Self-Organisation in Ant and Bacteria Colonies

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Contents

Abstract

Self-organisation, prevalent in many species, is pivotal in enhancing an individual's survival rate. Eusocial insects are a prime example of collective behaviour and self-organisation, while bacteria colonies also exhibit self-organised patterns stemming from mutual interactions, yielding increased complexity. Now, there needs to be more explanation of how the reciprocal interactions between individuals translate to the global structure. We introduce the concept of the internal state to address this. The internal state can represent certain physiological traits in a given context. We use the internal state to represent the ovary development of workers in *Diacamma cf. Indicum.* We explore how the reproductive division of labour is maintained via the patrol behaviour of the queen, whose movements are determined by her internal state. We used an agent-based simulation to model the internal state of the queen and workers and to include the spatial interactions between them. We extended the agent-based simulation to explain the development of altruistic behaviour in *Diacamma* workers. The findings showed that the coupling of the internal states of the queen and workers could explain the patrol behaviour of the queen and the observed colony size dependant behaviour in actual *Diacamma* colonies. The findings further showed that altruistic behaviour in workers gives a more significant inclusive fitness benefit than being selfish. It also offers an explanation for workers' re-

tention of functional ovaries in modern *Diacamma* colonies. Previous work has explored the self-organisation in bacteria colonies and the effect of the growth environment on morphology. We developed a morphological diagram to examine the landscape of observed morphologies in line with specific environmental conditions. We then used the fractal dimension to quantitatively measure the differences in morphology and use previous work to explore possible explanations. Using the concept of internal state coupling, we develop a framework for understanding the causal pathway from individual behaviour to collective behaviour.

Declaration of Original Authorship

Declaration: I confirm that this is my own work and the use of all material from other sources have been properly and fully acknowledged.

Simeon Adejumo

Chapter 1

Collective behaviour and Self-organisation

1.1 Introduction

1.1.1 Self-organisation vs collective behaviour

Self-organisation refers to a process where a system without central control or planner spontaneously develops order and structure from local interactions between its parts [1] [2]. This order can emerge from simple rules and doesn't require any external intervention. An example would be ants building complex paths without a leader [3] [4]. Self-organisation emphasises the emergent order and patterns that arise from individual interactions within a system [5]. This is not necessarily due to the conscious effort of the individuals. Instead, it is a result of their actions and interactions. Collective behaviour is how individuals interact in loosely organised groups [6]. It describes coordinated movements or activities of a group of individuals, and this coordination can arise from factors like shared information, common goals, or environmental cues. It relates to swarm intelligence, the study of distributed systems inspired by collective behaviour [7]. Swarms are made of simple "agents", individuals who interact (directly or indirectly) to give rise to the observed intelligent behaviour. This intelligent behaviour is an emergent phenomenon from the uncoordinated interactions of the individuals in the group.

Collective behaviour can be seen in birds [8] schooling fish [9] [10], and social insects [11]. This behaviour can be advantageous by increasing defence against predators [8] or enhanced foraging ability [12]. Group behaviour is seen extensively in flocking birds. Birds respond positively to their kind [13]. This behaviour is likely due to the positive outcomes of being part of a group. Birds also move together, forming magnificent aerial displays. The birds must consider their movements and neighbours to avoid collisions [14]. The most common formations are line formations (such as in the shape of a V) or cluster formations. Smaller birds are more likely to fly in clusters, giving rise to easily observed visual displays. In clusters, the velocity of one individual affects the velocity of others. The density of the cluster is affected by predation, with high predation leading to larger, denser flocks and low predation leading to sparser flocks [15]. Interactions between individuals allowed information to spread to all individuals in the flock, especially in the presence of a predator. Herbert-Read [16] showed that escape waves in fish always start from the position of the school closest to the predator, away from the origin.

In the same way, birds moved away from disturbances as a group initiated by a few individuals [17] [18]. Being in the group gave quicker perceptions of predators than individuals [8], giving individuals a much more extensive range of perception [19]. There was increased vigilance of individuals in the periphery of the group [20]. When not in the presence of a predator, individuals move more with respect to the group's centre of mass than to their neighbours. Cohesion helps the flock maintain its group advantage when avoiding predator attacks. These escape waves are easier to see in orientation changes, as shown by Hemelrijk [21]. The formation of waves is highly correlated with reduced predation risk [22].

Collective behaviour can also be seen in slime moulds. Slime moulds could be used to study collective behaviour in a more controlled setting as genetic and environmental factors can be more easily controlled [6]. The controlled setting makes linking individual interactions with larger-scale complex behaviour easier. Though in some cases, physics-like models can be sufficient to recreate (and possibly explain) collective behaviour without invoking new assumptions [23]. This gives a sort of Occam's razor where phenomena are attempted to be explained by current physical laws before developing new theories and assumptions. Self-organisation and collective behaviour are closely related concepts that help us understand how seemingly simple actions can lead to complex and coordinated group dynamics in nature [24] [25]. Collective behaviour relies on a level of self-organisation from the components of a system. In collective behaviour, self-organisation manifests through positive feedback, negative feedback and leader-follower dynamics.

1.1.2 Insect and ant colonies

Insects show collective behaviour and decision-making, with choosing the shortest path to a food source in ants [3] [4], to the strongly correlated movements of midges [26] [27]. These behaviours stay consistent over increases in swarm size. Eusociality describes a form of social behaviour found in animals characterised by three features: cooperative brood care, overlapping generations and division of labour [11] [28]. Ants and bees are the best-known examples. Eusocial insect species (specifically Hymenoptera) show complex group behaviours. For example, ants may need to emigrate to a new nest site due to various disturbances, which cause low mortality in workers. However, if mortality is high, colonies resist dispersal [29] [30].

Another example of collective behaviour in insects is the division of labour, which puts individuals into castes where each caste is slightly more specialised for specific tasks (e.g. foraging and nest defence). Reproduction is also divided, with some ants being part of the reproductive caste and others not. Typical castes include reproductive queens, workers and soldiers [11]. This can lead to physical (morphological) differences, such as soldier ants who defend the nest being larger than other ants. Task allocation is done through social interactions, directly via physical contact [31] or indirectly through pheromones [12]. Pheromones are usually used in foraging as a positive feedback method. However, crowding effects lead to less deposition of pheromones [32]. Studying how social insects behave and solve problems can help us develop algorithms to help us with crowding effects for alleviating traffic in autonomous vehicles [33].

Sumpter covers principles of collective behaviour [34] in his paper of the same name. These principles outline the processes by which these behaviours arise. These include the collective being more than the sum of its parts, individuals versus group complexity, integrity and variability, positive and negative feedback, redundancy, and selfishness. Feedback is vital for amplifying behaviours (such as pheromone deposition leading to more individuals going to a food source) and suppressing behaviours, stopping every forager from going to the same food source and preventing overcrowding. Redundancy is an advantage as it allows for a more robust system as units are replaceable. Selfishness brings up a curious contradiction: by natural selection, individuals will want to pass on their genes selfishly. Therefore, it would initially seem that group behaviour and cooperation are disadvantageous.

Richard Dawkins' book, "The Blind Watchmaker" [35], explains how evolution shapes organisms through a gradual, step-by-step process. Ant colonies are a great example of this, showing impressive organisation that arises from simple rules governing individual interactions. Dawkins' framework shows how ant colonies can achieve extraordinary things by the collective actions of individual ants, which would not be possible if they worked alone. This is similar to the "selfish gene" theory [36], where genes drive the evolution of traits, behaviours, and collective strategies. But an organism's influence is not limited to its physical form, as Dawkins argues in his book, "The Extended Phenotype" [37]. Ant colonies demonstrate this through their complex networks of tunnels and structures that reshape their environment, creating an extended phenotype that goes beyond the individual ant.

Different factors could lead to the evolution of group behaviour in social insects [11]. This includes delayed benefits for the individual. As for passing on genes, kin selection looks at how genes are passed on, either directly via offspring or indirectly via relatives. Suppose the indirect passing of genes is more effective than the direct method. In that case, more investment in this strategy will lead to the reproductive division of labour. Queller [38] discusses kin selection in its role in coloniality in social insects. He discusses how relatedness to relatives could explain the evolution of social behaviour, especially with haplodiploid females who are more related to their sisters than their own offspring, through Hamilton's rule [39]. However, there is still a conflict of interest as the increased relatedness applies to females, not males in social insect colonies. This means other mechanisms are needed to regulate the conflict between the individual and the group. Other mechanisms include worker and queen policing, such as that seen in bees. Workers police each other's reproduction [40] while in stingless bees, the queen polices the workers. There are advantages to living in groups, such as defence against predation and advantages over out-group competitors. However, there is increased competition between individuals in the group and the transmittance of disease.

Individual ants can be more complex than first thought [41]. This increased complexity and variability of individuals can give rise to emergent behaviour and division of labour [42]. However, individuals are still largely interchangeable [43]. Individual ants affect the colony's behaviour as a whole [9] [44] through their mutual interactions with others in the colony. Collective behaviour is seen in ants when determining the importance of specific tasks [45] when choosing food sources [12] [46] and avoiding overcrowding when a food source has been chosen [32]. Collective behaviour is also seen when choosing a new nest site [47] [48]. With relatively complex cognition, it is unsurprising that ants can create complex structures, such as in the paper by Franks and Deneubourg [49]. Consistent individual behaviour leads to complex structures, even if individuals do not know the global structure they are building [49]. Synchronicity in tasks is essential to ensure the tasks are executed efficiently [50] [51]. While ants do not sleep, they have active and inactive periods [50] and balancing individual and collective activity is vital to the colony's survival [52]. Humans have also been noted to go through active and inactive cycles [53].

A reproductive division of labour, where some individuals reproduce, and others do not, can cause conflict between queens and workers [54]. However, these conflicts can be mitigated in the interest of the group [55]. Reproductive division of labour can also give rise to individual variations in behaviour [56] leading to dominance hierarchies [57]. Workers at the top of the hierarchy are more aggressive to workers at the bottom. Resource allocation is biased when new nests are formed [58]. This can lead to different phenotypes where each caste has its own slightly specialised function [59] [60]. Due to caste differences [61] [62], the way individuals interact can be different depending on the status of the individuals involved and the purpose of the interaction. In dominance interactions, older females dominate younger females until the younger female takes over from the older alpha [63].

1.1.3 Diacamma

Dominance is essential in regulating reproduction portioning, and this dominance is determined through physical interactions in *Diacamma cf. Indicum* (from Japan). Pair interactions increase the transition probability from inactive to active [64], helping to recruit more workers and ensuring synchronicity. Another way ants interact with one another is through hostility. While nestmates accept sterile non-nestmates [65], non-nestmate queens can be violently attacked. Nestmate queens are always accepted, showing workers can recognise nestmates and non-nestmates. However, previous work has shown that *Diacamma cf. Indicum* were always aggressive to non-nestmates. This could be because *Diacamma* is a solitary forager, so non-nestmates are a threat [66]. In either case, workers police each other and enforce the group decision only to allow the queen to reproduce [67] [68] [69].

When it comes to ants, such as *Diacamma cf. Indicum* (from Japan), physical contact (the touching of antennae) [31], conveys the internal state of the individuals and gives each party something which affects their behaviour. Colony members are usually split into castes, such as queens who reproduce and workers who forage and construct the nest [11] [59]. In *Diacamma cf. Indicum*, workers maintain functional ovaries and can produce male eggs after their ovaries develop without mating. This allows workers to rear their sons who are more related than the queen's sons [38]. However, the queen suppresses the ovary development of workers [31], possibly by suppressing dopamine in the worker's brain [70]. Punishments for developing ovaries and laying eggs include eating the eggs and immobilising the rule breaker [71]. Previous work by Kikuchi *et. al* [72] describes how multiple mechanisms are needed to properly regulate workers' ovary development and keep the colony's homeostatic balance. These include the queen increasing the duration of her active period patrolling the colony with an increase in colony size and the increasing prevalence of policing by workers. This is due to the weakening effect of direct contact with the queen and self-restraint and self-restraint as the colony grows [72]. Worker policing and dominance interactions become more critical as the colony grows. Other species use "queen substances" to regulate reproduction, primarily in large colonies like bees [73] [74].

Reproduction in the colony must be regulated to ensure the colony does not grow too rapidly. The regulation of reproduction also ensures that the workers co-operate rather than selfishly produce and care for their offspring. There is a balance between workers allocated to different tasks needed to maintain the colony (brood care, foraging, defending the nest site and maintenance) [54]. In this way, the reproductive division of labour is a form of group homeostasis, which the queen and workers maintain.

When the gamergate (effective queen and henceforth referred to as simply "the queen") interacts with a worker, the queen gains information about the worker's internal state [31]. Here, the internal state of the worker represents its ovary development. On the other hand, the worker becomes aware of the queen's presence, and its ovary development is suppressed. Without the presence of a queen, workers begin to develop ovaries [72]. The queen's presence helps suppress the ovary development of the workers to maintain the size of the colony [31]. As such, the queen may have some awareness of the colony's sizes so that every worker is contacted to avoid developing their ovaries and laying eggs. While this is a more top-down approach to controlling a colony, when the colony size increases, the patrol time of the queen also increases [75]. This increase in patrol time shows a feedback loop based on the contact between the queen and the workers in the colony, which allows for the dynamic control of reproduction.

1.1.4 Bacterial behaviour

Bacteria colonies have similar characteristics to ant colonies in that they form communities where resources can be shared and regulated between the individuals in the community. The cells in these communities gain benefits for participating in this system, which increases their likelihood of survival [76] [77] [78] [79].

Unlike ants, the bacteria in these communities do not have a queen-like analogue that polices the colony. In *Bacillus*, it has been shown that the bacteria interact using electrical signalling, which is coupled to the metabolism of the individual cells [80]. Similar to the workers conveying information about their internal states or ovary developments, the *Bacillus* cells in a colony communicate their internal state (metabolism) to each other to stop the more active outer cells from taking up all the resources, allowing more of these to diffuse into the rest of the colony. This means that information processing occurs in the colony to understand the signals received from cells within a community [80]. The use of bioelectrochemical signalling likens this to the brain, where the connections of neurons allow the brain to process information about the environment. However, this could be considered a more primitive version of brain activity. Research in tadpoles shows that lack of signals from the developing brains of tadpoles causes abnormal growth in their tails and other structures [81] [82] [83]. This shows development in bio-electric signalling at different levels of complexity. It can be used to co-operate and self-organise bacterial cells, develop more complex organisms (in this case, tadpoles) and is most obvious in the complex connections of various animals' brains and nervous systems. The different levels of organisation show a relationship between electrical signalling and the morphology of a biological system.

Biofilms are surface-associated microbial communities encased in a self-produced extracellular polymeric substance (EPS) matrix, exhibiting enhanced cooperative behaviours and increased resistance to environmental stressors [84] [85]. Biofilms are organised communities containing billions of densely packed cells. Different authors can use the word community to mean the presence of different types of cells (such as a biofilm with cells from *Bacillus* and *E. coli*). In this text, we will use it to refer to a cluster of cells irrespective of whether there are different kinds of cells or the same cells present. Bacteria in a biofilm usually adhere to each other and the surfaces they grow on. The biofilm leads to a community with primitive homeostasis and a primitive circulatory system by which the individuals maintain the structure and integrity of the community [76]. These communities can also have subpopulations of different cell types, which interact in different ways [79]. Different cell types can lead to cooperative and antagonistic behaviours exhibited by these subpopulations, which affect the local biofilm composition. Antagonistic behaviour means some cells may restrict the growth rate of other strains in favour of their own. However, cooperative behaviours mean cells can also benefit from different strains, such as the release of a metabolic waste product of one cell, which is helpful to another cell [77]. This benefit means these subpopulations can work synergistically with each other and, as a group, confer advantages to the cells that reside within the biofilm. These advantages include social cooperation and enhanced survival when exposed to antimicrobials and antibiotics.

Social cooperation requires communication between the cells in the biofilm.

Bacteria can communicate through cell-cell physical interactions/short-ranged signalling [86] [87] and long-range chemical signalling [88]. While not as widespread as other forms of bacterial communication, electrical/electrochemical signalling in biofilms allows the bacteria to communicate over large distances within and between biofilm communities [88]. These signals are caused by the release of intracellular potassium (K+) ions. This release of potassium (K+) ions causes neighbouring cells to release K+ ions, which stops the degradation of the signal as the ions diffuse through the biofilm. The continual release means the signal's amplitude does not decay with the distance travelled. This active signalling allows cells inside a biofilm to communicate with cells closer to the edge of the biofilm. Oscillations in the membrane potential caused by the release of K+ of cells show communication between outer cells in the biofilm and inner cells. The APG-4 dye was used to look at the movement of potassium ions in the biofilm [80], and by using a potassium blocking dye [89], the formation of biofilms and signalling in biofilms was hindered. Prindle *et al.* [80] found that the signalling was linked to the metabolism of the cells, this means that a wave of hyperpolarisation coordinates metabolic states among the cells in the biofilm. This can enhance long-range metabolic co-dependence in biofilms where interior cells signal their need for greater access to nutrients to exterior cells. This signalling can also attract free-floating cells of the same or varying strains of bacteria. These are then incorporated into the biofilm [90], meaning the signal is species-independent. This allows two biofilms to timeshare resources to avoid competition [88]. The species-independent nature of the signalling also increases the biodiversity of the biofilm, which enhances their chances of survival.

Chemical potential also plays a role in biofilm formation. Within the biofilm micro-environment, chemical gradients arise due to variations in nutrient availability [80], waste accumulation, and metabolic byproducts. These gradients create spatial heterogeneity, dictating microbial localisation and activity within the biofilm matrix [88]. Chemical potential gradients influence microbial behaviour, fostering communication via quorum sensing [91] and guiding cellular responses such as motility and gene expression. How biofilms are formed depends on the environmental conditions and the strain attributes, which affect how nutrients diffuse to the cells in the biofilm. This can lead to different pattern formations, characterised into different morphotypes [92]. Morphotypes allow the grouping of different species of bacteria into groups that form similar patterns instead of grouping by species. Morphotypes are similar to snake venom, which can vary even within the same species and is better classified by venom type [93] [94].

Attempts to model the dynamics between the bacterial density, environmental nutrients and electrical signalling [95] have begun to capture some of the nature of the behaviour of bacterial colonies. These models start to take into account the signalling which coordinates this behaviour. Though previous work either focuses on how the environment affects the morphology of the colony [92] [96] or looks at the process of electrical signalling in the bacteria [80] [97] [98]. None look at all levels of the chain of events. These levels include how the environment and different inputs affect the electrical signalling in the bacteria colony, leading to a morphological change in the shape of the colony.

1.1.5 Bottom-up Approach

Collective behaviour requires a bottom-up approach to self-organisation. In the case of ants and bacteria, individuals communicate and have mutual interactions, which lead to collective action. By using the concept of an internal state, we can build an understanding of how emergent behaviours arise across species. The concept of internal states allows us to build a bottom-up approach to how emergent properties of groups arise. First, there is the individual internal state. This internal state represents some physiological property of the individual. In the case of ants, this internal state can represent the ovary development of the worker. For the queen, the internal state can regulate her activity cycle and patrol behaviour. In bacteria, the internal state can represent the cell's metabolic activity. In both cases, the individual must convey information about their internal state to others to elicit a behaviour change. This is done via mutual interactions with others. For ants, the exchange of internal state information regulates worker reproduction and the queen's patrol behaviour. For bacteria, the exchange of internal state information regulates the flow of nutrients through the colony, allowing access to resources. These internal states are essential during mutual interactions to coordinate the colony-level adaptive emergent behaviour observed in various species. Although each individual is unaware of the global structure, each contributes to it by participating in the network of interactions. Cornejo *et al.* [99] developed a model for the division of labour, which achieved near-optimal performance in time with respect to the colony size while implementing rest and active states.

Agent-based modelling can be beneficial for studying emergent phenomena in a way that does not directly try to replicate them. This allows the connection between micro and macro interactions to be more easily identified [100] [101]. An example of this is the work done by Sahin and Franks [102]. In this, they presented a behavioural model of the ant scout for assessing nest size and a simulation of the behaviour in an ant-bot. They compared the results of their simulation with real ants and were able to produce similar behaviour. The same thing can be seen in the work by Marshall *et al.* [103]. Agent-based modelling looks to replicate the patterns of collective and emergent behaviour seen in nature through the interaction of its agents. Therefore, if the individuals and the interactions are modelled correctly, it should give rise to emergent behaviour not explicitly built into the system [5]. While standard mathematical modelling can replicate system-level behaviour, agent-based modelling gives a kind of “on the ground” level information [101]. Modularity, flexibility and multi-scale analysis are further advantages added by agent-based simulation [104] [105]. An example would be how cells form tissue, which forms organs that all work together to keep an organism alive.

There are two methodological approaches: trial and error, and reproducing and simulating distributed living systems. Trial and error is an iterative process where agents within a simulation learn and adapt their behaviours based on the outcomes of their actions. The methodological approach in the PhD is closer to the second methodological approach with an approximate recreation of ant colony dynamics. The advantage of this approach is that it can rely on decades of research to inform it [100]. Starting with modelling individual agents and their interactions allows us to explore how macro-level patterns and trends emerge from the decentralised interactions of individual components. There are limitations [106], such as the lack of a standardised methodology and the calibration and validation of agent-based models due to the number of parameters and interactions. An example of an application of agent-based simulations is Boids [107] [108] [109]. ”Boids” is a play on the word ”birds,” reflecting the primary application of the concept: the simulation of bird flocking behaviour in a way that mirrors the observed biological phenomena. In summary, modelling and simulations are essential aspects of the scientific community. While they cannot give us a complete picture of the actual reality of nature, they are still helpful in gaining insights into natural phenomena.

Self-organisation and collective behaviour have many applications, including in swarm robotics. A swarm robotics system could be developed similarly to the ant cooperative framework. This system could be used for the autonomous mining of asteroids. The internal state of the individuals could reflect the amount of material they have collected and their run time so far. One of the individuals could act as a monitor, reflecting the queen's role and patrol behaviour. This would allow operations to run efficiently and with minimal input. A system developed more aligned with the bacteria framework could be used for search and rescue. Signals are communicated through the network to indicate signs of life or the presence of a target. Small drones for things such as home delivery or fireworks-like displays could increasingly use autonomous drones working together in this way.

1.1.6 Aim and Thesis structure

The PhD project aims to use a bottom-up approach, using the concept of the internal state, to explain different aspects of self-organisation observed in ants and bacteria. Specifically, the project aims to show how the concept of the internal state and the mutual interactions between agents can explain the following:

1) The ability of the queen in *Diacamma cf. Indicum* to control the ovary development of workers using her patrol strategy.

2) The evolution of the reproductive division of labour in *Diacamma cf. Indicum* and similar ant species.

3) The morphology of bacteria colonies and how mutual interactions can characterise these.

The first step is using an agent-based simulation to implement a model for the queen's patrol behaviour. The simulation will show how the negative feedback loop formed between the internal state of the queen and workers can be sufficient to regulate worker reproduction in the colony. The second step expands the model to investigate the origin of the development of the reproductive division of labour by introducing a genetic component to the agent-based simulation. Incorporating a genetic algorithm on top of the agent-based simulation forms an evolutionary agent-based simulation. This new simulation can be used to probe the landscape of evolutionary possibilities when determining the stability of altruistic behaviour in workers. The final step is to use the fractal dimension to quantify bacterial colony morphology changes under different environmental conditions. We aim to understand these morphological changes through the lens of internal states and electrochemical communication between the cells. All of this builds on previous work from Sugawara *et al.* [75], Ohtsuki and Tsuji [110], and Wakita *et al.* [111], as well as many others.

Sugawara *et al.* [75] introduced the concept of the internal state to explain the queen's patrol behaviour but did not use this to investigate the effect of the patrol behaviour on the internal states of the workers. The mathematical model Sugawara *et al.* developed also did not consider the spatial aspect of the interactions between the queen and workers. Here, we extend their work further by giving the queen and workers space to move and interact in an agent-based simulation. We monitor the queen's patrol behaviour and the worker's internal state to discover novel insights into the control mechanism of the reproductive division of labour in *Diacamma cf. Indicum.* Using agent-based simulations allows individual behaviour and pair interactions to be the fundamental aspect of emergent swarm intelligence. They mimic the underlying mechanisms of the biological system. With the simulation, we can find how information is transferred in the system (i.e. how agents interact) by monitoring the encounters between the queen and the workers. Does the hypothesis of the internal state explain observed behaviour? All of this is covered in chapter 2 of the thesis.

Ohtsuki and Tsuji [110] used game theory and the inclusive fitness function to show the different stages of colony development. They also showed the best social strategies for the queen and the workers to maximise their inclusive fitness. Their model also did not consider the spatial aspect of the interactions between the queen and workers. More importantly, rather than attempting to maximise the inclusive fitness of the queen and workers, in this project, we introduce a genetic predisposition towards altruism and use inclusive fitness as a selection method for determining the propagation of this genetic component. This is where aspects of the genetic algorithm are implemented. Using this method, we can investigate the evolution of altruistic behaviour in workers from a natural history perspective. We can modify various components in the model to view their effects on the inclusive fitness of the queen and workers over time. We characterise how this information transfer is related to observed emergent behaviour, such as colony size dependant behavioural changes in the queen and workers. These aspects are covered in the third chapter.

For the bacteria colonies, it is a combination of the work by Prindle *et al.* [80], Ben-Jacob [92], and Wakita *et al.* [111]. Wakita *et al.* developed a morphology diagram showing the changes in bacteria colony morphology with environmental conditions. Ben-Jacob used the fractal dimension to quantify changes in morphology to develop morphological groupings called morphotypes. Prindle *et al.* showed that bacteria communicate via potassium (K+) ions and can communicate their metabolic state to other cells to get nutrients for themselves. This project uses the fractal dimension to quantify morphology changes caused by different environmental conditions. The ultimate goal is to use the electrochemical communication seen in Prindle *et al.* to explain the causal chain, which ultimately leads to the changes in morphology seen on a macro scale. This will be covered in the 4^{th} chapter.

This shows that the internal state is a versatile concept that can explain the emergence of self-organised behaviour in different organisms and can be adapted appropriately to fit the context. Using agent-based modelling can give us a tool for analysing the behaviour of different collective systems and how information spreads within the network of interactions between individuals. These individuals can be ants or bacteria cells. All of this will be brought together in the final chapter.

Chapter 2

A real-time feedback system stabilises the regulation of worker reproduction under various colony sizes

2.1 Contributions

This chapter is a reformulated form of the paper published in PLOS Computational Biology. The experiments were conducted by Tomonori Kikuchi, Kazuki Tsuji and Kana Maruyama-Onda. The agent-based simulation was developed and worked on by myself, with input from my supervisor and Ken Sugawara.

2.2 Introduction

In the realm of social insects, the orchestration of various collective behaviours is often governed by self-organisation [59]. While extensive research has delved into understanding the autonomous control of short-term collective behaviours, such as foraging and movement dynamics, a relative dearth of knowledge concerning the homeostatic mechanisms that govern societies exists. While the autonomous regulation of individual organismal functions like breathing, thermoregulation, and osmoregulation has been thoroughly explored, the autonomous maintenance of society has garnered less attention.

Each species or population of social insects typically exhibits characteristic social traits, such as colony size and caste ratios. However, colony size can undergo significant fluctuations over time as the colony grows, akin to the changes in body size seen in multicellular organisms [112]. Moreover, caste and age compositions can exhibit shorter-term fluctuations [113] [114] [115]. The persistence of the reproductive division of labour, a hallmark of insect eusociality, amidst such changes implies the existence of a size-independent autonomous control mechanism. However, the detailed mechanisms behind these phenomena remain elusive [116].

From a phylogenetic perspective, the ancestral state of insect eusociality is considered to involve physical suppression through dominance behaviour, transitioning to chemical suppression using queen pheromones as colony size increases [12]. However, challenges in control mechanisms may arise with the growth of colony size, and addressing these challenges during colony development (colonial ontogeny), where physical suppression remains practical, demands further investigation.

While extensive studies have explored mechanisms of reproductive division of labour, such as dominance behaviour, queen pheromones, and worker policing

[117] [118] [119], less attention has been devoted to understanding the robustness and effectiveness of these regulation mechanisms against increases in colony size [120] [121] [122] [28].

In this chapter, we leverage a computer simulation model to scrutinise the robustness of the regulation system governing worker reproduction in the face of changes in colony size. Many Hymenoptera workers are known to refrain from laying eggs in the presence of the queen, engaging instead in non-reproductive work—a phenomenon considered an adaptive tactic for inclusive fitness optimisation [110] [123].

In the event of the queen's death or absence, workers' ovaries initiate development, eventually laying male-destined haploid eggs. In this situation, worker offspring can contribute to the colony's survival by contributing to contribute to the workforce [124]. The switch in reproduction is triggered by the perception of the queen's presence. Thus, understanding the mechanism of reproductive division of labour hinges on deciphering the transmission of information about the queen's existence [119] [125] [126] [127].

Empirical studies suggest that queen pheromones, particularly low-volatility cuticular hydrocarbons (CHCs), play a crucial role in transmitting information about the queen's existence in social Hymenopteran taxa like ants, bees, and wasps [128]. In environments with low-volatility queen pheromones, direct physical contact between the queen and nest mates is hypothesised to be necessary for effective transmission.

In this chapter, we turn our attention to *Diacamma* ants, specifically the Japanese *Diacamma*, *Diacamma. cf. Indicum*, the only Japanese species, as a model system [129]. The information transmission mechanism of the gamergate worker (referred to as the queen hereafter) in this species has been well-established [72]. CHCs code this information, and the transmission occurs through direct contact between the queen and workers [31]. Notably, the queen's patrol activity, which describes the queen's frequent movement within the nest [130], becomes more active in larger colonies [72].

This heightened patrol in large colonies is viewed as the queen's strategy to counter the potential decrease in the transmission efficiency of the queen pheromone due to increased colony size. Given that the physiological effects of the queen pheromone last only around 3 hours [72], maintaining stable control of worker reproduction necessitates the queen's awareness of the ever-changing colony size, allowing her to adjust her patrol effort. However, the specific mechanisms through which the queen obtains and utilises information about colony size remain elusive.

In ants, the frequency of contacts between individuals serves as a local proxy for population size, and such contact frequency plays a role in behavioural switching in various contexts, such as nest relocation [131] [132]. Yet, the fundamental mechanisms linking different types of perception, including contact frequency, local density perception, and colony size perception, remain largely unclear.

Feedback mechanisms are integral for system stability and are identified in various self-organised collective actions [133] [134] [135]. In *Diacamma*, the queen exhibits aggressive behaviour towards reproductive workers, engaging in queen policing by stealing and destroying eggs laid by worker ants [69]. We hypothesise that the queen can detect the reproductive status of workers during her patrol, as suggested by circumstantial evidence [70], laying the groundwork for subsequent dominance interactions that suppress worker reproduction.

A previous theoretical model by Sugawara *et al.* [75] proposed a feedback mechanism influencing *Diacamma* queen patrol behaviour based on three key assumptions: (1) Workers losing contact with the queen are released from the inhibitory effects of the queen pheromone; (2) such workers initiate ovarian development and emit chemicals associated with reproduction; (3) the queen, upon contacting a worker emitting these chemicals, increases her future patrol effort based on the perceived chemical concentration.

As colony size increases, the contact efficiency of the queen decreases, providing workers with more opportunities to develop ovaries and emit associated chemicals. The resulting colony-size-dependent behaviour of the queen is posited to be a consequence of feedback mechanisms responding to changes in worker physiological conditions (Fig. 2.1).

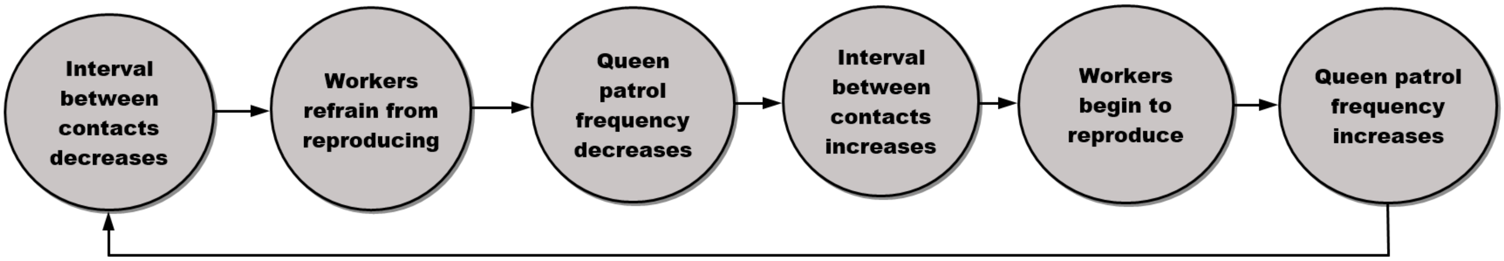


Fig 2.1. Feedback Loop Diagram A feedback loop between the queen's patrol behaviour and the reproductive activity of workers

Notably, Sugawara *et al.*'s model assumed a constant rate of queen-worker contacts as colony size changes, with the contact rate decreasing linearly as colony size grows, prompting an increase in queen patrol frequency (Fig. 2.1). However, the constant queen-worker contact rate assumption is not inherently evident. Worker behaviour might shift as worker density increases in a given space, potentially responding to changes in contact frequency to reduce the likelihood of inter-individual contact [136].

While average individual density within an ant nest may remain relatively stable despite changes in colony size, adjustments to the nest space size and worker density regulation have been observed [137] [138] [139]. Using an agent-based simulation in our study provides a novel approach, considering spatial aspects and assuming constant worker density per nest space regardless of colony size. This contrasts with Sugawara *et al.*'s model, which focused solely on queen patrol without investigating changes in worker reproductive status.

This study marks the first utilisation of an agent-based simulation to assess the efficacy of queen patrol behaviour by tracking the internal states of workers. Unlike other biological simulations, such as Boids, where individuals follow simple rules to replicate complex flocking patterns [107], our simulation incorporates feedback between individual agents. Past simulations have successfully replicated nest quality assessment behaviour in ants [140], trail formation [103] [135] [141], and reproductive labour division dynamics [75]. Our agent-based simulation, emphasising internal states and interactions, establishes a negative feedback loop, shedding light on controlling reproductive labour division in colonies. Our model could be used to determine the effectiveness of dominance interactions in other eusocial insects [142].

By leveraging an agent-based simulation, we delve into how colony size changes impact the reproductive state of workers (ovarian development) and queen patrol behaviour. Our simulation assumes constant individual density per nest space, a condition supported by experimental evidence presented in this chapter. We propose a feedback mechanism linking internal states and queen patrol behaviour, offering insights into how this mechanism ensures stable suppression of worker reproduction as colony size increases.

2.3 Materials and Methods

2.3.1 Maintenance and Experimental Procedure

The taxonomic status of species of genus *Diacamma* is still under revision. Since it is known that there are only one species of this genus in Japan that is very closely related to the Indian species *Diacamma Indicum*, we tentatively use the new name *Diacamma cf. Indicum* (from Japan) following Fujioka *et al.* [143] (previously described as *Diacamma sp.* from Japan). The species has no morphological castes among females, that is, all females are wingless and monomorphic. In each colony, a single mated female (queen) functions as the reproductive queen that produces female eggs, whereas the other females play the helper-worker role [144].

New colonies are founded via colony fission. When the queen is absent after fission or due to queen mortality, among the cohort of newly emerged females the most dominant individual (usually the first emerged) copulates and becomes the next queen. In the field, colonies contain 20–300 workers, and alates (males) are produced in large monogynous colonies (where there is only one reproductive female) and orphan colonies [67] [145]. Unmated workers can potentially lay male-destined haploid eggs. However, in colonies at the ergonomic (growing) stage (i.e., ones with fewer than 100 workers), worker reproduction is suppressed by queen pheromone and, queen and worker policing [31] [67] [69] [71]. Whereas in colonies at the reproductive stage (containing 100 workers or more) such suppression is relaxed, and males are produced by worker reproduction [67].

We used colonies of *Diacamma cf. indicum* collected on the main island of Okinawa during 2001–2014. Those colonies were maintained in a laboratory at 25 ±1°C with a light: dark cycle of 12 h:12 h. Each colony was kept in a plastic container (26.5 cm length × 18.5 cm width × 5 cm height) with a plaster floor (1.5 cm thick), however, in the natural environment, ants would explore the surrounding environments to expand the colony space whenever there is an opportunity. In the middle of the floor, a 13 × 9 cm depression (1 cm deep) covered with a glass plate was prepared for the ants as an artificial nest. Ants were fed honey water and mealworms ad libitum three or four times a week.

Ants were kept in the laboratory. First, all workers and the queen in each of the 15 colonies were marked with enamel paint for individual identification. The number of workers (colony size) was 58, 69, 81, 110, 125, 128, 131, 144, 149, 151, 162, 169, 174, 181, and 214, respectively (mean ± SD = 137.9 ± 42.7). For the video recording, each colony was moved to another artificial nest, which was a plastic container (26.5 cm length × 18.5 cm width × 5 cm height) with a plaster floor (1.5 cm thick). In the middle of the floor, a depression (8 cm length × 16 cm width × 1 cm depth) covered with a glass plate was prepared for the ants as an artificial nest. After acclimatisation for a day, we video-recorded each colony for 12h. By using that video data, we were able to track all queen–worker contacts.

2.3.2 Agent-Based Simulations

Overview

To validate our hypothesis of the negative feedback loop between the queen and workers, we ran the agent-based simulations in which the queen and workers move randomly within the grid space and contact each other. The internal state of the workers is defined as the hypothetical physiological condition, such as the hormone level, which controls the ovary development in workers and queen pheromone secretion in the queen. Within an ant colony, the queen's perception of the internal state via contact is largely dependent on the frequency of her contacts with workers as a function of time. Thus, along with the internal dynamics of the queen and workers, the spatial distribution of the workers and the queen as a function of time should play an important role in the patrol behaviour of queens and the reproductive behaviour of workers. Note that the density of workers was kept the same though the number of workers (colony size) increased. This can distinguish the mechanism based on the negative feedback loop from those dependent on the perception of density [136]. To include the spatial degree of freedom, we used an agent-based simulation to model the behaviour of the queen and individual workers within a certain space representing the nest.

We first assumed that the internal state of the workers and the queen would operate differently. For the worker, the internal state would represent their ovary development and demonstrate a steady increase over time. This could be suppressed by the queen via direct contact (perception of the queen pheromone). For the queen, the internal state would represent the probability to become active. That is, the likelihood that the queen will go from an inactive state to an active state, at which point she will begin to patrol the colony. The queen's internal state steadily decreases (increasing her resting period) but increases when interacting with workers. This increase is proportional to the internal state of the worker who has been contacted. Meaning, a worker with a low internal state has minimal effect on the queen's internal state but a worker with a high internal state increases the queen's internal state and therefore her likelihood to begin patrolling the colony.

The queen's movement [64] [146] around the nest was based on her temporal behaviour: when she is in the active state, she moves around the space, whereas in the inactive state, she halts within the grid she had moved in. The contact behaviour of the queen depended on these temporal behavioural patterns of active–inactive cycles. In this case, the workers also have active-inactive cycles which determined their movement around the space and were determined a priori.

The movement of the queen and workers around the colony was a simple random walk around the nest space. The next position of the agents is generated randomly from one of 4 directions, North, South, East and West. The movements of the agents are asynchronous, with agents only moving when they are in an active state. The queen walks around the nest space to contact all workers in the colony to suppress their internal states. The duration of the simulation was determined by how long it took the queen to contact all workers at least once. The simulation was then terminated. This was done because the purpose of the simulation is to determine the effectiveness of the queen's patrol behaviour. If the queen were to miss a worker, this would be a hole in her patrol strategy as that worker can then develop their ovaries. Various variables were recorded for analysis, including the queen's active and inactive periods and the contacts between the queen and workers.

Internal State Dynamics

The rhythmic cycle of the active–inactive state was simplified into the two time periods of the active time (t_a) and the inactive time (t_r). For the worker agents, t_a and t_r were kept constant ($t_{a_{constant}} = 20$ steps, $t_{r_{constant}} = 100$ steps). For the queen agent, t_a was kept constant ($t_{a_{constant}} = 20$ steps), but $t_r(t)$ was modulated by her internal state, $I_q(t)$, using the dynamics of:

$$t_r(t) = t_{r_{constant}} \cdot e^{-\delta \cdot I_q(t)} \tag{2.1}$$

where $t_{r_{constant}}$ and δ were constant ($t_{r_{constant}} = 100$ steps, $\delta = 20.0$). This was implemented by having the queen switch from inactive to active if the queen was inactive and had been in such a state for $t_r(t)$. For example, if at time t the queen became inactive, then the queen would become active at time $t + t_r$. $I_q(t)$ represents the internal state of the queen. This affects the rest time of the queen and, therefore, determines how long the queen will spend patrolling. Increases in $I_q(t)$ lead to a decrease in the inactive time, $t_r(t)$, of the queen.

If the queen has a prolonged period where her internal state is low, then $t_r(t)$ approaches $t_{r_{constant}}$.

To test our hypothesis about the feedback mechanisms of internal state and behaviour, we model the dynamics of the internal states of the queen and workers in the following manner. The dynamics of the internal state of the queen are given by:

$$I_q(t+1) = (1-\epsilon) \cdot I_q(t) + \alpha \cdot \delta(\overrightarrow{x_q} - \overrightarrow{x_w}) \cdot I_w(t) \tag{2.2}$$

where time is discrete, $(1-\epsilon) \cdot I_q(t)$ is a damping factor, $\epsilon = 0.01$ and is constant. As the reproductive division of labour enables the queen to be the main producer of offspring in the colony, there is a compromise between patrolling the colony and laying eggs. As the internal state of the queen represents the likelihood she will become active, we use the damping factor to decrease the queen's internal state over time naturally. This allows the queen to move to a more restful state with minimal patrol, assuming workers have a low internal state. The second term, $\alpha \cdot \delta(\overrightarrow{x_q} - \overrightarrow{x_w}) \cdot I_w(t)$, is an activation factor. The activation factor increases the probability of the queen becoming active when the queen contacts a worker with a high internal state. It is also proportional to the number of contacts with the workers. $\alpha = 0.1$ and is constant. $\overrightarrow{x_g}$ and $\overrightarrow{x_w}$ denote the [X, Y] position of the queen and worker respectively, in the 2D nest space, with each agent being a finite size. The term $\delta(r)$ denotes Kronecker's delta, i.e., its value is zero except when the distance between $\overrightarrow{x_g}$ and $\overrightarrow{x_{W_i}}$ is zero, then $\delta(r) = 1$.

The dynamics of the internal state of the workers was given by:

$$I_w(t+1) = (1-\beta) \cdot I_w(t) + \gamma - \kappa \cdot \delta(\overrightarrow{x_q} - \overrightarrow{x_w}) \cdot I_w(t) \tag{2.3}$$

γ represents an activation factor that increases the internal state of the worker over time as a function of time. This levels off over time as $I_w(t)$ approaches 1, depending on your choice of γ and β. In this case, γ and β were set to 0.0001. Here we chose the constants of the activation factor to reflect the pace of ovary development in workers observed in previous work where the queen was removed from the colony [72]. The next term only functions to decrease the internal state of the worker, $I_w(t)$, when the worker is contacted by the queen with $\kappa = 0.9009$, representing an approximately 90% decrease in the worker's internal state.

Spatial Behavioural Dynamics

While we understand that the movement of ants in a real colony are less than random, for simplicity, we implemented spatial dynamics in the following way:

1. The virtual nest was set with a grid size of $L \times L$ (Fig.2.2A) with distance measured in arbitrary units (simply referred to as units). Each agent was set to be 5 units long. The ants (agents) were distributed randomly throughout the nest space at the start of the simulation. The size of the nest space was dependent on the colony size to keep the density ($\frac{N}{L^2}$) approximately constant (L was set proportional to the square root of N). For example, when $N = 20$, $L = 100$ units. We controlled L to keep the ant density per space constant (this assumption was based on empirical evidence, as shown in SI 3 - Experiments and SI 4 - Experiments).

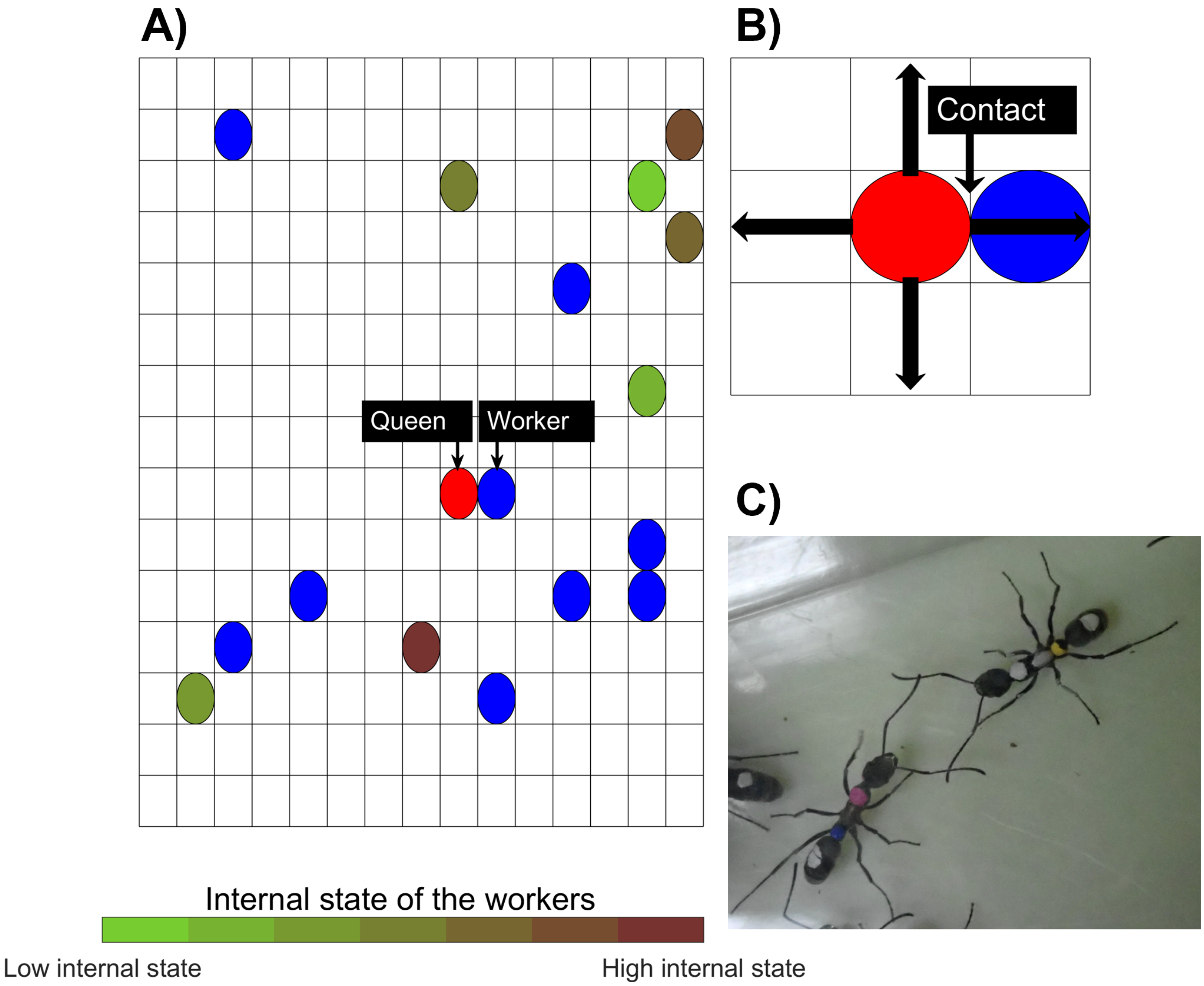


Fig 2.2. Schematic picture of the simulation environment A) Grid structure with the queen and workers. The scale of green to brown represents how low or high the internal state of the worker is. A blue worker is one which has been contacted by the queen. B) Contact is established when workers are within range of the queen. In this instance, the threshold was set to 5 units of length. C) Two ants contacting each other by touching antennae

2. Every time step the agents move randomly in one of four directions: north, south, east, or west (Fig.2.2B) in the grid. The agents are prevented from going outside of the virtual nest space with a simple check of their next position vs the position of the boundaries of the space. The agents move at a constant velocity but when they are inactive they do not move at all.

3. Agents are unable to overlap each other within the single grid.

4. A contact is determined when a worker is close to the queen, within the length of 5 units (see evidence of the ant's morphology Fig.2.2C). The queen can only contact one worker in each time step. Therefore we decided that the queen would not contact the same worker twice in a row. This is to decrease the prospect of a worker who has already been contacted recently monopolising contact with the queen despite other workers being in range in a short period of time. When the queen contacts the worker, the internal state of the queen and the worker increase and decrease respectively. The increase in the queen's internal state is proportional to the internal state of the worker, while the decrease in the worker's internal state is constant (approx. a 90% decrease). Contacts are checked in the same order as these are stored in an array and associated with the same worker throughout the simulation. This may introduce a bias to the simulation. However, as each new simulation has randomly generated positions for the workers and the queen, any bias' introduced should be cancelled out by running the simulation multiple times.

Initial Conditions and Analysis

Conditions of workers and the queen were initialised with parameters that represent the position, direction, velocity and internal state. The internal states of the workers were randomly assigned a value between 0 and 0.5. The queen was given an initial internal state of 0.1. The number of ant workers, N, was predefined to sample the different colony sizes. The initial rest time of the queen is set to the maximum rest time ($t_{r_{constant}} = 100$). The status of the workers and queen (whether it is active or inactive) were randomly assigned at the beginning of the simulation. Each agent, either a worker or the queen, has its internal state, I_w and I_q, respectively. I_w, the internal state, is assumed to decrease when the worker contacts the queen but to increase otherwise (Eq. 2.3). I_q is assumed to increase when the queen encounters a worker with high I_w and to decrease in the absence of such an encounter (Eq. 2.2). The queen's internal state is assumed to be correlated with her patrol behaviour, i.e., a higher I_q leads to a shorter resting time, t_r.

The simulation ended when the queen had contacted all the workers in the colony at least once. The simulation was repeated 50 times for each of the colony sizes $N = 20 - 200$ increasing N in increments of 20. The colony size coincides with the range of natural *Diacamma cf. Indicum* colony sizes [72]. In every trial, the positions of the workers are reset to another random value (different initial conditions for spatial distributions of workers). The total time of the simulation, patrol frequency and length of the rest time were recorded. The internal states of the workers and the contacts between agents were recorded. Using these variables, the effect of the queen's patrol behaviour could be analysed by calculating the average internal state of workers over time, as well as the distribution of these internal states. Contact rates between the queen and workers were also calculated based on the number of contacts made between the queen and the workers within the simulation time. All variables used in the simulation are shown in Table 2.1.

We tested the robustness of our model in several ways. Firstly, we checked the initialisation of parameters. By increasing the initial internal of the queen, we assessed its effect on the system. We found that it had little affect and returned to similar values seen in our original initialisation (see SI 8 - Simulations, S8 Fig, S9 Fig). We then standardised the time across the colony sizes which were investigated to mitigate possible effects due to the system not being in a steady state. We also found this to have little effect (possibly strengthening our results, see SI 9 - Simulations, S10 Fig). Finally, we increased β and γ to 10x their original values. While we found a significant difference in the results (see SI 10 - Simulations, S11 Fig), the dynamics of the system were unchanged.

Table 2.1. Variables, constants, and initial conditions used in the agent-based simulation

Variable Symbol	**Variable Name**	**Value**
L	Grid length and width	100(when N=20)
N	Number of workers (colony size)	20,40,60,80,100,120,140,160,180,200
$t_r(t=0)$	Resting time for the queen	Randomly assigned between 0 and $t_{r_{constant}}$
$t_{r_{constant}}$	Maximum resting time	100
$t_{a_{constant}}$	Maximum active time	20
δ	Delta(constant)	20
ϵ	Epsilon(constant)	0.01
α	Alpha(constant)	0.1
γ	Gamma(constant)	0.0001
β	Beta(constant)	0.0001
κ	Kappa(constant)	0.9009
$I_q(t=0)$	Queen internal state	0.1
$I_w(t=0)$	Worker internal state	Randomly assigned between 0 and 0.5
$\overrightarrow{x_q}$	Queen position	Randomly assigned between 0 and L for the [X, Y] position
$\overrightarrow{x_w}$	Worker position	Randomly assigned between 0 and L for the [X, Y] position

2.4 Results

In the experimental results, overall in small colonies with fewer than 100 workers, the queen was able to contact more than 80% of workers in the 20 bouts of patrols, whereas, in large colonies with more than 100 workers, the queens' per worker contact frequency dramatically decreased (Fig.2.3). These results suggest that although the queen increased her patrol effort with increasing colony size, the efficiency of making contacts between the queen and workers dropped in the large colonies.

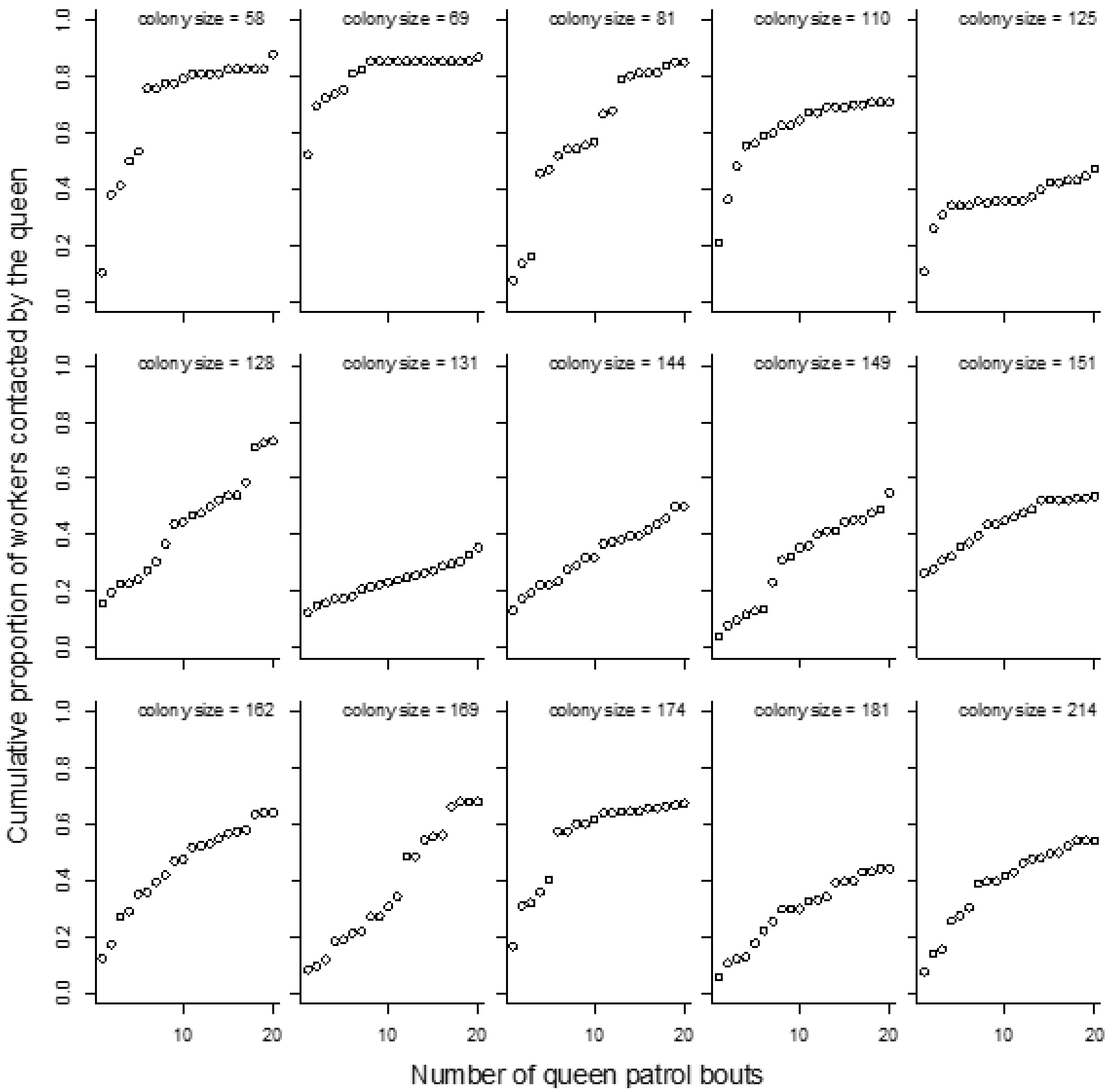


Fig 2.3. Cumulative proportion of workers contacted by the queen during patrols at various colony sizes during laboratory experiments

The first set of simulation results displays colony size dependent features of the queen's patrol behaviour. Fig.2.4 shows the patrol frequency and rest time of the queen with respect to colony size. In this case, the patrol frequency refers to how often the queen patrols the colony at a given colony size. The rest time is the total amount of time the queen spends inactive for a given colony size. Fig.2.4A showed an increase in the patrol frequency of the queen with respect to colony size (see also SI 6 - Simulations, S6 Fig) due to the increased internal state of workers. A larger colony size could also mean it takes longer for the queen to contact each worker. However, the increase in patrol frequency is driven primarily by the internal state of the queen, due to its effect on her rest time (see 2.1). Inversely, Fig.2.4B showed that the mean resting time for the queen decreased with colony size, i.e., as the colony size increases, the queen increases her patrol effort to contact an increasing number of workers in the colony. The increase in patrol frequency does not lead to constant patrolling by the queen at large colony sizes, which would be impossible for a real queen due to physical restrictions.

These results qualitatively agreed with the experimental data reported by Kikuchi *et al.* [72]. Kikuchi *et al.*, through colony size manipulation, also showed that the queen increased her patrol effort with increasing colony size. This was also confirmed through our own experiments (SI 1 - Experiments, S1 Fig). As a next step, let us determine the effectiveness of the queen's patrol behaviour in controlling the internal state of workers in the colony.

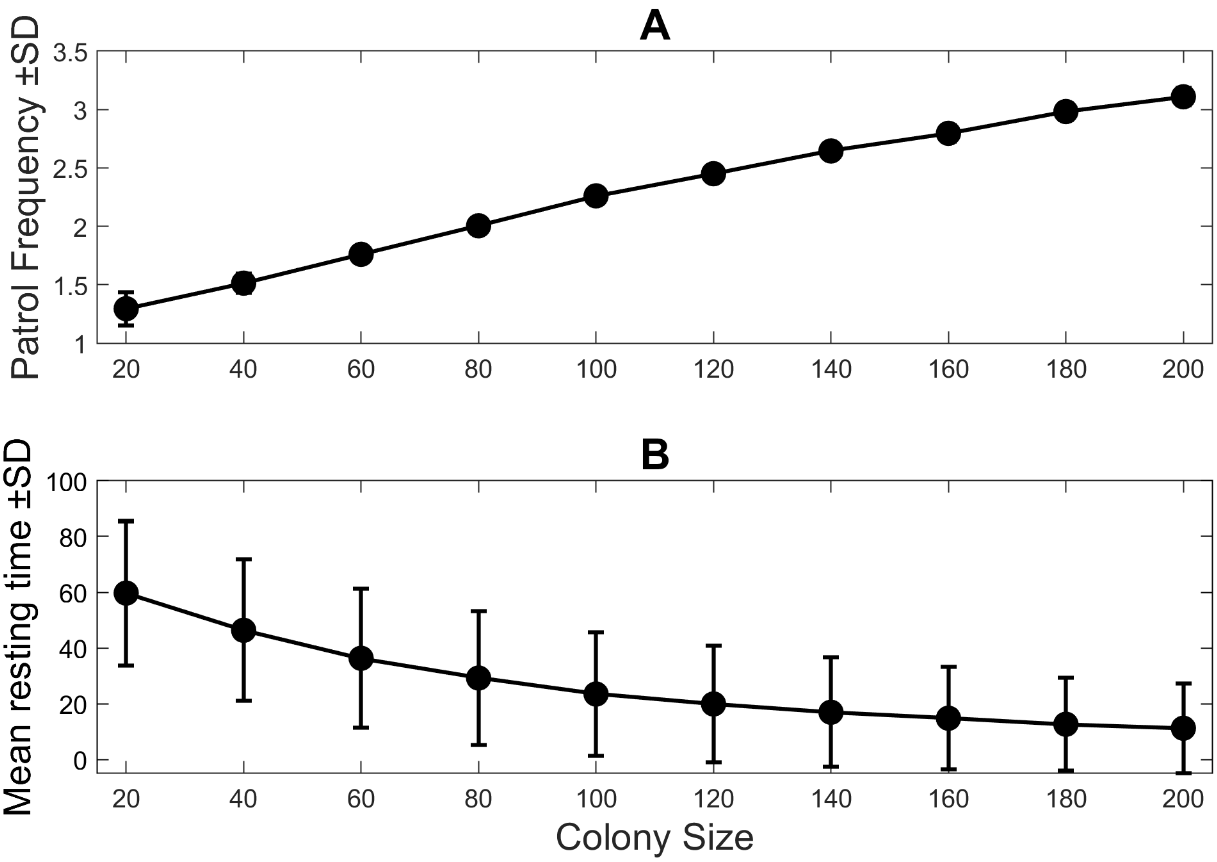
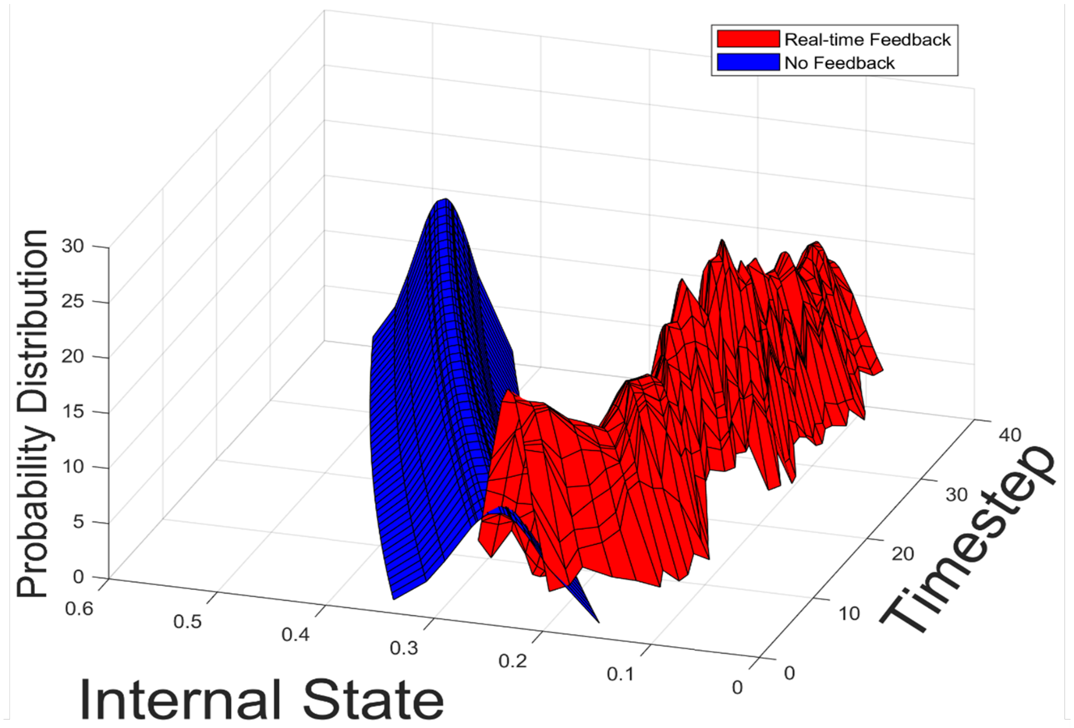


Fig 2.4. Patrol Frequency and Encounters (A) Patrol frequency of the queen as a function of the colony size. The patrol frequency of the queen increases as the colony size increases. This shows an increase in patrol effort by the queen. (B) Resting time as a function of the colony size shows an inverse relationship to the patrol frequency. The mean resting time decreases with colony size as the queen spends more time patrolling

To determine the effectiveness of the queen's patrol behaviour, distributions of worker internal states were calculated over time. Figure 2.5 shows the distributions of the internal states of workers (when $N = 20$), comparing two cases where real-time feedback was implemented and not implemented (contact with the queen had no impact on worker state as a control). Here, we could quantify the internal states as a function of time in the agent-based simulations, which cannot be obtained experimentally.

These results indicate that the real-time feedback model of the queen's patrol behaviour suppresses the internal states of the workers effectively with smaller variance than the case when there is no feedback. Results for a larger colony size, $N = 120$, show that the feedback model can be effective in controlling the internal state of workers when compared to no the feedback case (S5 Fig). However, there appears to be an increase in the mean internal state and the variance of the distribution. Therefore, as colony size increases, the effectiveness of the queen's patrol behaviour decreases.


Real-time Feedback
No Feedback
Probability Distribution
30
25
20
15
10
5
0
0.6
0.5
0.4
0.3
0.2
0.1
0
Internal State
0
10
20
30
40
Timestep

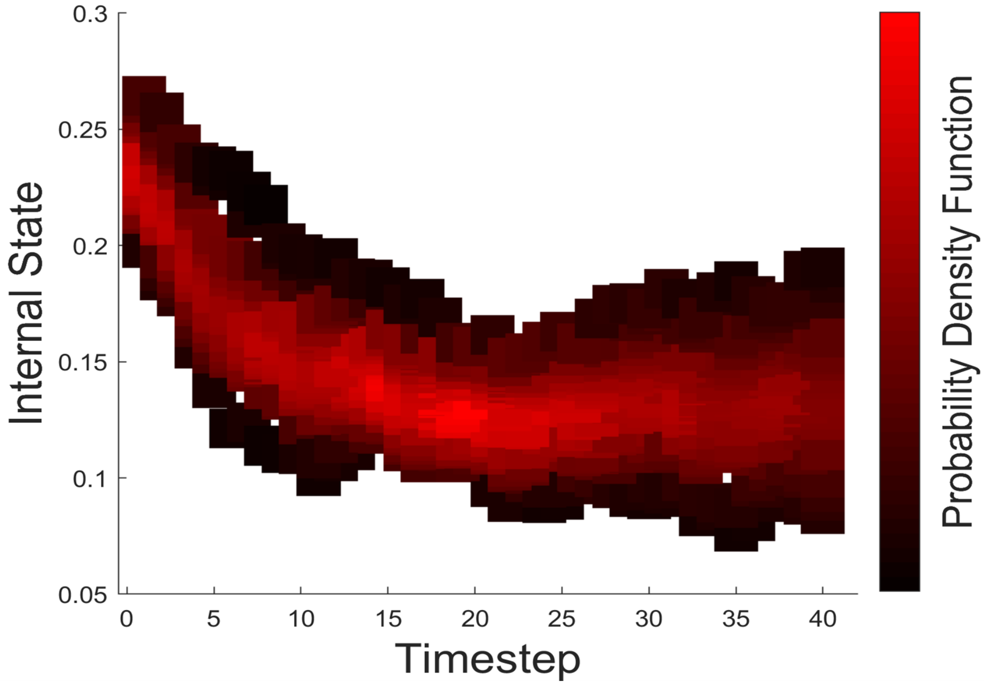
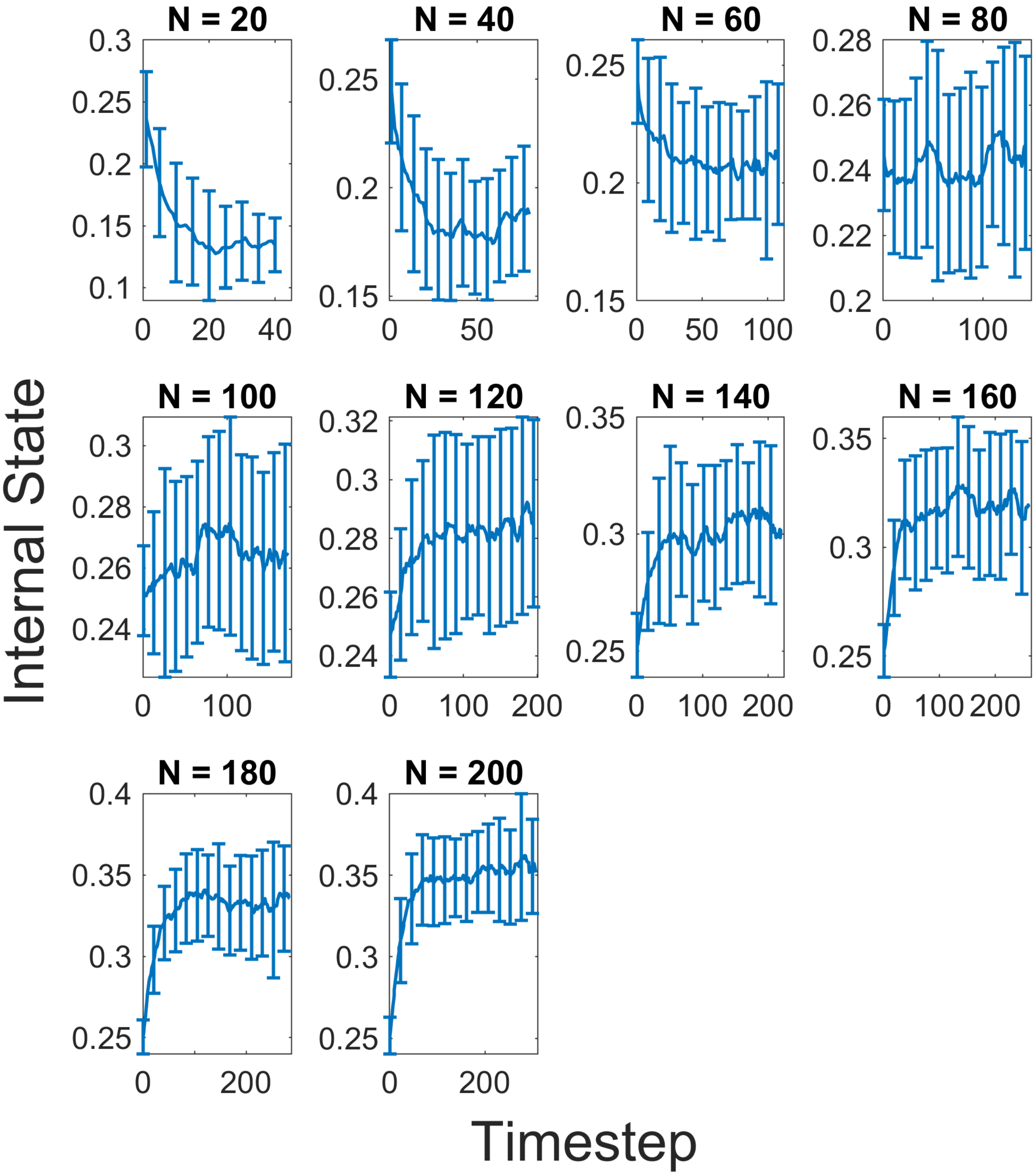

0.3
0.25
0.2
0.15
0.1
0.05
Internal State
0
5
10
15
20
25
30
35
40
Timestep
Probability Density Function

Fig 2.5. Probability distribution of worker's internal state (N=20) (Top) Probability distribution of workers' internal states over time in a colony of 20 workers with (red) and without (blue) real-time feedback. We compared the results of simulations with real-time feedback and with a no-feedback case to study how queen patrol behaviour suppresses the internal state of workers. No feedback (blue) causes a continuous increase in the internal state of workers over time, both at small colony sizes and larger colony sizes (see 2.6.11). With real-time feedback in relatively small colonies, the average value of the workers' internal states decreases from 0.2361 to 0.1344. (Bottom) This can be seen more clearly in the bottom plot, with the decrease in the internal state of workers from the initial value. The intensity of the colour shows the probability density function, with a larger proportion of workers being close to the mean. The shift of the distribution for real-time feedback demonstrates that the patrol behaviour is successful in suppressing the internal states of workers.

The decrease in the efficiency of the queen's patrol behaviour can be shown more clearly in Fig. 2.6 which shows the mean internal states of workers over time for different colony sizes ranging from $N = 20$ to $N = 200$. In smaller colony sizes, we observe a decrease in the mean internal state of workers as a function of time from the initial random values of the internal states.

This confirms that the suppression of the internal states was realised sufficiently via physical contact by the queen. This indicates that the feedback loops between the perception of the internal states and the decrease of the rest time in patrol worked effectively. Also, in the spatial degree of freedom, the queen (through her random walk) was able to contact all the workers who were also walking around randomly even though the colony size increases.

When the colony size increases further, there appears to be an inflexion point, between $N = 80$ and $N = 100$ (Fig.2.6), where the mean internal state begins to increase rather than decrease as a function of time. This shows a decrease in the effectiveness of the queen's suppression of worker internal states or the start of the failure of the patrol behaviour. This can be seen more clearly in the larger colony sizes ($N = 120$ to $N = 200$). The lack of suppression at this stage is due to the lack of contact between the queen and workers and not the physical limitation of the queen i.e. exhaustion from prolonged periods of patrolling. While there is an increase in the mean internal state with colony size, there appears to be relative stabilisation in the mean after some time. As a next step, let us interrogate the mechanism in the spatial degree of freedom, namely the number of contacts from the queen to the workers, and vice versa.



N = 20
N = 40
N = 60
N = 80
N = 100
N = 120
N = 140
N = 160
N = 180
N = 200
Internal State
Timestep
0.3
0.25
0.2
0.15
0.1
0
20
40
0.15
50
100
0.28
0.26
0.24
0.22
0.32
0.35
0.4
200

Fig 2.6. Average internal state of the colony for various colony sizes (N = 20–200 at increments of 20 workers) As the internal states of workers were initialised randomly between 0 and 0.5, the average initial value for all colony sizes is approximately 0.25. Due to each run of the simulation having different end times, the average internal state was calculated by taking the average of the internal states for simulations that had a run time equal to or more than the average simulation time. This decreases the precision of the average closer to the end of the simulation. The error bars were calculated using the 'std' MATLAB function, which calculated the standard deviation for the average internal state of the workers. At smaller colony sizes ($N = 20$, 40, and 60), the average internal state of workers decreases as a function of time and stabilises at a point which is lower than the initial value. As the colony size becomes larger, there appears to be a transition (at $N = 80$ and 100) where there is greater fluctuation in the average values over time. At the largest colony sizes ($N = 140$ to 200), there is an increase in the average internal state of workers over time. Here too there is stabilisation but at a point higher than the initial value.

By logging the number of contacts that occurred during the simulation, various contact rates could be calculated. These are the queen contact rate, the per-worker contact rate and the contact rate between workers. The contact rate is defined as the number of contacts per unit time. Therefore, the queen contact rate is the rate at which the queen contacts workers per unit time. While the per-worker contact rate is the average contact rate of a worker in the colony. These values were calculated separately, with contacts logged for the queen and individual workers. Theoretically, assuming an even distribution of contacts between workers, the per-worker contact rate is equivalent to the queen contact rate divided by the number of workers. However, the per-worker contact rate conveys the contact efficiency of the queen and, therefore, the effectiveness of the queen's patrol behaviour.

Fig.2.7 shows various contact rates between the queen and workers as a function of the colony size. Distinctions were made for the contact rate during the rest cycles (Fig.2.7A) and patrol cycles (Fig.2.7B) of the queen. This was to demonstrate that the majority of the contacts by the queen were made when the queen was patrolling. Fig.2.7C shows the overall contact rate for the queen increasing (black line) while the per-worker contact rate decreases (blue line).

Despite the increased patrol effort by the queen (shown in Fig.2.4), the contact efficiency of the queen decreases with colony size. This is due to the insufficient increase in the queen contact rate.

Note here that all the results obtained in the agent-based simulations were predicated on constant density. The results so far indicate that while the queen contacts more workers in larger colonies, based on more frequent patrols, the lower contact per worker leads to an increase in the mean internal state of workers in the colony due to the decrease in contact efficiency.

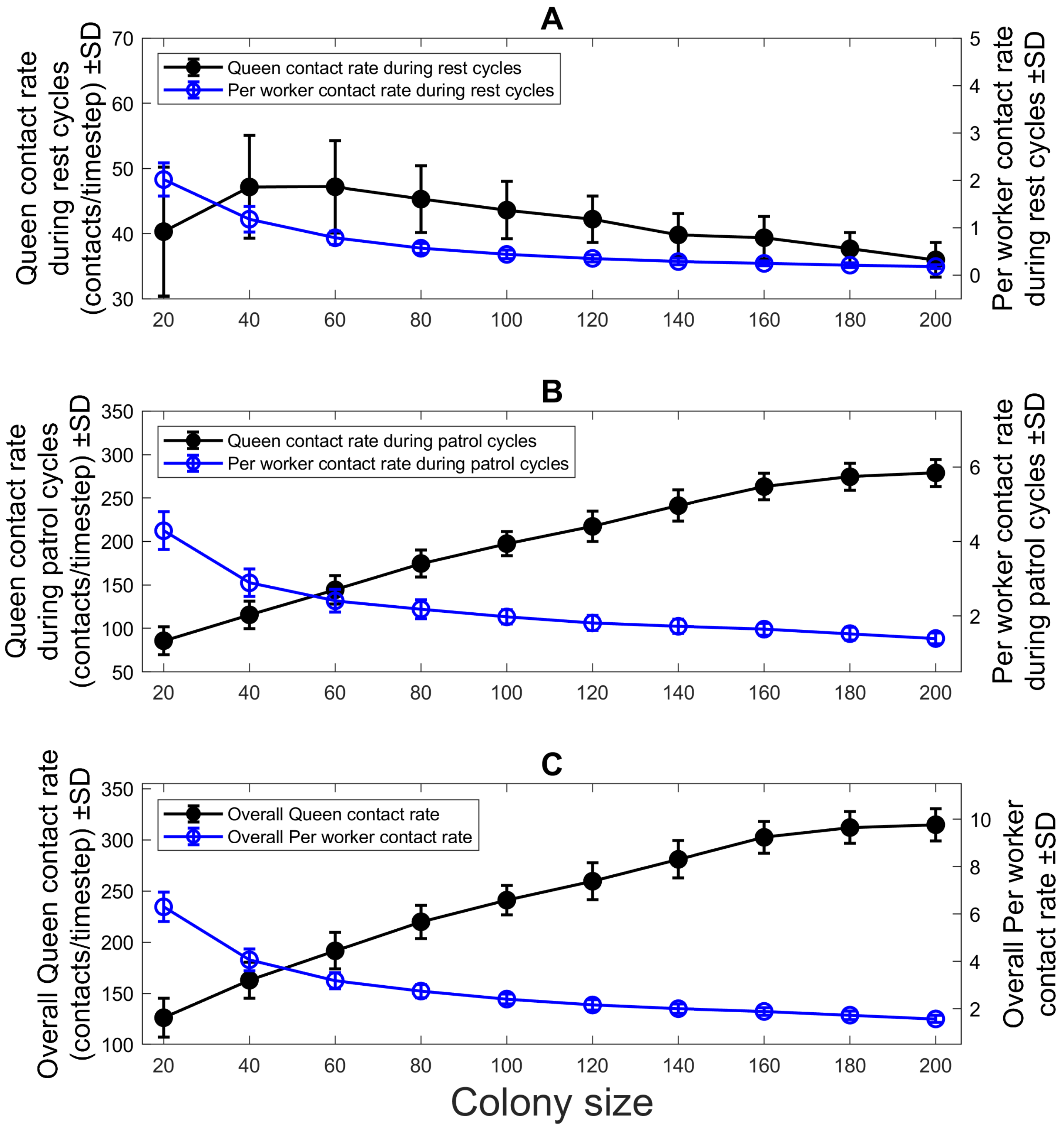

A
Queen contact rate during rest cycles (contacts/timestep) ±SD
Per worker contact rate during rest cycles ±SD
Queen contact rate during rest cycles
Per worker contact rate during rest cycles
30
40
50
60
70
0
1
2
3
4
5
B
Queen contact rate during patrol cycles (contacts/timestep) ±SD
Per worker contact rate during patrol cycles ±SD
Queen contact rate during patrol cycles
Per worker contact rate during patrol cycles
50
100
150
200
250
300
350
2
4
6
C
Overall Queen contact rate (contacts/timestep) ±SD
Overall Per worker contact rate ±SD
Overall Queen contact rate
Overall Per worker contact rate
100
150
200
250
300
350
2
4
6
8
10
20
40
60
80
100
120
140
160
180
200
Colony size

Fig 2.7. Contact rates between the queen and workers (A) The queen contact rate and per worker contact rate during the rest cycles of the queen. While there is a slight increase in the queen contact rate, overall the trend is a decrease in both the queen and per-worker contact rate with colony size. (B) The queen contact rate and per worker contact rate during the patrol cycles of the queen. There is an increase in the queen contact rate, showing that more workers are contacted during the patrol cycles of the queen than during the rest cycles. However, there is still a decrease in the per-worker contact rate, similar to Fig.2.7A & 2.7C. (C) The overall queen contact rate and the per-worker contact rate. The overall queen contact rate increases with colony size. This reflects an increase in the patrol effort as well as the increase in colony size. However, the per-worker contact rate decreases with colony size, showing a decrease in the contact efficiency of the queen.

The loss of contact efficiency may be due to a colony size dependent effect on the patrol behaviour of the queen.

This should not affect how workers contact each other. To test this, we quantified the contact rate between workers, shown in Fig.2.8. This contact rate reflects the general contact rate between workers, i.e. how many times workers contact each other. The per-worker contact rate here is the contact rate that worker A has with other workers in the colony. Similar to Fig.2.7C, the contact rate between workers increases as a function of colony size, but the per-worker contact rate between workers decreases. Note again that the results were obtained based on constant density. The per-worker contact rate decreases for the same reason it decreases for the queen. The contact efficiency is lost at larger colonies because the agent's movement is insufficient to cover the space. However, for the queen, we can relate this to her behaviour because her internal state is related to her movement.

In comparison, the worker's movement stays the same at all times. Hence, the loss in per-worker contact rate between workers is likely more significant than the drop in per-worker contact rate with the queen. Although there are more workers than the queen and as the simulation ends once the queen has contacted every worker at least once, there is a more considerable drop in the per-worker contact rate with the queen in Fig.2.7C than in Fig.2.8. The workers do not have to have unique contact with other workers. In contrast, the queen does have unique contacts because she needs to address every worker individually.

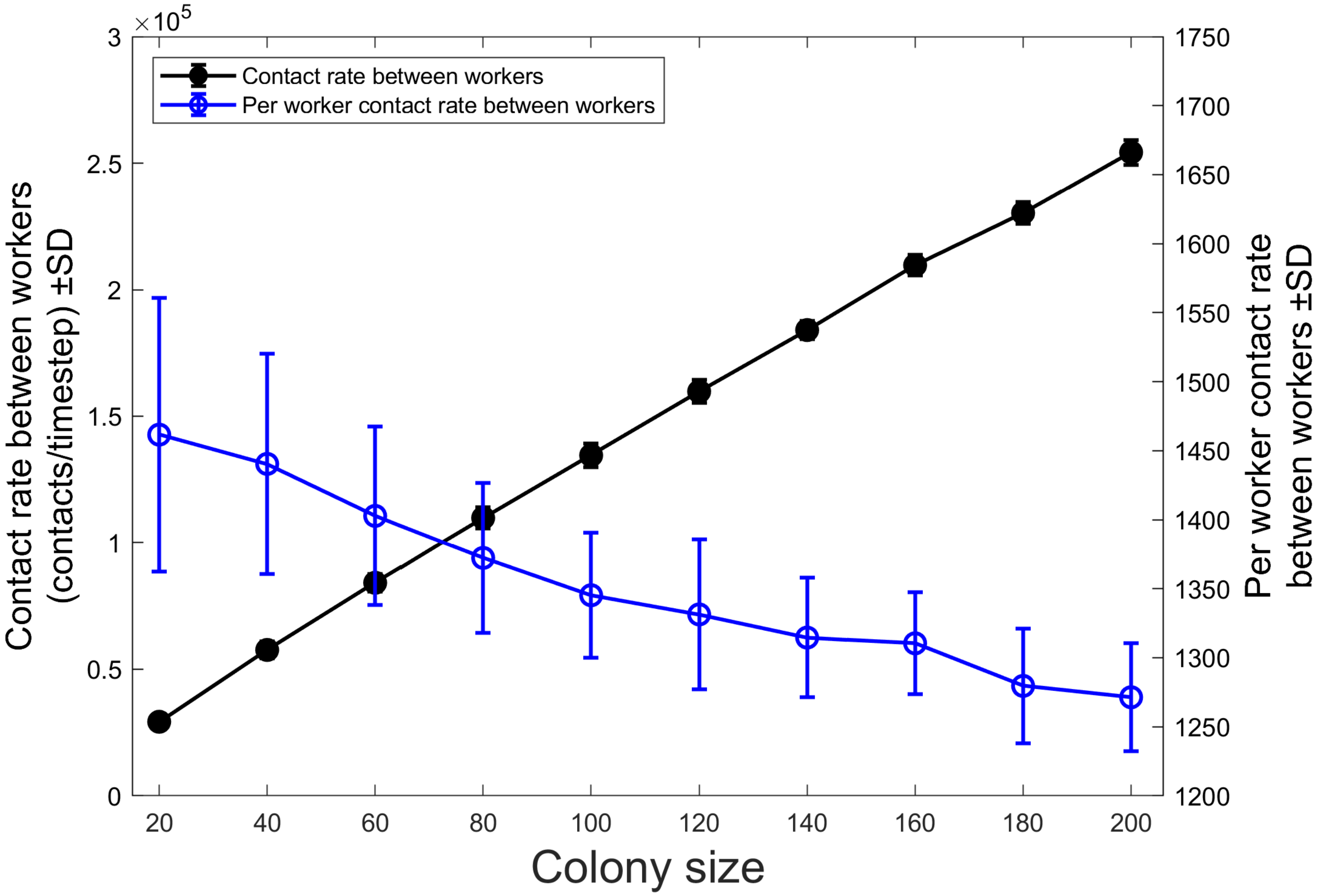


Fig 2.8. Contact rate between workers The contact rate between workers (black line) increases with colony size. An increased colony means more workers so there will be more contacts in general. When looking at the per-worker contact rate (blue line), there is a decrease with colony size similar to that seen in Fig.2.7C. There appears to be a decrease in the contact efficiency not just between the queen and workers but also between workers.

2.5 Discussion

Our results from the agent-based simulations revealed that the real-time feedback system between a queen and workers can have an influential role in maintaining and stabilising the internal states of the workers under various colony sizes. The simulations showed that, with a constant density, the queen increased her patrol frequency as the colony size grew (Fig.2.4A), and as a result, she could suppress the internal states of workers effectively (Fig.2.5). The underlying feedback mechanism is as follows: When the average internal state of workers increases, the queen frequently perceives a larger internal state, leading to an increase in the queen's internal state (2.2). This increase in the queen's internal state in turn leads to an increase in the queen's patrol frequency by decreasing her resting time (2.1). In short, as the colony size increases, the per-worker contact rate (Fig.2.7C, blue line) decreases, which triggers an increase in the queen's patrol frequency (Fig.2.4A). Hence, the queen's sterility-maintaining behaviour in response to an increasing colony size is revealed. However, this was only the case until the colony reached certain colony sizes. In larger colonies, $N = 120$ to $N = 200$, the queen contact efficiency became low (Fig.2.7C), and consequently, the internal states of workers were no longer effectively suppressed, i.e., the average internal states increased as a function of time (Fig.2.6) and at the end of the run many workers were ready to perform self-reproduction.

This simulation result was qualitatively consistent with what was observed in real *Diacamma* colonies. Namely, a positive association between the queen patrol effort and colony size (SI 1 - Experiments, S1 Fig, see also [72]), and the effective suppression of worker reproduction in small colonies and less effective suppression in larger colonies [145] [67]. The feedback loops through physical contact between queens and workers are sufficient to suppress the internal state of workers in small colonies (Fig.2.5). In theory, such colony size dependent worker reproduction is beneficial in terms of the inclusive fitness of workers in monogynous and monandrous hymenopteran colonies [110]. Suppression of worker's reproduction when the colony is small (ergonomic stage) contributes to rapid colony growth. When the colony is large (reproductive stage), worker-produced eggs are less policed and more likely to survive [67], which can imply that the selfish option (worker reproduction) may benefit workers.

We are the first to explicitly state a hypothetical proximate mechanism generating the colony size dependent character expression and the shift from ergonomic to reproductive stages. More importantly, both the reproductive division of labour among a queen and workers and the switch in the colony stages (from ergonomic to reproductive) are simply achieved by the decision-making of member individuals who just rely on personally acquired local information of recently encountered individuals. Decentralised control and self-organisation are thought to be the mechanisms that give rise to various functions of social insect colonies, such as the allocation of the workforce to various tasks that the colony needs, and selective recruitment of foragers to better food sources among the food sources available [147] [148] [149]. These theories commonly argue that single colony members have access to only limited "local" information, but they perform adaptively as a whole [3] [150].

So far, the "overall" frequency of encounters with other individuals related to local density in a nest has been often discussed as a piece of effective colony-size information for each colony member to decide their behaviour [132] [136]. However, in this study, we assumed that the individual density per nest space is constant even if the colony size changes. This minimises changes in local density being the primary cause of the contact frequency. We consider that in real ants a positive correlation of individual density per space with colony size can occur. This can occur in situations in which ants have physical difficulty in expanding their own nest space. However, in the absence of such a spatial constraint, it would be more natural to assume that ants extend the housing architecture of the nest as the colony grows. For this reason, we consider that local density, or the simple frequency of encounters, does not generally serve as reliable information on total colony size. Actually, in *Diacamma* (SI 4 - Experiments, S4 Fig) individual density per nest space is likely regulated to be more or less constant. Also, in some ants, workers change their behaviours depending on density, thereby contact frequency does not linearly increase with density [136].

In this computational study, we show that even at a constant individual density per nest space, colony size dependent behaviours both in queens and workers emerged. This demonstrates that the behavioural changes caused by the feedback loop (which couples the internal state of the queen and workers) code the information regarding the contact rate of the individual worker by the queen. Note that in our simulations all the agents are assumed to exhibit a random walk, i.e., no grouping or clustering, in a constant individual density per space. This demonstrates that it is not the simple overall frequency of encounters, but instead, the two types of specific contact rates that play a role; the contact rate of the queen with reproductive workers and the contact rate of the worker with the queen. The former contact rate is a measure of the inverse of how completely the queen can make contact with workers. The latter is how often individual workers can be contacted by the queen. Due to the contact rate of the individual worker decreasing with colony size, the internal state of the worker increases. Through the resulting change in the internal states, the queen's patrol behaviour is controlled as if she perceives the colony size as discussed previously. Furthermore, the queen patrol efficiency decreases in very large colonies presumably due to some constraints (see later), which leads to the colony stage shifting from the ergonomic stage to the reproductive one, a general phenomenon considered to be adaptive. This discovery is quite novel in that it reveals a single real-time feedback system can control both suppression of worker reproduction in small colonies and its release in large colonies. In monogynous colonies, where there is only one reproductive female (queen), when the queen pheromone is transmitted by direct physical contact between the queen and workers, we consider that this mechanism can generally operate. When these situations arise, the queen-to-worker ratio in the group can be of key importance.

Now we consider the generality of the model presented in this thesis in relation to both *Diacamma cf. Indicum* and other social insects. As we have shown, our model is able to replicate the patrol behaviour observed in the queen for *Diacamma cf. Indicum*, with increases in the patrol frequency as a function of colony size. Additionally, previous work [72] has shown the colony size distribution for *Diacamma* with most colonies containing less than 120 workers. From our results (Fig.2.6), we show that there is a transition between $N = 80$ and $N = 120$ where the queen's control on worker internal state weakens, with an increase in the internal state of workers. Thus, our model adds value in its explanation of the field observations of real *Diacamma* colonies. With regards to other social insects, the applicability of our model is dependent on the way information is transmitted across the colony. For Bumblebees and Honeybees [72] where queen presence is transmitted through low volatility CHCs, our model could be relevant and adapted to investigate the effectiveness of the queen presence in those colonies and the suppression of worker reproduction. Other eusocial insects such as *Pachycondyla* and *Dinoponera* use dominance interactions from the queen to control worker reproduction in the colony [151] [152]. For such insects, our model could be applicable as a mechanism for the enforcement of the reproductive division of labour. However, for social insects with much larger colonies (such as leaf cutter ants) it would likely be impractical given decreases in queen patrol effectiveness shown in this thesis. Queen patrol behaviour would have to be observed in such colonies and other mechanisms would have to be taken into account when determining the importance of such a behaviour in the dynamics of the colony.

2.6 Supporting Information

2.6.1 SI 1 - Experiments

The frequency of patrols in 12h was positively associated with the colony size (GLMM, $\chi^2 = 9.396$, $P = 0.002$, S1 Fig A). The mean resting time (time between two serial patrols) was negatively correlated with the colony size ($\chi^2 = 11.202$, $P = 0.0008$, S1 Fig B). This finding confirms the results of Kikuchi *et al.* [72].

2.6.2 SI 2 - Experiments

We focused on the first 20 patrol bouts for each queen. The mean patrol duration was 40.6 ± 36.0 sec (SD), and each queen contacted on average 13.1 workers per patrol. The longer the patrol duration, the more workers were encountered during the patrol (GLMM, $\chi^2 = 475.42$, P < 0.001). However, the mean patrol duration was not significantly correlated with colony size (GLMM, $\chi^2 = 1.148$, $P = 0.264$). The cumulative percentage of workers that a queen encountered in 20 patrols was negatively associated with colony size (GLM, colony size: $z = -5.93$, $P < 0.001$, S2 Fig).

2.6.3 SI 3 - Experiments

Finally, we analysed the spatial distribution of workers and the queen, because we had an impression that worker density in the vicinity of the queen is regulated to be relatively constant. Note that we provided an artificial nest of the same design to all 15 colonies. The nest space (the depression of the plaster floor) seemed wide enough for even the largest colony containing 214 workers. Inside the nest, workers tended to aggregate around the queen. Within such an aggregation, spacing between workers seemed more or less constant irrespective of the colony size: in large colonies, a wide space within the nest was occupied by such an aggregation, whereas in small colonies, the aggregation used only a small portion of the nest space (S3 Fig).

2.6.4 SI 4 - Experiments

To test this observation statistically, using the video data for the 15 colonies of *Diacamma*, we made a snapshot of the inside of a nest every 2h, for a total of five times for each colony. We counted the number of workers inside the circle of 2.5-cm radius, the centre of which was positioned on the petiole of the queen. We only counted workers who had over 50% of the body area inside the circle. We excluded snapshot data in which the queen stayed near the wall (within 2.5-cm). The worker density in the circle was not significantly correlated with the colony size (GLMM, $\chi^2 = 1.302$, $P = 0.24354$, S4 Fig), suggesting that the local worker density around the queen was kept roughly constant regardless of colony size (mean ± SD: 7.04 ± 1.89 workers). Thus, for the queen, a simple encounter frequency with workers is not a reliable proxy of the colony size.

2.6.5 SI 5 - Simulations

Using data we collected from the agent-based simulation, we plotted the probability distribution of worker internal states for a larger colony size ($N = 120$) to observe the effect of colony size on the distribution over time. Our real-time feedback was effective in controlling the internal state of workers over time (S5 Fig). With no feedback, workers' internal states simply increase. Although, at the larger colony size, there is an increase in the mean internal state and the variance of the distribution. This reflects a weakening of the control the queen has on the reproduction of workers at larger colony sizes as opposed to smaller colonies.

2.6.6 SI 6 - Simulations

Previous work of Kikuchi *et al.* [72] found that while the rest time of the queen decreased with colony size, the patrol time did not seem to significantly increase. We reflected this in the simulation by setting the patrol time of the queen to be constant. However, as shown in Fig 2.4A, the patrol frequency of the queen increases with colony size. S6 Fig shows the queen's activity cycle for $N = 20$ and $N = 200$. In the simulation code, when the queen is active (and therefore patrolling) the variable "QueenActive" is set to 1, otherwise, it is set to 0. Though the active time of the queen stays the same, the decrease in the rest time causes shorter delays between each patrol when the colony size is large. This is demonstrated in S6 Fig where the increased closeness of the patrols can be seen for $N = 200$.

2.6.7 SI 7 - Simulations

The reason for this increase in patrol frequency is the internal state of the queen. The queen's internal state is a proxy for a transition probability. If the queen is inactive and interacts with workers of a high internal state, it increases the probability that the queen will become active and patrol the colony. S7 Fig shows the queen's internal state over time for colony sizes $N = 20, 100$ and 200. As the colony size increases, the internal state of the queen also increases, with a similar trend for colony sizes. There are periods where the queen's internal state is high, followed by significant drops. The larger colony size means that there are more workers to interact with and patrol. From Fig 2.6 we see that higher colony sizes have a higher average internal state for the workers. This is reflected in the queen also as a higher internal state as she interacts with workers which have an average higher internal state in larger colonies.

2.6.8 SI 8 - Simulations

To check that the dynamics are not influenced by the initialisation of the internal state of the queen, we also ran simulations where the internal state of the queen was initialised at 0.8 (instead of the default 0.1). S8 Fig shows that, despite a different initial value, the queen's internal state mirrors the trends shown in the previous figure, with an increase in the queens internal state as the colony size increases. We also compared directly the internal state of the queen at $N = 20$ when the initial value was 0.1 and 0.8 (S9 Fig). This confirmed that the queen's internal state returns to similar values despite the increase in the initial value showing the robustness of the dynamics of the system.

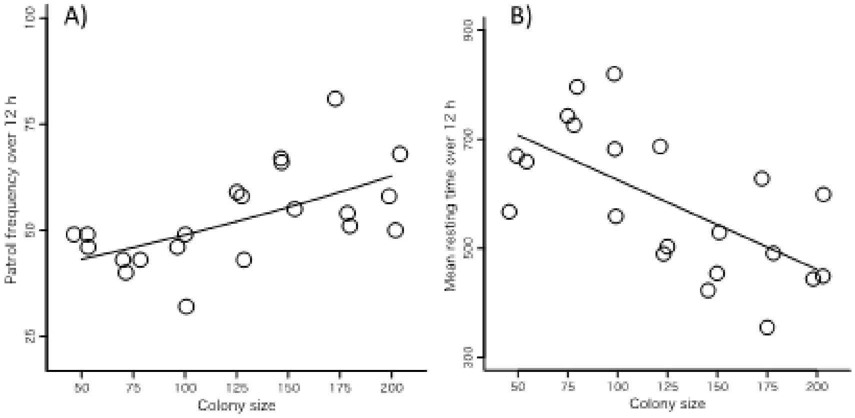
2.6.9 SI 9 - Simulations

To further confirm our results, we changed the end criteria of the simulation. Initially, the simulation would end when the queen had contacted all workers in a colony. The time it took to accomplish this differed between colony sizes. Thus, the results may not be reflective of the system at a steady state. To account for this, we simulated 300 timesteps across all colony sizes (shown in S10 Fig). We found that the effectiveness of the queen's patrol is strengthened. While there is still an increase in the average internal state of workers as the colony size increases, the suppression of the internal state of workers continues for larger colony sizes than what is shown in Fig 2.6. This means that our results may underestimate the effectiveness of the queen's patrol behaviour.

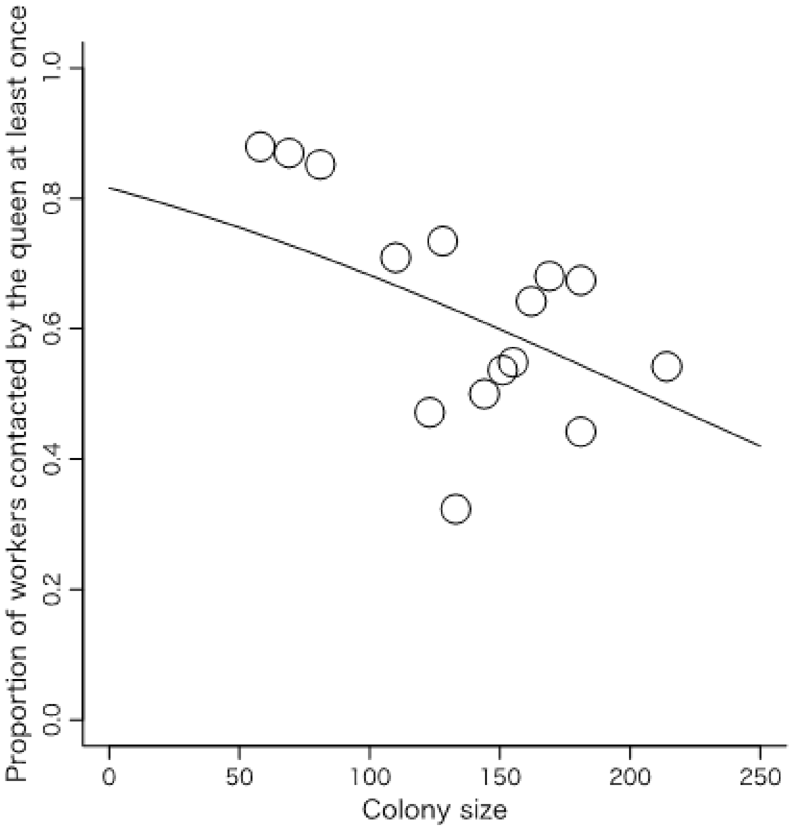
2.6.10 SI 10 - Simulations

However, the effectiveness of the queen's patrol behaviour is also linked to how the workers develop their internal state. What would happen if workers developed their internal state more rapidly? To answer this, we increased the constants β and γ. From Eq. 2.3, β and γ control the rate at which workers develop their internal state. By increasing their value to 10x the original value, S11 Fig showed that the effectiveness of the queen's patrol behaviour in this model is reliant on the rate that workers develop their internal state. The dynamics are similar, with weaker control as the colony size increases, but total loss of control occurs at smaller colony sizes. In the main results β and γ were set to approximate the development rate found in the previous work of Kikuchi *et al.* [72].

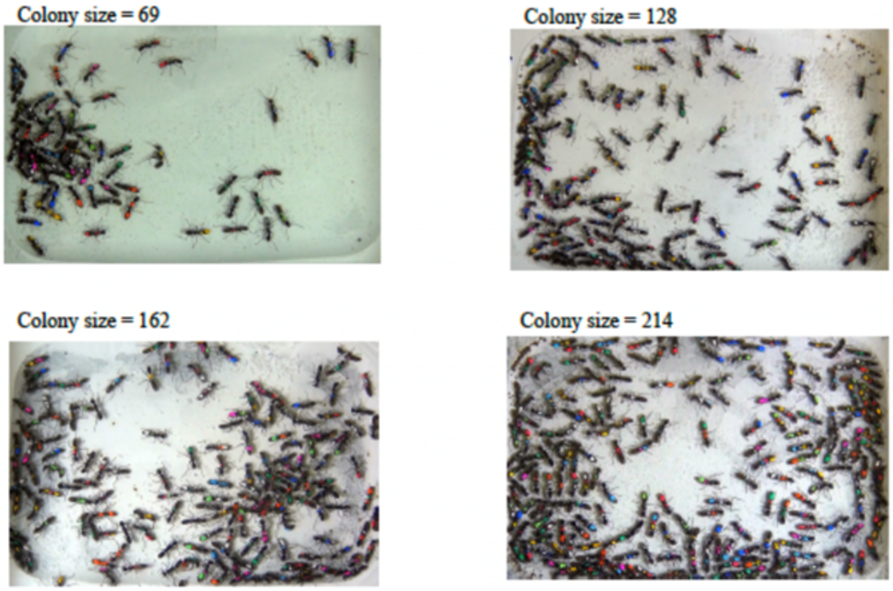
2.6.11 SI Figures



S1 Fig. Changes in the (A) frequency of patrol behaviour and (B) mean resting time of the queen at various colony sizes (N = 3 colonies per colony size)



S2 Fig. Proportion of workers contacted by the queen at least once in 20 patrol bouts at various colony sizes (N = 15)



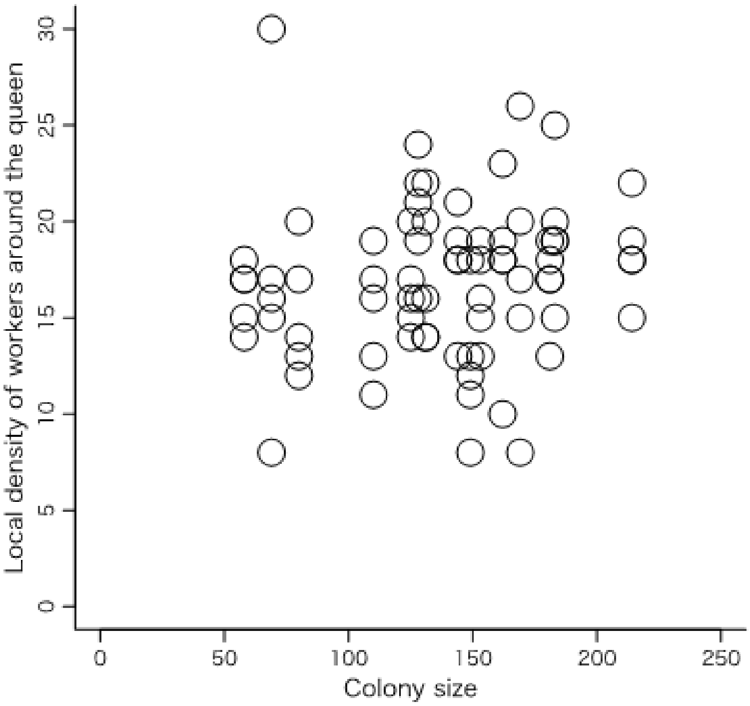
Colony size = 69

Colony size = 128

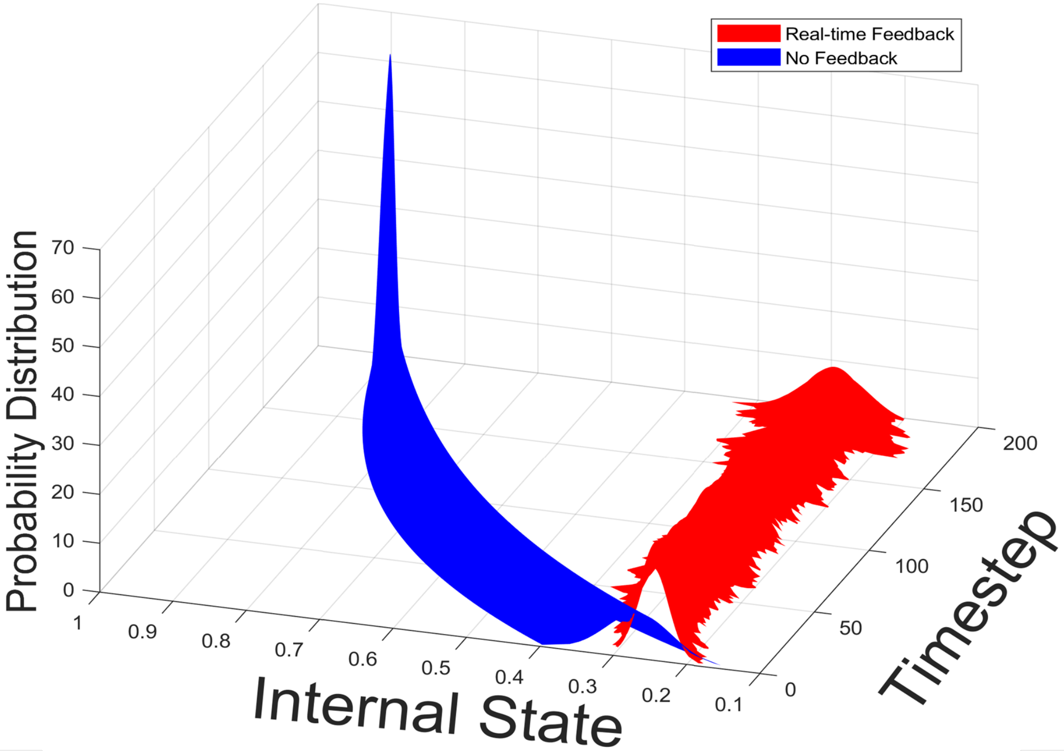
Colony size = 162

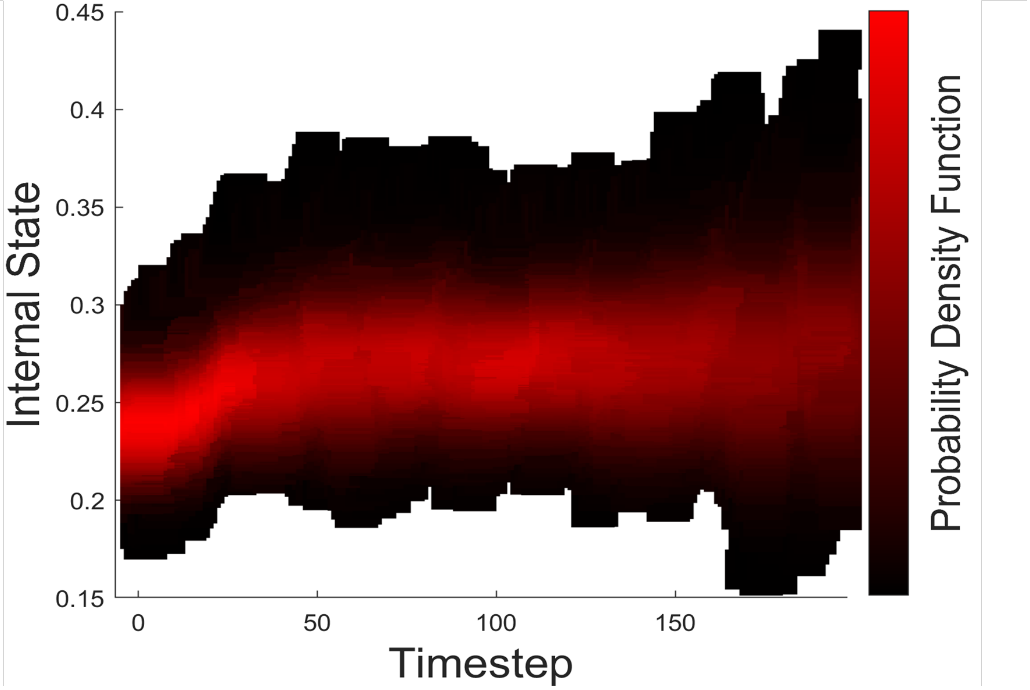
Colony size = 214

S3 Fig. Aggregation patterns of *Diacamma* individuals at various colony sizes in the artificial nest

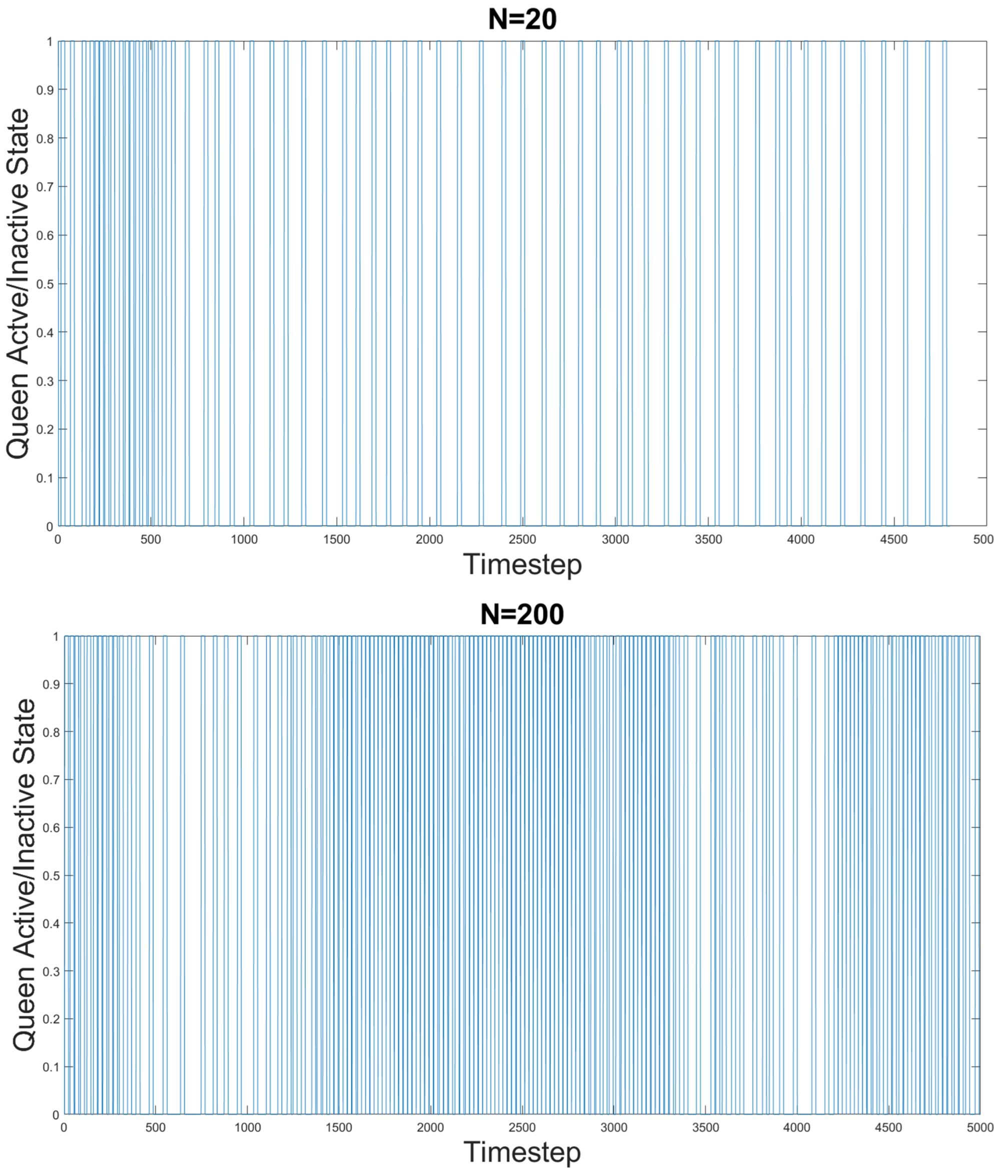


S4 Fig. Density of workers within a 2.5-cm radius of the queen at various colony sizes (N = 15)

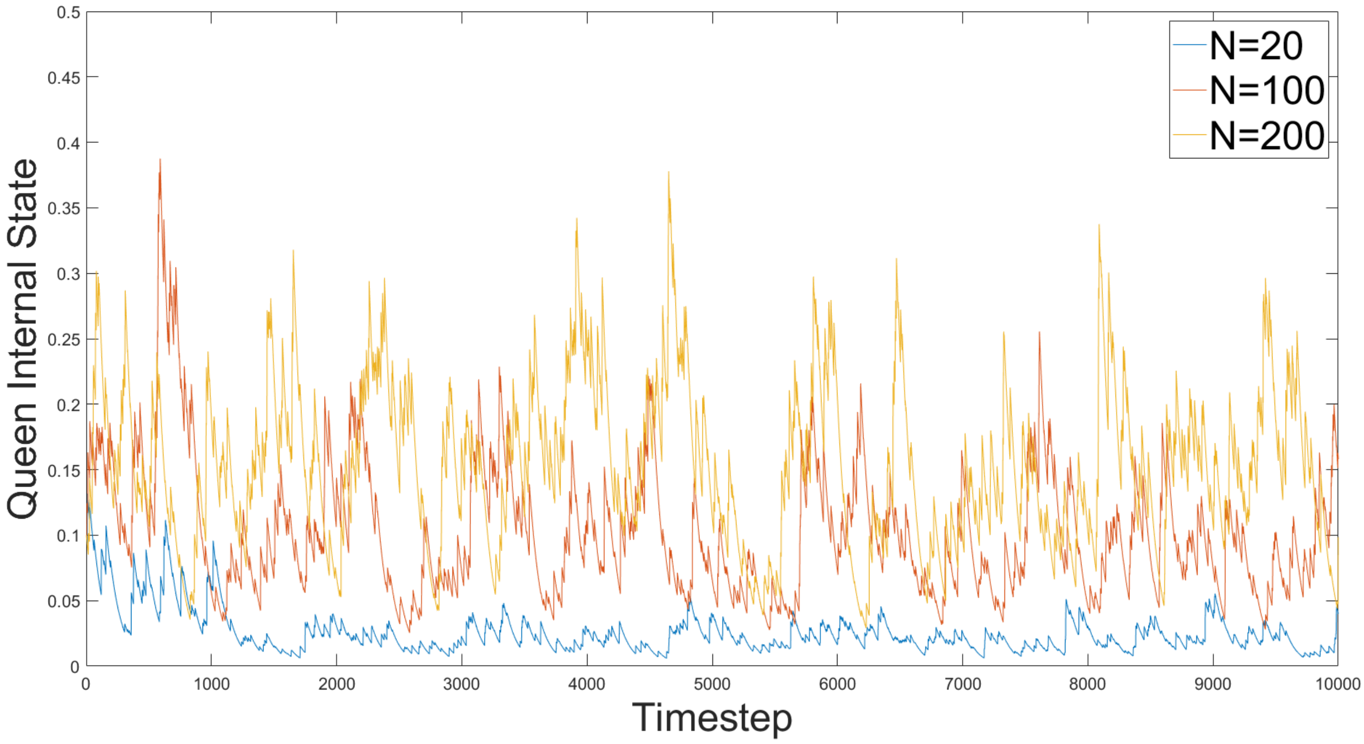

Real-time Feedback
No Feedback
Probability Distribution
70
60
50
40
30
20
10
0
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Internal State
0
50
100
150
200
Timestep


0.45
0.4
0.35
0.3
0.25
0.2
0.15
Internal State
0
50
100
150
Timestep
Probability Density Function

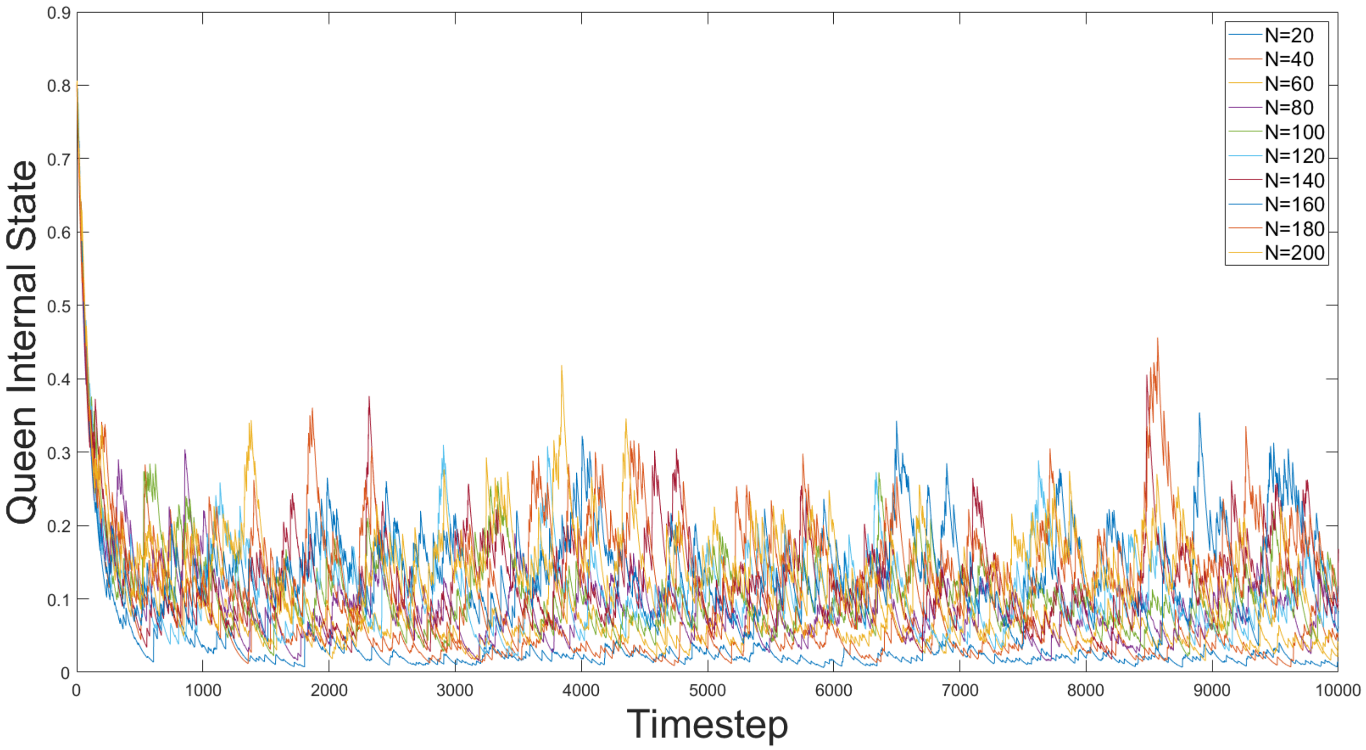
S5 Fig. Probability distribution of worker's internal state ($N = 120$) (Top) The probability distribution of workers' internal states over time in a colony with 120 workers with (red) and without (blue) real-time feedback. The average value increases from 0.2473 to 0.2888. (Bottom) The mean and variance are greater at $N = 120$ compared to $N = 20$ seen in 2.5. This shows a decrease in the effectiveness of the queen's patrol behaviour at larger colony sizes.



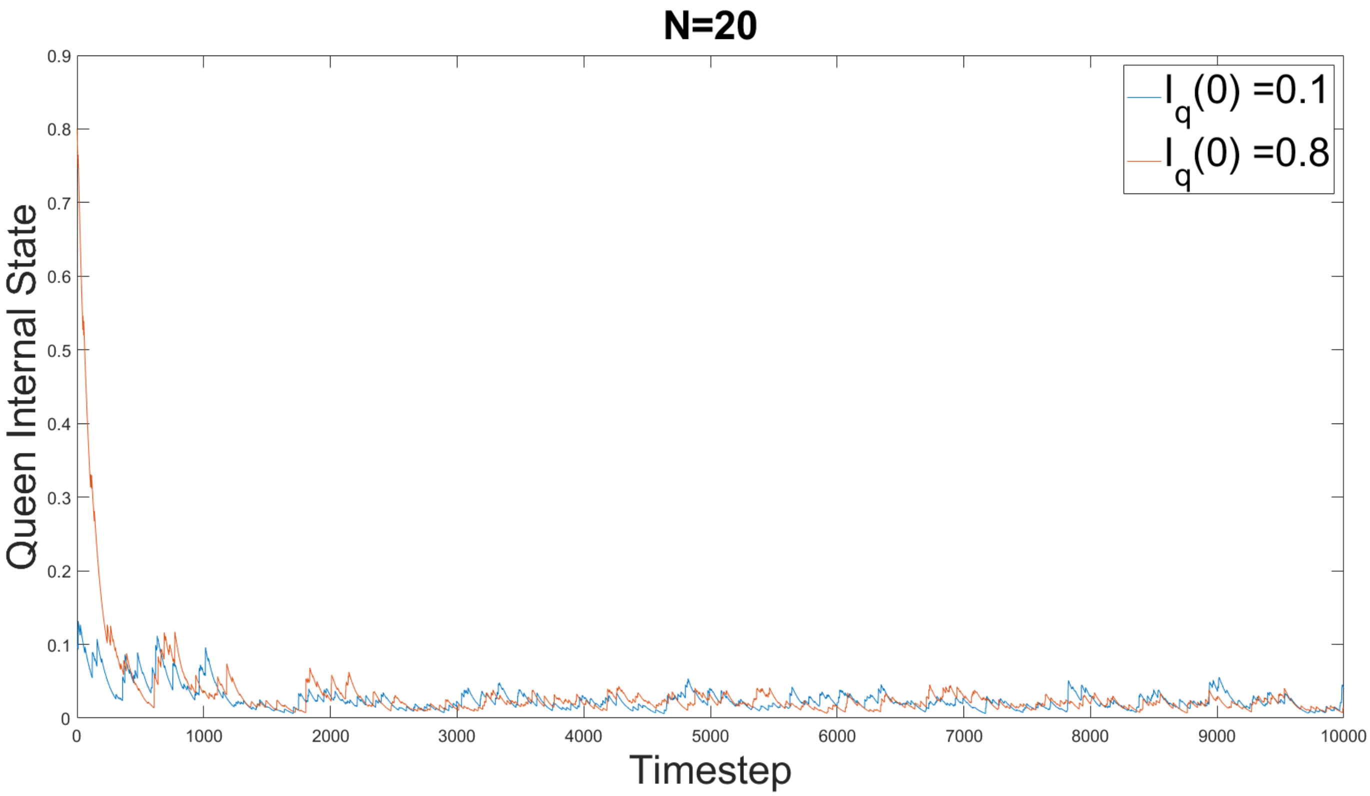
S6 Fig. Activity cycle of the queen at N=20 and N=200 The activity cycle of the queen changes with colony size. As the colony size increases, the rest time of the queen decreases. This increases the frequency of patrol for the queen at larger colony sizes. Here this is seen as clusters of blue lines, with more clusters when $N = 200$.



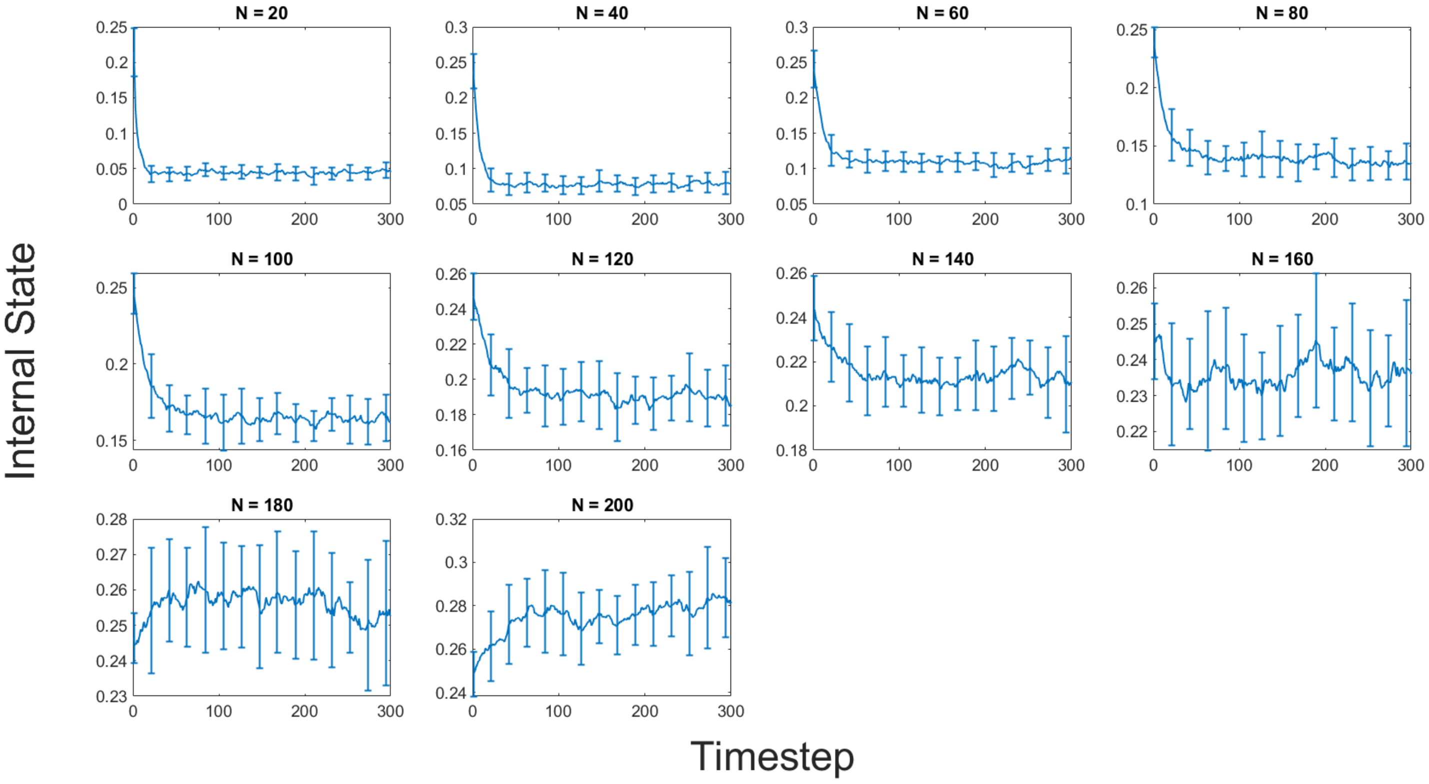
S7 Fig. Queen internal state over timestep The queen's internal state changes over time and is coupled with the internal state of workers. Workers in a larger colony have a higher average internal state, causing the internal state of the queen to increase with colony size.



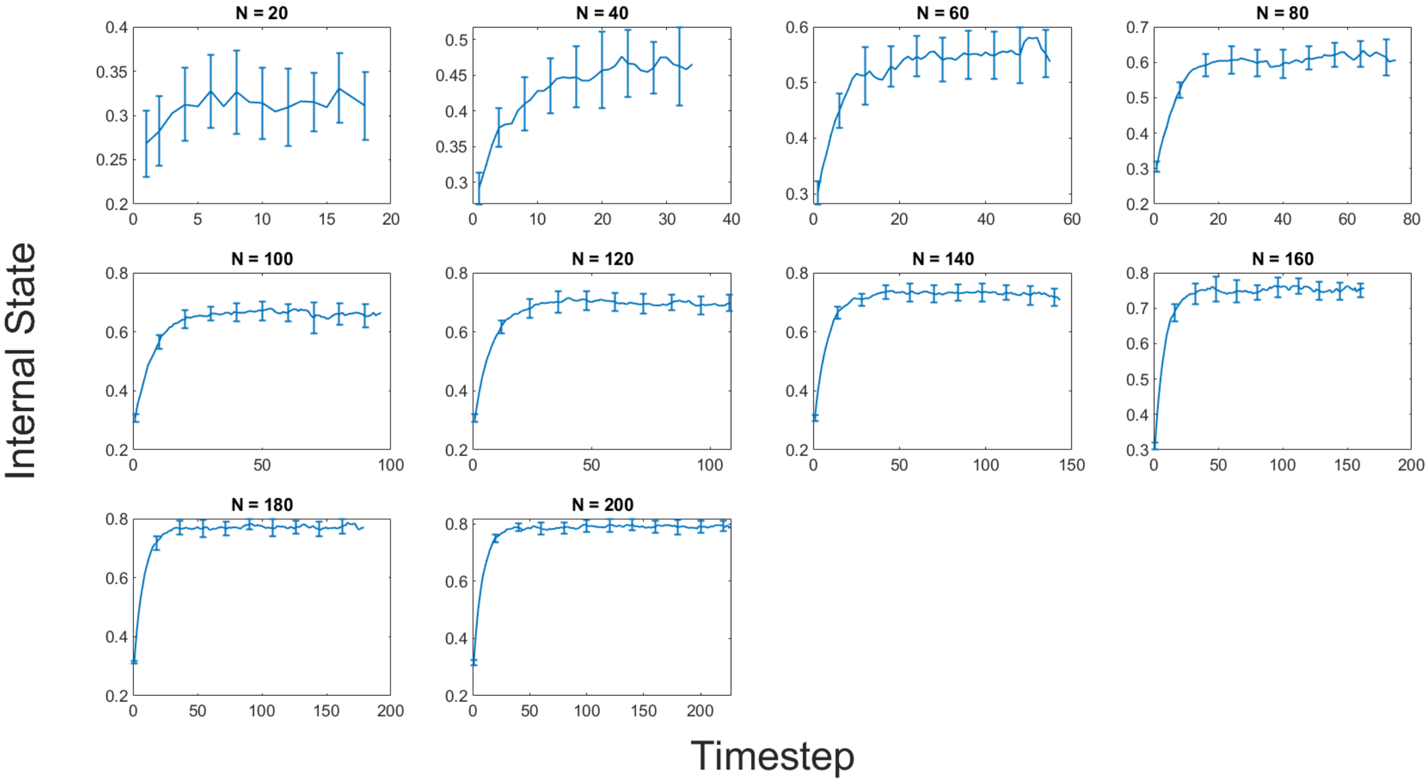
S8 Fig. Initialising the queen's internal state at a higher value We initialised the internal state of the queen at 0.8 rather than 0.1. The initial value of the queen's internal state does not affect the dynamics of the system. Given a higher initial value, the queen's internal state returns to the normal range observed in the previous figure, with larger colony sizes causing an increased internal state as before.



S9 Fig. Comparison between different initialised value Comparing the internal state of the queen for the same colony size with different initial values, we find that there is a convergence in the queen's internal state after approximately 400 time steps. This shows that the initialisation of the queen's internal state does not affect the dynamics.



S10 Fig. Running simulation for longer period of time By running the simulation for a consistent period of time for each colony size, it is more likely that a steady state is reached during the course of the simulation. With this, we see greater control by the queen over the internal state of workers. There is still weakening in the effectiveness of the queen's patrol behaviour but the reversal of the suppression occurs much later at the largest colony sizes.



S11 Fig. Increasing β and γ By increasing β and γ, the rate that workers develop their internal state, we showed that the queen has weaker control over the reproduction of workers. Loss of control begins even at smaller colony sizes such as $N = 40$.

Chapter 3

Investigating the evolution of altruistic behaviour in ant colonies using an evolutionary agent-based simulation

3.1 Introduction

In eusocial insects, such as ants, there is a division of labour between reproductive and non-reproductive individuals in a colony [42]. In some ant species, nestmates accept sterile non-nestmates [65], while non-nestmate queens can be violently attacked. Workers also police each other and enforce the collective decision not to produce eggs individually. They allow the queen to make eggs for the colony instead [67] [68] [69]. In Chapter 2, we focused on the queen's role in maintaining the division of labour in Diacamma through her patrol behaviour. In the following chapter, we will be looking at the worker's role and how the reproductive division of labour benefits them. The evolution of altruistic behaviour displayed by non-reproductive individuals is mysterious, given that direct fitness usually entails passing on one's genes via reproduction. In eusocial insects, members of the colony are typically split into different castes. These include queens who reproduce and workers who forage, construct the nest and raise the queen's offspring [11] [59].

Due to caste differences [61] [62], interactions between individuals are dependent on the status of the individuals, with some nestmates being dominant over others. Dominance is vital in regulating reproduction portioning, and this dominance is determined through physical interactions. In Diacamma cf. indicum (from Japan), workers retain the ability to produce eggs [31]. The queen suppresses the ovary development of workers [31] by making physical contact with the worker and repressing dopamine levels in the worker's brain [70], which has a gonadotropic function i.e. suppresses the ovary development of the worker. When the queen interacts with a worker, the queen gains information about the worker's internal state [31]. Simultaneously, the worker senses the queen's presence and suppresses the worker's ovary development. As the reproductive division of labour can cause the conflict between the queen and workers [54], policing is needed to enable cooperation and prevent selfish behaviour [153] [154].

To this end, policing by workers and refraining from reproducing in the queen's presence were studied [153]. Worker policing and self-restraint lead to the inhibition of direct reproduction. These are useful for maintaining the homeostatic equilibrium of the colony. The queen must regulate reproduction to ensure a balance in resource allocation, with workers allocated to different tasks needed to sustain and increase the survivability of the colony. This balance includes brood care, foraging, defending the nest site and nest maintenance [54]. Punishments for workers developing ovaries and laying eggs include eating the eggs of the rule breaker and immobilising the rule breaker [71].

In Diacamma, the cost hypothesis suggests that worker policing behaviours in ant colonies have evolved as a response to the potential costs associated with the reproduction of other workers [153] [155]. The cost hypothesis proposes that worker policing behaviour arises because the colony as a whole may suffer if too many worker-laid eggs develop into males. This is because male ants do not contribute to tasks such as foraging, nursing, or colony defence. The relatedness hypothesis is another explanation for the occurrence of worker policing. This hypothesis is based on the idea that worker policing evolved as a mechanism to enhance the relatedness between individuals within the colony [156] [157]. Both of these seek to explain the development of worker policing in Diacamma because, without a queen, workers begin to develop ovaries [72]. It is worth noting that the cost and relatedness hypotheses are not mutually exclusive.

Darwinian fitness is based on the survival and reproduction of an individual [158]. An individual's offspring inherits its characteristics with slight variation, which leads to an accumulation of traits that increase the individual's survival in a given environment over evolutionary time [159] [160]. Experimental evidence has shown the different mechanisms which can affect the evolution of different species and how it may even be possible to predict evolutionary outcomes [161] [162] [163] [164] [165] [166].

However, Darwinian fitness does not explain the behaviour of workers refraining from reproducing. It seems counterintuitive that workers would refrain from reproducing to assist the queen's reproduction. If workers largely refrain from reproducing, they cannot produce offspring that inherit their trait of not reproducing. The lack of offspring would lead to the prediction that individuals work selfishly to maximise their fitness and pass on their genes via reproduction.

The concept of inclusive fitness, introduced by W.D Hamilton [167] [39], solves this contradiction. Inclusive fitness accrues benefits beyond the individual. The rule (known as Hamilton's Rule) posits that a worker should perform a costly action if the benefit to a related individual outweighs the cost. Algebraically, this can be represented as $C < r \cdot B$. Where C is the fitness cost to the individual, r is the genetic relatedness between the actor and the recipient, and B is the benefit to the recipient. Further work has shown how inclusive fitness provides a conceptual framework for understanding social organisation [168] [169].

Such organisation includes population structure [170], observed cooperative behaviours [171] and the balance between cooperation and selfishness [172]. There has been work to relate inclusive fitness to gene propagation and the mechanisms by which inclusive fitness could affect the genes and phenotype of organisms [173] [174]. This applies Hamilton's theoretical work to experimental work. Inclusive fitness means that if raising the queen's offspring (highly related to workers) is more beneficial in terms of their inclusive fitness, workers contribute to the colony's maintenance, which increases the colony's breeding capacity.

Part of our integrated simulation involves a genetic algorithm. A genetic algorithm (GA) is a method of optimising some attributes using evolutionary and genetics principles [175] [176] [177] [178]). There is a genetic component to the expression of social behaviours in workers. This genetic component is affected by the propagation of genes in the workers over the generations. Therefore, monitoring this change over time is necessary, noting its influence on the worker's inclusive fitness. The genetic algorithm then allows for the maximisation of inclusive fitness and the ability to study the evolution of altruistic behaviour from the ground up.

In work by [179], authors provide a set of criteria that could be used for recognising and analysing genes for altruism. These provide a set of testable hypotheses which assist in the description of the evolution of genes for altruism. The first of these criteria is satisfying Hamilton's rule. The rule, named after W.D Hamilton [167] [39], is related to the number of offspring equivalents the individual saves or raises. The benefits to related individuals must outweigh the cost of altruistic behaviour. The next criterion is that altruistic genes should be environmentally sensitive. Meaning the genes are expressed differently depending on the social context. In the case of social insects, different expressions could be found between the reproductive queen and supposedly non-reproductive workers. The third criterion involves the increasing complexity of the altruistic genes with increased social-behavioural sophistication. The increased complexity could entail morphological changes that accommodate increased performance in a particular role.

The next criterion entails the co-evolution with/dependence on the evolution of genes for kin recognition. Kin recognition is also a prerequisite of fulfilling Hamilton's rule as the benefits of altruistic behaviour should, preferably, go to the kin of the selfless individual. Next is the at least partial additive nature of the altruistic genes. Altruism requires heritable variation, meaning the genes for altruism should be responsive to kin-mediated selection. The penultimate criterion is the exhibition of strong pleiotropy of the genes. The final of these criteria is the genes underlying altruism may reside in regions of low recombination, exhibit co-expression and show modular genetic architecture. The genes may become linked into gene complexes that collectively determine worker-like and queen-like traits. The criteria laid out will assist in searching for genes that could code for altruistic behaviour observed in various social insects, including Diacamma cf. indicum from Japan. Though these are not strict criteria.

In this thesis, we studied the balance in the evolution context between selfless and selfish genes linked with social behavioural patterns in ant colonies. To this end, we integrated a genetic algorithm and agent-based simulation to realise reproductive allocation conflict from the perspective of the fitness function, when workers can directly produce offspring or contribute to the maintenance of colonies. Our approach in computational biology is to use the fitness functions as an optimisation function in the genetic algorithm over colony generations, and reflecting the phenotype of genes, use the agent-base simulations and population dynamics to include the role of queen and workers such as patrol behaviour, ovary developments, colony maintenance, and so on. Notably, we investigated the evolution of altruistic social behaviour in workers over generations of colonies, which leads to behaviours such as worker policing, caring for the queen's brood and nest maintenance. The propagation of altruistic genes over generations could then be observed to study the stability of the genes under the optimisation of inclusive fitness functions [173] [174] [110].

3.2 Methods

Table 3.1. Variables and Constants

Variable Symbol	Variable Name
x	Constant in Eq.3.1b
t_t	Time since queen contact
T_{W_i}	Uncertainty
M_i	Memory of worker i
I_{W_i}	Internal state of worker i
t_{r_i}	Rest time of worker i
t_{a_i}	Active time of worker i
$t_{r_{max}}$	Maximum rest time
$t_{a_{max}}$	Maximum active time
ρ	Genetic predisposition towards altruism
s_i	Social strategy of worker i
x	Constant in Eq.3.7
I_g	Internal state of the queen
t_r	Rest time of the queen
t_a	Active time of the queen
σ	Breeding capacity
Z	Eggs which grow into adulthood per unit work
q	Relative fertility of the queen
W	Number of workers
V	Modulating variable determining when workers lay eggs
m_i	Worker derived offspring
m_j	Offspring derived from other workers
V_A^B	Kin value
φ^{W_i}	Worker inclusive fitness
φ^Q	Queen inclusive fitness
k	Queen effective paternity
ρ_i^{Mut}	Mutated genetic predisposition towards altruism
S_i	Probability of adding or taking away from the gene
r_i	Mutation range
a_i	Mutation step
T	Final simulation timestep

3.2.1 Simulation Design

Exploring the Phenomenon of Altruism

For the current work, we introduced a variable representing the genetic predisposition, ρ, towards altruism. The genetic predisposition for altruistic behaviour affects time spent on social/selfish behaviour. Workers were assigned a genetic trait ρ where $0 \leq \rho \leq 1$. If $\rho = 0$, the worker is entirely selfish while if $\rho = 1$, the worker is completely altruistic. In this case, selfishness means the worker forgoes nestmate responsibility so it can reproduce for itself. On the other hand, perfect altruism means the worker spends all their active time on social behaviours (nest maintenance, worker policing, etc.) and does not reproduce. The genetic predisposition towards altruism is shared between every worker in a colony and uniformly distributed between the initial colonies generated at the beginning of the simulation, in the range [0,1].

We integrated this along with equations from previous work by [110] to form an evolutionary agent-based simulation. [110] used dynamic game analysis to show how workers could maximise their inclusive fitness by changing their social strategy. The inclusive fitness function was utilised to optimise the social strategy of the queen and workers during the calculation process [110]. Their work included calculating the marginal value of new workers, representing the indirect future benefit of producing new workers. The model by [110] also allowed the individuals to know the colony's lifespan, and so the marginal value of new workers decreases until the end of the colony's lifespan.

In our study, however, we used the inclusive fitness function as an evaluator to select colonies with the highest inclusive fitness. This high inclusive fitness results from the cumulative effects of the mutual interaction between agents in the colony that are influenced by their genetic predisposition. Our study is driven by social and genetic factors that determine the selection of colonies based on their inclusive fitness. The model does not require the specificity of particular altruistic genes that code for altruistic behaviour. Altruism could be due to specific genes or the cumulative minor effects of many genes seen in complex traits such as intelligence [180]. It's important to note that ρ does not necessarily represent a gene "for" altruistic behaviour. Like there are no genes "for" each part of the body, as argued by [181], an organism's genetics mediate the development of the body's features. In this context, ρ represents a cumulative genetic propensity towards altruistic behaviour. It can be viewed as a variable that describes the continuity between the two evolutionary strategies of selfishness and altruism, with each colony having a different balance.

While the work of [110] included terms which consider the proportion of males and females, there is no explicit inclusion of males in this simulation, so those terms were excluded from the equations to follow. This was done as this study is interested in the development of altruistic behaviour in workers, who are female, and does not directly deal with the conflict between the queen and workers in terms of male parentage. This is rolled into the dynamics of the interactions between the queen and workers and how that affects their behaviour both over time and over the generations. We believe the consequences of this exclusion to be minor due to the limited role of males with regard to nest maintenance and brood care. The terms introduced in this chapter are necessary when investigating the benefit to the worker. Analysing this through the lens of inclusive fitness requires a genetic component that can be optimised to increase the inclusive fitness of workers to the maximal extent. This can then be used to determine what type of behaviour should be expected from workers to maximise their inclusive fitness. The model in this chapter is an extension of what was presented in Chapter 2, designed to keep the aspect of the previous work which showed the effect of the queen's patrol behaviour on worker reproduction but now includes more dynamic behaviour for workers, whose behaviour changes based on social interactions.

Internal state

Here, we used the population dynamics to allow the agent-based simulations to consider the workers' social strategy. The internal state of the worker determines the social strategy. The internal state of a worker represents its ovary development. The internal state of the worker is defined as:

$$I_{W_i}(t+1) = (1-\beta) \cdot I_{W_i}(t) + \gamma + M_i(t) - (\kappa \cdot \delta(\overrightarrow{x_g} - \overrightarrow{x_{W_i}}) \cdot I_{W_i}(t)) \tag{3.1}$$

where time is discrete and $I_{W_i}(t)$ is the internal state of worker i. β and γ are constants and $M_i(t)$ is the memory of the worker. This formulation takes into account multiple components. First, without the queen's contact, workers increase their internal state over time. Constants β and γ determine the rate at which the internal state of the worker develops. The constants were chosen to reflect a rate similar to observations made in previous work [72].

Second, after being recently contacted by the queen, workers exercise restraint and do not increase their internal state for some time. This means workers have a sense of memory concerning the queen's presence. Ants have been shown to possess some capacity for learning and cognition [41], so including a variable that represents this is not unfounded. This is reflected in the parameter $M_i(t)$. $M_i(t)$ represents the memory of the worker and is defined as:

$$M_i(t) = \frac{I_{W_i}(t) \cdot \beta - \gamma}{T_{W_i}(t)} \tag{3.1a}$$

where $T_{W_i}(t)$ determines how long it takes until the worker forgets the queen's presence. This effectively cancels out the terms $(1-\beta)\cdot I_{W_i}(t)+\gamma$ in Eq.3.1 to give a delay before the internal state of the worker begins to increase. Previous work [182] has explored the role of memory in the foraging *Drosophila* and defined memory loss as increasing uncertainty. Here, the uncertainty relates to the queen's presence i.e. if the queen is still present in the colony. If enough time has passed the queen's presence is forgotten and the worker begins to develop ovaries. This uncertainty is captured in $T_{W_i}(t)$ and is defined as:

$$T_{W_i}(t) = 1 + e^{-x+t_t} \tag{3.1b}$$

where t_t is the elapsed time since the previous contact with the queen. x is a sufficiently large constant such that at time $t_t = 0, e^{-x+t_t}$ is small. The internal state of the worker, with memory, means when the queen has recently contacted the worker, there is a delay before the worker increases their internal state. This reflects the restraint shown while the worker is aware of the queen. The delay can be adjusted by adjusting the constant x in Eq.3.1b.

The final component is the interaction effects with the queen. When workers interact with the queen, the queen suppresses their ovary development. The variable κ is a constant representing the effectiveness of suppressing the internal state of the worker by the queen. In this case, $\kappa = 0.9009$. This leads to a 90% decrease in the worker's internal state, making the queen's suppression highly effective individually. $\overrightarrow{x_g}$ and $\overrightarrow{x_{W_i}}$ are the positions of the queen and worker i respectively. $\delta(r)$ denotes Kronecker's delta function, i.e. its value is zero except when the distance between $\overrightarrow{x_g}$ and $\overrightarrow{x_{W_i}}$ is zero, then $\delta(r) = 1$. This means that when the queen and worker are close together, an interaction takes place, which decreases the internal state of the worker, thereby suppressing their reproductive capability.

In the simulation, workers in each colony were initialised with internal states uniformly distributed in the range [0,1]. Contact between the queen and the worker is only established when their distance is within a specific range. This reflects the physical communication, which would typically be done by extending the antennae between two ants.

For the queen, the internal state represents the queen's likelihood of becoming active. The queen patrols the colony when active. This means the queen will patrol more when her internal state is high; when her internal state is low, the queen will patrol less. The internal state of the queen is affected by her interaction with workers in the colony. The internal state of the queen is determined by:

$$I_g(t+1) = (1-\epsilon) \cdot I_g(t) + (\alpha \cdot \delta(\overrightarrow{x_g} - \overrightarrow{x_{W_i}}) \cdot I_{W_i}(t)) \tag{3.2}$$

where ϵ and α are constants (0.01 and 0.1 respectively). The internal state of the queen generally decreases with no contact with workers, meaning the queen can spend more time being inactive. When the queen is inactive, she lays more eggs and produces more workers as necessary. This is captured in the damping factor $(1-\epsilon) \cdot I_g(t)$. When contact with workers is established, the queen's internal state increases and the queen becomes more active in patrolling the colony and suppressing the development of the worker's internal state. This is proportional to the level of development sensed by the queen from the worker. So, if the queen encountered a worker with a high internal state, that would make the queen more active than encountering a worker with a low internal state. This is captured in the delta function, taking into account the positions of the queen ($\overrightarrow{x_g}$) and worker ($\overrightarrow{x_{W_i}}$). α acts as the sensitivity parameter for the queen to the internal state of the worker.

Active and Inactive time

Previous work by [134] has shown the rate of interactions can be a way for workers to regulate foraging behaviour. Here, rather than the interaction rate, the interaction itself regulates worker activity, with the inactive time and active time being linked to the internal state, $I_{W_i}(t)$, of the worker.

The inactive time is defined as:

$$t_{r_i}(t) = t_{r_{max}} \cdot e^{-\delta \cdot I_{W_i}(t)} \tag{3.3}$$

and the active time:

$$t_{a_i}(t) = t_{a_{max}} - t_{a_{max}} \cdot e^{-\delta \cdot I_{W_i}(t)} \tag{3.4}$$

where δ is a constant.

$t_{r_{max}}$ and $t_{a_{max}}$ are constants that determine the maximum time spent being inactive and active, respectively. This formulation means that as the internal state of the worker increases, the inactive time decreases. On the other hand, when the internal state of the worker increases, the active time also increases. When the worker is active, the worker will move around the nest and engage in various activities. These include both altruistic and selfish behaviours, such as nest maintenance and taking care of the worker's offspring respectively. When the worker is inactive the worker is in a state of rest. While the worker's internal state affects the worker's activity cycle, it also influences worker offspring production. As the queen can influence the internal state of the worker, this means the activity cycle of the queen is intertwined with the activity cycle of the worker and, therefore worker offspring production.

For the queen, the inactive and active time is calculated similarly to the workers' and is linked to the queen's internal state.

Inactive time:

$$t_r(t) = t_{r_{max}} \cdot e^{-\delta \cdot I_g(t)} \tag{3.5}$$

Active time:

$$t_a(t) = t_{a_{max}} - t_{a_{max}} \cdot e^{-\delta \cdot I_g(t)} \tag{3.6}$$

$I_g(t)$ is the internal state of the queen. During her active time, the queen patrols the colony to suppress the internal state of workers. The queen's inactive/rest time is spent laying eggs, which are reared to maturity by the workers in the colony.

Social strategy of the workers

With the internal state of the workers now established, we integrate this into the social strategy of the workers. Each worker had a social strategy that determined the resources the worker invested towards social tasks in the colony. As workers' internal state develops and they get closer to laying their eggs, they will change their social strategy to be less social. This is because, as the worker gets closer to laying its eggs, it will become more selfish and want to take care of its offspring. Therefore, the worker social strategy is determined as follows:

$$s_i(t) = \frac{1}{1 + e^{-x(1-2 \cdot I_{W_i}(t))}} \tag{3.7}$$

where x is a constant (unrelated to x in Eq.3.1b). x is greater than zero and is sufficiently large such that $\frac{1}{1+e^{-x}} = 1$ when $I_{W_i}(t) = 0$. When the worker has a low internal state it will be very social, while a high internal state will lead it to be antisocial. This leads to the next section on population dynamics.

Population dynamics

The population dynamics determine how the colony changes over the course of the simulation and the way the interactions between the queen and the workers affect the production of new workers and worker-derived offspring. This takes into account the social strategy of workers and the genetic predisposition of the workers. The genetic predisposition is introduced when determining the rate of worker production and worker-derived offspring. This is because it allows us to link the genetic predisposition more directly to the production of related individuals who have an inclusive fitness benefit to the workers. Higher predilection towards altruism should lead to a higher rate of worker production, while more selfish genetic predispositions should lead to a higher rate of worker-derived offspring.

Breeding Capacity

The breeding capacity is the number of new workers that could be added to the colony. It is affected by the amount of effort (social strategy) workers in the colony use towards social activity, in this case, brood care. This means the breeding capacity can be determined as:

$$\sigma(t) = Z \cdot \sum_{i} s_i(t) \tag{3.8}$$

where Z is a constant that represents the number of eggs that grow into adulthood per unit of work. $s_i(t)$ is the social strategy of worker i. If all workers are 100% social and work towards the good of the colony, the number of new workers the queen could produce is equal to $Z \cdot Numberofworkers$.

Worker production by the queen (queen derived offspring)

Taking all of this into account, the rate of workers produced is determined as:

$$\frac{dW}{dt} = (\frac{q}{C}\sigma(t)) \cdot \rho \cdot s(t) \tag{3.9}$$

where $\sigma(t)$ is the breeding capacity and C is a normalisation constant. ρ represents the mean genetic predisposition of workers in the colony and $s(t)$ represents the average social strategy of workers in the colony. This means $\rho \cdot s(t)$ is the proportion of resources used to produce more workers. Higher levels of altruism and more social behaviour in the colony led to the production of more workers. q is the relative fertility of the queen. q is relative to that of a worker ovipositing at her maximum speed.

ρ affects the population dynamics and worker policing behaviour in workers. It also affects their selfish behaviour in terms of their rate of egg production. This effect can be seen in the extreme cases of $\rho = 0$ and ρ =1. When $\rho = 0$, the workers are selfish, meaning the equation of the production of new workers is zero (Eq.3.9). Hence, there is no colony growth at this level. Meaning the output of worker offspring is determined solely by social interactions. When $\rho = 1$, the workers are non-selfish, and the pattern is reversed, meaning the production of new workers is determined solely by social interactions. Workers, in this situation, do not produce their offspring. Note that worker offspring do not contribute to the colony size because workers can only produce males. As workers (who are female) carry out the tasks inside and outside the nest, worker offspring do not contribute to the production of new workers.

Worker derived offspring

When workers are not 100% social the breeding capacity is split between the workers and the queen. This means the rate of worker i derived offspring can be determined as:

$$\frac{dm_i}{dt} = V(t) \cdot \frac{(1 - s_i(t)) \cdot (1 - \rho_i) \cdot (1 - s_{-i}(t) \cdot \rho_{-i})}{C} \sigma \tag{3.10}$$

If s_i represents the social strategy of the worker, where a larger s_i means more time spent on social behaviour, $(1 - s_i(t))$ is the time spent on selfish behaviour. Further, if ρ represents the genetic predisposition towards altruism, $(1 - \rho_i)$ represents the genetic predisposition towards selfishness. Here, we use the notation of $-i$ to indicate a complement of i. Therefore, $(1 - s_{-i}(t) \cdot \rho_{-i})$ represents the gap in the social behaviour (in particular worker policing) of all other workers in the colony. Meaning if workers are both perfectly social and perfectly altruistic, the amount of worker policing would be perfectly applied to all workers, and no worker would reproduce.

Within a generation, 1-ρ represents the selfishness of a worker. This selfishness concerns how much resources it is inclined to put towards its offspring. The social interactions in the agent-based simulation mitigate this. Within a given agent-based simulation, 1-ρ is a constant since the genetic predispositions of the workers do not change. Only the social strategy, s, changes per the social interactions. In essence, ρ is the innate tendency of a worker, determined by its genetic predisposition. Meanwhile, the social strategy, s, is determined by social interactions in the colony. Both contribute to the production of new workers and worker-derived offspring, which drives the queen's and workers' inclusive fitness functions. $V(t)$ is introduced as a modulating variable that links the worker's internal state to the amount of worker offspring that is produced. The modulating variable is defined as:

$$V(t) = \frac{1}{1 + e^{-c_u(t) + c_d(t)}} \tag{3.11}$$

where

$$c_u(t) = const - const \cdot e^{-\delta \cdot I_{W_i}(t)} \tag{3.11a}$$

and

$$c_d(t) = const \cdot e^{-\delta \cdot I_{W_i}(t)} \tag{3.11b}$$

$const$ is a constant. $c_u(t)$ and $c_d(t)$ are used to ensure that workers will only begin to produce offspring when their internal state is very high or close to/equal to 1. This reflects the worker having fully developed ovaries and beginning to lay eggs.

The genetic predisposition trait is implemented in the population dynamics of the simulation. This is because the effects of the behavioural changes which would occur due to differences in the genes of workers, in relation to fitness, would occur at the population level. Worker production is determined by how much work is put into the raising of the eggs laid by the queen. The genetic predisposition towards altruism affects worker's willingness to take part in this social behaviour as well as their social strategy. The same is true for worker-derived offspring. When workers have a high internal state their social strategy and genetic predisposition will determine whether they lay eggs as well as the social strategy and genetic predisposition of other workers in the colony. A worker with a high internal state in a selfish colony will lay eggs because they are more selfish and other workers do not police their reproduction. Having the genetic component implemented at the level of, say, the internal state of the worker, would lead to the same effect. This would be more in line biologically, however, this would add the additional effect of having different rates of internal state development for selfish and selfless workers. Here we assume that all workers have the same rate of internal state development. Taken together, the internal state of the queen and workers drive their behaviour in the agent-based simulation in conjunction with the genetic predisposition.

Inclusive Fitness of the Workers and the Queen

To test the stability of the genetic predisposition (balance between selfish and altruistic), we used the inclusive fitness function as an optimisation function in a genetic algorithm (GA). The genetic algorithm determines which genetic predisposition is the fittest, i.e. what level of altruism has the highest inclusive fitness. Colonies compete against each other as all workers have the same genetic predisposition. The current variable ρ, representing the genetic predisposition towards altruism, encapsulates four of the points in the criteria listed previously by [179]. These include satisfying Hamilton's rule, being environmentally sensitive, a dependence on kin recognition and the partial additive nature of the genes. The genetic predisposition reflects innate tendencies toward altruistic social behaviours such as nest maintenance, taking care of the queen's brood and refraining from developing ovaries in the queen's presence. The higher the value assigned to ρ, the more time is spent on those behaviours and less time spent laying eggs. Altruism increases inclusive fitness with the production of more workers. Selfishness increases inclusive fitness with the production of more worker offspring.

The inclusive fitness function assesses the inclusive fitness of each genetic predisposition, ρ. The pattern for the average genetic predisposition and the inclusive fitness is similar due to this link. The simulation's genetic algorithm stage optimises ρ to maximise the workers' inclusive fitness function. With this integration of the agent-based simulation and the genetic algorithm, we can investigate genetic influences on the behaviour of workers and the queen and how stable the altruistic gene is over the generations while optimising the inclusive fitness function. Modifying the equations from [110] we defined the inclusive of the workers and the queen as:

$$\varphi^{W_i} = V_{W_j}^{W_i} W_j(T) + V_{m_i}^{W_i} m_i(T) + \sum_j V_{m_j}^{W_i} m_j(T) \tag{3.12}$$

$$\varphi^{Q} = V_{W_j}^{Q} W_j(T) + \sum_j V_{m_j}^{Q} m_j(T) \tag{3.13}$$

where φ^{W_i} and φ^{Q} represent the inclusive fitness of workers and the queen respectively. V_A^B is a kin value which describes the inclusive fitness return to per capita production of caste A for the individual B [110]. $W_j(T)$ is the number of workers produced up to time T in the simulation where T represents the end time of the simulation. $m_i(T)$ is the number of worker i derived offspring. $m_j(T)$ is the number of offspring derived from other workers. While workers produced by the queen and worker-derived offspring both contribute to the inclusive fitness of workers and the queen, there is tension between them as the increase in the rate of production of one leads to a decrease in the rate of production of the other due to the allocation of resources by workers. To maximise inclusive fitness, given some parameters, what amount of altruism vs. selfishness is needed to get the maximum inclusive fitness return? The higher the inclusive fitness, the more effectively genes have been passed on to future generations. As the range for the genetic predisposition is [0, 1], if the average increases above 0.5, it can be said that a colony is more altruistic. Otherwise, the colony is more selfish.

Differences in genetic predisposition are accounted for when calculating the inclusive fitness of the individuals (the individual worker or the queen). It captures the success of workers with different genetic predispositions to produce related individuals. Selfish or selfless genes in workers influence behaviour patterns such as the production of their eggs and contributing to the maintenance of the colony. On the other hand, the mutual interactions between the queen and workers change the breeding capacity of the colony.

3.2.2 Simulation Overview

Agent-based Simulation

The simulation begins by forming several colonies which make up the initial generation. The difference in these colonies is the worker's genetic predisposition towards altruistic behaviour, ρ. ρ determines how innately selfish or selfless a worker is. All workers in a colony have the same genetic predisposition. In this context, selfishness refers to behaviours which primarily benefit the individual at the cost of another. This includes not taking care of the queen's brood and laying eggs.

In contrast, selflessness or altruism are behaviours that do not immediately benefit and may negatively impact the individual but benefit others. This includes spending time on nest maintenance but not laying one's eggs. When a colony is initially formed at time $t = 0$, there is a single queen with a small population of workers $N = 20$. These workers have an internal state, I_{W_i}. The internal state of the workers is defined as the hypothetical physiological condition, such as the hormone level, which controls the ovary development in workers and queen pheromone secretion in the queen.

Three main factors determine the rate of production of new workers. These are the relative fertility of the queen (q), the breeding capacity (σ) and $\rho \cdot s(t)$, which is the proportion of resources allocated to produce more workers. The workers determine the balance of resources allocated to produce more workers as they are the ones who take care of the brood and ensure their survival and maturation. The feedback loop controls the social strategy as the interactions with the queen make workers more social to support the colony members by suppressing their internal states. The feedback loop influences the population dynamics of the agent-based simulation by causing changes in workers' internal states, which affect the social strategy of workers. This then affects the breeding capacity, directly affecting the production rate of new workers and worker-derived offspring. These are also affected by the genetic predisposition of the workers in the colony. A worker's social strategy and genetic predisposition toward altruistic behaviour determine how much time they will spend assisting in producing new workers.

On the other hand, the production rate of worker offspring is determined by the relative absence of social behaviour and altruistic genes and the absence of these in other workers (Eq.3.10). Workers who have a selfish social strategy and selfish genes produce more offspring. Though the behaviour of other workers limits this as policing is not restricted to just the queen, workers also police each other.

As there is a trade-off between policing other workers and laying eggs in workers, more selfish workers will produce more offspring if other workers are also selfish. An internal trade-off exists between patrolling and laying eggs for the queen. The time spent patrolling may increase the social behaviour of workers by suppressing their internal state. Still, it is also time that the queen could have spent laying eggs that would become new workers. The mutual interactions between the queen and workers determine the balance between selfish and altruistic behaviour. The agent-based simulation can present these delicate balances in their natural life, which runs from time $t = 0$ to time T, representing the end of the agent-based simulation.

A crucial factor in enhancing the fitness function lies in the efficacy of the individual in transmitting their genetic material to subsequent generations. Either directly via their offspring or indirectly via the queen's offspring, the inclusive fitness of the queen is determined by the number of workers and the number of worker offspring (to a lesser extent) at the end of the agent-based simulation (time T). In comparison, the number of workers determines the inclusive fitness of workers, i.e., the number of their offspring directly produced and the number of offspring produced by other workers.

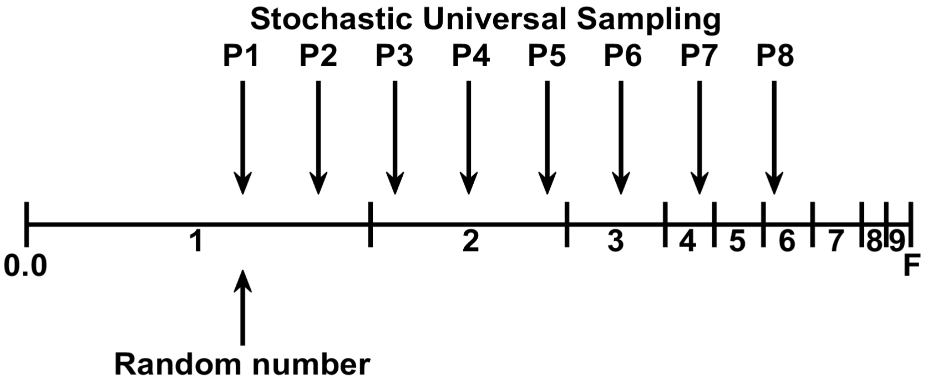


Fig 3.1. Stochastic universal sampling Stochastic universal sampling is used to select the colonies that will be used to generate the next generation of colonies. Each colony is assigned a fitness score equal to the average fitness of workers. The scale represents the total fitness of the population, i.e. all of the colonies, where F is the total fitness. Pointers are placed at equal distances from each other, with the first pointer being placed randomly between zero and $\frac{1}{N}$ where N is the number of pointers. In this case, the number of pointers indicated the number of colonies which were selected to produce more colonies

Genetic Algorithm

The method for choosing these colonies is stochastic universal sampling (SUS). This was chosen because, unlike elitism-prone methods like roulette wheel selection, SUS mitigates bias against individuals with lower fitness scores and minimises the risk of premature convergence, where the population quickly stagnates due to repeated selection of the fittest individuals [183]. Stochastic universal sampling (shown in Fig.3.1) works by first choosing a random number in the range $0 \leq P1 \leq \frac{1}{N}$. N represents the number of pointers (number of colonies which will produce future colonies). $\frac{1}{N}$ represents the distance between the pointers. Each colony is given space on a line proportional to its fitness. From Fig.3.1, we see nine colonies and eight pointers. Colony 1 has two pointers, Colony 2 has three pointers and so on. Two future colonies will initially have genes from Colony 1, three from Colony 2 and one from Colony 3, 4 and 6. Once the number of future colonies each present colony will produce has been found, the genes are transferred from the present colony to the corresponding future colonies.

There is a 50% chance a mutation will occur. This mutation can either be small or large, making future colonies more or less altruistic. Once this process is finished, new colonies are formed with an agent-based simulation as described above and the process repeats. For this thesis's results, 11 colonies and five pointers were used unless stated otherwise. The colonies chosen by the pointers produce two colonies in the next generation. For the number of colonies to remain constant, the fittest colony produces an extra colony to keep the number of colonies at 11. The number of iterations was 30 generations. This can be summarised as follows:

1. Regulation Mechanisms $\Longrightarrow$ Social Strategy
2. Social Strategy + ρ = Population Dynamics
3. Fitness Function = Cumulative population dynamics effects
4. Colonies with higher fitness functions are selected, and some are mutated
5. Go back to 1

3.2.3 Adjusted variables

Here, we discuss the different parameters of the simulation which could have an effect on the propagation of ρ based on a prediction of how the model behaves. In this current simulation, these can be categorised at two levels: the agent-based simulation level and the genetic algorithm level. At the lower agent-based simulation level, this refers to variables such as the rate of worker internal state development, amount of worker memory, effective paternity of the queen, the effectiveness of queen suppression and breeding capacity. The effective paternity of the queen is the number of males she has mated with. At the higher genetic algorithm level, this refers to variables such as colony selection, mutation and the initial distribution of genes in the population. We will first discuss the way the agent-based simulation variables could affect the results then discuss the genetic algorithm effects.

The way the worker's internal state develops in the agent-based simulation is the most basic way to influence the results of the simulation (Fig.3.2). The rate at which worker internal states develop determines how quickly workers begin to reproduce. Given a faster or slower rate of reproduction, this could lead to different end states of the simulation i.e. whether colonies become more altruistic after 30 generations. Faster development would lead to more worker offspring whereas slower development would lead to less worker offspring. Different development rates could be given in line with the genetic predisposition towards altruism. So more selfish workers may increase their internal state faster than more selfless workers. Worker memory is also something that could affect the results of the simulation. Workers exercise restraint in the presence of the queen. When workers are aware of the queen they do not increase their internal state. As time passes this fades and they begin to increase their internal state. This effectively acts as a delay to the development of the worker's internal state.

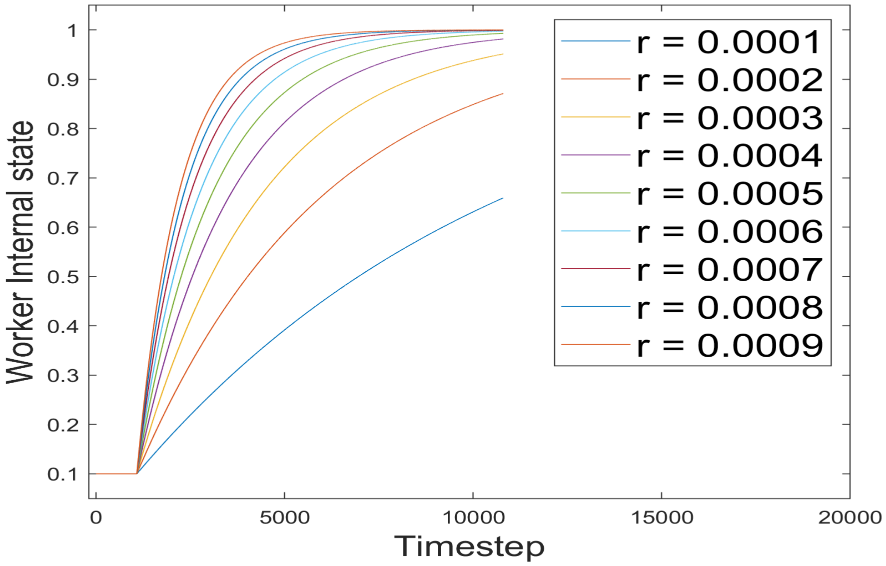


Fig 3.2. Varying rates of internal state development obtained by running a numerical simulation of the worker's internal state with varying rates, r, where r is the value of γ and β in Eq.3.1. Varying rates of internal state development could affect the effectiveness of the queen's patrol and the number of offspring workers can produce. Slower internal state development means the queen can more easily manage the internal state of the workers and the number of offspring workers produce

Changing the delay will also affect the development rate of workers. As with the internal state development rate, more selfish workers could have shorter or no delay when contacted by the queen as opposed to more selfless workers with longer delays.

The effectiveness of queen suppression is achieved through physical contact between the queen and the worker (possibly through the transfer of cuticular methyl alkanes [184]). The effectiveness of queen suppression in this thesis is assumed to be constant in its effects on all workers (in this case worker's internal state is decreased by 90%). This choice implicitly assumes workers are equally affected by contact with the queen.

The effective paternity of the queen changes the fitness return to workers of other workers. We assume (initially) a singly mated queen, resulting in an effective paternity close to one. The per capita fitness return to worker W_i for the production of another worker is determined as:

$$V_{W_j}^{W_i} = 0.25 + \frac{0.5}{k} \tag{3.14}$$

where k is the effective paternity of the queen. This is adapted from the work of [110]. As the effective paternity of the queen increases, there should be a decrease in the incentive (from a fitness perspective) to workers to produce other workers as there is a diminished return to producing new workers. However, in [110], they note the marginal return of a new worker may be more than a worker's child when the colony is small. Specifically, in the short term, it is better to assist in the creation of new workers. This is because, at this early stage of the colony, more workers mean more agents capable of doing work, which means more resources for the colony. This is also linked to the breeding capacity. A greater number of workers means there is a greater breeding capacity. When workers are perfectly social, the queen can produce the maximum amount of new workers but when they are not perfectly social the breeding capacity is split between the queen and more selfish workers.

On the genetic algorithm level, colony selection is a factor that can significantly affect the distribution of genes in the population. In this case, colony selection refers to the number of colonies allowed to reproduce (i.e. produce future colonies). When colony selection is high, there is a greater convergence in the gene pool as only the fittest colonies produce future colonies, leading to a lack of genetic diversity. If colony selection is too low, there is a lack of competition as more colonies pass on their genes to future generations.

While group selection is seen as controversial ([185], [186]), [185] and [187] argue that formulations of group selection can be seen as equivalent to kin selection. They explicitly propose ways previous group selection models could be converted to kin selection models. [185] through the application of inclusive fitness theory and [187] through the proposition of a ”K-G space”, which conceptualises kin and group selection as overlapping regions. The authors would argue that the formulation of this evolutionary agent-based simulation would be classed in this way. In our simulation, though selection takes place on the colony level, the colonies are selected based on the average inclusive fitness of the workers. The calculation of inclusive fitness considers the individual's direct benefit and assesses the colony's performance as a whole from a fitness perspective ([188]). In this way, the simulation allows us to analyse the evolution of altruistic behaviour in a species (Diacamma) where individuals (other than the queen), largely refrain from reproducing. [189] analysed microbial data by applying both group and kin selection. They found that together, they had greater explanatory power in revealing fitness outcomes that were not captured when only using one approach. This was done to show that the use of both group and kin selection is needed for useful applications of theory when interpreting experimental results. Our simulation offers flexibility as it can be adapted to combine both approaches.

Mutation is the factor which can introduce new genetic variants due to various factors such as errors during DNA replication and errors in recombination. Mutation has two aspects that can affect the individual: the mutation step and the mutation rate. The mutation step is the size of the variation that occurs when a mutation takes place, while the mutation rate refers to the probability of a mutation taking place. Over time, the accumulation of small genetic changes can lead to new traits which were not initially present. So, even in a perfectly altruistic environment, selfishness can come about. It may even thrive by taking advantage of its altruistic neighbours. Altruistic behaviour could also arise in a selfish environment, though the number of other altruistic individuals to cooperate with could affect the spread of the trait leading to this behaviour.

In this case, mutations were randomly created values that were added/subtracted to the genetic predisposition variable with some probability. This is to assist in the optimisation of the fitness function. The operator produces small mutation steps with a higher probability and large mutation steps with a lower probability. The work of [190] proposes a mutation operator for their Breeder Genetic Algorithm which is used here in a simplified form. The mutated form of the genetic predisposition ρ_i^{Mut} is determined by the equation:

$$\rho_i^{Mut} = \rho_i + S_i \cdot r_i \cdot a_i \tag{3.15}$$

where ρ_i is the genetic predisposition shared by workers in colony i before the mutation has occurred. The parameter S_i is the probability of increasing or decreasing ρ_i ($S_i = \pm 1$, randomly distributed). The parameter r_i is the mutation range. This represents the maximum value of the size of the mutation (mutation step). a_i is calculated as $2^{-u \cdot k}$ and is related to k, the mutation precision, where u is a random number in the range [0, 1]. The smallest relative mutation step-size is 2^{-k} (where $u = 1$) ,with the largest being $2^0 = 1$ (where $u = 0$). This means the mutation steps are created inside the interval $[r_i, r_i \cdot 2^{-k}]$. Whether or not ρ_i increases/decreases and by how much is determined by $S_i \cdot r_i \cdot a_i$. It simulates mutation in a way that allows small mutations to occur more frequently than large mutations. It also has an equal chance of increasing or decreasing ρ_i, meaning it's possible for more selfless or selfish genetic predisposition to arise given enough time.

The initial environment, the initial distribution of genes in a population can affect how a future population might look. If there is a uniform distribution of altruistic and selfish traits it is more likely altruistic behaviour will win out, as cooperation between individuals leads to better outcomes. If there is, however, an asymmetry in the number of selfish and selfless traits then this could impact how the traits spread through a population. An initial population of selfish individuals will find it more difficult for cooperation to thrive. A selfless population may allow some level of selfish behaviour from individuals as long as it does not affect their survivability.

The interaction between the two levels and the context of the simulation should be considered. Currently, workers in a colony were initialised with internal states uniformly distributed in the range [0,1]. All workers in a colony had the same genetic predisposition towards altruism which was uniformly distributed between the colonies in the range [0,1]. The agent-based simulation runs until time $T = 1080$. This was chosen as a nod to previous work by [72] which showed that workers could detect the absence of the queen after three hours. At this point, colony fitness was determined (using average worker fitness), and, using stochastic universal sampling, colonies were chosen to produce new colonies with a related genetic predisposition. For example, if a colony where $\rho = 0.4$ was chosen to produce two further colonies, the genetic predisposition of these colonies would be ρ_i^{Mut}. These new colonies would then start with one queen and 20 workers. For each of the variables that have been previously outlined, the simulation was run to test the effects of some variables deemed likely to have the most impact. As these effects can be systematically documented, they can be used to construct "scenarios". Scenarios involve implementing multiple parameter changes to view their cumulative effect on the results. This includes selfish workers having an increased rate for the development of their internal state, shorter delays before beginning to develop their internal state (showing less restraint) and having a lower suppression of their internal state when they were contacted by the queen.

3.3 Results

3.3.1 Conceptual Framework

[191] developed an agent-based simulation that showed how the coupling of the internal states between the queen and workers could explain the patrol behaviour of the queen. The internal state of the workers represents their ovary development. The internal state of the queen controls her activity cycle. By including the spatial aspect of the interactions between the queen and workers, [191] showed that the feedback loop formed from the coupling of the internal states drives the behaviour of the patrol behaviour of the queen. Real-time feedback allows the queen to control the internal state of the workers, especially when the colony is small.

The simulation can be broken down into two stages, seen in Fig.3.3 and Fig.3.4. The first is the agent-based section which involves the internal states of the queen and workers and the population dynamics. We assumed that the internal state of the workers and the queen would operate differently. For the worker, the internal state would represent their ovary development and increase over time. The queen could suppress this via direct contact. For the queen, the internal state would represent the probability of becoming active. That is, the queen will likely go from an inactive state to an active state, at which point she will begin to patrol the colony. The queen's internal state, I_g, steadily decreases (increasing her resting period) but increases when interacting with workers. This increase is proportional to the internal state of the contacted worker. A worker with a low internal state has minimal effect on the queen's internal state. However, a worker with a high internal state increases the queen's internal state and, therefore, her likelihood to begin patrolling the colony.

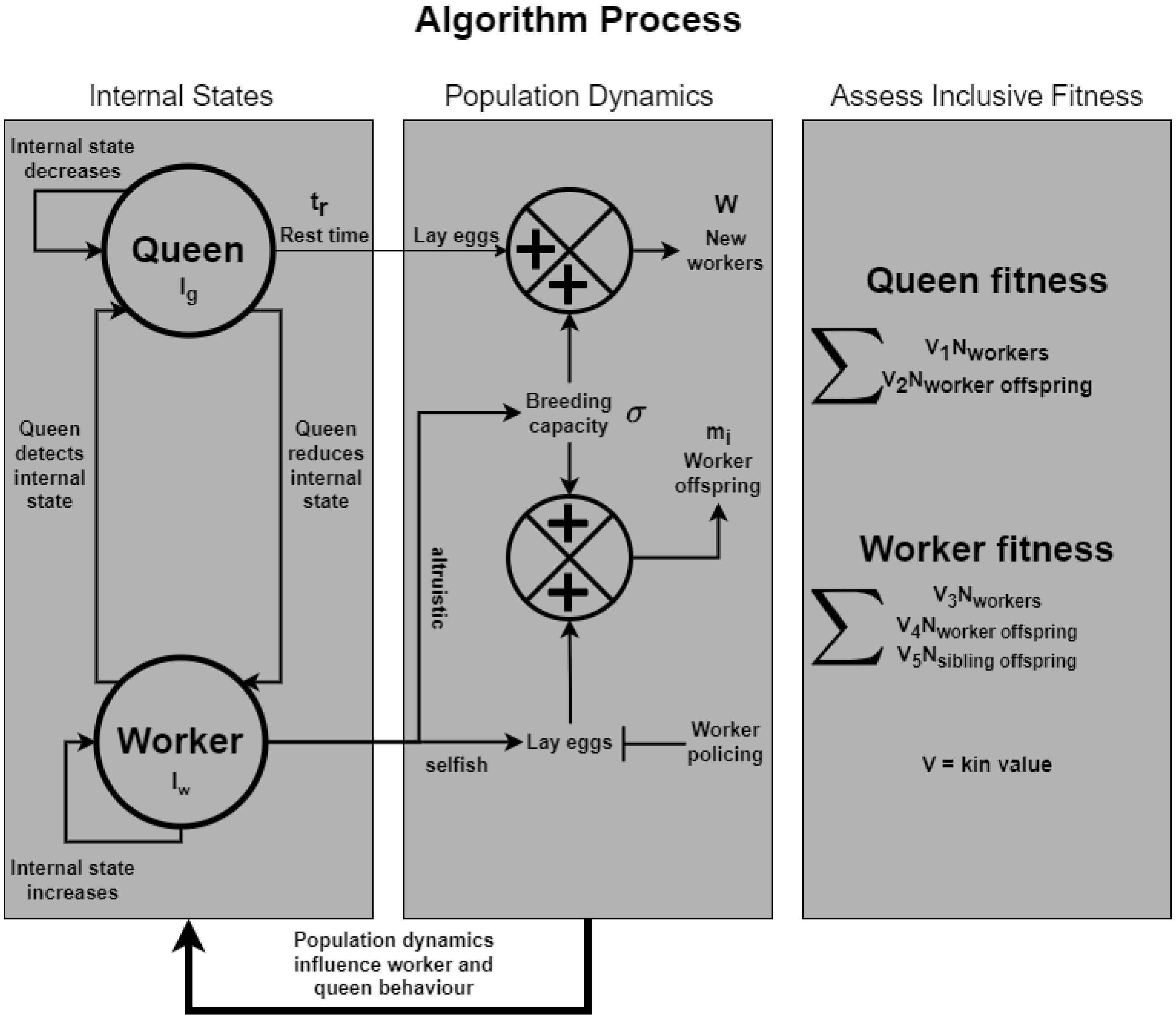


Fig 3.3. Algorithm Process This diagram visually represents the different aspects of the agent-based simulation. First, beginning with the feedback loop based on the coupling of the internal states of the worker and the queen. Mutual social interactions regulate the internal states of the queen by making her more active and patrolling the colony and the workers by suppressing their internal state, representing their ovary development. This leads to the population dynamics, which determine the production rate of new workers and worker-derived offspring, taking into account the resources dedicated by workers (the breeding capacity) into each type. Policing by other workers also suppresses the number of worker-derived offspring. The agent-based simulation consists of cycling between these two aspects. When the agent-based simulation ends at time T, the inclusive fitness of workers and the queen is assessed. The genetic algorithm used inclusive fitness as the basis for the selection of the colonies, which will propagate their genes into the next generation.

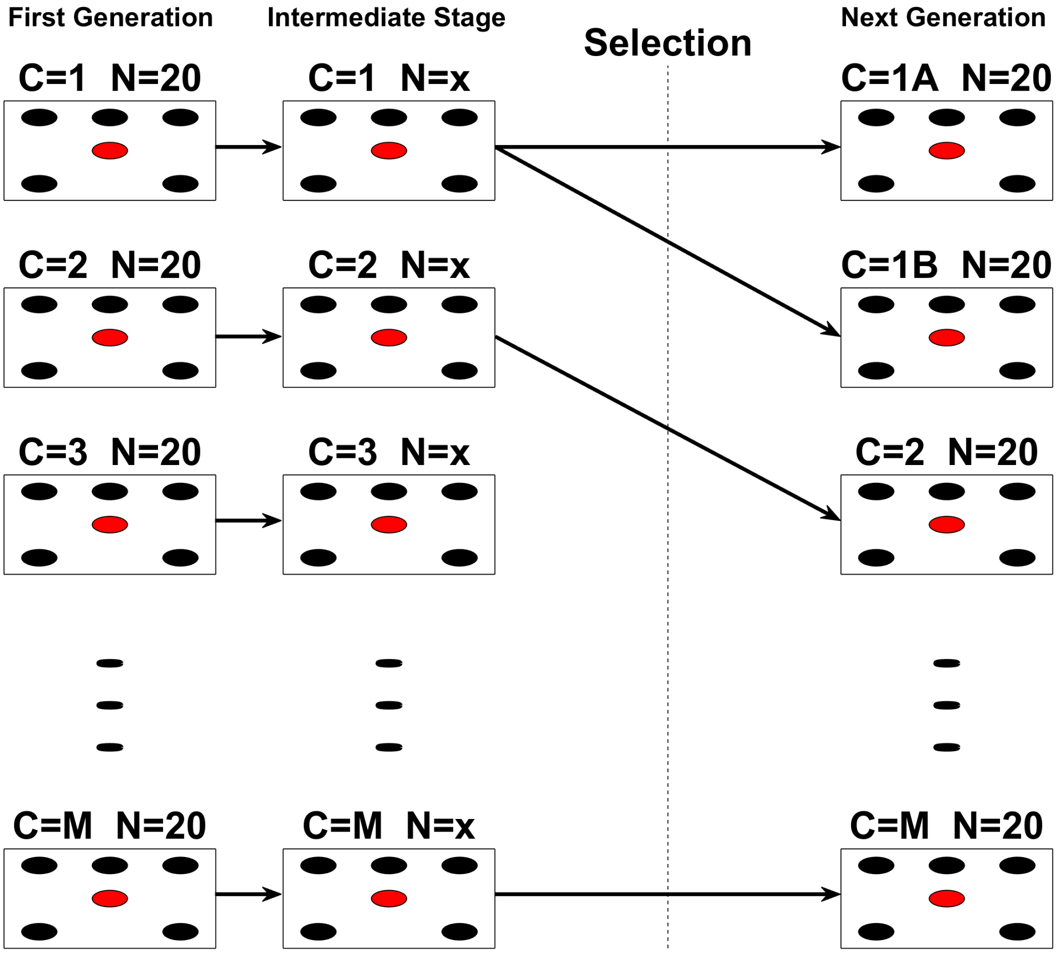


Fig 3.4. Evolutionary Agent-based simulation, Genetic Algorithm stage. Colonies are initialised with the same number of initial workers and each is an agent-based simulation which has the workers and the queen interact up to time T. At this point, the inclusive fitness of workers and the queen is calculated and the average fitness of the colony is used to select which colonies will produce the next generation. This is then repeated over 30 generations.

The worker's internal state can also affect a variety of social behaviours in workers. The social behaviours include their inactive time (t_{r_i}), spent resting, and active time (t_{a_i}), which workers can spend on selfish or selfless activities. Whether or not workers spend their active time on selfish or selfless actions is the social strategy (s_i) employed by workers, also affected by their internal state. The higher the internal state of a worker, the more likely they will be more active, spend active time engaging in selfish activities and lay more eggs. As the queen and workers can affect each other's internal state, this serves as a feedback loop where pairwise interactions can influence individual behaviour [64]. These interactions then change the population dynamics of the colony. We calculated the fitness functions for the queen and workers at the end of the agent-based simulation. Inclusive fitness refers to the measure of an individual's evolutionary success based on both direct reproductive success and the indirect impact on the reproductive success of related individuals.

The second stage of the simulation is the genetic algorithm. A genetic algorithm is an optimisation technique that uses principles of genetics and natural selection to evolve solutions to a problem over multiple generations. By using a genetic algorithm, we can look at how average genetic predispositions of the colonies change over the generations and how this affects their fitness. The range of the genetic predisposition toward altruistic behaviour is $[0, 1]$ where 0 is entirely selfish, and 1 is completely selfless. If being more altruistic is more beneficial from an inclusive fitness standpoint, the average genetic predisposition will be greater than 0.5. Otherwise, it will be less than 0.5. The average inclusive fitness of workers in a colony is calculated for each colony to determine this. The colonies with higher inclusive fitness were chosen. These selected colonies then make future colonies with genes resembling the parent colony. A mutation operator is used to alter the genes slightly from parent colony to child colony with some probability.

3.3.2 Results of the simulation

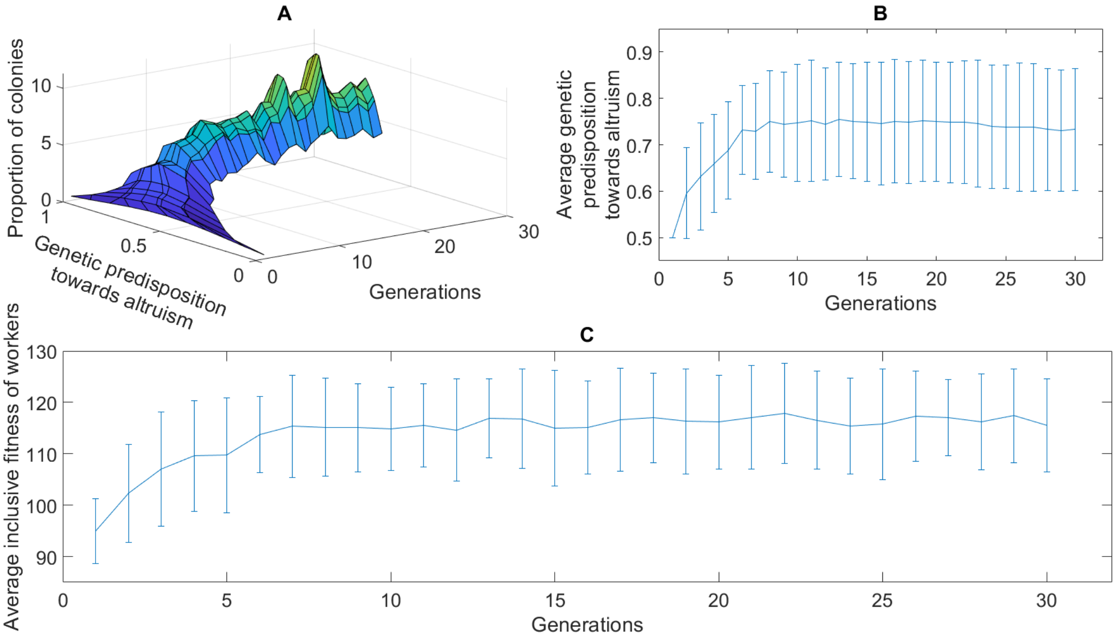


Fig 3.5. Rise of altruism A) The distribution of the genetic predisposition over the generations. There is a shift in the distribution of the genetic predisposition towards more altruistic behaviour. However, workers did not reach perfect altruistic behaviour. B) The average predisposition towards altruism shows an increase from the middle point of 0.5, stabilising around 0.75, showing an increase in the prevalence of altruistic genes over time. C) The average inclusive fitness over the generations. There is an increase in inclusive fitness from the first to 6^{th} generation, after which the inclusive fitness seems to stabilise, following a similar pattern seen in B. The error bars are the standard deviation.

Figure 3.5 shows an increase in the genetic predisposition towards altruistic behaviour over time. Inclusive fitness increased with the propagation of more altruistic genes. The average inclusive fitness converges, indicating that the colony's inclusive fitness benefits from altruistic behaviour. More altruistic workers help the queen produce more workers and engage in more social behaviours, increasing their inclusive fitness over more selfish workers. More selfish workers split the breeding capacity and, therefore, decrease the production rate of new workers. While these workers produce offspring, this does not give them a greater inclusive fitness benefit. Perfect altruism also does not give a more significant inclusive fitness benefit. Part of the explanation for the lack of perfect altruism involves the production of worker offspring. Worker offspring adds to the inclusive fitness of workers and the queen.

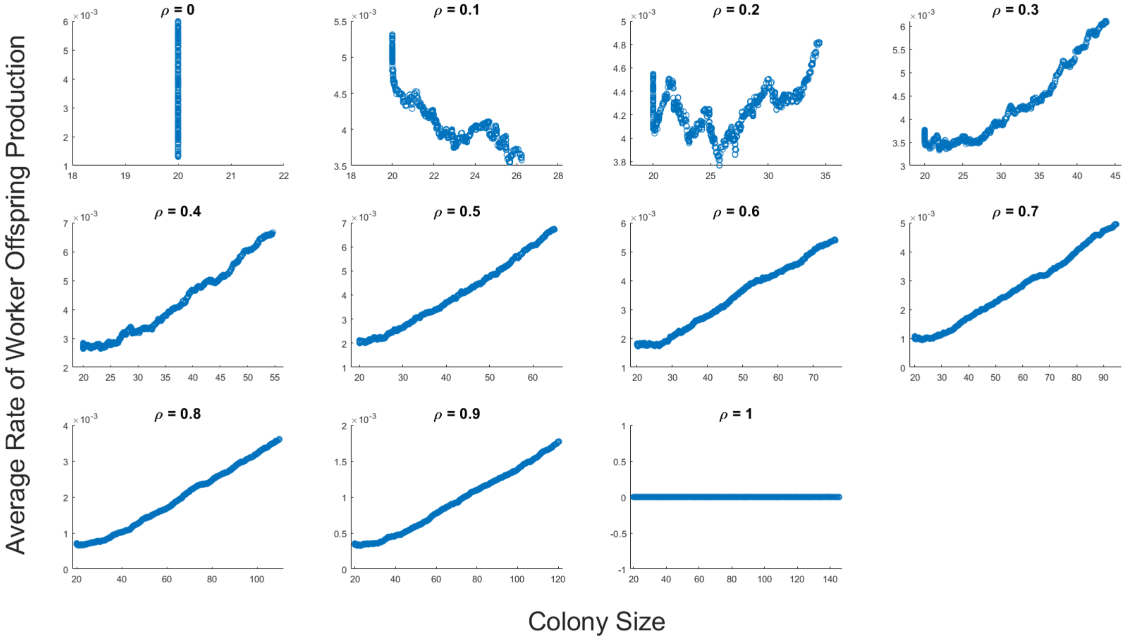


Fig 3.6. Rate of worker offspring production against colony size ρ represents the different worker predispositions ranging from 0 to 1. The colony size and worker offspring production evolve with time. The colony size of each genetic predisposition is different due to the differing rates of worker production in each condition. Larger colonies have a greater breeding capacity, which lends itself to greater worker offspring production. The extremes of $\rho = 0$ and $\rho = 1$ show drastically different behaviour due to the characteristics of these extremes. When $\rho = 0$, the workers are totally selfish and do not assist in colony work. This means the colony does not grow, and each fends for themselves. Conversely, when $\rho = 1$, workers do not produce offspring, irrespective of colony size, meaning they never have offspring. Barring the extremes, when $\rho = 0.1$, there is a decrease in the rate of worker offspring production. $\rho = 0.2$ shows more noisy fluctuations in the production rate with an increase in worker offspring production as the colony size reaches 30. However, it could be seen as an inflexion point where the effectiveness of the queen's patrol is mixed. $\rho = 0.3$ to $\rho = 0.9$ generally show similar trends, with increasing worker offspring production as the colony size increases.

Figure 3.6 shows the average rate of worker offspring production at various genetic predispositions (ρ). From $\rho = 0.3$ to $\rho = 0.9$, there is a linear increase in the rate of worker offspring production. The extremes of $\rho = 0$ and $\rho = 1$ are exceptions due to the characteristics of these extremes, i.e. workers at $\rho = 0$ do not help to produce more workers and workers at $\rho = 1$ never produce offspring. $\rho = 0.1$ and $\rho = 0.2$ differ slightly due to the smaller colony sizes.

In these smaller colonies, the queen can patrol the colony and stop workers from developing their ovaries [191]. However, in larger colonies, the queen is less able to control worker reproduction, leading to a linear increase in worker offspring over time. Perfect altruism hinders inclusive fitness because workers do not produce offspring. Workers maximise their inclusive fitness by assisting the queen at smaller colony sizes and producing more offspring at larger colony sizes.

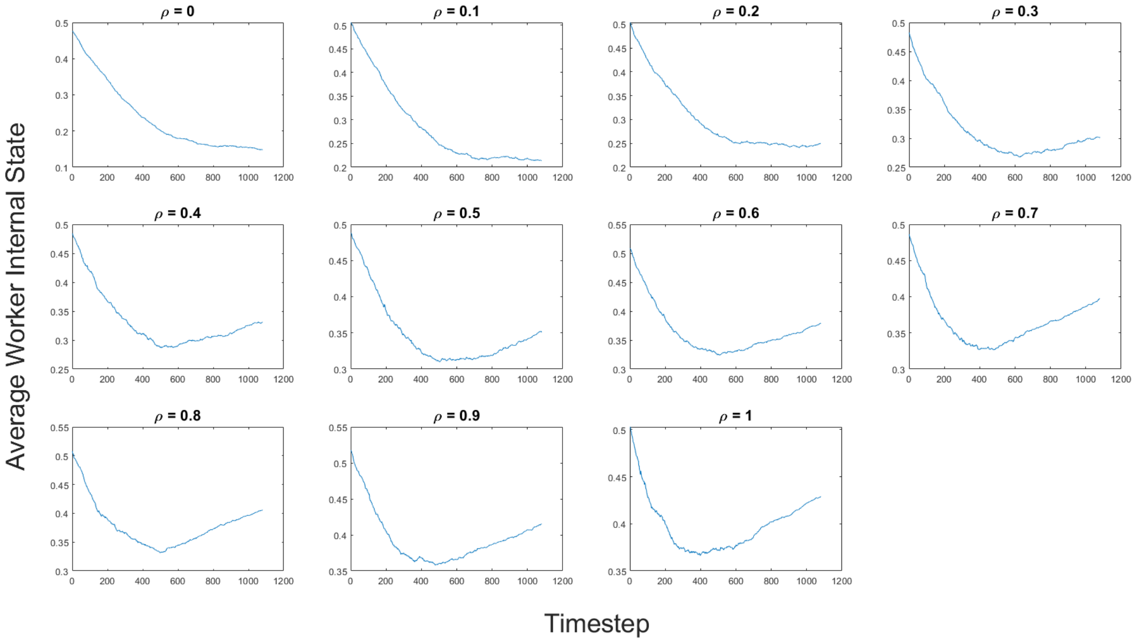


Fig 3.7. Average Internal state workers The internal state of the workers throughout the agent-based simulation in the first generation gives an insight into the effectiveness of the queen's patrol behaviour. This allows us to investigate colony size effects. Generally, there is a decrease in the internal state of workers for the first 600 time steps of the simulation. Then, the genetic predispositions (ρ) from $\rho = 0.3$ to $\rho = 1$ show an increase in the internal state of workers until the end of the simulation. There is an increase in the colony sizes with the genetic predispositions (shown in Fig.3.6). The increasing colony size means a decrease in the effectiveness of the queen's patrol, reflected in the increased internal state of workers.

Figure 3.7 shows the average internal state of workers throughout the simulation for various worker predispositions. There is a general decrease in the internal state of workers for the first half of the simulation. As the genetic predisposition increases, there is a more significant increase in the internal states of workers in the second half of the simulation. This increase is due to the increase in colony size, with the increase in worker internal states being greater in more altruistic colonies.

Increasing colony size causes the effectiveness of the queen's patrol to decrease as workers are contacted less frequently by the queen. More altruistic colonies have greater production of new workers due to the increased resources workers invest in the queen's eggs vs. their own. Though at larger colony sizes, workers can benefit from producing their offspring due to a general increase in the breeding capacity of the colony and a weakening of the enforcement of the reproductive division of labour.

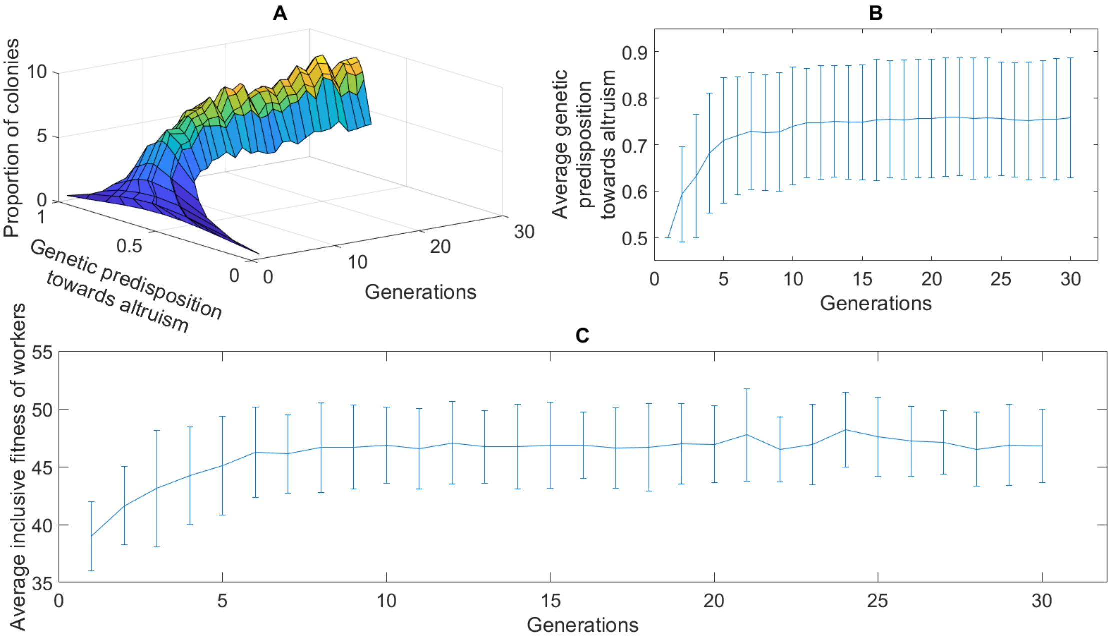


Fig 3.8. Altruism and worker relatedness The effective paternity of the queen affects the relatedness between workers. By increasing the effective paternity of the queen, workers become less related to each other. In an extreme example, we set the effective paternity of the queen to 10 (as opposed to 1 previously). Changing the effective paternity of the queen does not have a general effect on A) The distribution of the genetic predisposition over the generations and B) The average genetic predisposition over the generations. These appear to be similar to Fig.3.5. The main difference is in C) The average inclusive fitness over the generations. While the trend is the same, the value of the inclusive fitness of workers is less than half of what is seen in Fig.3.5C. This is due to the lower relatedness between workers, which means the per unit fitness return of new workers is much less from this perspective. The error bars are the standard deviation.

When looking at adjusted variables affecting inclusive fitness, the first point is the relatedness between workers. In this case, the effective paternity of the queen, k, determines the relatedness between workers. In Fig.3.5, the effective paternity of the queen is set to 1, meaning that the workers have a high relatedness. Figure 3.8 shows the genetic predisposition and inclusive fitness when the relatedness between the workers is lower ($k = 10$). There is still an increase in the genetic propensity towards altruism over the generations, despite a decrease in workers' per unit fitness return. Figure 3.8C shows that the inclusive fitness of workers is lower than in Fig.3.5C but still increases from the initial value. This means a lower relatedness between workers does not necessarily lead to more selfish behaviour from workers.

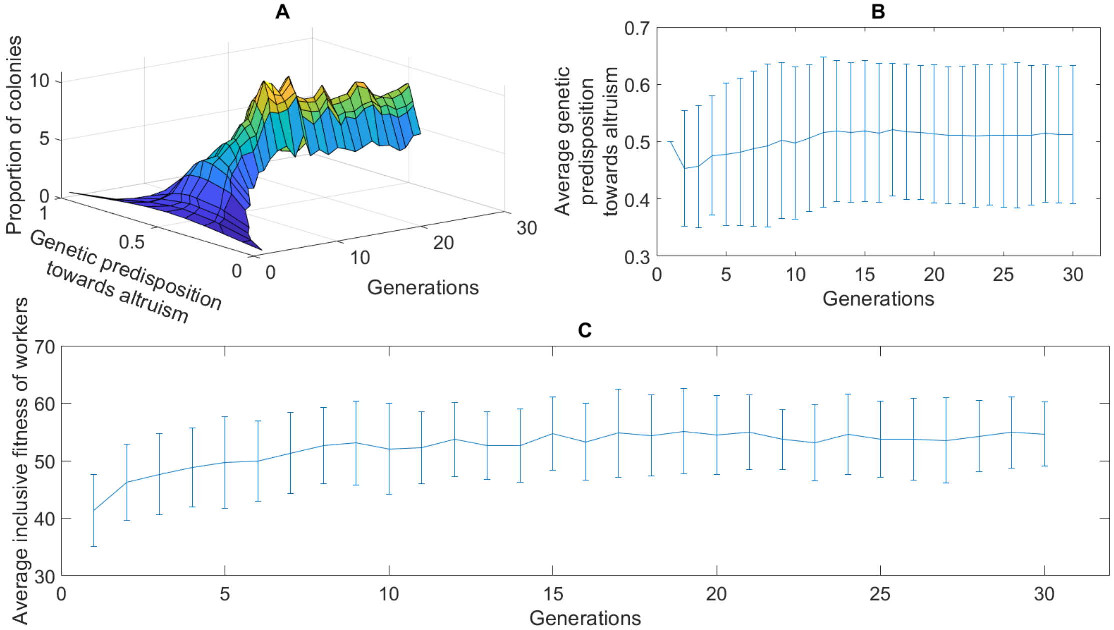


Fig 3.9. Altruism and relatedness The relatedness between workers is a factor in determining the level of altruistic behaviour. Here, the relatedness (as determined by the kin value) between workers is set to zero. A) The distribution of the genetic predisposition over the generation shows a convergence of the genetic predisposition. The average does not move much above the initial average of 0.5. B) The average genetic predisposition over the generations is seen more clearly. The average over the generations is similar to the initial genetic predisposition. C) The average inclusive fitness over the generations is lower than in Fig.3.5 (but similar to Fig.3.8). There is an increase in the inclusive fitness of workers, but again this increase is less than in Fig.3.5. Due to the relatedness being set to zero, the inclusive fitness of workers is more reliant on the production of worker offspring. The error bars are the standard deviation.

However, relatedness between workers is still important for inclusive fitness. Figure 3.9 shows when the relatedness between workers is zero. The average genetic predisposition does not increase above 0.5 (Fig.3.9B). In the case of zero relatedness, the workers' inclusive fitness depends on their offspring and the offspring of other workers. This situation does not lead to a more selfish predisposition due to the breeding capacity being dependent on the number of workers produced by the queen. As this splits the breeding capacity between worker offspring and the production of new workers, the genetic predisposition remains balanced. However, worker offspring have a lower kin value, which lowers the weighting of the inclusive fitness. This means that while the inclusive fitness increases over the generations (Fig.3.9C), the absolute inclusive fitness is lower than the default configuration in Fig.3.5.

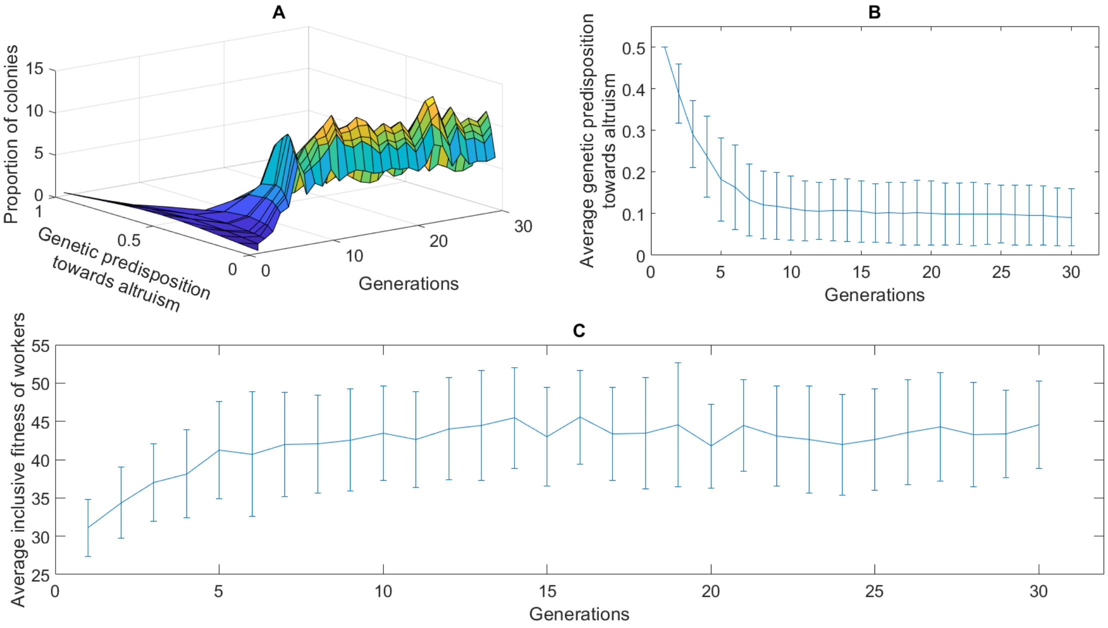


Fig 3.10. Altruism and fertility The rate of worker production is determined by the number of resources workers use to raise the queen's brood and by the relative fertility of the queen. By decreasing the relative fertility of the queen (from 1 to 0.1, with $Z = 1$), the rate of worker production slowed. This slowdown causes a significant shift in A) The distribution of genetic predisposition toward altruistic behaviour towards the more selfish side and B) The average genetic predisposition, which decreases over the generations. C) The average inclusive fitness of workers over the generations increases, though the inclusive fitness is lower than in Fig.3.5C. The error bars are the standard deviation.

While higher relatedness does increase the propagation of altruistic genes, high relatedness also does not guarantee more altruistic behaviour from workers.

The relative fertility of the queen changes the rate of worker production. When the rate of worker production is too low, this affects the propagation of altruistic genes, as seen in Fig.3.10. A drastic decrease in the rate of worker production causes a reduction in the genetic predisposition towards altruism (Fig.3.10B). While there is a similar trend in Fig.3.10C as in previous results, the inclusive fitness is lower than in Fig.3.5C despite a higher per unit fitness return for the production of workers than Fig.3.8. This shows high relatedness alone is not enough to increase the altruistic behaviour of the workers. The queen must also produce new workers at a specific rate for altruistic behaviour to yield inclusive fitness benefits.

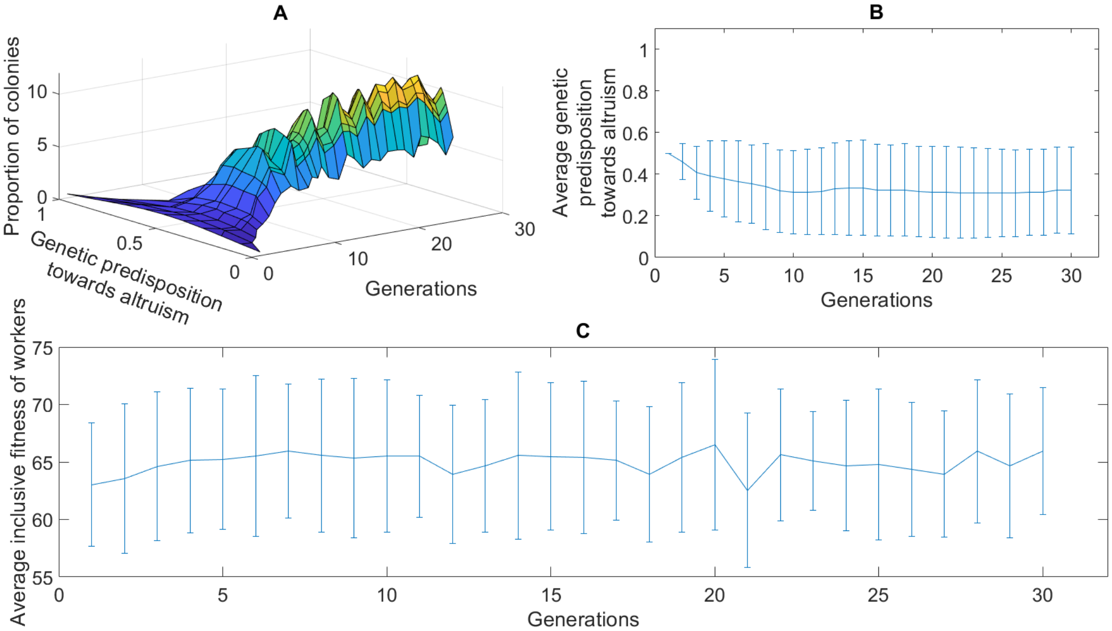


Fig 3.11. Altruism and queen suppression The queen plays a significant role in propagating the genetic predisposition towards altruism. Specifically, the degree to which the queen can suppress the internal state of workers. By decreasing κ, which determines the level of worker internal state suppression, to zero, the workers are free to develop their internal state. κ affects A) The distribution of genetic predisposition over time. As in previous figures, there is a convergence in the genetic predisposition. The average genetic predisposition decreases below 0.5, showing the development of more selfish colonies. B) The average genetic predisposition can be seen more clearly with a decrease over time. The workers in the colonies became more selfish over the generations. Still, not wholly selfish. C) Inclusive fitness does not increase significantly over the generations. There appears to be a stagnation in inclusive fitness as workers get most of their inclusive benefits from their offspring despite a lower kin value and, thus, a lower inclusive fitness return. The error bars are the standard deviation.

Increasing altruism is not just dependent on the queen's ability to produce workers but also the ability to suppress worker reproduction. Figure 3.11 shows the genetic predisposition when κ is zero. This effectively translates to the queen's inability to suppress worker reproduction in the colony. Due to this, the genetic predisposition decreases over the generations (Fig.3.11B) as workers become more selfish. Likely because workers can produce offspring as their internal state increases without the interruption of queen suppression.

Inclusive fitness does not appear to increase significantly over time but stays relatively similar. While there is no notable increase in inclusive fitness, the inclusive fitness is higher than the inclusive fitness in previous figures (except Fig. 3.5). This is likely due to the maintenance of the relatedness with other workers. Despite the lower level of altruism likely leading to the production of fewer workers, there are enough to boost the selfish workers' inclusive fitness. These results highlight the queen's role in the propagation of altruistic genes. Though very weak suppression may not be enough, we found that only some suppression of worker reproduction was needed to reverse the trend in the genetic predisposition, linking natural history with the evolutionary process.

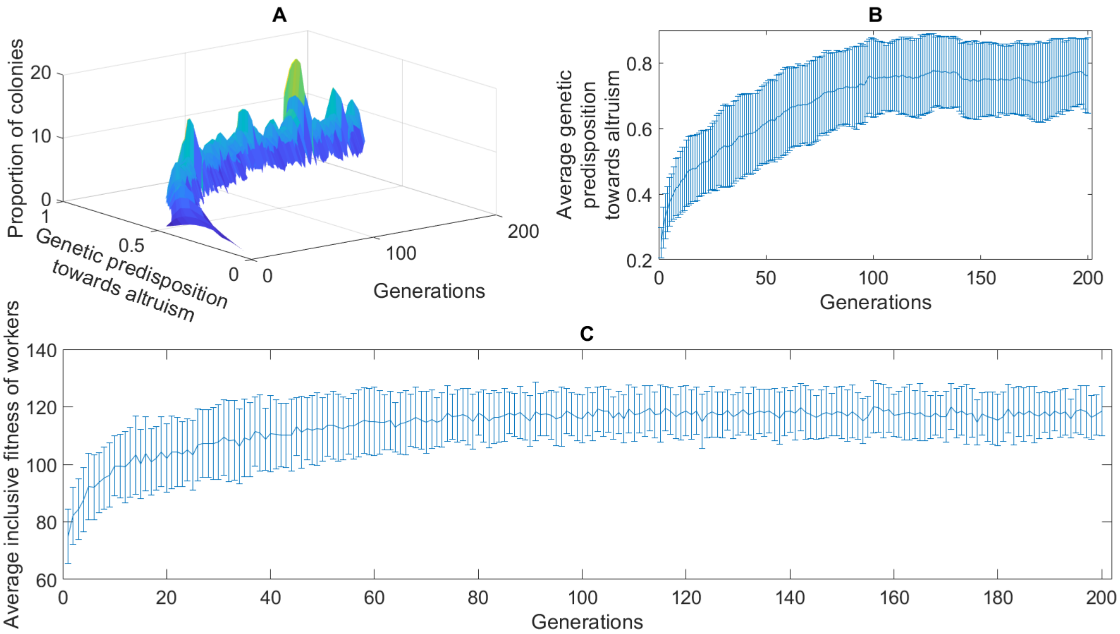


Fig 3.12. Changes in initial conditions When the initial set of colonies is selfish (with a genetic predisposition below 0.5), the genetic predisposition increases over the generations, which can be seen in A) The distribution of the average genetic predisposition over the generations and B) The average genetic predisposition over the generations. In both cases, there is an increase in altruistic genes before reaching a stable point similar to Fig.3.5. However, reaching this stable point takes approximately 100 generations due to the lower initial genetic predisposition of the colonies. This suggests the stable point found in Fig.3.5 is not subject to the initial conditions. C) The average inclusive fitness over the generations shows the same trend and reaches the same level as seen in Fig.3.5C. The error bars are the standard deviation.

So far, the initial distribution of the genetic predispositions of the colonies has been the same. Given a lower initial predisposition towards altruism, it may be possible that colonies do not reach the level of altruism shown in previous figures. The initial colonies were initialised with lower genetic predispositions. Figure 3.12 shows the result of an initial distribution of more selfish colonies.

Though there is initially a collection of more selfish colonies, the colonies become more altruistic. This indicates more selfless colonies had a consistently higher inclusive fitness than other less selfless colonies as shown by the increase in inclusive fitness in 3.12C. Despite the longer timeline, the average genetic predisposition reaches above and out of the range of the initial values. It stabilises at a similar point to Fig.3.5. This suggests robustness in the results and that more altruistic colonies have higher inclusive fitness. Still, perfect altruism does not offer additional gains.

The results suggest a possible evolutionary stable strategy [192], which shows an equilibrium between selfishness and altruism, demonstrating the inclusive fitness effects in a social environment. This evolutionary agent-based simulation could investigate different parameters and their impact on the propagation of altruistic genes throughout the generations. In summary, we created a multi-layered simulation which included interactions between the genetic predisposition, the behavioural algorithm, and population dynamics to examine the balance between selfish and non-selfish genes during the evolution process.

3.4 Discussion

Darwinian fitness describes the survival and reproduction of an individual [158]. Workers largely refrain from reproducing, meaning they do not produce offspring that inherit their traits. Standard Darwinian fitness does not explain the behaviour of workers refraining from reproducing. Inclusive fitness addresses the gene contributions of evolutionary success [167] [39]. Using the concept of inclusive fitness, Ohtsuki *et al.* used dynamic game analysis to show how workers could maximise their inclusive fitness by changing their social strategy [110].

As a new approach, in this chapter, we suggested that the inclusive fitness functions can be used as an optimisation function in the genetic algorithm, aiming at the evolutionary success of gene types. Furthermore, we included a natural history perspective, integrating the agent-based simulation with inclusive fitness. By adding a genetic component for selfish and non-selfish behaviour in the genetic algorithm, we could show the stability of altruistic genes over the generations. There is an inclusive fitness benefit to workers when they help produce more workers, and it also benefits them to produce offspring directly. This chapter investigates whether this advantage is significant enough for workers to forgo personal reproduction to help the queen produce her offspring.

The iterative process of the evolutionary agent-based simulation makes it possible to track workers' genetic predispositions over generations. We can then determine how altruistic behaviour spreads over generations and if a certain balance of genes can be the most effective fitness strategy.

From the results, colonies with more altruistic workers give a higher fitness return when compared to colonies that have more selfish workers. ρ initially starts at 0.5. Over the generations, ρ converges to approximately 0.74, a higher genetic predisposition towards altruism (Fig.3.5). Altruistic workers allow the colony to grow and increase colony productivity, improving the colony's survival. This means more genes were passed on indirectly through the queen by the creation of other workers, which are highly related to them. It also allows workers some selfishness by enabling them to contribute to their inclusive fitness. Workers can contribute to inclusive fitness by producing eggs when the colony size is large and queen patrol behaviour is weaker. Their genes were passed on directly via their offspring and indirectly via the number of workers in the colony. The results suggest that the optimal level of altruism is not perfect for all colony members within the number of generations in the simulation (Fig.3.5B). A level of selfish behaviour is accepted in individuals.

The findings in this chapter were consistent with the conclusions of [110], which showed that policing is most effective and well-implemented when the colony is small but becomes less effective or less implemented when the colony becomes larger. At this stage (which [110] referred to as the "reproductive stage"), workers begin to produce their offspring or at least produce more of them, as seen in Fig.3.6. The results were also consistent in that there is greater worker-derived offspring when the colony is large (Fig.3.6). However, the approach of the current thesis uses a more bottom-up approach, as the feedback loops and genetic predispositions affect the workers' behaviour before selection occurs based on their fitness functions.

The interaction between social or environmental factors and the genetic predispositions of the workers determines their behaviour. There is an implicit trade-off between laying eggs and the queen's patrol behaviour [191]. When the queen is not patrolling, then, with the help of workers, she lays eggs, which become new workers [31]. The average internal state decreases in the first half of the agent-based simulation seen in Fig.3.7. The queen's patrol behaviour is effective at this stage. Workers' internal states begin to increase closer to the end of the simulation even as worker genes become more altruistic (Fig.3.7). This is likely due to the weakening effect of the queen's patrol behaviour [72] [191]. The ability to produce offspring is necessary for situations where the queen is removed from the colony. In these situations, an entirely altruistic colony would die due to workers refraining from producing offspring even without the queen's presence, which would lead to fewer workers (seen in Fig.3.6 for $\rho = 1$). Fewer workers would then lead to lower colony survivability due to lower colony capability in terms of foraging. Even in the simulation, where there are workers who do not die over time, a larger colony is more productive than a smaller colony.

Some factors would be assumed to change the genetic predisposition towards altruism. The first would be the effective paternity of the queen, k. k affects workers' per capita fitness return to other workers (and the queen). When $k = 10$ (Fig.3.8), there appears to be no initial change when looking at the distribution and the mean for genetic predisposition over the generations when comparing Fig.3.8A and Fig.3.5B to Fig.3.5A and Fig.3.8B. However, when we look at the average inclusive fitness (Fig.3.8C), there is a significant drop when compared to Fig.3.5C. While workers are less related to each other in this instance, the production of workers still benefits the colony overall. It is still better for colonies that can produce more workers (at least initially) than to be more selfish. At the start of the simulation, all colonies start with 20 workers and are therefore small and need to expand by producing more workers. More altruistic colonies produce more workers than selfish colonies (Fig.3.6). There is a lower need for patrolling by the queen, meaning the queen has more time to make her offspring. Ultimately, this leads to the proliferation of more altruistic colonies.

Secondly, the per capita fitness return to the workers is important as it contributes to the inclusive fitness of the workers. When per capita fitness is lowered to zero (Fig.3.9), the workers do not become more altruistic, as seen in Fig.3.8. In this case, there is still an advantage to helping the queen and producing more workers. With more workers able to help rear offspring, the breeding capacity is increased (3.8) when there are more workers in the colony. In this case, workers gain no inclusive fitness benefit from assisting the queen to produce more workers. However, the increase in the breeding capacity helps the workers with their offspring (3.9), leading to the genetic predisposition to balance selflessness and selfishness. However, there is once again a lower absolute inclusive fitness compared to the default configuration (Fig.3.5C). However, there has been an increase in inclusive fitness over the generations.

Third, a factor that decreases the genetic predisposition is the queen's relative fertility, q. When $q = 0.1$, the queen is 10 times slower at producing eggs than a worker. Figure 3.10 shows the distribution (A) and mean (B). There is a sharp decrease in genetic predisposition over the generations from $\rho = 0.5$ to approx. $\rho = 0.09$. The average inclusive fitness is lower than previous results (approx. 44 rather than 116), though it shows the same trend of generally increasing over time. The decrease in altruistic behaviour is because of the speed at which the queen produced new workers.

While workers, in this case ($q = 0.1$), were more related to other workers, it was not worth the sacrifice to forgo their reproduction to take care of the queen's brood. Without the increase in productivity that comes with the increase in colony size, workers can more effectively increase their inclusive fitness by increasing their internal state and laying eggs. This is despite the higher per capita fitness return from new workers.

Another factor that decreases the genetic predisposition is the queen's ability to suppress worker reproduction. Repressing worker reproduction is crucial as it shapes the workers' social strategy and interactions with the queen. When we removed queen suppression of worker reproduction (Fig.3.11), there was a decrease in altruism over the generations. Here, selfish workers are not punished for developing their ovaries and laying eggs, producing more offspring than altruistic workers. However, this does not translate clearly to an inclusive fitness benefit. Unlike other configurations, there is no apparent increase in inclusive over the generations (Fig.3.11C). This may be because workers can be more selfish and boost their inclusive fitness in the short term. However, in the long term, selfishness restricts the number of new workers created in the colony and, therefore, limits the inclusive fitness of workers.

Mutation is a crucial aspect of genetic variation. Here, we introduced mutation through the use of a mutation operator. The benefit of using a mutation operator is that there are no constraints to the initial values chosen at the beginning of the simulation. It is possible to start from any point and reach an equilibrium. While some scenarios decrease the genetic predisposition over the generations, the equilibrium found in the initial results is stable. We lowered the initial range of values for the genetic predisposition ([0, 0.5] rather than [0, 1]). After starting from more selfish colonies, the genetic predisposition can become more altruistic (Fig.3.12). The stable point in Fig. 3.5 is consistent. The results show high stability in the genetic predisposition and explain the prevalence of altruistic behaviour in workers, at least in the earlier stages of colony development.

The present chapter presents an agent-based simulation examining the regulation of reproduction in a nest by investigating the feedback loop between the queen and the workers' internal states and their interactions [191]. Note that regulating foraging behaviour may differ from regulating worker reproduction, distinguishing their study from previous work on foraging regulation [132]. Our simulation results reveal that, unlike previous studies where density/encounter rate was the primary driver of behaviour, interactions and their influence on the queen and workers' internal state drive behaviour in the current study (Fig.3.7). Maintaining the global density in the simulation showed that density/encounter rate is not the primary driver of behaviour across colony sizes.

By integrating the agent-based model summarised in the paragraph above, the genetic algorithm determines which genetic predisposition has the highest inclusive fitness. Inclusive fitness determines the individual's reproductive success (see Eq.3.12,3.13), and we took the colony average. When competing, colonies with the genetic predisposition for more altruistic behaviour increase over the generations (Fig.3.5). Perfect altruism is not needed. A certain level of selfish behaviour is accepted within a colony (Fig.3.5). From a biological perspective, selfish behaviour is helpful in situations when a colony is orphaned, and the queen is no longer available to produce workers. In this situation, an utterly altruistic colony would need more individuals to make workers for the colony. From a modelling perspective, there may be no greater inclusive fitness to being more selfless than necessary, or the marginal fitness return diminishes.

On the other hand, if all the workers are selfish, the colony does not grow. While the queen affects the social strategy of the workers by suppressing their internal states (see Eq.3.2), the selfish genes lead to the situation where the workers still do not assist in the production of new workers (Fig.3.6). In effect, the queen only changes the rate of worker reproduction in this instance. Since the workers use no resources to produce new workers, the colony does not grow. However, this is an extreme case.

Different factors can affect the propagation of altruistic genes. These include the effective paternity of the queen and the relative fertility of the queen. Altruistic behaviour may not seem helpful in terms of direct reproduction. In this case, the abdication of reproductive responsibility to the queen appears antithetical to the direct propagation of one's genes [160]. On closer inspection, though, altruistic behaviour confers an inclusive fitness advantage to the individual, which the individual cannot gain if they acted independently. This highlights the evolutionary intricacies of altruistic behaviour [67] [68] [69]. Moreover, the rise of altruistic behaviour in the results supports Hamilton's theory of inclusive fitness, providing a robust framework for understanding these social dynamics [167] [39]. Our data further corroborate the role of patrolling and policing in sustaining the colony's social structure, acting as a regulatory mechanism that aligns with inclusive fitness benefits [153].

The workers' genes are passed on indirectly through the queen by their higher relatedness to their siblings. Even in cases of lower relatedness, there is an advantage to altruistic behaviour, because of the increase in productivity at the early stage of the colony. Workers can then produce their offspring at a later stage in colony growth (see Fig. 3.6, Fig. 3.7). This means that the right balance of altruistic and selfish genes can maximise the inclusive fitness function (see 3.12, 3.13) by producing the maximum amount of related offspring.

As our integrated GA agent-based simulation was set for all individuals in a colony to have the same genetic predisposition, in the future, the algorithm could be adapted to work at the individual level. From the perspective of inclusive fitness functions, workers would compete directly. While workers do not compete directly with each other, there is a conflict with the queen over male parentage which was not directly tested here [110]. By tweaking the parameters of the simulation, we can test the effect of more direct interaction between selfish and selfless individuals. In this framework, the positive impact of altruistic behaviour, i.e., in which workers can share between equally altruistic nestmates, may be diminished due to selfish individuals taking advantage of the altruism of less selfish individuals. The simulation gives a tool to investigate different scenarios and evaluate how these scenarios can affect individuals' inclusive fitness and help elucidate the evolutionary pathway to the modern behaviours we see in present social insect species.

Our simulation introduces a methodology that integrates natural history and evolution over generations, which allows proof-of-concept modelling to fill in the conceptual gaps that may be difficult to grapple with empirically [193] [194].

The simulation allows us to study the evolution of this behaviour at the relevant time scales within a natural history perspective. First, there is the real-time perspective on the behaviour of colony members. Real-time covers, first, immediate interactions between the queen and workers and how these interactions affect the internal state of the agents. There is then the mid-term time scale looking at the population dynamics of the colony. These involve the social strategy and how the internal states of workers affect the production of new workers versus the production of worker offspring. Finally, there is the evolutionary time scale, using inclusive fitness as a metric for selecting the propagation of the genes over the generations.

Chapter 4

Bacteria biofilms - Morphology and Electric Fields

4.1 Introduction

In this chapter, we will investigate the changes in the morphology of the species *Bacillus subtilis* using different media conditions. This builds on previous work by Wakita *et al.* [111], who developed a morphological diagram. A morphological diagram shows how different environmental conditions affect the morphology of the bacteria colony. This allows us to view a landscape of morphology and the different patterns that can be generated. Going beyond the phase diagram as a function of conventional environmental factors, in this study, we employed a new environmental factor, the application of electric fields to bacteria colonies. Applications of electric fields and direct stimulation to biofilms, which examine how they affect the biofilm, have been done to some extent. For example, previous work has shown that depending on the stimulation [195], exposure to an electric field or current can increase the effectiveness of antibiotics. Exposure to an electric field or current can also decrease the number of bacteria which grow on a surface and change the direction of movement for bacteria [196].

We will also take this further by characterising the changes in morphology using the fractal dimension. Fractals have been used to describe living and non-living systems [197]. The fractal dimension (D_f) refers to a measure of the complexity or irregularity of the shape of bacterial colonies. Fractal dimension quantifies the degree of self-similarity or repeating patterns at different scales [198] and can be used to characterise the intricate, irregular shapes of bacterial colonies. There are multiple methods for calculating the fractal dimension of an object, including box-counting, Bouligand-Minkowski and Fourier Transform [198]. The box-counting method was used to analyse the change in morphology for this project. The box-counting method is based on dividing the space of the object to be analysed into a grid of squares (boxes). At each iteration of the method, a different side length, r, for the boxes in the grid is used. The fractal dimension is the slope of a straight line fitted to the curve of the function: N(r) x r in a log-log scale where N(r) is the number of boxes of length r which intersect the fractal object. This means that the fractal dimension can be written as $D_f = \frac{log(N)}{log(r)}$.

Ben-Jacob *et al.* [92] use the fractal dimension to describe changes in the morphology of the bacteria colony when exposed to antibiotics. The morphology of the bacterial colony can show the effect of stress on the colony when exposed to external forces. Ben-Jacob *et al.* explore these changes concerning antibiotic stress [92]. They exposed the bacteria *Paenibacillus dendritiformis* to a non-lethal concentration of antibiotics. They found a change in their chemotactic signalling, bacteria length and increased metabolic load. The change in chemotactic signalling relates to the electrochemical signalling in Prindle *et al.* [80]. The bacteria communicate about their environment (the presence of low levels of antibiotics) and form different growth patterns than they would without the presence of antibiotics. These growth patterns are similar to fractal patterns seen in non-living systems. In that way, they grouped bacteria by the bacteria colony's pattern formation (morphotype). They looked at how the fractal dimension was affected by the presence of antibiotics in the media. There was a decrease in the fractal dimension due to a decrease in the complexity of the self-organised colony. By using a non-linear diffusion model to replicate the growth patterns of one of the morphotypes, Ben-Jacob *et al.* show that an increase in maintenance cost for the bacteria leads to lower complexity. This is an example of how the fractal dimension can be used to investigate changes in the morphology of biofilms and bacterial communities when exposed to different stimuli.

While it is interesting to view the patterns generated by different environmental conditions, we can characterise more quantitatively the changes in morphology brought about by the changing environment using the fractal dimension. In this way, we develop the morphological diagram based on different environmental factors and a fractal dimensional morphology diagram showing the consistent effect of one environmental change versus another. Little has been done on how the application of an electric field affects electrochemical signalling in a biofilm [80]. Based on previous works, we hypothesise that the electric field can also affect the biofilm's morphology because of its potential effects on the bacterial membrane of individual cells, which disturbs the formation of biofilms. Direct effects include bacterial membrane damage and bacterial proliferation inhibition [195]. It is vital to consider the indirect ways biofilm growth can be affected. This includes exposure to an electric current, change in pH and toxic electrolysis products (H_2O_2), which impede bacteria growth [196].

All variables should be considered when exploring how they affect the electrical signalling in the biofilm and the subsequent morphological changes which occur. Different bacteria move to different electrodes when exposed to the electric field [199]. Large amounts of potassium were put opposite the biofilm [90]. Free cells were attracted to the potassium rather than the biofilm. The bacterial biofilm can be somewhat directed to move using an electric field. By considering how the cells communicate within the biofilm, the way the directional change information is distributed through the biofilm might be determined.

4.2 Methods

4.2.1 Experimental Procedure

Experiments were done using *B.subtilis*, type 3610 and PY79. Dr Asally Munehiro from the University of Warwick kindly provided this. Liquid Lysogeny Broth (LB) was used to produce an overnight culture kept at 37 degrees Celcius (plus/minus 1). This liquid culture inoculated the media with a (theoretically) consistent number of bacteria cells. The solid media on which the bacteria were grown was produced using a method similar to that described by Wakita *et al.* [111]. A phosphate buffer was used, which used a mix of potassium hydrogen phosphate mono-basic and di-basic to maintain the pH at 7.1 after being adjusted by adding 1M sodium hydroxide. The phosphate buffer also included 5 g/litre of sodium chloride. The solution was then mixed with various agar and peptone concentrations, which determined the growth environment. The solution was autoclaved, and 20 ml was poured into sterilised 90 mm plastic Petri dishes. After solidification at room temperature, the agar plates were dried in the incubator for 60 mins at 37 degrees.

3 μl of the liquid culture was inoculated at the centre of each agar plate. The liquid droplet was allowed to dry before being wrapped in parafilm and incubated for 3-4 days at 37 degrees. The concentrations of agar used included 0.75%, 1%, 1.5%, 1.75% and 2%. Only three peptone concentrations were used, 1 g/l, 2 g/l and 5 g/l. These were chosen as they are similar to Ben-Jacob *et al.* [92]. The bacteria were exposed to an electric field ranging from 1 V/cm to 8 V/cm using copper electrodes on either side of the media. The field strength was calculated as the voltage divided by the space between the electrodes. The cells were exposed to the electric field for their entire growth period. In this case, the growth period of the cells was at least seven days at room temperature.

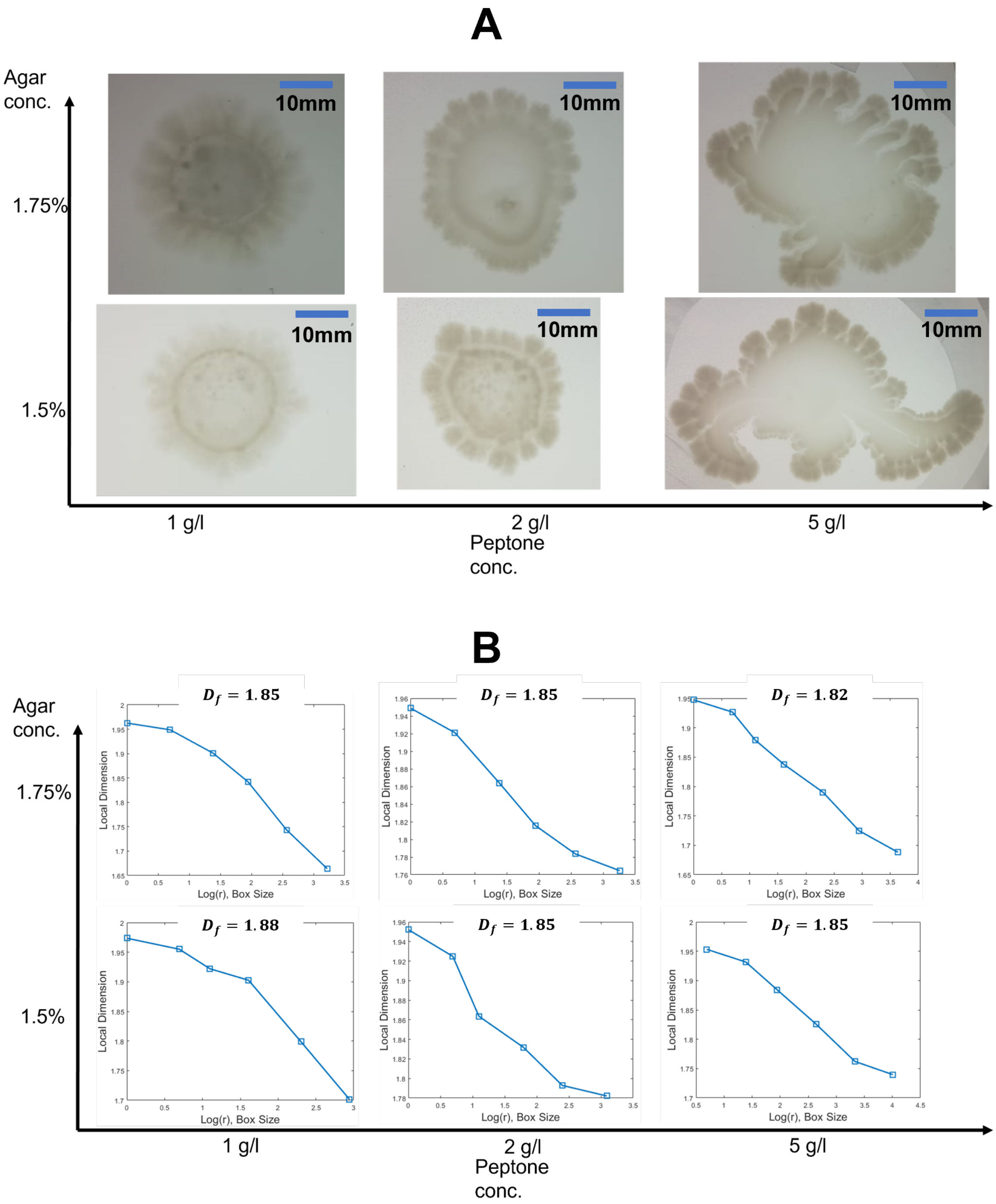
4.2.2 Fractal Dimension and Image Analysis

A program was developed in MATLAB to calculate the fractal dimension of bacteria using the box-counting method described above. Firstly, to check that the code written for calculating the fractal dimension worked correctly, the bacteria images (Fig 12A and 12B) from Ben-Jacob *et al.* [92] were used for testing.

A program was developed in MATLAB to calculate the fractal dimension of bacteria colonies using the box-counting method described above. To test the accuracy of the program, images from Ben-Jacob *et al.* [92] were used. Specifically, Fig. 1. The fractal dimensions for other figures in Ben-Jacob *et al.* [92] were not given, so it was assumed that the fractal dimensions reported were consistent across all of their samples. The method of measuring the fractal dimension was also not given. For the bacteria they used, (*Paenibacillus dendritiformis*), the fractal dimension, D_f, is 2 without antibiotics. In contrast, in the presence of antibiotics, D_f is approximately equal to 1.8. From our own measure of the fractal dimension of *Paenibacillus dendritiformis*, a D_f of 1.91 was found without antibiotics a D_f of 1.79 with antibiotics. This difference in the measured fractal dimension could be due to differences in image processing before fractal analysis (cropping the image and the contrast of the image) and a difference between the methods for measuring the fractal dimension [198]. Applying the same program to other figures in Ben-Jacob *et al.*, we found a consistent effect in terms of the lowering of the fractal dimension with the presence of antibiotics. This was taken as an indication that the program worked in the intended manner. After the bacteria were allowed to grow, the fractal dimension was assessed using the code. The images were taken using the Samsung Galaxy S21 Ultra camera system. Petri dishes were laid on a light pad set to the highest brightness, and the images were taken from above.

4.3 Results

First, we developed a morphological diagram, following in the footsteps of Wakita *et al.* [111]. Figure 4.1A shows the morphological diagram for *B.subtilis* 3610. The results of Wakita *et al.* have been replicated with increasing colony size occurring with increased peptone concentration and different morphologies seen under different environmental conditions. Their work is taken further by the fractal morphological diagram seen in Fig. 4.1B. The local dimension reflects the local fractal dimension at each point on the curve of the function: N(r) x r in a log-log scale. This was calculated using the gradient MATLAB function. The "overall" fractal dimension, D_f, is given for environmental conditions and is again calculated specifically as $D_f = (Log(N)2 - Log(N)1)/(r2 - r1)$. Here we find that the fractal dimension appears to decrease with increasing peptone concentration, from $D_f = 1.85$ to $D_f = 1.82$ at 1.75% agar concentration and from $D_f = 1.88$ to $D_f = 1.85$ at 1.5% agar concentration. The fractal dimension also seems to decrease with an increase in the agar concentration for 1 g/l peptone and 5 g/l peptone, although this was only tested using two agar concentrations. Also, we can show that the fractal dimension can distinguish differences in morphology and act as a more quantitative measure of those differences.



A
Agar conc.
1.75%
1.5%
10mm
1 g/l
2 g/l
5 g/l
Peptone conc.
B
$D_f = 1.85$
$D_f = 1.85$
$D_f = 1.82$
$D_f = 1.88$
$D_f = 1.85$
$D_f = 1.85$
Local Dimension
Log(r), Box Size

Fig 4.1. Bacteria morphology and fractal dimension: Sample 1 A) *B.subtilis* 3610 strain grown under different environmental conditions where $N = 5$ for each condition. The colonies chosen are meant to represent the replicates of each condition. The peptone concentration ranges from 1 g/l to 5 g/l. The agar concentrations used were 1.5% and 1.75%. The growth period was 10 days at approximately 20 degrees. As peptone was used as the source of nutrients in the colony the greater the peptone concentration in the media the larger the bacteria colony grew during the growth period B) The fractal dimension of the corresponding peptone and agar concentrations. Increasing peptone concentration appeared to decrease the fractal dimension of the colony. This is consistent for both agar concentrations. Increasing agar concentration also decreased the fractal dimension of the colony. This is also consistent at a given peptone concentration

Figure 4.2 shows another morphological diagram for *B.subtilis* 3610. Here, the growth conditions are slightly different, with the bacteria being grown at higher temperatures in the incubator rather than on the temperature-controlled lab bench. Again, we see increased colony size occurring with increased peptone concentration. As seen in the previous figure, we also see a decrease in the fractal dimension with increasing peptone concentration, from $D_f = 1.87$ to $D_f = 1.83$ at 1.75% agar concentration and from $D_f = 1.90$ to $D_f = 1.86$ at 1.5% agar concentration. This shows a consistent result across the samples. While the fractal dimension varies across samples, there is a consistency in the effects of peptone and agar concentration on the fractal dimension of the colony.

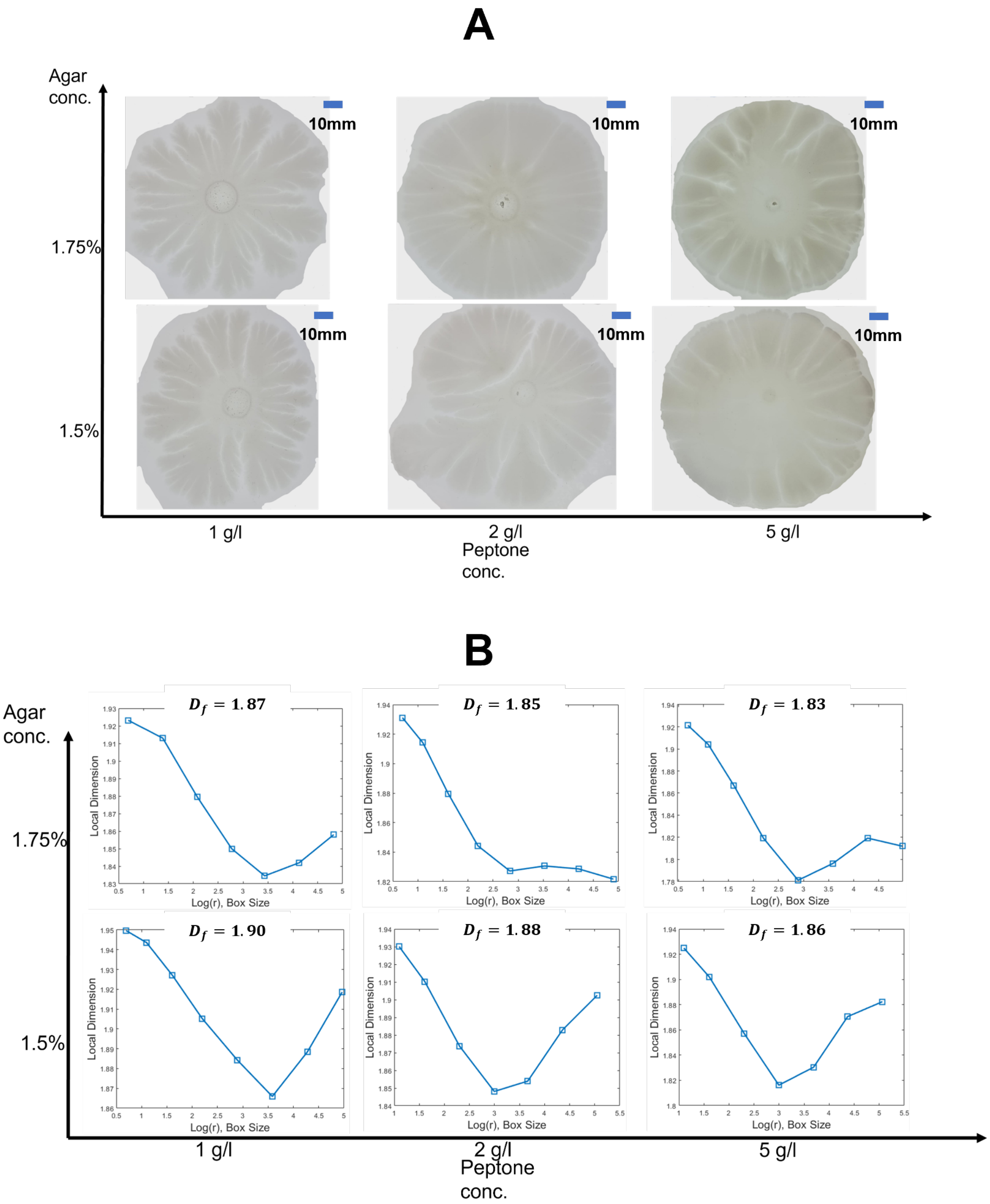

A
Agar conc.
1.75%
1.5%
10mm
1 g/l
2 g/l
5 g/l
Peptone conc.
B
Agar conc.
1.75%
1.5%
$D_f = 1.87$
$D_f = 1.85$
$D_f = 1.83$
$D_f = 1.90$
$D_f = 1.88$
$D_f = 1.86$
Local Dimension
Log(r), Box Size
1 g/l
2 g/l
5 g/l
Peptone conc.

Fig 4.2. Bacteria morphology and fractal dimension: Sample 2 A) *B.subtilis* 3610 strain grown under different environmental conditions where $N = 5$ for each condition. The colonies chosen are meant to represent the replicates of each condition. The peptone concentration ranges from 1 g/l to 5 g/l. The agar concentrations used were 1.5% and 1.75%. The growth period was 5 days at approximately 35 degrees. Peptone concentration increases the growth rate of the bacteria. Also, the higher temperature used for this growth period increases the growth rate compared to 4.1 B) The fractal dimension of the bacteria at different peptone and agar concentrations. Increasing peptone concentration appeared to decrease the fractal dimension of the colony. Increasing agar concentration also appeared to decrease the fractal dimension of the colony. As there is variation between samples there is a lack of consistency in the fractal dimension. However, the changes in the fractal dimension appear to be consistent across samples

The PY79 strain was also cultivated, showing different growth patterns in different parts of the colony (Fig.4.3). When comparing the fractal dimensions of different regions, we find differences based on the observed growth patterns. Figure 4.3B shows a region of the colony where the growth is more branching. This region shows a higher fractal dimension than the colony overall ($D_f = 1.95$ compared to $D_f = 1.92$). The second region, shown in Fig. 4.3C also shows a higher fractal dimension, $D_f = 1.95$, when compared to the whole colony, $D_f = 1.92$. The fractal dimension can be used to characterise changes in morphology between different environments and regions of an established bacteria colony when different growth patterns are observed.

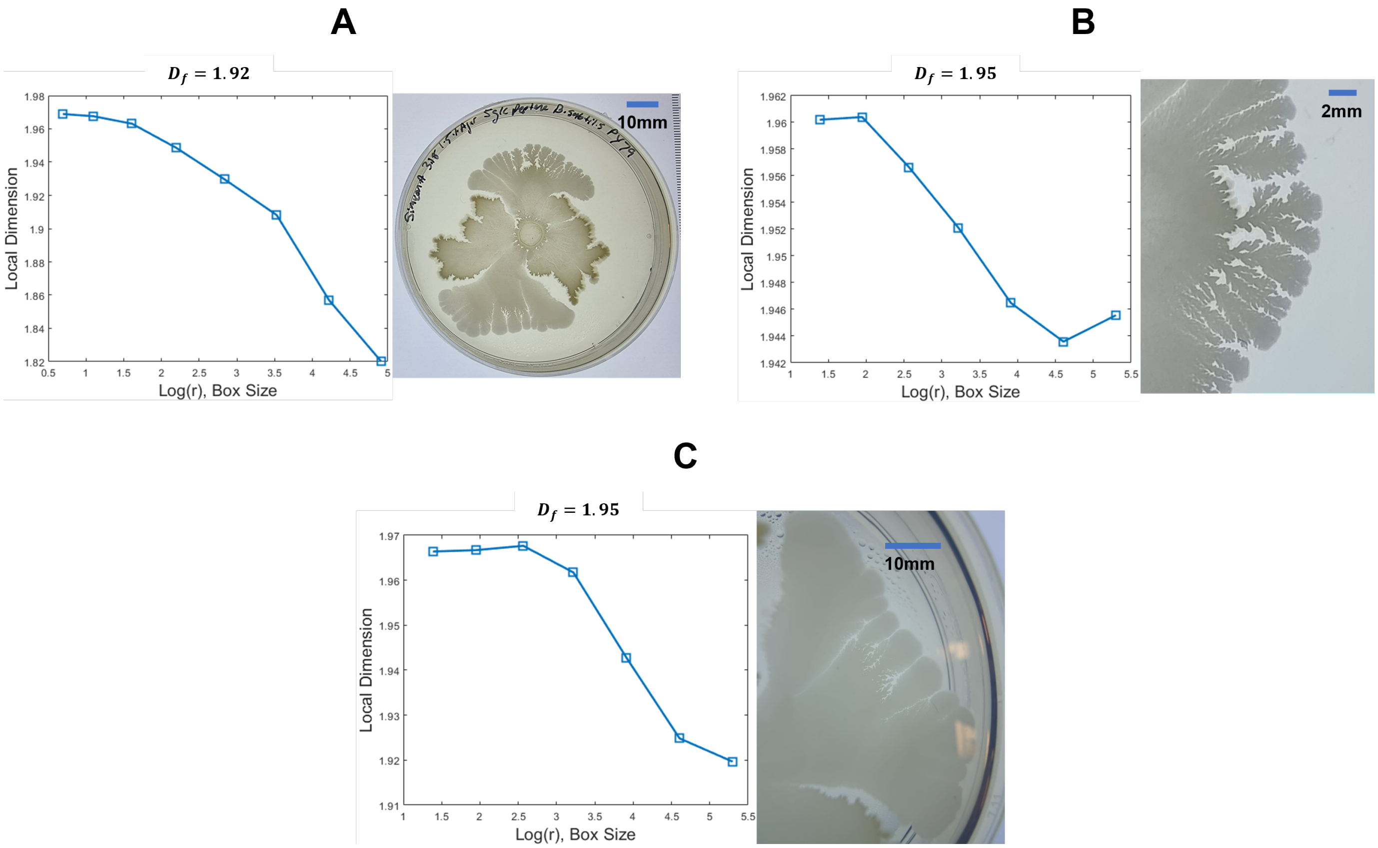


Fig 4.3. Regional fractal dimension When looking at the PY79 strain of *B.subtilis*, there are different growth patterns compared to the 3610 strain. The growth period was 5 days at 35 degrees. The agar concentration was 1.5%, and the peptone concentration was 5 g/l A) PY79 has a more branching structure than the 3610 strain. Different regions of the colony show different growth patterns and these give different fractal dimensions B) Region 1 shows greater branching and gives a higher fractal dimension when compared to the fractal dimension of the entire colony C) Region 2 also gives a higher fractal dimension when compared to the whole colony but similar to the fractal dimension seen in B

B.subtilis has been know to respond to electrical stimulation [200] [201]. Here, we applied an electric field to investigate potential morphology differences and determine if a fractal dimension change could capture those differences.

Figure 4.4 shows the bacteria grown with and without the presence of the electric field (A and B respectively). The colony grown without the field shows a layering in the growth pattern while the colony grown with the field appears much more smooth. Previous work has shown the ability to manipulate cell behaviour to generate interesting patterns (such as forming a smiley face [202] [203]). In this work, the field appears to be causing a smoothing in the growth pattern of the bacteria. This smoothing effect was not consistent across the samples or different agar and peptone concentrations. Although it did appear the most at the highest peptone concentration. The field also appears to increase the fractal dimension of the colony. This increase is likely due to the smoothing of the colony.

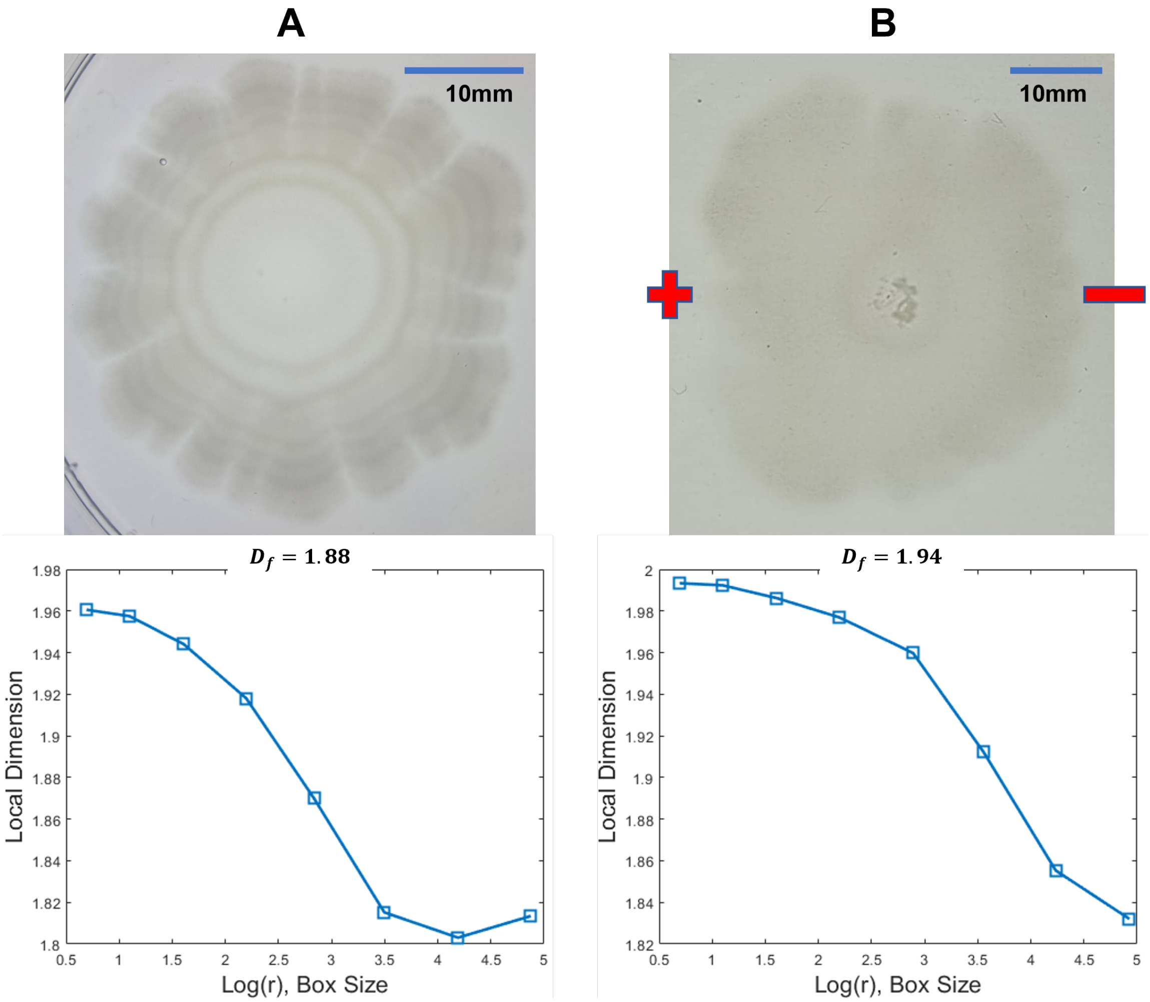


Fig 4.4. Effects of the electric field: Sample 1 Strain 3610. $N = 4$ and the images shown are meant to represent the replicates of each condition. The agar concentration is 2% and the peptone concentration is 5 g/l A) Concentric rings appear during the growth of *B.subtilis* strain 3610. Different environmental conditions can give rise to different growth patterns [204]. B) We exposed the bacteria to an electric field with a field strength of 4 V/cm for the entire growth period which in this case was 5 days at approximately 20 degrees. It was assumed that the field lines ran parallel to each other from one electrode to another. The electric field appears to have caused a smoothing effect in the growth pattern of the bacteria colony and increased the fractal dimension of the colony

When investigating the effects of the electric field on morphology there is the question of the growth asymmetry and also the field lines. This asymmetry was observed in some of the growth samples, one of which is shown in Fig.4.5. These bacteria were grown in a 3D-printed petri dish, which was printed to be $60W$ x $20H$ x $60D$. This was used to decrease the distance between the electrodes to increase the field strength. Fig.4.5B shows asymmetric growth of the colony. This growth is perpendicular (i.e., downward) to the electrodes placed on the left and right sides of the dish just out of frame. Again, the electric field did not cause consistent differences in observed morphology (there does not appear to be a smoothing effect). Although again the field caused an increase in the fractal dimension, albeit a smaller increase than in Fig.4.4. The field lines were assumed to be straight, as shown in the top part of Fig.4.5B. However, given the circular nature of the agar, it is possible for the field lines to be curved or slightly distorted as they travel from one electrode to another. This could mean that the effect of the electric field is felt differently at different places on the agar rather than uniformly as pictured. This would then lead to different colony morphology.

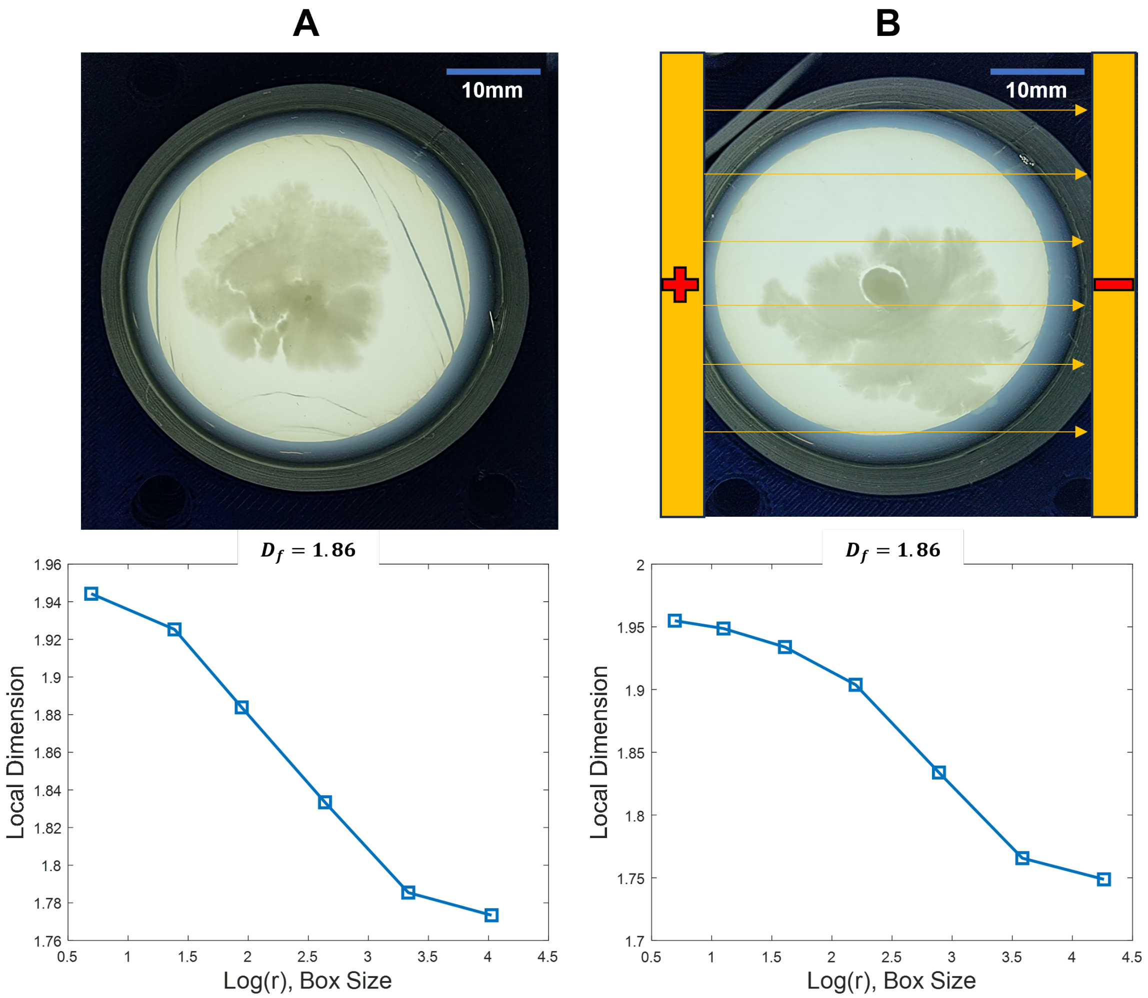


Fig 4.5. Effects of the electric field: Sample 2 Bacteria were also grown in a 3D printed petri dish, which was designed to be smaller to increase the field strength of the electric field. Here, we see strain 3610 grown over a period of 6 days at room temperature. $N = 4$ and the images shown are meant to represent the replicates of each condition. The agar concentration used was 1% with a peptone concentration of 5 g/l A) The colony grows as expected without the influence of the electric field B) The bacteria were exposed to a field strength of 8 V/cm over the course of the growth period. There appears to be an asymmetry in the growth of the bacteria colony, with the growth occurring perpendicular to the field

4.4 Discussion

Previous work has investigated morphological differences in bacteria growth with respect to changing environmental conditions. From this they developed a morphological diagram which shows the effects of nutrient concentration (in the form of peptone) and agar concentration on the growth pattern of the bacteria. Previous research has also used the fractal dimension to characterise changes in morphology, but not to the extent of this project. From the results, we developed a morphological diagram which shows the changes in morphology with changing agar and peptone concentration (Fig.4.1A & Fig.4.2A). Increasing peptone concentration increases the growth of bacteria, as expected, with higher concentration agar impeding the growth of the bacteria, possibly due to the increased hardness of the media. The fractal dimension more qualitatively measures the changes in morphology, giving us the fractal morphological diagram seen in Fig.4.1B & Fig.4.2B. The effects of the changing environmental conditions appear consistent across the samples, with increasing peptone concentration decreasing the fractal dimension. Increasing agar concentration also decreased the fractal dimension of the colony.

Different strains are known to have different growth patterns. Hence, Ben-Jacobs use of morphotypes as a method of categorisation [92]. The *B.subtilis* strain PY79 showed different growth patterns in different regions of the colony (Fig.4.3). There was variation in the fractal dimension based on the region of the colony that was analysed. There were observable differences in regions of the bacteria colony. One of the regions analysed had a more branching structure forming more intricate patterns (Fig.4.3B) while another region had thicker branches with more intricate sections. The fractal dimension of these regions varied from that of the overall colony, showing that even with one bacteria colony, there can be regional differences in the propagation of cells through the media. Previous work has investigated this ”sector formation” [205] with models developed to describe their formation. With the use of the fractal dimension, the differences in morphology for these sectors can be catalogued. Combining this with numerical simulations would yield new ways of predicting changes in morphology due to different factors and environmental conditions, allowing us more control over how the colony grows.

Electrical stimulation can be used to control how the colony grows [195] [200] [206]. Here, the colony was under constant exposure to the electric field, as opposed to previous work where the colony was only exposed to electrical stimulation for a certain time during the colony's growth period. The effects of the field on colony morphology were not consistent when looking at the observed differences in bacteria growth from one sample to another (Fig.4.4 to Fig. 4.5). One sample showed a smoothing effect of the electric field on colony growth (Fig.4.4). Another sample showed an asymmetry in the morphology of the colony exposed to the field (Fig.4.5). However, in both cases, the fractal dimension was determined to increase when the colony was exposed to the electric field. As there was no consistency in the observed colony morphology, it is possible that the increase in fractal dimension is coincidental. Previous work by Rajnicek *et al.* [200] showed that electric fields induced curved growth in the cells of various bacteria species, including *B.subtilis.* Rajnicek *et al.* exposed the bacteria to the field for 1-1.5 hours and showed that the cells returned to normal when the field was off. With this curved growth in mind, it would not be surprising to see an asymmetry in the growth of the bacteria colony when exposed to a constant electric field. However, the curved growth observed by Rajnicek was toward the anode. This was confirmed when the polarity of the field was switched and the cells began to bend in the direction of the new anode. Here, the asymmetrical growth occurred perpendicular (i.e. downward) to the electrodes, which were placed on the left and right side of the dish just out of the frame and is therefore unlikely to be caused solely by the curved growth of the cells. Symmetry breaking is also occurring, with the growth only occurring in one direction. This could be due to a stronger form of the chirality which was observed by Ben-Jacob *et al.* [92]. However, the weak chirality they found was due to the elongation of the bacterial cells. Here, it is unclear if elongation is the cause of the symmetry-breaking growth seen in Fig.4.5.

Multiple mechanisms could be at play when determining how the growth pattern of the colony should change based on the colony's exposure to the electric field. First, there is the aforementioned curvature of the cells when exposed to the electric field. One would imagine that curved cells divide and lead to more curved cells, eventually leading to an asymmetric growth pattern. Even so, the curved growth observed in Rajnicek *et al.* occurred in cells which were more spaced apart. The colonies observed in the figures here would contain many more cells which are more closely packed together. It is currently unknown what effects the curved growth of the cells would have on the morphology of the colony at a macro scale when the cells are more closely packed. The curvature effect could be mitigated by all the cells attempting to curve the same way, with some in different orientations causing differences in the amount of curvature experienced by different cells. Another mechanism is the electrochemical communication that can occur between the cells [90]. Disruptions in this signalling caused by the electric field [199] would change the morphology of the colony. Whether this would conflict with the curved growth of the cells is yet to be determined. In either case, the strength of the effect of curvature vs communication disruption would need to be measured. It is possible that curved growth also causes disruptions in signalling between cells, further exacerbating the expected change in morphology.

In this chapter, we investigated changes in the morphology of the species *B.subtilis* with different media environments. To demonstrate observed changes in morphology on the macro scale we used a morphological diagram, first developed by Wakita *et al.* The changes in morphology were then characterised using the fractal dimension to measure the differences in morphology between the media conditions. While there were some basic findings, the fractal dimension proved a useful tool in evaluating morphological change in a more quantitative manner. This was further applied to potential differences in morphology caused by exposure to an electric field. While there were no consistent observed differences between colonies exposed to the field and those that were not, there was a consistent increase in the fractal dimension when the colony had been exposed to the electric field.

Chapter 5

Discussion, Conclusion and Future work

5.1 Results Summary

5.1.1 Internal states and reproductive control

Social insects demonstrate adaptive behaviour for a given colony size. Remarkably, most species do this even without visual information in a dark environment. However, how they achieve this still needs to be discovered. In the ant *Diacamma cf. Indicum* (from Japan), the queen spends more effort on queen pheromone-transmitting behaviour (patrolling) in response to the growth of colony size to inhibit worker ovary development. We used an agent-based simulation to understand the mechanism of the colony size dependent behaviour of the queen. Through repeated physical contact between the queen and individual workers, individual colony members monitor the physiological states of others, reflecting such contact information in their physiology and behaviour.

We showed two things. First, the queen follows a feedback loop mediated by the mutual contact between her and the workers. In other words, the queen patrols the workers more often when she has recently encountered workers with developed ovaries. The queen can exhibit adaptive behaviour patterns for the increase in colony size. We found that this self-regulatory mechanism worked even when the worker density per space was constant. Second, we showed that physical constraints could underlie the adaptive switching of colony stages from successful patrol behaviour to unsuccessful patrol behaviour. This switch leads to constant ovary development in workers. Essentially, despite such feedback, the effectiveness of the queen patrol and, thus, the suppression of worker ovarian activity decreased with increasing colony size. This indicates a colonial phase shift from the ergonomic stage to the reproductive stage, a general phenomenon in social insect colonies, emerged as the colony grew.

Feedback between the sensing of physiological states and the corresponding behaviour patterns leads to self-organisation, with colonies shifting according to size. The feedback loops embedded in the queen between the perception of the internal states of the workers and behavioural patterns can explain the adaptive behaviour as a function of colony size. In this computational study, we show that even at a constant individual density per nest space, colony size dependent behaviours both in queens and workers emerged. This demonstrates that the behavioural changes caused by the feedback loop (which couples the internal state of the queen and workers) code the information regarding the contact rate of the individual worker by the queen.

5.1.2 Altruism from a Natural History perspective

Inclusive fitness, first introduced by W.D Hamilton, provides a framework for understanding the development of altruistic behaviour in related individuals.

Specifically, the development of altruistic behaviour in individuals who do not produce offspring directly but instead invest in the offspring of others. This study investigates the evolution and stability of ant workers' genetic predisposition toward altruism. By integrating an agent-based simulation and a genetic algorithm, the main result showed increased genetic predisposition towards altruistic behaviour over time (Fig.3.5). Inclusive fitness increased with the propagation of more altruistic genes. Still, it seemed to level off, indicating a ceiling to the colony's inclusive fitness benefits of altruistic behaviour. However, the inclusive fitness of more altruistic colonies is generally greater than that of more selfish colonies. More altruistic workers help the queen produce more workers and engage in more social behaviours, increasing their inclusive fitness over more selfish workers. More selfish workers split the breeding capacity and, therefore, decreased the production rate of new workers. While these workers produce offspring, this does not give them a greater inclusive fitness benefit.

The stable point of the genetic predisposition also did not require perfect altruism, leaving room for the expression of selfish behaviours by workers. This finding reveals how workers can continue to have functioning ovaries despite refraining from reproducing or being suppressed by the workers' interaction with the queen. By linking the genetic predisposition to the workers' behaviour in a digital nest space, the evolutionary agent-based simulation allows the exploration of parameters that can influence the propagation of altruistic genes. The findings in this thesis were consistent with the conclusions of Ohtsuki and Tsuji [110]. Policing is most effective and well-implemented when the colony is small but becomes less effective or less implemented when the colony becomes larger. At this stage (the "reproductive stage"), workers begin to produce their offspring or at least produce them more, as seen in Fig.3.6.

Different factors can affect the propagation of altruistic genes. These include the effective paternity of the queen and the relative fertility of the queen. Altruistic behaviour may not seem helpful in terms of reproduction. In this case, the abdication of reproductive responsibility to the queen appears antithetical to the direct propagation of one's genes. On closer inspection, though, we see altruistic behaviour, as presented here, confers an inclusive fitness advantage to the individual, which the individual cannot gain if they acted independently.

5.1.3 Morphology and fractal dimension

The fractal dimension is a value (D_f) which characterises the self-similarity of an object independent from the observation scale of the object [198]. There are multiple methods for calculating the fractal dimension of an object. A program was developed in MATLAB to calculate the fractal dimension of the bacteria colony. Here the box-counting method was used to analyse the change in morphology in *B.subtilis* colonies. The morphology of the bacterial colony can show the effect of stress on the colony when exposed to external forces. We developed a morphological diagram, following Wakita *et al.* [111]. Their work was extended by including a fractal morphological diagram (Fig. 4.1B), which was used to quantify the observed changes in morphology.

We found that the fractal dimension appears to decrease with increasing peptone concentration. The fractal dimension also decreases with an increase in the agar concentration. We hypothesised that the harder agar means bacteria cannot grow uniformly. Instead, branches grow to reflect the slight differences in the hardness of the media. In Ben-Jacob *et al.* [92], when looking more closely at the edges of the bacteria colony, the effect of antibiotics decreases the density and curvature of the branches. We know that cells communicate about their internal state to others using electrical signalling [90], which is linked to their metabolism [80]. A larger surface area for the boundary at the bacteria colony's edge effectively means greater sensory information for the colony. Increasing surface area could mean more cells are exposed to the environment and could communicate its effects to inner cells. A higher fractal dimension means a higher surface area to take up nutrients.

We exposed *B.subtilis* to an electric field to view its effects on the colony's growth. Multiple mechanisms could be involved when determining the growth pattern of the colony based on the colony's exposure to the electric field. First, there is the curvature of the cells when exposed to the electric field [200]. Curved cells would divide, and the colony would grow asymmetrically. However, such asymmetric growth was not consistently found in experiments. Previous work looking at the curvature of the cells [200] showed that different bacteria species respond and curve differently compared to each other. The curvature in *B.subtilis* is much lower at a given field strength. A much stronger electric field could be applied to see consistent asymmetric growth patterns in the bacteria colony. Second, disruptions in electrochemical signalling caused by the electric field could affect morphology [199]. The level of disruption could not be measured. However, it would no doubt affect the propagation of cells in the colony and, therefore, the macro morphology of the colony. In either case, exposure to the electric field appeared to increase the fractal dimension of the colony.

Overall, the morphological diagram demonstrated some primary findings regarding the environmental effects on colony morphology. The fractal dimension proved a helpful tool in evaluating morphological change more quantitatively.

5.2 Discussion

5.2.1 Self-organisation and Emergent functions

Self-organisation and collective behaviour is concerned with the interactions of individuals in loosely organised groups. Collective behaviour can have advantages both in the short and long term. These advantages require collective cohesion and stability, meaning group homeostasis is needed. Group homeostasis involves processes needed to maintain the group's survival. Eusocial insect species (specifically Hymenoptera) show complex group behaviours. In the case of eusocial insects, group homeostasis involves the division of labour which puts individuals into castes. Typical castes include reproductive queens and non-reproductive workers.

In the species *Diacamma cf. Indicum* (from Japan), the queen patrols the colony and suppresses worker ovary development to maintain the reproductive division of labour. The macroscopic details of policing behaviour are known [56] [57] [63] [67] [68] [69] but the details of how the policing behaviour is carried out are understudied. Sugawara *et al.* [75] introduced the concept of the internal state to explain the details of the queen's patrol behaviour. The PhD project aimed to describe the details of the reproductive division of labour. Using an agent-based simulation, we used a model to demonstrate that the queen could use information from interactions with workers to determine her patrol behaviour. We showed the colony's transition from the ergonomic stage, where more workers are produced, to the reproductive stage. The model was extended further to develop an evolutionary agent-based simulation which showed how altruistic behaviour could evolve. This altruistic behaviour leads to the reproductive division of labour seen in modern *Diacamma*. The model also captures the ability of workers to maintain their reproductive capacity even if they refrain from reproducing in the queen's presence. The agent-based simulation allows for incorporating the mutual interactions integral to self-organised systems.

Emergent behaviour arose from these mutual interactions using models based on experimental observations. As with any model, there were a few assumptions underlying the development of specific parameters. For example, the non-linear development of the worker's internal state. However, the model's parameters are grounded in the observed behaviours of *Diacamma*. The evolutionary agent-based simulation is more difficult to validate experimentally. The advantage of the simulation in being able to explore different variables is also its weakness. It is currently impossible to experimentally evolve a series of colonies with tunable traits as done here. Although, there is some basis for the validity of the model. It predicts the current state of worker behaviour observed in actual colonies. It was also consistent with previous findings on policing behaviour in the nest and the movement from one colonial development stage to another. It is an extension of the previous section, which looked at the enforcement of the reproductive division of labour but incorporated a Natural history perspective.

B.subtilis has shown similar co-operative behaviour with cells communicating and sharing resources [80]. Cells use electrochemical signalling to communicate their metabolic state to others [80]. The fractal dimension has been used to characterise changes in morphology caused by external stress [92]. We use the fractal dimension to quantify the differences in morphology and use previous research to explain differences in morphology based on environmental effects. One of these is the electric field, which affects the cells differently. While we could not show consistent effects, the conceptual framework for understanding the collective behaviour of ants and bacteria is the same. Individuals must communicate their inner state with others in the group; this affects their behaviour, shaping the colony. This leads to self-organisation in both cases. For ants, this self-organisation takes place in the form of the colony's reproductive division of labour and task allocation. For the bacteria, this self-organisation occurs in the form of observed macroscopic morphology.

5.2.2 Field Contributions and Novelty

There are several novel aspects of the work presented here. The first is using an agent-based simulation to investigate the reproductive division of labour and the patrol behaviour of the queen. Previous work that has looked at behavioural changes in workers focuses on the changes in density and encounter rate as a way of determining worker behaviour [45] [131] [132]. While density is important for regulating behaviour, it needs to consider a deeper information transfer that occurs during the interactions. Repeated interactions with workers can change worker behaviour as the sum of the interactions causes the worker to change their behaviour. However, this does not consider dominance interactions or the policing of workers by the queen. For any dominance interaction to occur, the individuals must recognise each other and convey information. Hence, the use of the internal state. By keeping the density the same, we show the effect of information transfer from the mutual interactions between the queen and workers. We are the first to state a proximate mechanism which generates colony size dependant behaviour and the shift from ergonomic to reproductive stages, with experimental results to validate the model.

Another novel aspect is the development of an evolutionary agent-based simulation, which showed the emergence of a non-selfish genetic predisposition toward altruistic behaviour. The simulation predicted that workers would be more altruistic and refrain from reproducing when the colony size is small but begin producing offspring when the colony size is large. This reflects what has been shown in previous work with live *Diacamma* colonies, where workers refrain from reproducing but begin developing their ovaries when they are unaware of the queen's presence [153] [31] [72]. Therefore, the simulation helps explain the rise in altruistic behaviour in workers and can be used to probe questions about inclusive fitness. The simulation allows for a unique look into the evolution of altruistic genes in workers, how they arise and become widespread, and the stability of altruism as an evolutionary strategy. While the work is based on Ohtsuki and Tsuji [110], there is a crucial distinction in the method for maximising inclusive fitness. There is an iterative process to the simulation, which allows for long-term evolutionary strategies. The evolutionary agent-based simulation allows tracking of altruistic genes' prevalence over time and determining the most effective fitness strategy for a given set of parameters.

The final novel point uses the fractal dimension to quantify the changes in bacteria colony morphology in response to different environmental factors. While colony morphology has been studied before, this usually involves striking images which look at the more easily observed differences in morphology. The fractal dimension has been used to study differences in morphology but only when looking at changes in one environmental factor. Here, we use it to view a landscape of morphological changes and also attempt to quantify the fractal dimension of the colony when exposed to the electric field for the first time. We then used findings from previous work on the effects of the electric field and electric signalling as possible explanations for changes in morphology, from the individual cell on the microscale to the entire colony on the macroscale. Overall, this shows that the internal state is a versatile concept that can explain the emergence of self-organised behaviour in different organisms. It can be adapted appropriately to fit the context. Using agent-based modelling can give us a tool for analysing the behaviour of different collective systems and how information spreads within the network of interactions between individuals. These individuals can be ants or bacteria cells.

5.3 Conclusion and Future work

A fundamental assumption of our real-time feedback model is that the queen can perceive a worker's reproductive status (an internal physiological state) when she contacts it. More importantly, the model also assumes that contact with a reproductive worker(s) increases the frequency of queen patrols. These are, however, necessary to empirically demonstrate in experiments using *Diacamma*. There is another issue that remains to be addressed. Why should the queen's patrol behaviour peak at a specific rate in real *Diacamma* colonies, even if the colony size expands further? The peak queen patrol time is only 20% to 30% of the total time available (S1 Fig). Thus, the queen could afford to increase her patrol effort further. If queens could significantly increase the frequency of their patrol behaviour, the suppression of worker reproduction would be achieved even in large colonies. To understand the adaptive strategies of queens, we must clarify the limiting factor of the queen's investment in patrolling large colonies. One hypothesis is that excessive investment in patrolling might have some fitness costs. These include diminished survival and fecundity, which should also be empirically studied.

The evolutionary agent-based simulation could be helpful for this in determining the fitness costs of increased patrol behaviour. Roulette wheel selection combined with elitism based on fitness parameters offers a mechanism for selecting individuals within a population for reproduction and genetic diversity maintenance [207]. Roulette wheel selection assigns probabilities to individuals based on their fitness values, allowing fitter individuals a higher chance of selection while still affording less fit individuals a probability of being chosen. This approach ensures that the selection process remains stochastic and unbiased, reflecting the diversity inherent in natural selection. By incorporating elitism, where the best individuals from each generation are preserved, the algorithm can maintain a high-performing subset of the population while still allowing for exploration and evolution.

Also, as to proximate mechanisms of reproductive division of labour in *Diacamma*, we have to consider other mechanisms, such as dominance behaviour between workers and worker policing. Dominance behaviour is a worker-worker aggressive interaction over the right to produce own male offspring. Dominance behaviour occurs both in the queen's absence [130] and in the queen's presence. Finally, it forms a linear hierarchy among workers [57] [208]. Interestingly, similar to the patrol behaviour of queens, ritualised aggressive behaviours by dominant individuals can have an inhibitory effect on the reproductive physiology of subordinate workers [70]. The frequency of dominance behaviours is known to increase with colony size in queen-right colonies [72]. This might have a complementary effect of suppressing worker reproduction when the efficiency of queen patrol declines. Worker policing, destruction of worker-produced eggs and aggression to an ovary-developed worker by other workers exist in *Diacamma* [69] [71]. The occurrence is also colony-size dependent [67]. Future studies need to develop a simulation model that involves these two mechanisms simultaneously operating.

The evolutionary agent-based simulation considers these behaviours in the form of the social strategy of the worker. However, it does not explicitly have dominance interactions when workers contact each other. Future research directions discussed above will further enhance our understanding of the mechanisms of the reproductive division of labour in social insects.

While observation and characterisation of the electric signalling in a bacteria colony are not novel, little has been done to see how the signalling might change due to external factors. Especially when investigating how the application of an electric field affects the signalling in a bacteria colony and, in turn, affects the morphology. All of this looks at how information is processed in the bacteria colony, where individuals signal their internal states via electrical signalling. This gives access to nutrients for cells that lack them and maintains the colony's homeostatic balance while leading to sustainable growth.

Future work in this area includes visualising membrane potential changes characteristic of electric signalling in a bacteria colony similar to Prindle *et al.* [80]. The fluorescent dye ThT could be used to visualise changes in electric signalling caused by environmental changes such as a nutrient gradient [209]. Fluorescence imaging would be needed to measure the fluorescence intensity in the dye. The fluorescence of ThT is proportional to its concentration in the cells. It acts as a Nernstian dye, which shows changes in the membrane potential. These experiments could be carried out using a microfluidic system developed using the PiFlow [210] pump. The fluorescence observed in Prindle *et al.* may only be possible in a microfluidic environment. In this case, developing a microfluidic system could be needed to refine the conditions with which the bacteria are grown. This could be done with the piezoelectric pump, a 3D printed static mixer [211], and a microfluidic bacteria plate.

This implementation is more cost-effective than buying a traditional microfluidic system. In this context, the fractal dimension could help dive deeper into colony morphology and reveal the causal chain from environmental stimuli to electrical signalling in the colony to the observed macroscopic morphology. Ways of disrupting and manipulating the electrical signalling could be explored, with the ability to shape the colony if the underlying morphology-forming mechanism is understood. Such disruptions include exposure to an electric field and exposure to antibiotics. Though it is likely to have a disruptive effect, it is unknown how antibiotic exposure affects the colony's electrical signalling.

Results could be improved by isolating cells at specific phases [212]. By isolating cells at specific phases we can study the behaviour, gene expression, and cellular processes that are characteristic of each growth phase in a controlled manner. This can lead to more consistent and reproducible experimental results, as the variability introduced by asynchronous cultures is minimised. This also allows for more accurate comparisons between different experiments and conditions. *Saccharomyces cerevisiae*, a model organism commonly used in yeast studies, may not be the most appropriate choice for synchronising cultures through centrifugation-based selection. While *S.cerevisiae* shares certain cellular processes and pathways with bacteria, its eukaryotic nature introduces additional complexities such as the use of chemical inhibitors [213] [214].

While bacteria in biofilms are relatively stationary, bacteria can spread over a moist surface via collective motion known as "surface swarming". This is usually studied by inoculating bacteria on a soft agar gel, which contains essential nutrients for their growth and proliferation and allows the bacteria to grow freely. Various things can affect this swarming behaviour. These include bacteria with the absence of pili (pilus is a hair-like appendage found on the surface of many bacteria) and viscous agents which decrease swarming [215] [216]. Surfactants which reduce surface and interfacial tension enhance swarming [216]. Water flow can account for the effects of swarming through a porous medium. This is known as Darcy's Law (reduced flow of water from the agar gets to the swarm front, causing a decrease in swarming) [216]. More needs to be done on the processes which lead to particular pattern formation.

After investigating various ways environmental factors can affect colony formation, an agent-based simulation could be developed. In the same way as previous simulations, it would model the communication in the bacteria colony. There would be input from the environment and other colony members. This would help us better understand the coupling between internal states and the global morphology of the colony and their adaptive nature, following in the footsteps of previous work [96] [217]. Work started by Mikami *et al.* [95] could be used as a guide for developing an agent-based model based on the internal state of individual cells.

Stephen Wolfram's work on cellular automata [218] could also be used as a point of reference for future research areas. Cellular automata are simple computational models consisting of a grid of cells, each of which can be in a finite number of states. The state of each cell evolves over discrete time steps according to a set of rules based on the states of neighbouring cells. Wolfram classified cellular automata into four classes based on their behaviour: Class 1, where patterns quickly stabilise into a homogeneous state; Class 2, where simple localised structures emerge; Class 3, where complex and chaotic patterns emerge; and Class 4, where complex and structured behaviour emerges that can support universal computation [218]. It is easy to see how this could be relevant in the investigation into self-organised behaviour in different systems, as cellular automata provide a framework for studying how simple local interactions between individual components can give rise to complex and emergent behaviour at the global level. It would be an alternative approach to the agent-based simulation presented in this thesis, as a method for exploring how local interactions and feedback mechanisms give rise to global patterns, structures, and dynamics. All of this would further our understanding of self-organisation in both ant and bacteria colonies.

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