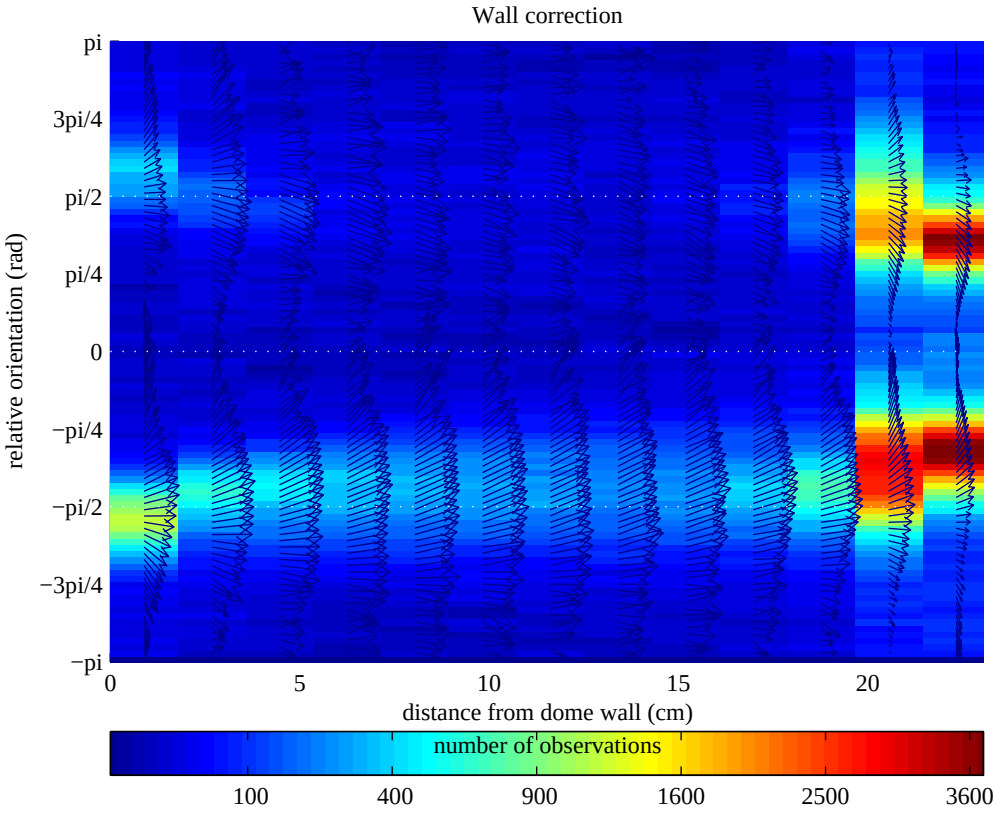


Figure 7.1 The effect of the distance from the dome and the orientation relative to the radius on the subsequent position of a locust. The vectors represent the position that a locust moves to one second later relative to its current orientation and location. The data is reoriented so that each individual is facing along the positive x-axis, thus an insect which moved straight forward for a second would be represented by a vector parallel to the x-axis and of length the distance which it moved in one second. The background colour indicates the square root of the number of observations of each combination of relative orientation and distance from the dome. This data is from the movement of all 45 individually tracked locusts obtained in chapter 5.

In chapter 5 I concentrated on the factors which activated locusts, the natural next step is to look at how locusts change their direction of movement in response to each other. This individual level data can be used to parameterise a model of the locust hopper behaviour. Initially this could be done in one dimension with individuals moving on a circle with instantaneous probability of starting or stopping depending on the proximity and orientation of their neighbours. Estimates of the probability of an individual probability of changing direction both within and between moving bouts would need to be extracted from the individually tracked data, we would expect this to depend on the motion of other individuals corresponding to alignment in Buhl et al. (2006). Such a model could be used to test if an individual's instantaneous reaction to

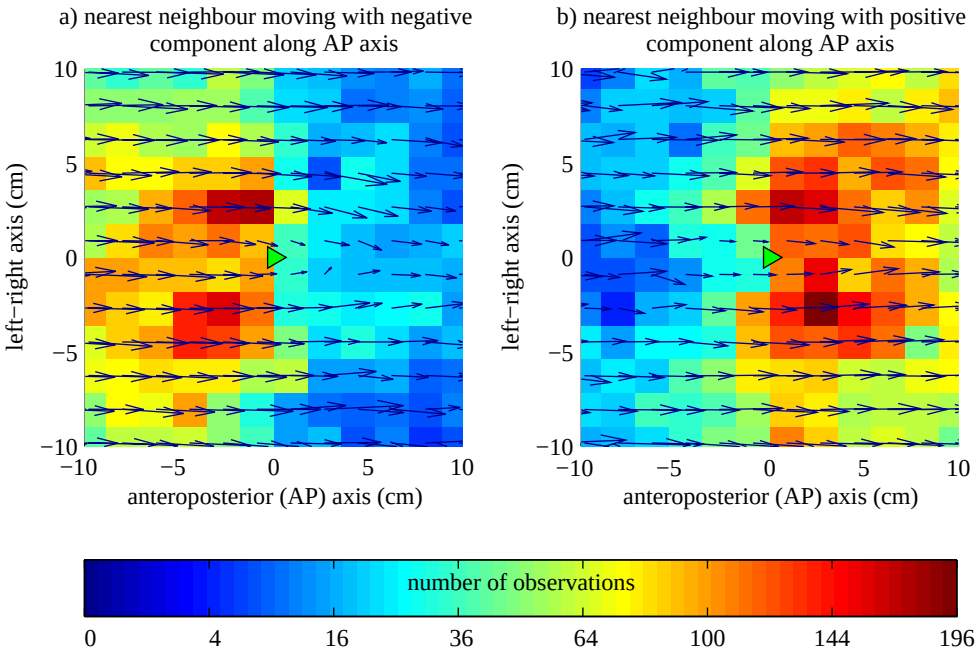


Figure 7.2 Effect of the relative spatial position (cm) of the nearest neighbour on a focal locust's subsequent motion given the focal locusts is moving after correction of the direction for effects of distance from the boundaries and orientation. Neighbours within one body length of the inner or outer walls were excluded. In each panel the focal locust is at the origin facing along the positive x-axis. The arrows indicate the mean velocity in cm s^{-1} of the focal individual given that it has its nearest neighbour is in the box edge one body length (take to be 1.8cm) centred at the base of the arrow. The background colour indicated the number of observation the mean direction is based on. (a) only considers moving nearest neighbours whose component along the AP axis of the focal locust is negative, so if the neighbour is ahead it is moving toward the focal locust and if the nearest neighbour is behind the focal locust it is moving away; (b) only considers nearest neighbours with a positive component of velocity along the focal locust's AP axis – that is neighbours which are moving towards the focal locust from behind or which are moving away from the focal locust ahead.

the orientation and motion state of a single neighbour is sufficient generate the behavioural patterns observed in the experiments. Assuming that the 1D model was able to capture the individually tracked system of 5 locusts it could then be tested against the experiments containing 30 individuals. This would provide the important link from individual to group behaviour.

Extending the model to two dimensions requires careful consideration of the effect of the edges of the experimental arena. The effect of an individual's distance from the

dome wall and their orientation relative to the radius is shown in Figure 7.1. This data gives the average direction taken by a locust a given distance from the wall. In some preliminary work I have used this to find out the deviation from this angle given the moving nearest neighbour at a specific position. Figure 7.2 shows the spatial distribution of the nearest neighbour and the average response of a focal individual given a neighbour at a point, corrected for the wall effects. This figure shows the avoidance of individuals moving towards them from in front and highlights the typical position of the nearest neighbour. This data requires further analysis.

7.2.2 Improving Methods of data collection and analysis

In this thesis we tracked data at different levels. The analysis of the movement of groups of interacting individuals has been greatly enhanced through the use of computer vision techniques (Buhl et al., 2006, Correll et al., 2006, Cavagna et al., 2008). It can be relatively easy to maintain tracks of the majority of the individuals across single frames. It rapidly becomes more difficult to keep track of individuals over extended periods of time or as the number of individuals increase. These difficulties arise due to the likelihood of losing the identity an individual when two individuals are sufficiently close. The number of such problematic encounters increases with time and the number of individuals. The magnitude of the problems also depends upon the average minimum distance that individuals maintain and the minimum level of separation that the recording system can detect.

It would be interesting to quantify the effects of tracking accuracy on the quality of information that can be derived from the tracked data. Ideally all individuals observed would be perfectly tracked. However, this is often impossible. We might expect that in a group of identical individuals behaving according to identical rules the

maintaining of individual identities whilst tracking is unnecessary. In reality we would expect all individuals to respond slightly differently. Differences between individuals have been shown to have an effect on the collective behaviour of a group (Couzin et al., 2002, Dussutour et al., 2008). As the differences between individual's responses increase, how does the quality of tracking affect the perception of the system? Some tracking errors may introduce systematic bias, for example if individuals are lost track of when they are at a certain distance from each other.

The effects of tracking accuracy could be analysed by adding noise to experimental data which has been completely tracked with high precision. However, this accurately tracked data is often very difficult to obtain. The use of simulated data would allow both a known underlying model and give control of factors such as different degrees of sociality and variation in behaviour between individuals. It would also be able to generate perfectly tracked data for arbitrarily large numbers of individuals. The effect of different levels of tracking accuracy could then be studied using this identical underlying data by adding noise to it. This could provide vital information for the design of experiments intended to test specific properties in large groups of interacting individuals.

In chapter 5 I started to investigate the detailed individual level behaviour of locusts. I was interested in being able to extract information for building an executable model of locust nymph behaviour, which would describe how an individual would react to its neighbours and environment both in how it moved and how its behavioural state changed. It would be useful to investigate the power of such analysis and develop the analysis further. In chapter 5 I did not find a significant effect of time an individual

had been stationary or moving on it changing its behaviour for example. The sensitivity of the analysis techniques used in chapter 5 could be assessed through simulation. Bout lengths of activity and inactivity could be taken from a Weibull distribution. The shape parameter of the Weibull distribution could be varied to represent different degrees of time dependence. The effect of different degrees of social activation and inhibition could also be incorporated by considering the effect of neighbours in different positions and movement states. The resulting data could then be generated for different numbers of individuals, different periods of observation and at different temporal scales. This data would then allow the sensitivity and power of the analysis methods to be assessed for different experimental conditions. Such information could then be used to guide the degree of experimental effort required for future experiments.

Simulated data would also be useful for testing other analysis methods. Hidden Markov Models (HMM) have been used to model the effect of starvation on locomotive behaviour in locusts (Macdonald and Raubenheimer, 1995). It has been suggested that an extension to HMM - the input-output HMM (Bengio and Frasconi, 1996) - would be suitable for generating executable models of behaviour from collections of observations (Balch et al., 2006). In these models the external (observed) state depends on the internal state and the external input, with the internal state depending on the previous state (or states) and the current external input. These models seem to provide a natural way to describe the transitions between an animal's behavioural states within a dynamic environment.

When individuals are moving, their acceleration as a function of nearest neighbour distances can be used to characterise attractive and repulsive 'social forces' between conspecifics (Tien et al., 2004). This method could be verified by applying it to data simulated by a classic Vicsek (1995) SPP model. It would also be interesting to explore these methods of analysis in an extended SPP models which included individuals becoming stationary and active due to combinations of external and internal factors.

7.3 Outlook

As technology improves and more advanced animal tracking techniques are developed we can expect the amount of data that biologists will be able to extract from their experiments to increase massively. In studies of group behaviour, the continuous tracking not just of every individual's position but also aspects of its physical state, such as temperature and hormonal levels, may well be recordable for example.

With the growth in available data comes the need to develop suitable analysis tools to make best use of it. The extraction of higher-level information through automatic recognition of an individual's behaviour will further enhance the quality and quantity of information available to biologists. Automatic behaviour recognition systems have already been developed for ants (Balch et al., 2006) and mice (Steele et al., 2007). Such advances require the close collaboration of people in a range of fields including biologists, computer scientists and statisticians. The development of more general analysis tools which could generate highly detailed profiles of each individual in an experiment and be accessible to biologists is needed. The ability to obtain such data

from large numbers of interacting individuals in both artificial and natural bands and swarms could provide vital insight into the behaviour of the locusts and other group living animals.

These techniques will allow for the development of increasingly sophisticated and accurate models of the organisms under study. Such models of locust behaviour combined with detailed environmental data and the continuing increases in computing power would facilitate massive-scale simulations of natural swarms. These models would be useful for the prediction of locust outbreaks and swarm behaviour, identifying key processes in the onset of locust swarms, and for testing different control strategies.

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