BUILDING MODELS: DEVELOPING THE BEHAVIOURAL MODEL OF *TEMNOTHORAX* COLLECTIVE WALL BUILDING TO STUDY THE EVOLUTIONARY ROBUSTNESS OF SELF-ORGANISED ALGORITHMS

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Building models: Developing the behavioural model of *Temnothorax* collective wall building to study the evolutionary robustness of self-organised algorithms

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I, Edith Invernizzi, do hereby certify that this thesis, submitted for the degree of PhD, which is approximately 52,800 words in length, has been written by me, and that it is the record of work carried out by me, or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for any degree. I confirm that any appendices included in my thesis contain only material permitted by the 'Assessment of Postgraduate Research Students' policy.

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Thanks to my father, because whatever I do is good for you.

Publications and contributions

Some sections of this thesis are based on research, developed during this Ph.D., that has already been published in peer-reviewed journals.

Section 1.2 was modified from:

- Invernizzi, E., & Ruxton, G.D. (2019). Deconstructing collective building in eusocial insects: implications for ecological adaptation and evolution. *Insectes Sociaux*, *66*(4), 507–518. https://doi.org/10.1007/s00040-019-00719-7

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For both publications, IE (the candidate) developed concept and research, created the figures and wrote the article, while RGD provided academic supervision.

Section 3.3 is based on collaborative research: credit goes to Dr Takao Sasaki (University of Georgia, who provided assistance with experimental design and laboratory equipment, and Dr Théo Michelot , who contributed his statistical expertise. In addition, Dr Kazutaka Soji (University of Tokyo) and Ben Taylor (B.Sc. Biology at UGA) offered precious insights regarding filming setup and video processing. Behavioural data and colony measurements used in this chapter were extracted, as well as by IE, also by N. Ng, A. Rouvière, E. Macqueen, M. Wright, R. Gorgon and L. Gray (all B.Sc. students at the University of St Andrews at the time). The input of these collaborators and helpers are reiterated in the introduction to **Chapter 3**. Of the work described in this chapter, IE was responsible for concept and agent-based model development, video processing, data collection and data collection methodology, statistical analysis (with assistance from MT), figures and writing.

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Research Data access statement

Research data and original scripts underpinning this thesis are available at: https://github.com/invernie/PhD-thesis.
The codes used for the publication *Updating a textbook model of collective behaviour* are found in the dedicated repository: https://github.com/invernie/UpdatingATextBookModelOfCollectiveBehaviour.

Abstract

This thesis focuses on wall building behaviour in *Temnothorax* ants as a case study of self-organised collective behaviour. It contains a progressing series of research packages, building towards one evolutionary question: how eusocial insect nest building algorithms successfully make the transition between two rule variants. I start by revising the existent behavioural model of *Temnothorax* wall building. By replicating the original agent-based model, I identify two issues: 1. the behavioural model performs poorly in conditions of low building material availability; and 2. the behavioural model lacks behavioural termination. I then introduce a revised version of the behavioural model (the *gradual* model) in which high stone density at building sites triggers a decrease in building activity, eventually leading to behavioural termination. I then compare the fit of both models to empirical data using laboratory observations of *T. rugatulus* wall building, applying a hidden Markov model framework to interpret the data. The *gradual* model provides the best match to the observed data. Finally, I use the revised model to test, in an agent-based model setting, how wall quality responds to different types of inter-worker variation in the building rule used: the presence of a mutant variant spreading within the colony; the co-existence of multiple variants; and widespread epigenetic individual variation. I find wall quality to be very robust to nearly any degree and frequency of variants. With additional simulations, I identify the two key elements of the building algorithm that provide robustness: the positive feedback effect, co-localising worker effort despite starting individual variation; and the existence of an area of overlap where activity occurs with high frequency under all variants (a *buffer zone*). I predict that these two components have been under selection for evolvability in wall building *Temnothorax* ants.

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Chapter 1: Introduction

This thesis contains a series of research items dedicated to one case study in social insect collective behaviour: nest wall building in *Temnothorax* ants. The focus of the chapters can be divided into two larger thematic sections: in the first (**Chapters 2-3**), I review the current behavioural model, propose a change to one of its components and test this new hypothesis against empirical data. In the second (**Chapter 4**), I use this updated behavioural model to ask how the components of a collective-behaviour self-organised algorithm can influence the evolution of the algorithm itself, particularly transition between mutations in its rules.

Before I begin, however, I will make a statement regarding the usefulness of this work. This is, after all, a single case study from a niche area of animal behaviour studies, and is at first glance devoid of practical applications. Why, then, should we be interested in nest construction in ants, and why in *Temnothorax* nest wall building? I can offer at least two reasons.

From a purely evolutionary perspective (and therefore from the perspective of fundamental science), we are interested in this process as a case of self-organisation, and specifically one apt at being investigated both experimentally and theoretically. Self-organisation is ubiquitous in biology, from multicellular organisation and embryonic development (*e.g.*, Painter, 2019) to collective movement (Sumpter, 2010). However, the evolutionary trajectories that cohesive and robust sets of mechanisms (*i.e.*, the toolkit for any successful self-organised process) take to evolve are still poorly understood. Understanding how self-organisation can be common in the living world is a three-step process: first, we must obtain accurate models of a sufficient number of self-organised processes; second, we must identify the common key factors enabling cohesion between mechanisms and robustness to perturbation (if a class of such shared mechanisms does indeed exist); third, we must attempt to model the trajectory that these sets of mechanisms must have taken to evolve. In this thesis, I focus on collective behaviour in animals as a type of self-organised process, and on nest wall building in *Temnothorax* as a specific case study that I will attempt to accurately model.

From a practical perspective, collective behaviour algorithms have found useful applications outside biology. For example, ant colony optimisation (ACO) algorithms, inspired by ant collective behaviour, have been applied to routing problems and antenna optimisation, among many other classes of search problems (*e.g.*, Dorigo & Gambardella, 1997). Swarm intelligence mechanisms more in general have been also applied to robotics, giving birth to the field of swarm robotics, with the aim of creating simpler and cheaper automata able to coordinate and make decisions efficiently through information sharing (*e.g.*, Tan & Zheng, 2013).

Below is a detailed breakdown of thesis content.

In **Chapter 1**, I briefly introduce the study of self-organisation in the context of animal collective behaviour (**Section 1.1**), followed by a more extensive overview of its properties applied to social insect nest building (**Section 1.2**). I also present a brief overview of the two study species used in the thesis, *Temnothorax albipennis* and *Temnothorax rugatulus* (**Section 1.3**).

In **Chapter 2**, I present a replication study of the existing behavioural model, developed by Franks and Deneubourg (Franks et al., 1992; Franks & Deneubourg, 1997). I identify some problems in the match between this model and the observed behaviour, including the fact that it lacks behavioural termination. This is expected, as the original model was meant to represent the initial phase of building. I propose a modification to the original model that includes negative feedback on building activity relying on the same stigmergic cue as the initial positive feedback phase: the amount of stones present at building sites. This change to the behavioural function leads to behavioural termination being incorporated in the model: this model can now be used to approximate the full length of building activity. The new function belongs to a type of response curve that seems widespread in social insect nest building. I test its performance using an agent-based simulation and find that the result is an accurate approximation of the observed structure.

In **Chapter 3**, I present a data analysis study using laboratory data of *T. rugatulus* wall building behaviour. I use a hidden Markov chain approach combined with statistical regression models to test whether the modified or the original model result in the best match to real data. My findings support the modified model (*gradual* model).

In **Chapter 4**, I use the more accurate *gradual* model to test the robustness of this building algorithm to the presence of individual variation in building rule parameter value in the colony, studying the impact of variant relative frequency and intensity of conflict among variants. I analyse four scenarios: 1. variant spread within an established colony following queen turnover, and worker replacement over time; 2. presence of multiple variants associated with distinct parental lines (which I identify as patrilines, for simplicity); 3. presence of multiple patrilines combined with patriline-specific building site fidelity; 4. epigenetic individual variation that results in normally-distributed worker-specific variants. I find that this building algorithm is robust to a high frequency of variation and to moderate-to-high levels of conflict. Two components of the building algorithm are responsible for maintaining a coherent and functional collective phenotype: the presence of a “convergence rule” (positive stigmergic feedback) and of a sufficiently wide “buffer zone” (the zone of overlap of the activities of the variants).

In **Chapter 5**, I conclude the thesis with a review of the literature on the evolution of building algorithms and I propose a first tentative hypothesis for the evolutionary path of *Temnothorax* wall building – with a suggested research approach to verify it.

1.1 Self-organisation and group behaviour

The term *self-organisation* encompasses a series of processes found both in biological and in non-living systems where an *organised* overall outcome is produced from the *local* interactions of individual components. The emphasis on the *localised* nature of these interactions is needed to differentiate these processes from those requiring oversight (in animal group behaviour) and to explain the organised structure of some living and non-living systems (such as embryo development and mineral crystallisation; Naka & Carney, 2007; Zhu & Zernicka-Goetz, 2020). The concept of self-organisation is often associated to that of *complexity*, as many of the interactions characterising these systems are non-linear (Wolf & Holvoet, 2004).

In the context of animal groups, self-organisation underlies all instances of collective coordination, such as group movement (*e.g.*, bird flocks), collective decision making (*e.g.*, nest site choice by eusocial insect colonies), and collective nest building (*e.g.*, nest excavation and mound building in termites and ants). The outcome of these group processes (coordinated group movements, accurate decision making, functional nest architectures) can be referred to as *collective phenotypes* (belonging to the class of extended phenotypes; Dawkins, 1999), because they result from the interaction among the individual activities of group members while holding properties that only exist at the group level.

Self-organised processes are nearly always characterised by feedback loops that either co-localise (if the feedback is positive) or separate (if negative) similar events in time and space, therefore generating an organised pattern. Positive feedback is particularly common in these systems (Camazine et al., 2001). In the case of collective behaviour, the cue generating the feedback must elicit the same response in all group members involved in the activity in order to produce group (rather than intra-individual) coordination. These feedback mechanisms are thereby essential to make the transition between individual and collective phenotype.

In the study of social insect colonies, most work on self-organisation has focused on three key collective behaviours, all central to the life of the colony: division of labour, decision making, and nest building. I discuss division of labour in **Chapter 3**. Research on collective decision making using eusocial insects has shown that colonies can make optimal decisions when presented with alternatives of different quality (*e.g.*, Franks et al., 2006; Pratt, 2009; Reid et al., 2012), surpassing the cognitive capacity of individuals (Sasaki & Pratt, 2011, 2012), and displaying, albeit with high failure rates, the ability to reverse their decision in the face of new incoming information (Franks et al., 2007; Latty &

Beekman, 2013; Sasaki et al., 2015). The ability to commit to a colony-level choice, often corresponding to optimality, is achieved by the assessment of multiple individual workers being propagated through the colony in a fashion proportional to the quality of the resource, thereby generating a positive feedback loop where high-quality options spread and low-quality options are dropped. The mechanism of information propagation is direct in some cases, through active nestmate recruitment by workers to their preferred option, and indirect in others, for example in the case of trail-laying on the way back from a successful foraging trip. In the first case, the positive feedback is generated by recruited workers engaging themselves in recruitment, while in the second one the trail exercises an attractive effect on co-workers, so that resources used by more workers attract a higher number of new workers. These self-organised mechanisms of decision-making are often accompanied by hard rules, such as the presence of a *quorum* for consensus, that speed up the process of convergence to one choice.

In the next section, I focus on the third most studied case of self-organised collective behaviour in the context of eusocial colonies: nest building.

The following section introduces nest building in eusocial insects and is based on a review article published in *Insectes Sociaux* (Invernizzi & Ruxton, 2019).

1.2 Deconstructing collective building in social insects: implications for ecological adaptation and evolution

1.2.1 Architectures of social and eusocial insects: complexity in the detail

The architectures built by insect collectives display a variety of forms. Here, I focus on two features that are common to all and that underlie the functionality and adaptiveness of these structures. The first one is functional organisation, where the nests of these species are organised into functionally distinct elements. The nest of leaf-cutting ants, for example, consists of a network of major tunnels leading deep into the soil (*Acromyrmex* sp) from which nest chambers housing the brood and fungus branch out, connected through a short peduncle (*Atta* sp and *Acromyrmex* sp). Despite inter-specific variety (Bollazzi et al., 2012; Moreira, Forti, Andrade, et al., 2004; Moreira, Forti, Boaretto, et al., 2004; Verza et al., 2007), this essential form of structural organisation is ubiquitous in the genus (Tschinkel, 2015). Similarly, ant nests across species share the same layout of one or more shafts laterally connected to chambers, with the number and angle of shafts, frequency and shape of chambers and relative distance between elements being identifiable species-specific features (Tschinkel, 2015). In *Atta* species, there is also fine-tuned functionality of specific structural elements: nest ventilation is passively driven by surface air currents flowing across the entrances to two distinct tunnel types, a central tunnel, for outflow, and several peripheral ones found lower in the structure, for inflow (Kleineidam et al., 2001). The same type of structural organisation and the exploitation of similar environmental forces for thermoregulation and gas exchange is also observed in termites (Bonabeau, Theraulaz, Deneubourg, et al., 1998; Korb & Linsenmair, 1998; Ocko et al., 2017). A different type of complex structure is found in social wasps and bees, where nests consist of geometrically-arranged cells creating a nest structure with species- or genus-specific characteristics. These structures are also likely to have thermoregulatory properties (*e.g.*, Höcherl, Kennedy, & Tautz, 2016). In wasp species living in larger colonies, the nest is often composed of repeated submodules built in sequence and linked through connecting structures (Starr, 1991; **Figure 1.1**).

Figure 1.1. Nest of the social wasp *Polybia nigripennis*, with cross-section.
The inside of the nest (exposed on the *right*) consists of connected repeated modular structures. © The Trustees of the Natural History Museum, London.

Second, besides finely developed functionality and structure subdivision and specialisation, a key feature of many eusocial insect nest architectures is flexibility that allows adaptation to both the local environment and to changing functional needs of the colony. In the crevice-dwelling ant *Temnothorax albipennis*, builders accumulate material (dirt granules) at a distance from the queen, brood and nursing ants that is proportional to colony size, creating a circular nest wall around the colony (**Figure 1.2**). If after construction the colony expands in number above a certain threshold, the existing wall is dismantled and a new one built at a greater distance from the centre of the brood (Franks et al., 1992). A similar phenomenon occurs for the royal chamber of *Macrotermes* (Bruinsma, 1979; Grassé, 1939). In leaf-cutter ants, again, the number of nest openings changes in response to external and internal humidity and temperature levels and airflow, to provide nest homeostasis (Bollazzi & Roces, 2007, 2010a, 2010b). A linear relationship between colony and nest size is indeed found in most ant species

(Tschinkel, 2015). Below I review the mechanisms proposed to underlie nest construction, focusing on the flexibility and functionality they provide to the nest.

Figure 1.2. Exposed *Temnothorax albipennis* nest.

Wall sections are visible internal to the contact marks left by the overlapping stones. The remains of the cluster of brood and nurses are visible (white arrow). Image courtesy of T. Sasaki.

1.2.3 Mechanisms of building

We will start by reviewing mechanisms used at the individual level and that can be applied also by solitary species. Only in the second section, I will shift focus to collective building specifically, discussing the problems, and potential solutions, of coordinating the parallel activity of multiple individuals toward one single structure.

1.2.3.1 Main hypotheses

The simplest proposed mechanism underlying complex building (Camazine et al., 2001) is the use of a series of actions in a fixed temporal or spatial sequence, a *recipe*. There is evidence of animals using a fixed sequence of building actions at some or all stages of construction, for example in spider web and moth cocoon weaving (Foelix, 2011; *e.g.*, Lounibos, 1975), but also in the initial phase of nest building in the primitively eusocial wasps *Polistes fuscatus* (Downing & Jeanne, 1988). A drawback of building strategies relying entirely on fixed progression is, however, the lack of flexibility in responding to unusual circumstances: the classic example of this is that of solitary potter wasps *Paralastor* sp, that are shown to respond to an artificially induced hole resembling one of the construction stages in their nest by replicating the construction sequence from that point onwards, even if this leads to an aberrant structure (A. P. Smith, 1978).

A second drawback of the recipe mechanism is that building Is isolated from the assessment of external factors (this limitation is shared by what has historically been another of the proposed mechanisms underlying complex building, the use of a *blueprint*, *i.e.*, a fully-explicit description of the target structure to be built; Thorpe, 1963). From the point of view of structure functionality, it follows that builders using this mechanism cannot take local environmental features into account and also cannot react flexibly to the environmental factors the structure exploits. This is in contrast with what observed for example, in *Macrotermitinae* and in leaf-cutter ant workers, whose activity relies on the continuous assessment of air currents, temperature and humidity gradients (Bollazzi et al., 2012; Bollazzi & Roces, 2007, 2010a, 2010b; Harris, 1956; King et al., 2015; Kleineidam et al., 2001; Roces & Kleineidam, 2000). Insensitivity to local cues is likely problematic especially in species whose habitat range covers a spectrum of relevant environmental conditions, where seeking to replicate a fixed blueprint or recipe might put the colony at risk of maladaptation to the local environment. In leaf-cutter ants, in particular, workers have been shown to open or seal nest outlets to the surface selectively, when the incoming air is dry or when the outgoing air is humid, respectively (Bollazzi & Roces, 2007, 2010a). The number of outlets increases with decreasing external humidity and increasing external temperature, hereby modulating nest humidity and temperature (Bollazzi & Roces, 2010b). Inter-colony variation has been studied and found in *Temnothorax rugatulus* ants, where 5-20% of this variation is explained by differing environmental conditions (DiRienzo & Dornhaus, 2017). Large intraspecific variation in nest structure is well-documented in *Macrotermitinae* (Coaton & Sheasby, 1972; Darlington, 1984; Grassé & Noirot, 1961; Harris, 1956; Pomeroy, 1977) and in leaf-cutting ants (Bollazzi et al., 2012; Moreira, Forti, Andrade, et al., 2004; Moreira, Forti, Boaretto, et al., 2004; Tschinkel, 2015; Verza et al., 2007) and evidence of structure adaptation that matches macroscopic environmental conditions exists at least in some insect species. *Macrotermes bellicosus* mounds found in forests have a dome-like structure and reduced external ridges, providing reduced ventilation but increased thermal homeostatic capacity compared to the ridged pinnacle mounds built in open savannah (Korb & Linsenmair, 1998). The leaf-cutting ant *Atta vollenweideri* controls structural stability and wall porosity when presented with different building materials by combining substrates with different physical properties at different ratios (Cosarinsky & Roces, 2012). From the point of view of collective behaviour, moreover, recipes (or blueprints) cannot guarantee coordination of the independent activity of thousands of individuals: even when considering sensory feedback from neighbouring worker activity, different worker groups might develop the structure at the same time in different directions and the same structure be built multiple times by workers tending to distant sections–- especially in large structures. Coordination of large, complex structures using a blueprint would require a degree of labour division *within* building activity not documented so far in any social insect.

The use of rules exploiting *templates*–- reference points from which to measure the distance and shape of deposition of the building material–- allows building with respect to environmental elements, other features in the same construction or even focal individuals (Theraulaz et al., 1998). Template-based building enables both the incorporation of cues with adaptive value and of cues on the geometry of the structure. Structure size, shape and position can be adaptively fitted this way. In *Polistes fuscatus*, for example, the position on the pedicel where construction of the first nest layer starts is carefully evaluated as a function of distance from the substrate, as proximity to the tree is crucial for nest access by predating ants (Downing & Jeanne, 1988). Workers of the Formosan subterranean termite orientate the excavation of new galleries through reference vectors from the tunnel of origin (Bardunias & Su, 2009a). Possibly more importantly, templates also allow directly-guided, flexible, immediate adaptation of the architecture to functional needs. An example occurs in *Macrotermes subhyalinus*, where workers deposit building material in relation to distance to the queen (whose size changes as her abdomen expands) and modify chamber size accordingly throughout the life span of the colony (Bruinsma, 1979; Grassé, 1939). Direct feedback of this type between function and structure construction, however, does not provide information on any elements other than the template, that is, each builder's knowledge is local and not global. This might be an important limitation. For example, in *Temnothorax albipennis*, nest walls (**Figure 1.2**) need to suit the size of the brood cluster, but also that of the colony population size. While there is evidence that wall construction, similarly to *Macrotermes*, occurs in relation to distance to the brood, use of a physical template alone cannot explain rebuilding in response to colony size expansion. In this sense, a non-obvious type of spatial or temporal template is that of the builder's activity itself, in terms of spatial or temporal units elapsed since a particular action was performed, or of templates informative of nest mate density, such as chemical templates or the mechanical template generated by impact with other workers (Camazine et al., 2001).

Physical structures, activity-based reference points, volatile elements such as chemicals, and environmental factors have all been shown to act as templates. Queen-released pheromone in termites is the most well-studied case (Bruinsma, 1979; Bonabeau et al., 1998; Camazine et al., 2001), while instances of humidity, light and temperature gradients, as well as air currents (Bollazzi & Roces, 2007, 2010b, 2010a; Ceusters, 1986; Jost et al., 2007; Kleineidam et al., 2001; Ocko et al., 2017; Roces & Kleineidam, 2000; Sudd, 1972; Tohmé, 1972) have also been identified. Template mechanisms based on environmental conditions or features can directly feedback on the suitability of the structure to local space and guide its shaping, explaining how local factors can be taken into account in detail.

For example, temperature-driven removal and deposition of soil in *Macrotermes* has been proposed as a driver of mound shape adjustment to sun exposure (Turner, 2000). The nest structures of termites and leaf-cutting ants cited above, in particular, rely on environmental conditions for their functions. Gas exchange, for example, occurs through ventilation generated through the tunnels on the basis of daily temperature oscillations, in *Odontotermes obesus* (King et al., 2015), such that the underlying building mechanism needs to be tightly linked to local condition sensing.

1.2.3.2 Collective Building

Collective building shows two apparently contradicting features. Firstly, despite the emerging structure being coherent, individual behavioural events appear random to some extent (Camazine et al., 2001). These instances of stochastic behaviour are a consequence of probabilistic, rather than deterministic, building rules in the use of templates. Secondly, the coherence of the final structure implies a degree of behavioural coordination that cannot be achieved by the use, alone, of the building mechanisms proposed above. Actions from a fixed spatial or temporal sequence performed by multiple individuals will clash and result in an unstructured architecture unless division of labour is extremely fine. The use of templates does guide individuals to construct the same architecture. However, a complex structure includes a high number of features, which would in turn require a large number of templates and of rules guiding the shift between templates. The cognitive capacity for such a high number of rules is expected to be large. The minimisation of both the amount of high-level organisation required and the number of rules a worker needs for a complex structure can be achieved with the help one or more of three mechanisms: stigmergy, self-assembly and self-organisation (Camazine et al., 2001; Sumpter, 2010; Theraulaz et al., 1998, 2003).

Stigmergy

Stigmergy (Grassé, 1959) can be summarised as the recognition and consequent response to selected (stimulating) spatial configurations found in the environment or arising as intermediate stages in the building process. Such response manifests in a change in the quantitative (in intensity of) or qualitative (in the form of) type of building activity and is stimulus-specific; so that different stimulating configurations produce different responses. This type of building rule is similar to recipes in that they recognise previous structures and respond with pre-established actions, but have, in contrast, the advantage of being unbound to a temporal or spatial sequence. As long as individuals do not discriminate between building done by themselves and others, they can collectively contribute to the same structure by applying the same set of rules. Theraulaz *et al.* (1998) give the following helpful description of a (potential) stigmergic sequence:

"[ ] a stimulating configuration triggers a response, a building action, by a termite worker, transforming the configuration into another configuration that might trigger in turn another (possibly different) action performed by the same termite or any other worker in the colony."

We can see that stigmergic rules can provide start and termination points to the building activity: construction starts as a stimulating configuration or heterogeneity is found in the environment and ends when the last performed action leads to a non-stimulating configuration, causing all activity to stop without the need for external input. Also in this sense, stigmergy has been interpreted (Camazine et al., 2001; Theraulaz et al., 1998) as a form of indirect communication among workers, through modification of their environment (sematectonic communication; Wilson, 1971), on the state of the ongoing construction.

However, stigmergic mechanisms do not necessarily result in behavioural coordination, even when workers are assumed to use the same rule set. In qualitative stigmergic building, unless all emerging configurations are unique, multiple stimulating configurations will become available at the same time, creating a landscape of multiple potential building states that are the result of the stochastic choices made by each worker at each step. In quantitative stigmergy, stochastic differences in the actions of individual workers will result in incoherence in the final structure that may put structure stability and efficiency (*e.g.*, the thickness or evenness of a duct wall) at risk. How then might coherence be achieved?

Self-assembly

Some sets of qualitative stigmergic rules can guarantee structural coherence. Theraulaz and Bonabeau (Bonabeau et al., 2000a; Theraulaz & Bonabeau, 1995) explored the space of potential qualitative rule sets (building algorithms) identifying those that give rise to coordinated, coherent structures and called these sets *coordinated algorithms.* The resulting structures resemble nests built by social wasps. The authors identify at least one property common to all such rule sets: these algorithms produce only stimulating configurations that are non-overlapping, that is, that are unique to a single building stage, so that no more than one state can follow from a previous one (an emerging fixed sequence). The use of these rule sets by a group of builders leads to the creation of a geometrically complex and coherent structure, without the aid of other guiding mechanisms, and is termed *self-assembly*.

The coherence necessary to produce organised structures means that coordinated algorithms often produce modularity. Similarly to what is observed in social wasp nests (**Figure 1.1**), the final structure consists of repeated, relatively less complex substructures in a semi-fixed arrangement. Moreover, these algorithms allow for variation in the final architecture (most commonly in the size and spatial organisation of substructures) arising from stochastic choices of individual builders when multiple stimulating configurations are active at any one step.

Interestingly, from an evolutionary perspective, these algorithms occupy a restricted and compact area of the space of all possible sets of rules. Similar rule sets give rise to similar architectures, so that mutations in one set can potentially switch the building to a neighbouring architecture in the configuration space (Theraulaz & Bonabeau, 1995). Although Theraulaz and Bonabeau do not offer enough detail to understand the implications, it is possible that the algorithms' rules are such that the use of two neighbouring rule sets within one group of builders can still result in a coherent final structure. That is, the similarity between the two sets might imply that even the minority of rules that differ can be integrated without creating discontinuities. This hypothesis has important implications when considering mutations in building rules in an evolutionary scenario. Mutations carried by a sub-group of workers might be likely to shift rules to a neighbouring set, rather than a fully different one, and thus still produce a coherent structure when used jointly with the old set. Furthermore, the effect of random mutations can be overridden if we add one more implication of coordinated rule sets: the type of configurations emerging during construction is partially or fully constrained, so that only a small subset of randomly mutated rules are likely to ever be implemented (Theraulaz & Bonabeau, 1995).

Although self-assembly seems to provide a sufficient explanatory mechanism for most architectural elements of composed wasp nests, architectures which are not obviously composed by the geometrical arrangement of building units and with higher complexity in their internal organisation can better be explained through self-organisation dynamics.

Self-organisation

Self-organisation is the process by which an organised pattern emerges from the *quantitative* modulation and interaction of behaviours (as opposed to the qualitative character of self-assembly). The key quantitative aspect involved here is the probability of performing a behaviour. In this sense, self-organisation is potentially cognitively cheaper than self-assembly: because the same rule can produce different intensities or frequencies of one behaviour and thus result in different outcomes at the collective level, fewer rules might be required and applicable at different construction stages, as long as the stimuli in each differ in the response triggered.

There are two main features that underlie self-organised dynamics:

1. the probabilistic, as opposed to deterministic, nature of the key behaviours is at the basis of most dynamics and of building initiation;
2. feedback mechanisms often drive the spatial and temporal organisation of the behaviour.

Feedback mechanisms regulate the spatial and temporal coordination of workers by positively or negatively reinforcing a stimulus. Positive feedback directs the activity of an increasing number of workers towards the same location as others, thus co-localising effort. An example of a substructure emerging purely by self-organisation is the opening of lateral tunnels in Formosan subterranean termites (Bardunias & Su, 2009b): because most unladen termites are travelling towards the excavation front, lateral tunnel branches tend to be initiated and develop in the direction away from the origin of the tunnel system. Stigmergic positive feedback driven by the incipient depression in the lateral wall then promotes the excavation of the tunnel in the right direction, while pellet deposition, being independent from this stimulus, remains occurring at constant rate and prevents the closing of the main and developing tunnels by chance. Feedback can also provide an indirect means of optimising aspects of pattern efficiency linked to the feedback signal, as experimental evidence with ant foraging trail formation shows. In this case, foragers are drawn to a path by the increasing concentration of pheromone released by other ants. As this pheromone is volatile and falls below the ants' response threshold after a few minutes, the path taken by the majority of ants over time will be the one for which passage frequency is highest, *i.e.*, the shortest: time-related features of the signal optimise time efficiency in the pattern (Beckers et al., 1992a, 1992b).

Initiation of building in self-organised systems is also often reliant on positive feedback: from random events triggered by probabilistic behaviour, a series of events close enough in time or space must occur to hit a stimulus threshold generating positive feedback (Theraulaz et al., 2003). For example, in termite and ant wall building, independent material collection and deposition by workers is often observed, but building is not initiated until a sufficient co-localised number of these events occurs. Below a certain population size threshold, building is not initiated in termites (although this effect might also be a consequence of aggregation being a necessary stimulus; Green et al., 2017), while increase in colony size below a certain proportion does not trigger wall expansion in *T. albipennis* nests (Franks & Deneubourg, 1997). Initiation may also be guided by stigmergic rules or by other external stimuli that may carry adaptive information on the choice of building site or timing, as in Formosan subterranean termites, where depressions in the terrain also drive material pick-up and deposition initiation (Bardunias & Su, 2009b). In the ant *Acromyrmex lundi*, chamber construction and enlargement follow the relocation of brood (Römer & Roces, 2014).

Convergence of the behaviour of the majority to a single state (*e.g.*, one trail) driven by feedback occurs over time and passes through intermediate stages where a decreasing number of alternative states (*e.g.*, the number of active trails) are active. This is driven by competition between positive feedbacks of nearby stimuli (two equally utilised paths), resulting in long-range reciprocal inhibition (after a distance threshold, both paths are equally attractive, while the chance of a new path forming in-between is extremely low: negative feedback).

The switching point between states is reached when the intensity of the behaviour-triggering stimulus is great enough to become an attraction point over neighbouring stimuli. At points at which the switch is non-reversible, we have bifurcation points, where the system takes a turn towards the formation of what will become the final pattern. The direction taken at these points might be driven by external dynamics, such as the higher attraction force exercised by a better food source in decision making for foraging trails, or population dynamics intrinsic to the system, such as group size at different locations, and might allow for adjustment to external circumstances. Because of the stochastic component of behaviour, moreover, the same system might develop towards different final states according to the path taken at bifurcation points: multiple stationary states exist, allowing for variation in the range of final patterns observed. When the path taken is the result of stochastic fluctuations, the result is stochastic variation in the outcome.

Feedback-generating stimuli might be chemicals released by the workers, such as pheromones, but might also rely on indirect communication through stimulating stigmergic conformations (*e.g.*, pick-up at nest outlets, in *Acromyrmex heyeri*; Bollazzi & Roces, 2010a), or even derive from spatial dynamics. *T. albipennis* nest wall building, for example, is believed to rely on the physical hindrance created by stone piles to ant movement, so that ants laden with sand are more likely to drop their pellet in the presence of stone heaps, ultimately – in combination with a template – creating a wall (Franks and Deneubourg, 1997).

Termination of the building activity is usually achieved through negative feedback by causes such as depletion of building material combined with long-range inhibition dynamics: one example is the attractive effect of stone piles or pillars of pellets that makes removal of material from these locations and deposition at other locations a very rare event.

In biological systems, the patterns emerging from self-organisation dynamics are often under fitness-driven selection. For this reason, in these systems self-organisation dynamics nearly always interact with cues providing information on the neighbouring environment and on the effectiveness of the pattern, in a combined mechanism likely to have emerged under selection. These cues are the templates described above. In *Acromyrmex heyeri*, for example, the regulation of nest outlets' opening and sealing is the result of the self-organisation of environmental template- and stigmergy-driven behaviour. The baseline pick-up rate of an ant worker is higher than its baseline deposition, maintaining the outlets open within the optimal range of environmental conditions. As the humidity of outflowing air increases, it acts as a template on deposition at the site, triggering an increase in its rate. Increasing deposition is counterbalanced by the stigmergic stimulus created by the growing heap of sand granules, which increases pick-up behaviour. The ratio of deposition to pick-up rate is what maintains the system in balance and prevents total closure of the outlets unless the escaping humidity is extremely high (Bollazzi & Roces, 2010a).

1.2.4 Consequences of self-organisation for complex nest building, adaptation and evolution

1.2.4.1 Transition between substructures emerges from environmental cues and local rules

Self-organisation dynamics are likely to be at the basis of transitions between nest elements in the building of a nest, because they provide a simple mechanism for the creation of complex structures that does not require new behavioural rules. The emergence of distinct substructures can follow from the use of the same rule set consisting of few, simple building rules, that give rise to different shapes based only on the interaction with the physical or chemical properties of the environment where the behaviour is performed. For example, Bonabeau *et al.* (1998) present a computational model of *Macrotermes subhyalinus* galleries and chamber construction where the same diffusion principle that leads to wall formation from termite trails creates arch-shaped isoconcentrations of pheromonal template when termites walk on pillars or walls. Following this new gradient, building activity shifts from the production of pillars to that of gallery roofs. Similarly, the intersection of different trails generates a round-shaped inhibition effect due to high trail pheromone concentrations, leading to wider construction-free spaces: chambers.

Transition between substructures might also be modulated by the interaction of different templates, where neighbouring areas of the nest emerge from the interaction of two neighbouring templates. In this case, the shape of the area at the border between the two structures can be modelled by the quality and intensity of the interaction. In particular, we can envisage two scenarios (**Figure 1.3**). Let's imagine two templates, template A (T_A) and template B (T_B), with an overlapping area of influence H where both can be perceived by a worker at an intensity above its behavioural response threshold. In a scenario where T_A and T_B regulate the same behavioural rule, in the hybrid zone H the probability of performing the behaviour is either (1.) determined by a combination of the two template effects or (2.) controlled by the stronger template at that point in space. In case 1, the effect on the behaviour within H will be higher than the effect of the behaviour within the one gradient area of either T_A or T_B. The outcome will thus be an enhanced structural feature, such as a larger excavation area or thicker walls. In case 2, where the stronger template at each spatial location dominates, simple merging of the two structures developing in opposite directions should be expected across H.

In a scenario in which T_A and T_B modulate different behaviours, in contrast, the two behavioural mechanisms will merge within H. If they are compatible, an equilibrium structure that is the product of mechanism combination is reached, possibly generating new substructures. Conversely, if the competition between mechanisms cannot be resolved, this should be expected to result in an area of ongoing building activity, with builders removing material where deposition has just occurred.

Transition between substructures might alternatively be organised through stigmergic cues: qualitative stigmergy might trigger the shift between building behaviours as a stimulating conformation is reached and self-organisation might underlie the formation of such configurations. An example is the construction of the royal chamber by *M. subhyalinus*, which develops through shifts between substructures (Bruinsma, 1979). Pillar construction around the queen shifts to expansion into lateral lamellae after a certain height is reached, followed by joining of lamellae into a roof and pillars merging into homogeneous walls. Here, the initiation of building activity is stimulated by the heterogeneities created by building material pellets scattered across the nest's floor.

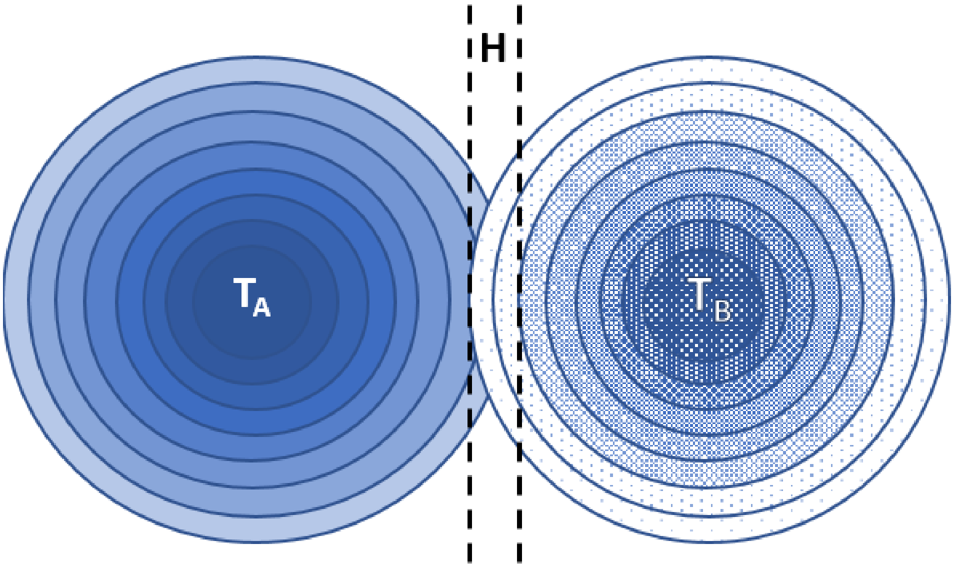

Figure 1.3. Interaction of two building templates modulating transition between neighbouring structures. Proximity between two templates (T_A and T_B) creates a hybrid zone *H* where their gradients overlap. The effect of T_A and T_B on behaviour, the relative intensity of each gradient in the hybrid zone and the compatibility between the two behavioural products determines what collective phenotype emerges in *H*.

It must be noticed that, from an evolutionary perspective, and independently from the mechanism we consider, what we recognise as specialised substructures might have emerged by chance from building dynamics initially evolved to meet different building needs. These new structures could then have been co-opted for specialised purposes, with scope for potential additional selection. As an example, we may imagine the hypothetical case of a ground-excavating termite species that exhibits faster digging behaviour in humid terrain, as in *Coptotermes frenchi* (Evans, 2003). During queue formation at the front of tunnel excavation, any lateral excavation (Bardunias & Su, 2010) that meets an area of high humidity will lead to quick expansion of the new digging site and full exploration of the humid patch (**Figure 1.4, panels 1-4**). As a consequence, chambers siding the main tunnel may emerge (**Figure 1.4, panel 5**) that present the right humidity conditions to be later co-opted for other functions, such as hosting fungal symbionts. Overall, new experimental and modelling work is necessary to test these hypotheses. Additionally, structural elements might be the product of evolution under selective pressures other than structural efficiency. For example, Oldroyd and Pratt (2015) propose the hypothesis that the different types of comb organisation observed in the Australian stingless bee genus *Tetragonula* are the by-product of selection on the cell provisioning and oviposition process (*i.e.*, the provisioning of brood cells and subsequent egg positioning and cell sealing). According to their scenario, selection for avoidance of queen-worker and worker-worker reproductive conflict would promote the emergence of different building algorithms in which brood cells are built either one at a time or in parallel, and either at close-by or at far-away nest locations, depending on the degree of conflict. In turn, the resulting building rules would result in different, species-specific brood cell arrangements: comb, spiral and cluster. Understanding the building algorithm(s) of *Tetragonula* species, coupled with simulation modelling, is necessary to test this hypothesis.

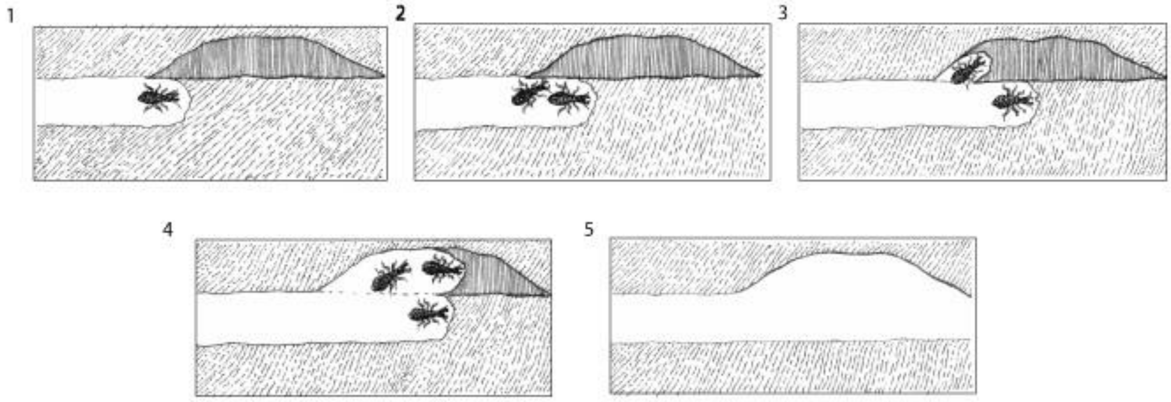

Figure 1.4 Emergence of lateral chambers from tunnel digging dynamics.

The figure shows the progression of events during tunnel building that could, in a hypothetical scenario, lead to the formation of high-humidity chambers, without rules specific to the formation of this chambers being required. The example illustrated is based on the behaviour displayed by *Coptotermes frenchi* and Formosan subterranean termites. Darker areas in the figure indicate zones of higher humidity in the digging substrate. Tunnel digging often leads to worker queue formation when the excavation front is occupied (**Panels 1-2**). Workers awaiting access have a constant probability of instead initiating lateral excavation along the queue (**Panel 3**). If excavation speed is higher in humid than dry terrain, as it is the case in *Coptotermes frenchi*, then lateral digging will proceed laterally until the whole humid patch is excavated, producing a lateral chamber (**Panels 4-5**). Image drawn by James Wilson.

1.2.4.2 Flexibility and the adaptiveness of nest structures

The reliance of self-organisation on the convergence of probabilistic behaviours, or of inter-individual variation (Dussutour et al., 2009; Yamamoto & Hasegawa, 2017), provides an additional advantage:

flexibility. Beside the assessment of environmental cues that allow adaptation to the specific *fixed* characteristics of the local environment (*i.e.*, exposure to sunlight, direction of air currents, heterogeneities on terrain surface, *etc.*), the structure also dynamically responds to short-term changes, both to the environment and to the colony (Perna & Theraulaz, 2017; Turner, 2007). Nest size, for example, is known to correlate to colony size (Tschinkel, 2015), a phenomenon explained by the increase in building events and rate as the number of workers increases. Similarly, the intensity of worker activity provides a mechanism regulating structure size: the size of galleries increases in response to worker traffic rate.

These events in collective building are effectively a case of collective decision making. The competition between different possible cues of the same type (*e.g.*, two air currents) attracting workers means that the colony is assessing multiple building alternatives at any one time. Probabilistic individual behaviour can be regarded as a means of exploration of the phenotypic landscape of the structure, with feedback dynamics ultimately creating convergence to one solution. Convergence will lead to the strongest template or the one with the most favourable spatial position (positive feedback driven by ease of access), if the difference in quality (the relative attraction basin) is large enough. It can, for example, be argued that the “winning” template emerging from the dynamics above can often coincide with an optimal decision for structure functionality (Beckers, 1992; Franks, Dornhaus, Fitzsimmons, & Stevens, 2003; Sasaki, Colling, Sonnenschein, Boggess, & Pratt, 2015). Recent studies suggest that self-organisation can provide flexible adaptation to changes in the environment (Latty & Beekman, 2013; Ramsch, Reid, Beekman, & Middendorf, 2012; Reid, Sumpter, & Beekman, 2011; Vittori, Gautrais, Araújo, Fourcassié, & Theraulaz, 2004; but see Beckers, Deneubourg, & Goss, 1992), although the effectiveness of the change decreases if only a subset of individuals actively monitor cues (Sasaki et al., 2015). Fine-tuning feedback mechanisms to adjust the rate of increase as well as decrease in activity might be necessary for feedback dynamics to be triggered and efficient across colony sizes (Latty & Beekman, 2013; Nicolis et al., 2003; Planqué et al., 2010). Often, self-organisation dynamics also need to account for trade-offs between different requirements of structure function (*e.g.*, Bollazzi & Roces, 2010b; Buhl et al., 2009; Farji-Brener et al., 2015; Perna, Jost, et al., 2008; Perna, Valverde, et al., 2008). Fine-tuning probabilistic behaviour could also be under evolutionary selective pressure for optimal exploitation in environments with different degrees of dynamicity (Deneubourg, Pasteels, & Verhaeghe, 1983).

The response of structure shape to environmental conditions might not necessarily be adaptive. Rather, it might be a by-product of the physical properties of the environment (*e.g.*, the higher digging speed of *Coptotermes frenchi* in damp in contrast to dry soil might reflect a preference of the termites for humid environments, but it might also result from this type of substrate being easier to excavate;

Evans, 2003) or of rules that are adaptive in a different context (*e.g.*, corpse pile formation in *Messor sanctus* is sensitive to wind speed, a response that is likely to have evolved for brood pick-up and deposition preferences; Jost et al., 2007). Similarly, computer simulations have shown that many aspects of the shape of insect nests are likely the result of the interaction with physical properties of the substrate and other features in the environment, with templates contributing to the shaping of smaller structures only (Khuong et al., 2011; Ocko et al., 2019). Generally, while some self-organising mechanisms might have evolved under selection for architectural or behavioural optimisation, others might simply have emerged from the properties of cognitive networks or of physical and chemical laws and display features that, coincidentally, confer an evolutionary advantage For example, in the ant *Monomorium pharaonis*, foraging networks form so that branching angles at junctions result in optimal polarity for the network (Jackson, Holcombe, & Ratnieks, 2004; but see also, in *Messor sanctus*, Garnier, Combe, Jost, & Theraulaz, 2013). Whether this is the result of selection or a side-effect of the cognitive and neural mechanisms underlying trail laying and orientation.

1.2.4.3 Robustness

Feedback dynamics enable individuals to gather information about the level and location of the activity concomitantly performed by others. This concept has interesting implications in terms of mutations in building rules. Let’s imagine a colony that responds to a template gradient with intensity a, so that building converges towards a point at distance R_a from the template. Let’s now imagine that a genetic mutation arises that changes the intensity of the response to the template to b, resulting in a preferred building distance R_b from the template. What impact would this mutation have on construction? Most social insect species in which collective building occurs are eusocial, that is, with a particular colony genetic structure where the worker population is the offspring of one or a few breeding pairs, or of queens mated to one or few males. In these systems with high genetic similarity, genetic mutations can spread very quickly and create conflict among the building rules used by workers. In the presence of multiple genetic lines (polygynous colonies or multiply-mated queen), workers abiding to different building rules are contributing to the same structure. Even when colonies are monogynous and the queen mated to a single male, dependent colony foundation or queen replacement means that a mutation carried by a member of the breeding pair spreads in the colony following worker turnover. In both scenarios, when a large enough proportion of workers responds to a template with the new variant (b), the expected effect is structural incoherence, resulting in parts of the structure being built at different distances – or is it?

Where feedback mechanisms exist that guide individual activity based on collective activity, we might observe the collective-level phenotype converge to an intermediate form between all variants of the building rule used. The exact shape of this intermediate phenotype depends on the relative influence of each variant on the intensity of positive and negative feedback that its product generates. The frequency at which variants are expressed and the discrepancy between their product are also likely to determine whether convergence can occur.

An illustration of how this process might unfold may be given using the example below (**Figure 1.5**). Let's suppose that, in addition to a distance-from-a-template rule, the building product (sand deposited) also generates an attractive effect on workers, so that even more activity occurs in the presence of the product. Negative feedback dynamics that decrease the level of activity where the product is rarer also exist. At low frequency of variant *b*, the collective phenotype is likely to converge to variant *a*, because the *b* product is rare (**Figure 1.5**, **left**). When frequencies of *a* and *b* are similar, the two competing variants have areas of overlap in their template gradient. If there is a subarea of the overlap where the sum of the probability of deposition from both rules is higher than the single probabilities, then this is the area of highest attractiveness in the gradient. Group deposition converges towards this point and a collective phenotype intermediate between *a* and *b* emerges. The existence of such an area depends on the difference between R_a and R_b (the discrepancy between the variants) and on the shape of the gradient. A similar process can be envisaged in self-assembly when the new variant can be integrated with coherence with the existing rule set (*i.e.*, the neighbouring coordinated algorithms described by Theraulaz and Bonabeau, 1995).

A process of this type has important consequences for the evolutionary robustness of the building phenotype. It enables transition between changes in rule sets that are neutral to the adaptiveness of the structure when used by the collective (the nest survives the transition). It also lengthens the survival period of the nest when the mutation creates a non-adaptive architecture, as long as the mutation can be overridden at low frequency and the intermediate collective phenotype is adaptive. Furthermore, it has interesting implications for mutations in building rules that increase nest fitness. An adaptive mutation that allows, for example, better match to a template by increasing sensitivity to it will also generate higher attractiveness, leading to adjustment of the group-level building phenotype before all workers in the colony express the mutation. Conversely, adaptive rules that cannot influence feedback dynamics will be masked by other rules, until they reach high frequency among workers.

In **Chapter 4** of this thesis, I test the role of self-organisation in providing robustness to mutations using *Temnothorax* wall building behaviour as a case study, and I identify the key behavioural parameters that enable this phenomenon. The scenarios described above are of genetic mutation. However, the same principle means that these dynamics might enable individual variation in behaviour to be supported in a collective process. There is some indication that self-organising systems might be robust to individual error (Ramsch et al., 2012). I test this possibility further in **Chapter 4**.

It is likely that minimising the impact of one disruptive mutation on the stability of the whole architecture remains an evolutionary problem. The full set of building rules used to generate a complete architecture needs to both minimise the total number of rules (parsimony) and to enable feedback between components, while maintaining as much independence as possible between rule subsets used in different structures. Separate ant nest features such as chamber shape, area, volume and spacing seem to evolve independently across the ant genus, pointing at a large degree of independence between the underlying rule sets (Tschinkel, 2015).

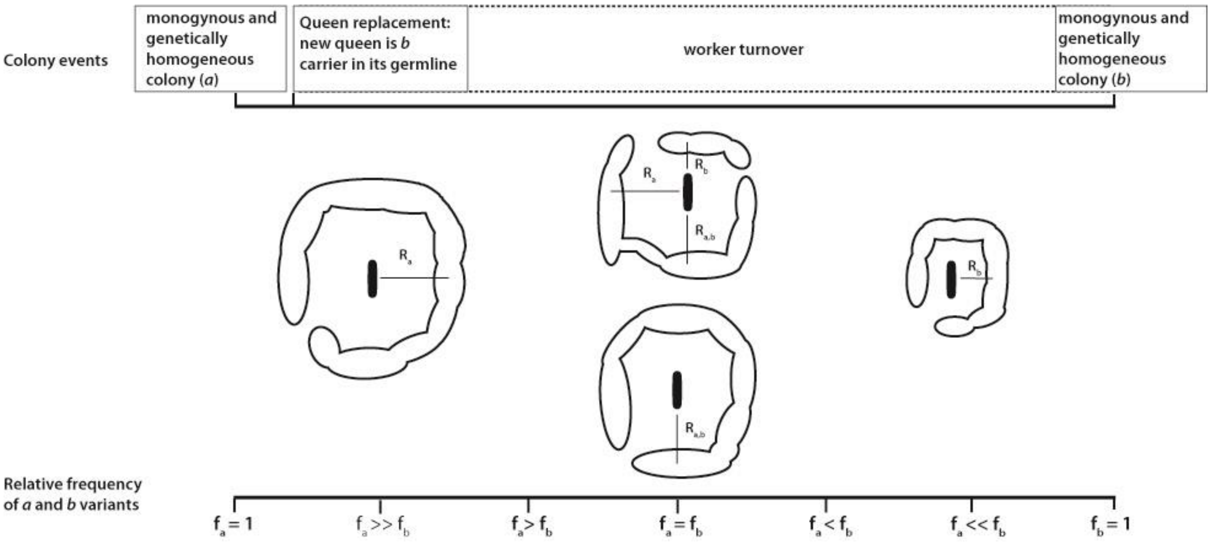

Figure 1.5. Transition between building rules after genetic mutation in a monogynous colony.
The figure shows change in the collective phenotype (*centre*) as the ratio between the frequencies of a and b variants of the building rule R changes (x-axis, *bottom*). Top: the corresponding events in the colony's life history are shown. The rule describes sensitivity to a template (*black line* in the centre of the structure) that creates a gradient of individual deposition and pick-up probability as distance from the template increases. Deposition and pick-up probability increase and decrease respectively with distance from the template; the mechanism is identical to the model of royal chamber wall construction in termites (Bonabeau et al., 1998). From left to right: the colony is genetically homogeneous for rule a and group deposition focuses at distance R_a from the template (*left*). Queen replacement introduces variant b in the worker population via mutation in the germline and the frequency of b increases with worker turnover. When the frequency of b starts approaching that of a, some wall sections will emerge at distance R_a, some at distance R_b and some in areas of the overlap between gradients a and b where total deposition probability summed across both variants is high, $R_{a,b}$ (*centre, top*). Over time, collective activity will converge towards $R_{a,b}$ if this total probability is higher than individual probabilities at R_a and R_b (*centre, bottom*). The collective phenotype converges towards R_b as b workers become the predominant genotype in the colony.

The current literature on self-organisation and self-assembly in eusocial insect nest building provides in-detail evidence and models of how these mechanisms result in functional adaptation to environmental conditions. New studies are beginning to emerge that show how the response of the nest to the environment includes features that are an indirect response of dynamics and not functionally adaptive. I take these observations as a cue for discussing self-organisation from an evolutionary perspective. The emergence of even primitive forms of seemingly functionally fine-tuned nest substructures might be the by-product of other building dynamics, or might instead result from selective pressure to meet the structure's purpose. In this second case, theoretical studies of self-organisation should address the question of under which circumstances, premises and mechanisms can coordinated rule sets evolve. At the same time, self-organisation dynamics separate collective phenotype from the linear sum of individual phenotypes that govern baseline building activity, because individual variation in rules underlying feedback dynamics are a crucial element in determining the collective outcome. Therefore, the evolution of collective behaviour and of collective building in particular might display a pattern of phenotypic adaptation at the collective level that overrides individual-level mutations. Again, theoretical studies can enquire about the evolutionary trajectories followed by collective phenotypes under different eusocial structures, under drift and under adaptive selective pressures. Short term adaptation to the immediate environment might occur through the exploration of collective phenotypic space mediated by convergence of individual choices to a collective decision. Experimental studies can investigate to which extent this parallel holds true. There is a need for new research, both experimental and theoretical, to approach self-organisation in collective building also from this evolutionary perspective.

1.3 A brief overview of the genus *Temnothorax* and of the study species, *Temnothorax albipennis* and *Temnothorax rugatulus*

Temnothorax (Order: *Hymenoptera*, Family: *Formicidae*, Subfamily: *Myrmicinae*) is a hyper-diverse genus comprising approximately 390 species, distributed across the globe (**Figure 1.6A**). The physical appearance of these species is inconspicuous: all *Temnothorax* ants are monomorphic (see ***Box 1.1*** for terminology), with typically small (< 4 mm) and cryptically coloured workers. Colonies are typically of small size (< 100 workers). Colonies are usually monogynous, with facultative polygyny found in some species. Most species average one queen per colony (but see *T. tuscaloosae* at the other extreme, averaging 10.4; Alloway et al., 1982; Foitzik & Heinze, 2001; Guénard et al., 2016; Rüppell et al., 1998). Queen-less colonies are also not rare (*e.g.*, Alloway et al., 1982) and polydomy is typical of some species (Alloway et al., 1982; Foitzik & Heinze, 2001; Partridge et al., 1997). Many species in the genus are affected by social parasitism and by slave-making (*e.g.*, Delattre et al., 2012; Seifert et al., 2014).

Ecologically, *Temnothorax* are adapted to a variety of environments. Their distribution encompasses habitats as diverse as deserts and tropical forests and altitudes ranging from sea level to 4000 m, although they are most-commonly found in mid-to-high elevation forests of the Northern hemisphere. Trophic generalists, they are scavengers of several resource types, ranging from other insects to seeds. There are no documented active predators in the genus. For many species, nests are arboreal, often exploiting pre-existing cavities created by termites and beetles. The small size typical of the genus enables species to specialise on niches as small as rock crevices or acorns for dwelling purposes (Prebus, 2017; Snelling et al., 2014).

The morphological similarity with the sister genus *Leptothorax* has caused *Temnothorax* species to be, until recently (Bolton, 2003), classified as member of various subgenera of leptothoracines (particularly *Myrmoxenus*) and confusion remains in the literature regarding some species names. Recent genetic and phylogenetic analysis has confirmed that *Temnothorax* is a natural group (Prebus, 2017).

Temnothorax albipennis

T. albipennis (formerly known as *Leptothorax tuberointerruptus* or as *Leptothorax albipennis*) is found in the grassland and light scrubs of central and Southern Europe (**Figure 1.6B**), usually nesting inside rock crevices and always preferentially in dark environments. Workers are 1.2-1.5 mm long, with the smallest known karyotypes among leptothoracines and temnothoracines (n = 8; Orledge, 1998). This *Temnothorax* species is believed to be obligately monogynous with singly-mated queens. The species exhibits seasonal polydomy (Partridge et al., 1997). Colonies hibernate (usually from the end of September to the end of March) to survive the winter. The nesting behaviour, which is the focus of **Chapter 2**, consists in the erection of a wall using local material or, alternatively, in the excavation of a cavity in the detritus found within a crevice, leaving enough material to surround the nest with a protective ring-shaped wall (**Figure 1.2**). Because this species is host to the slave-making ant *T. ravouxi* (Czechowski & Czechowska, 2000), hypotheses on the function of the wall include a defensive purpose against this enemy, but also a homeostatic purpose, for the preservation of optimal temperature and humidity conditions for brood raising.

Temnothorax rugatulus

T. rugatulus is a North American species (**Figure 1.6C**) inhabiting moist habitats such as grasslands. Its 2.6-3.8 mm sized workers are large compared to *T. albipennis*, with which it shares wall building behaviour (**Figure 1.7**) and rock-crevice dwelling habits. The life histories of the two species, however, partially differ. *T. rugatulus* is facultatively polygynous (with an average of 2.7 queens/colony and only half of its nests found to contain a single queen) and the two alternative modalities of colony foundation are associated with different queen morphs: a microgyne, slightly smaller than the size of a worker, is found in polygynous colonies, and a macrogyne, approximately twice the size of a worker, in monogynous ones. Moreover, 11% of colonies are queen-less (Choppin et al., 2021; Rüppell et al., 1998). Similarly to *T. albipennis*, polydomy is also common in this species, where it is thought to be triggered by the colony of origin when a threshold density is exceeded (Cao, 2013).

In the next chapter, I will focus on the nest wall building behaviour displayed by these two species and introduce the behavioural model of self-organisation that has been proposed to underlie this case of collective behaviour.

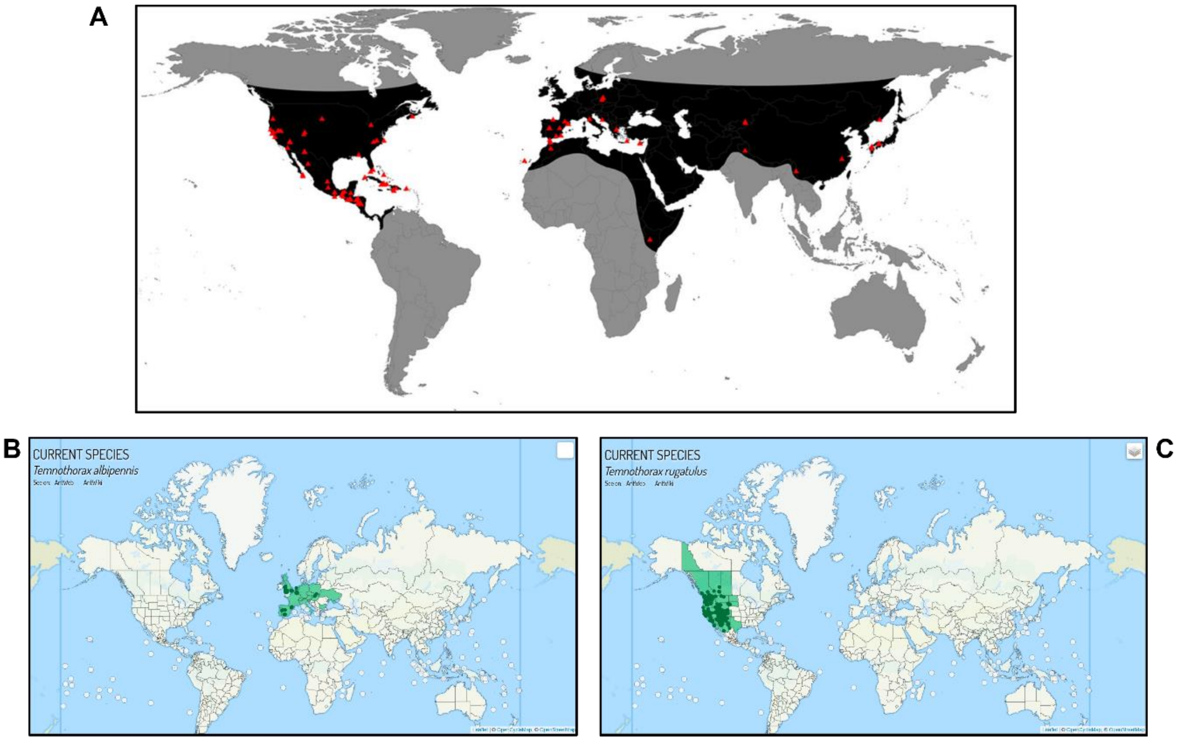

Figure 1.6. Distribution of genus *Temnothorax* and of the two study species, *T. albipennis* and *T. rugatulus*. **Panel A** shows the genus world distribution. *Red triangles* represent the collection locations of the source study (Prebus, 2017). **Panel B** shows the distribution of *T. albipennis* and **Panel C** of *T. rugatulus*. *Green* indicates that the range is native (no record of introduction in alien environments is known for either species). *Dark green dots* indicate the specific locations of database records for this species. Sources: **A.** Prebus (2017; use permitted under Creative Commons Attributions License 4.0), **B-C.** AntMaps (Guénard et al., 2017; Janicki et al., 2016).

Figure 1.7. Nest built by a *T. rugatulus* colony under laboratory conditions.

The photo shows the nest wall built by one of the colonies used in the study described in **Chapter 3**. In a laboratory setting, *T. albipennis* and *T. rugatulus* ants can dwell in artificial nests built using two microscope slides separated by cardboard corners, to create a narrow cavity. The top slide is usually lightly obscured, because these ants prefer darker spaces. When provided with a cavity and with building material, the workers will migrate the colony to the new nest (by carrying queen, brood items and nestmates) and subsequently build a wall surrounding the brood. The picture was taken after 39h of building activity, when the wall is complete.

Box 1.1: Ant terminology

Caste: morphologically distinct group of workers specialised in one or more nest activity; the term is also used with reference to reproductive behaviour, where the reproductive caste (queen and male) is opposed to the sterile worker caste

Colony foundation: the founding stage of a colony by a newly-mated queen (see **Chapter 4** for a more in-detail review of this ant life history trait)

Monogyny: presence of one single queen per nest

Monomorphic: with no morphologically differentiated castes

Social parasitism: (in ants) parasitic behaviour used by the queens of species lacking a worker caste, consisting in infiltrating a host nest and laying there their eggs, which are then raised by the workers of the host species

Polydomy: inhabiting of multiple (usually connected) nests by the same colony

Polygyny: presence of multiple reproductively active queens within the same nest

Slave-making ants: ant species exhibiting a raiding behaviour whereby they take the brood from other nests to replenish their own workforce

Chapter 2: Investigating and extending the Franks and Deneubourg model

In the 1990s, a research group at the University of Bath, led by Nigel Franks, produced two papers based on laboratory data, in which the building behaviour exhibited by the ant *T. albipennis* was decomposed into what were thought to be the underlying mechanisms (Franks et al., 1992; Franks & Deneubourg, 1997). The papers in question described a set of individual behavioural rules that, the authors proposed, generate the collective phenotype through self-organisation. The first article, in particular, applied this rule set in a spatially explicit agent-based model, one of the first to describe self-organised collective animal behaviour and a precursor to a later entry (including a fuller description of model details) in the highly influential Camazine *et al.* textbook (2003), "Self-organization in biological systems". The rule set applied this way successfully reproduced some observations made on laboratory colonies.

The papers, while foundational, lack some of the necessary detail to make the model reproducible and to understand the behaviour of the system over time and across conditions. These limitations are important because *Temnothorax* nest building is an exceptional case study for collective building: these species inhabit narrow rock crevices in which they build a wall to separate the brood from the entrance of the fissure and the resulting architecture can be approximated by a 2-dimensional representation, which simplifies the study and the spatial modelling of the behaviour; colonies are small, cheap to maintain and easily nest between microscope slides, so that the behaviour is directly observable in the laboratory without the need of expensive technology; *Temnothorax* have a limited number of workers without a morphological caste system and the behaviour can be easily broken down into individual rules.

Bringing this agent-based model to a reproducible state means that it can be used by any researcher to simulate how this type of nest building responds to changes in the environment, or in colony composition, or how it evolves.

The behavioural model proposed by Franks and colleagues of *Temnothorax* nest building consists of a template component and of a stigmergic component. Workers are assumed to use a reference to estimate their distance from the cluster of brood and nurses that is located at the centre of the nest, according to which distance they vary their deposition and pick-up probability. The optimal distance at which to build the wall around the brood is one that leaves sufficient space for the workforce to move about and get on with their tasks but is close enough to be easily built and maintained (and

perhaps to keep the right level of humidity and the right within-nest temperature). Nest area (that is, the area included by the wall) should therefore be responsive to colony size.
I have replicated the model here with the advantage of today's computational power. I add some observations.

Section 2.1 is based on the replication report *Updating a textbook model of collective behaviour: nest wall building in Temnothorax albipennis*, published in *Animal Behavior and Cognition* (Invernizzi and Ruxton, 2021).

Section 2.2 discusses the empirical evidence supporting the behavioural rules used in the model.

Because the original study only used modelling parameters estimated from the data, **Section 2.3** contains the results of a sensitivity analysis of the model over (what is a biologically meaningful) larger parameter space. Using this approach, we can test whether the relatively good match between the model and the structures observed in nature can be replicated if we assume measurement error or variation between colonies. From a more evolutionarily focused perspective, it asks how much we can stray away from the parameters before the collective phenotype collapses.

In **Section 2.4**, I propose a modification to the original Deneubourg *et al.* behavioural model, using a more subtly regulated relationship between stone density and deposition that allows for behavioural termination. I call this *the gradual model*. By plugging the *gradual* model into the same agent-based simulation reproduced in **Section 2.1**, I show that this alternative predicts a better-quality nest structure than the original. This model is also robust to variation in its parameter values, based on the same sensitivity analysis method used in **Section 2.3**.

Note that across the thesis I will use *Franks and Deneubourg model* to refer to the behavioural model and to the set of pick-up and deposition probability equations that mathematically represent it and *Deneubourg et al. model* to refer to the agent-based model (first used in Camazine *et al.* 2001) that represents the use of this behavioural model by individual agents moving in 2D space.

2.1 Updating a textbook model of collective behaviour: nest wall building in *Temnothorax albipennis*

In 1992, one of the first papers on *Temnothorax albipennis* (then *Leptothorax tuberointerruptus*) nest wall building also established one of the first computational models of animal collective behaviour (Franks et al., 1992). The paper contained the results of a spatially-explicit agent-based model without describing the model structure in detail. Later, in 1997, an analytical version of the same behavioural model was published (Franks & Deneubourg, 1997), followed by a simpler version of the agent-based model that became, literally, a text-book example of self-organised collective behaviour (Camazine et al., 2001). This later version of the agent-based model is described in more detail than the one in Franks et al. (1992). However, even this later description is missing important information: details on how to interpret time in the model, as well as data on the repeatability of the outcome over replicate runs of simulation, are not reported, which means that we are unsure of the building time scale predicted by the model and whether it corresponds to realistic building times, as well as how much variability we find among the structures created by this model. Here, I replicate the model based on the details in Camazine *et al.* (2001) to answer these two questions. I find that the prediction (also supported by the analytical model; Franks & Deneubourg, 1997) that a finished wall may not expand over time to accommodate a colony size increase does not hold over longer time scales. I explain why this is a consequence of the stochastic dynamics that are expected in both agent-based models and real life and why this conclusion is not at odds with the analytical model.

The behaviour described is a case of animal architecture: the wall built by workers of *T. albipennis* ants to protect their nest (possibly having a homeostatic function in maintaining nest temperature and humidity levels stable, as well as to prevent flooding; the wall might also act as a defence from *T. albipennis* parasitic queens; Franks et al., 1992; Camazine et al., 2001). This *Temnothorax* species nests within rock crevices or other narrow cavities, where workers use soil particles or other light building material to erect walls. The characteristic spatial organisation of this species has the cluster of queen, brood and nurse workers in the middle of the nest and the walls approximately equidistant around these walls (Franks et al., 1992). Because of the preference of *T. albipennis* for narrow cavities, the wall is an unusual example of nearly-2D architecture, this simplicity making it a perfect case study for early computational models of collective animal building. The models (Franks et al., 1992; Camazine et al., 2001; Franks & Deneubourg, 1997) describe individual building rules that are independently applied by each worker and lead to the construction of a coherent wall structure when used by the collective, through indirect feedback mechanisms (self-organisation; Camazine et al., 2001; Invernizzi & Ruxton, 2019; Theraulaz et al., 1998, 2003) and the use of spatial reference points (a template). Effectively, the rules explain how behavioural coordination allowing the cost of building the nest wall to be split among multiple individuals, as well as how constant updating of nest size to respond to changes in colony size is possible. The model hypothesises the effect of two elements on building behaviour: a distance template, that affects worker pick-up and deposition probability as a function of distance from the brood cluster (*i.e.*, the centre of the nest), and a stigmergic feedback effect of the number of neighbouring stones (*i.e.,* the number of stones in the local spatial vicinity of an ant) on each probability function, that affects worker deposition and pick-up probability as a function of the number of nearby stones.

The agent-based model adds stochasticity and an explicit representation of the effect of space to the behavioural rules already described by the analytical model. Therefore, this modelling strategy is an important tool for evaluating how well the rules reproduce the behavioural patterns and emergent architectures observed in nature. It also generates additional predictions to the analytical model that can be tested on real data.

2.1.1 Replication methods

Model Details

I replicated the model according to the description offered in Camazine *et al.* (2001). The agent-based model was time-discrete and space-discrete and contained 30 identical agents (the workers). Space was designed as an 80x80 matrix. I worked bearing in mind the units used in Camazine *et al.* (2001), which defined each node as corresponding to 1 mm in the physical world, and therefore the modelled environment represented a square arena of edge length 8 cm (this length corresponds to approximately 53 times the average body length of a *T. albipennis* worker). Movement in space was random: from each node on the matrix, a worker could move to any other node in the next time step. That is, for each individual, its new node was selected stochastically, with all of the 1600 possible nodes being equally likely (independent of the current position of that individual, or the presence of other workers or stones at the selected location).

A single time step consisted of one round. At each round, the following process was repeated 30 times, one for each worker (because workers are identical and do not carry any information from previous rounds, iterating the process 30 times is sufficient to simulate a workforce of such size). Each worker started by moving to a new random node in the matrix. If there was no stone present at that node, then the individual's turn was over and evaluation moved to the next ant. If at least one stone was present, the focal ant was given one chance to pick up a stone (according to probability function *P(r)*, where *r* is the distance of the worker from the centre of the matrix; see below) from the chosen node. If it failed to pick up a stone, then its turn was over, otherwise it moved to new randomly-chosen positions, sequentially, until it deposited the stone at one of them (at each node, the probability of depositing depended on the probability function *D(r)*). Unless otherwise specified, I ran the simulation for a total of 5000 rounds. The use of rounds as a time unit differs from the time formulation in Camazine *et al.* (2001), which used hours. Their time specification is unclear (and potentially misleading): if they found a means of conversion between one round of simulation and ant building time in the physical world, they did not report it; if they used computational time as a physical-world equivalent, this is not reproducible because it is dependent on machine specifications. Critically, computational time in the early 1990s was several orders of magnitude slower than current standards.

By specifying the time in number of rounds, I provide a comparable unit that can be used for replication and further work based on this model.

The model was initialised with stones and ants randomly scattered with uniform distribution throughout the matrix, subject to the constraint of the node's stone carrying capacity. Each node had a maximum carrying capacity of three stones. Workers could pick up but not deposit at nodes that had reached their carrying capacity.

Behavioural Probability Functions

I used the functions described in Camazine *et al.* (2001) to define the probability of pick-up, *P(r)*, and deposition, *D(r),* where *r* is the shortest distance of that matrix node from the matrix centre. These probabilities described a relationship between behaviour and distance from the centre of the brood cluster (identified with the centre of the matrix) and the total number of stones at the chosen node and four neighbouring locations (*i.e.*, the nodes North, South, East and West of the focal node).

The probability of pick-up was

$$P(r) = P_{\mathrm{M}} \left(1 - \frac{1}{1 + \tau(r - r_{\mathrm{O}})^2}\right) F(S)$$

Here, r is the distance from the centre of the brood cluster, r_{O} is an optimal distance value known to workers at which the wall should be built, *S* is the total number of stones at the five locations, P_{M} is the maximum possible probability of deposition if we ignore the effect of the local number of stones, τ is the parameter defining wall thickness and $F(S)$ the effect of the stones (the stigmergic component):

$$F(S) = \begin{cases} F_{\mathrm{M}}, \text{ for } S < S_{\mathrm{c}} \\ F_{\mathrm{m}}, \text{ for } S \geq S_{\mathrm{c}} \end{cases}$$

where S_{c} is a critical number of stones value after which the behaviour changes and with $F_{\mathrm{m}} < F_{\mathrm{M}}$. The constants P_{M}, r_{O}, F_{M}, F_{m} and S_{c} were the same for all ants. The two key aspects of this function are that an ant is more likely to pick up a stone if it is currently far from the optimal distance for the wall from the centre of the brood, and if there are few stones in the immediate vicinity. In this equation, the part within brackets represents the template component.

The probability of deposition function describes the opposite relationship to distance and number of stones: being higher if the ant is positioned in space near the optimal distance for the wall from the brood centre, and if there are many stones in the immediate vicinity. The particular formulation used was

$$D(r) = D_{\mathrm{M}} \frac{1}{1 + \tau(r - r_{\mathrm{O}})^2} G(S)Q$$

where $Q = 0$ if the maximum carrying capacity for stones at that node had already been reached and $Q = 1$ otherwise. The effect of stones on deposition (the stigmergic component) was

$$G(S) = \begin{cases} G_{\mathrm{m}}\ for\ S < S_{\mathrm{c}} \\ G_{\mathrm{M}}\ for\ S > S_{\mathrm{c}} \end{cases}$$

with $G_{\mathrm{m}} < G_{\mathrm{M}}$. Again, all these constants were the same for all ants. The fraction corresponds to the template component in this equation.

I ran all simulations with the same parameter values used in Camazine *et al.* (2001): $P_{\mathrm{M}} = 0.35$, $D_{\mathrm{M}} = 0.5$, $G_{\mathrm{m}} = F_{\mathrm{m}} = 0.01$, $G_{\mathrm{M}} = F_{\mathrm{M}} = 0.55$, $S_{\mathrm{c}} = 6$, $\tau = 0.025$ and $r_{\mathrm{O}} = 18$.

The distance of each matrix node to the centre was calculated by converting the matrix to cartesian coordinates (centred on the central node) and applying Pythagoras's theorem, assuming a one-unit distance between two immediately adjacent locations.

Delayed Colony Size Increase

Part of the original Camazine *et al.* (2001) chapter consisted of testing whether the behavioural model proposed reproduced the failure of colonies to adjust the nest area to small-to-intermediate increases in population size, once the wall is completed, that is observed under laboratory conditions. I replicated this test using their original approach.

The simulations testing the effect of a delayed increase in colony-size on wall location were run for 75000 rounds. At *t* = 5000, r_O was switched from 18 to 24, to reproduce a change in template effect caused by a doubling of colony size. The authors do not explain how they arrive to the conversion between a two-fold increase in colony size and a change in expected preferred building distance from 18 to 24 mm; I have reconstructed their reasoning is as follows. By making a two-fold increase in colony size correspond to a two-fold increase in nest area, I obtain

$$\frac{A_1}{A_2} = \frac{1}{2} = \frac{2\pi {r_1}^2}{2\pi {r_2}^2}$$

$$\frac{2\pi {r_1}^2}{2\pi {r_2}^2} = \frac{1}{2}$$

$$\frac{r_1}{r_2} \approx \frac{10}{14}$$

Given that $r_1 = 18$, $r_2 = 25.2 \ \cong 24$.

Data on wall location and quality were extracted at time points *t* = 6000, *t* = 10000 and *t* = 75000.

Number of Depositions per Round

To estimate the number of depositions in each round, I tracked stone positions in space round by round in a set of 20 simulations with 3000 stones. I calculated the number of locations where the number of stones had changed, between consecutive rounds, and divided it by two to calculate the rate, because for each moved stone, both the pick-up and the deposition location changed in value. Note that this value is only an estimate of the real value, because multiple stones that are moved from or to the same location are tracked only once. The average for each round was the calculated across simulations for an averaged estimate.

Software

The model was built in Python 3.7.9 (Van Rossum & Drake, 2009) and simulations run using Spyder 4.1.1.

Statistical Measures of Stone Dispersion

In order to understand how much variation there is in the structure of the walls generated by the behavioural model, I needed to quantify this variation. Here, I chose to measure variation in two characteristics that encapsulate different aspects of the quality of the structure: the presence of gaps and the density of building material and thickness of the wall at each section. This second characteristic also informs us on whether the behavioural model succeeds in concentrating the available building material at building sites and how quickly. Both characteristics can be measured as dispersal of the deposition locations across the matrix. These two measures were not present in the original book chapter and were chosen by me because considered appropriate measures for quantifying wall structure. Given that the expected shape of the final wall is a circle centred around the brood cluster, it is possible to measure these two dispersion values (at the end of the simulation) as dispersion over the two dimensions of a circular plane relative to the central position: θ, the angle, and *r*, the radius (distance from the cluster). I defined a deposition location as any location in the matrix that has at least one stone. I calculated dispersion along the θ axis as circular dispersion (or spread) $\bar{R}$ (Pewsey et al., 2013):

$$\bar{R} = \frac{1}{M}\sqrt{\left(\sum_{i=1}^{M}\cos\theta_i\right)^2 + \left(\sum_{i=1}^{M}\sin\theta_i\right)^2}$$

with M being all the deposition locations and $\theta_i$the angle of each location i measured in radians.
I measured dispersion (or spread) in radius as

$$\text{distance dispersion} = \frac{SD(r)}{\bar{r}},$$

the standard deviation of the distance from the centre (radius) *r* of all locations with at least one stone, i, divided by the average distance of these locations, $\bar{r}$.

2.1.2 Replication outcome

I reconstructed the agent-based model described in Camazine *et al.* (2001). My results successfully replicate those of the original study, showing that the model reproduces the structural patterns made by ant behaviour.

I compared the output of the model without (*template only*) or with (*stigmergy + template*) the effect of neighbouring stones. The difference between these two models, in terms of parameter values, is that in the *template only* model F and G are replaced by a constant of 1. I made the comparison across three different values of stone availability: 1000, 3000 and 5000 stones (**Figure 2.1A**). The model including stigmergy best reproduced the nest wall structure built by the ants under laboratory conditions (compare with **Figure 2.1B**, Figure 1 from Franks & Deneubourg, 1997), resulting in a more cohesive structure.

I additionally observe that, when the available material is limited (1000 stones instead of 3000), the feedback effect of stigmergy leads to the formation of local stone piles (centred at an optimal template-guided distance from the cluster) and prevents the formation of a complete wall, which would instead be built under a template-only mechanism. The material is not redistributed over a longer time scale (**Figure 2.1C**) and the wall remains incomplete. The wall built at low stone availability under this behavioural model is always low quality. **Figure 2.2A**, **top panel**, shows how dispersed deposition locations are along the distance-from-cluster axis. I used standard deviation in distance of the stones to the central cluster divided by mean distance as a measure. This definition allows the spread to be compared between behavioural models, given an equal number of stones, while simulations with higher number of stones result in higher values. I call walls with low dispersion (given equal stones) *compact*, meaning that they are high stone density. **Figure 2.2A**, **bottom panel**, shows how uniformly distributed the angles of these locations are on a circular plane, a measure of how many gaps there are in the wall. The closer these two measures are to zero, the more uniform the wall and the less dispersed the stones from the optimal distance. We can see that the more stones are present, the more variation in the distance from the cluster is visible in both models, because wall thickness increases, but (given the same building time) the *stigmergy + template* model produces a more compact wall than the *template only* model. However, in this model the wall formed at low stone availability is largely non-uniform, *i.e.*, it contains gaps.

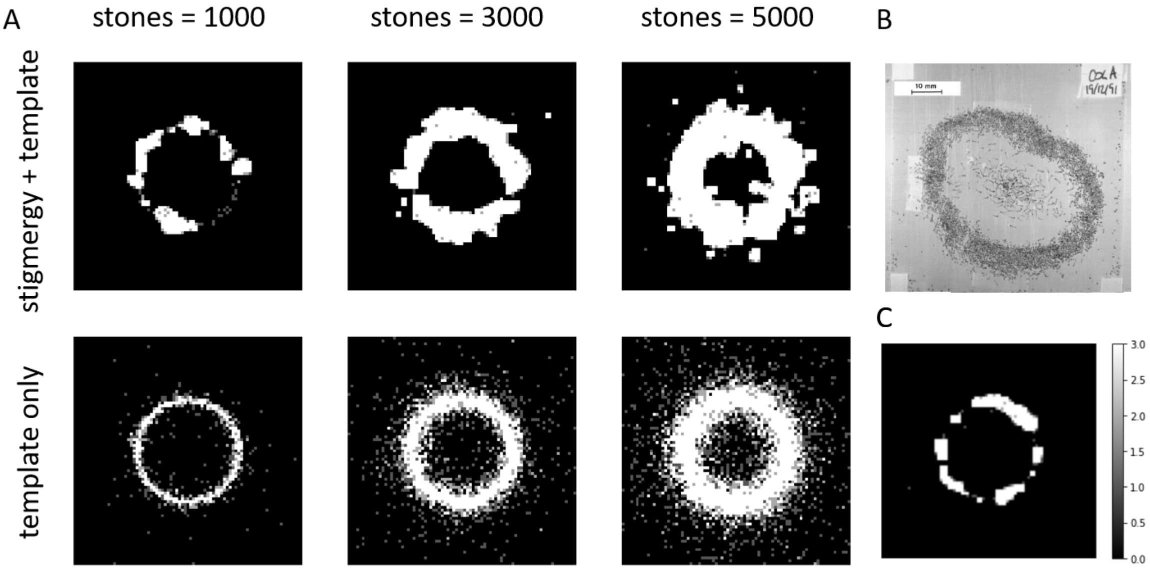

Figure 2.1. Model output across different levels of stone availability.

The figure shows the output of the *template only* and of the *stigmergy + template* model, with a photo of a nest built under laboratory conditions for comparison. The *template only* model has $F = G = 1$ independently from the amount of neighbouring stones. **Panel A** shows how the output of the same simulation run changes if the model includes (*top row*) the feedback effect of deposited stones on future depositions and pick-ups or if it contains the template effect only (*bottom row*) and with different stone availabilities (this remains a constant throughout the simulation). Each simulation was run for 5000 rounds. **Panel B** shows Figure 1 in Franks and Deneubourg (1997), a picture of a nest wall built by *T. albipennis* under laboratory conditions. Image reproduced by courtesy of N. Franks (From “Self-organising nest construction in ants: individual worker behaviour and the nest’s dynamics,” by N. R. Franks and J-L. Deneubourg, 1997, Animal Behaviour, 54(4), 779-796. Elsevier. Reprinted with permission.) **Panel C** shows the output of the same run as the top left subplot of panel A, after 20000 rounds. The *grayscale* reflects the number of stones at each location (corresponding numerical scale to the right of the figure).

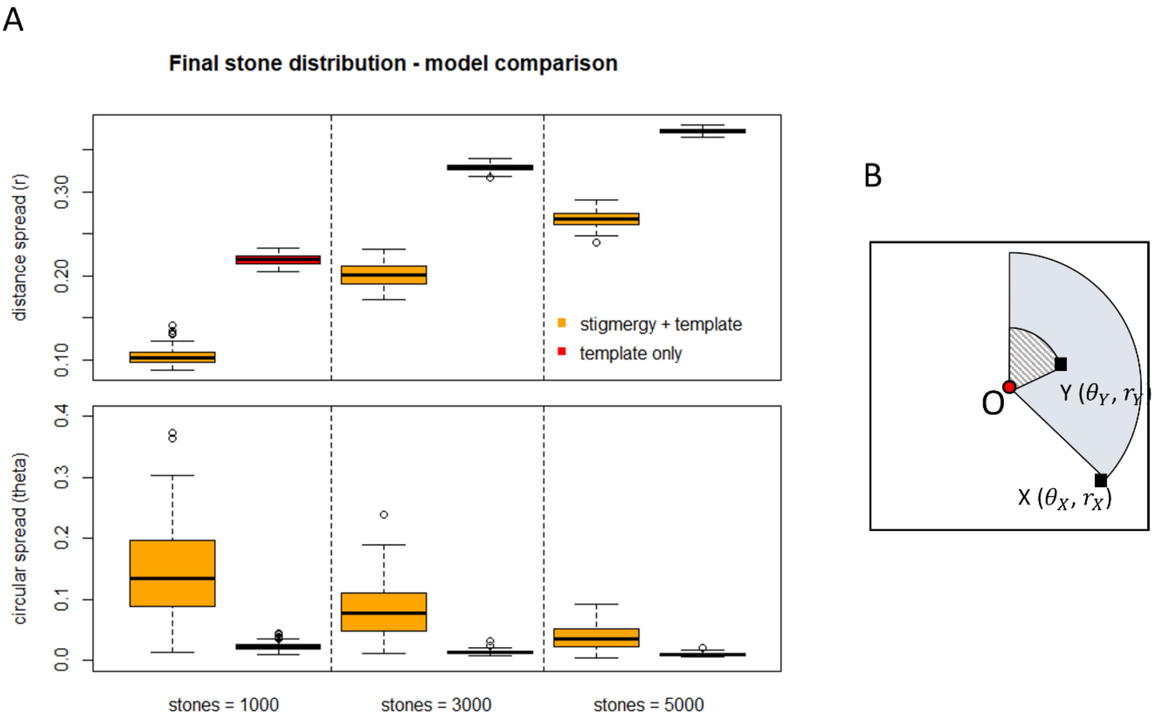

Figure 2.2. Measuring final stone distribution.

Panel A shows how the spread of building locations on the two axes of a circular plane varies over 100 simulations. The two axes are: distance from the cluster (distance spread or spread in *r*; *top panel*) and angular axis (circular spread or spread in $\boldsymbol{\theta}$; *bottom panel*). Details of the statistical measures used are found in *Methods*. **Panel B** shows what *r* and $\boldsymbol{\theta}$ represent. Two deposition locations, X and Y, can be represented on a circular plane (with origin in the brood cluster *O*) using two coordinates: the distance from the cluster *r* and the angle $\boldsymbol{\theta}$, measured from 12 o'clock.

Empirically, real *T. albipennis* colonies have been observed to fail to adjust nest size when colony size is suddenly doubled (by re-introducing the previously removed half of the laboratory colony) after a complete wall has already been built (Franks & Deneubourg, 1997). In terms of model parameters, this translates to the fact that, if r_0 is increased after the wall has reached a steady state, the average location of stone deposition remains close to the original distance: building is not re-initiated and the old wall is not dismantled to move to a distance more appropriate to the new colony size. The *stigmergy + template* model is shown, in the original work, to successfully reproduce this effect (Camazine et al., 2001). When replicating these simulations, I find that this is true, but only over short-term time scales (**Figures 2.3A** and **2.3B**, first panel). If the wall is observed for a longer time after reintroduction of the missing colony half, we see it progressively move outwards, approaching the new expected nest size (**Figures 2.3A** and **2.3B**, second and third panels).

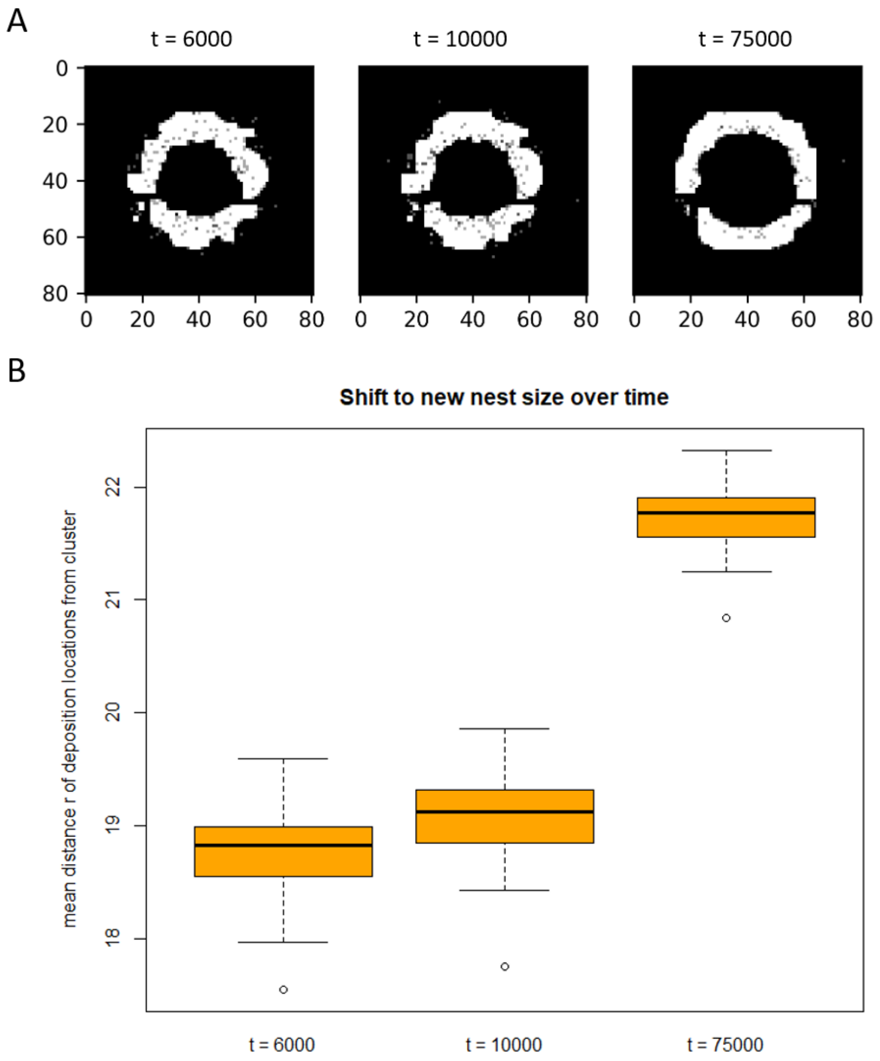

Figure 2.3. Shift to new nest size over time.

The figure shows what happens to the wall given enough time after colony size increase. Simulations are run for 75000 time steps, starting with $r_0 = 18$ and switching to $r_0 = 24$ at time step 5000. In **panel A**, three sample outputs from three time points in the simulation, *t* = 6000 (corresponding to short-term change after a population increase – see main text), *t* = 10000 and *t* = 75000, are shown. On the axes, each unit corresponds to one node in simulated space, 1 mm in real space. The centre of the cluster is modelled to be at coordinates (41,41). **Panel B** shows the distribution of the mean distance *r* of deposition locations from the centre over 100 simulation runs, at the same time points.

The lack of adjustment of nest area to population size observed in real colonies is the effect of hysteresis, the existence of multiple stationary states given the same parameter values, where the final state reached is influenced by the history of the system (this is better explained in the analytical model presented in Franks & Deneubourg, 1997). Why, then, do the simulations show progressive expansion? The results from this model do not contradict real data, nor the Franks and Deneubourg

(1997) model. While, on average, the wall is stable (that is, deposition and pick-up activity are balanced), complex systems are noisy, resulting in locally imbalanced activity. Because the amount of building material is finite, over time this noise causes continuous relocation of material towards the location in the nest that is now favoured by the template. The wall slowly moves towards the new optimal value. This means that, in real systems and given enough time (and the same template effect as used in the model), we expect the wall to slowly expand to accommodate the larger colony.

We can attempt to convert computational time into estimated real time. Time conversion of this type of simulation should be treated with extreme caution: the goal of the simulation is to assess, qualitatively, whether the behavioural paradigm reproduced can generate walls similar to the one observed in nature, and not to accurately reproduce the system. This means that some simulation details are unrealistic when looked at from a time perspective. In addition, the model does not incorporate details of the system that do not modify the qualitative outcome but may alter its time scale. Many ant species, for example, show oscillations in colony-level activity, meaning that, periodically and synchronously, the majority of the colony is found in a state of low activity (Cole, 1991). These patterns have a key influence on the overall activity rate, including building rate, so that rates calculated over short-term periods differ from those calculated over the full length of the building activity (this issue will become of key importance in **Chapter 3**). Franks and Deneubourg (1997) provide a "'global' rate of stone deposition", but do not offer details on how this is calculated, or on the number of building workers in the colonies, so that we do not know how this may compare to the simulation. While attempting a conversion here, this should only be considered a rough estimate with no pretension of accuracy. Franks and Deneubourg (1997) state that a wall is approximately completed in 24h. In my simulations, the estimated number of depositions per round levels to almost zero around the 2000th round (**Figure 2.4**). We can consider this point as the time point in the model at which the wall is completed (*i.e.*, the system reaches a point of equilibrium after which pick-ups and deposition are balanced in both number and location and the wall is not substantially altered), meaning that the correspondence between one round and real time is approximately 43 s/round. Therefore, in **Figure 2.3**, the first time point (6000 rounds) corresponds to 12h after colony size increase. At this stage, we see little change in nest size. The second and third time points (10000 and 75000 rounds) correspond to 4.5 and 37 days after colony size increase, respectively. This indicates that accommodation of the wall to the new equilibrium point is a slow process.

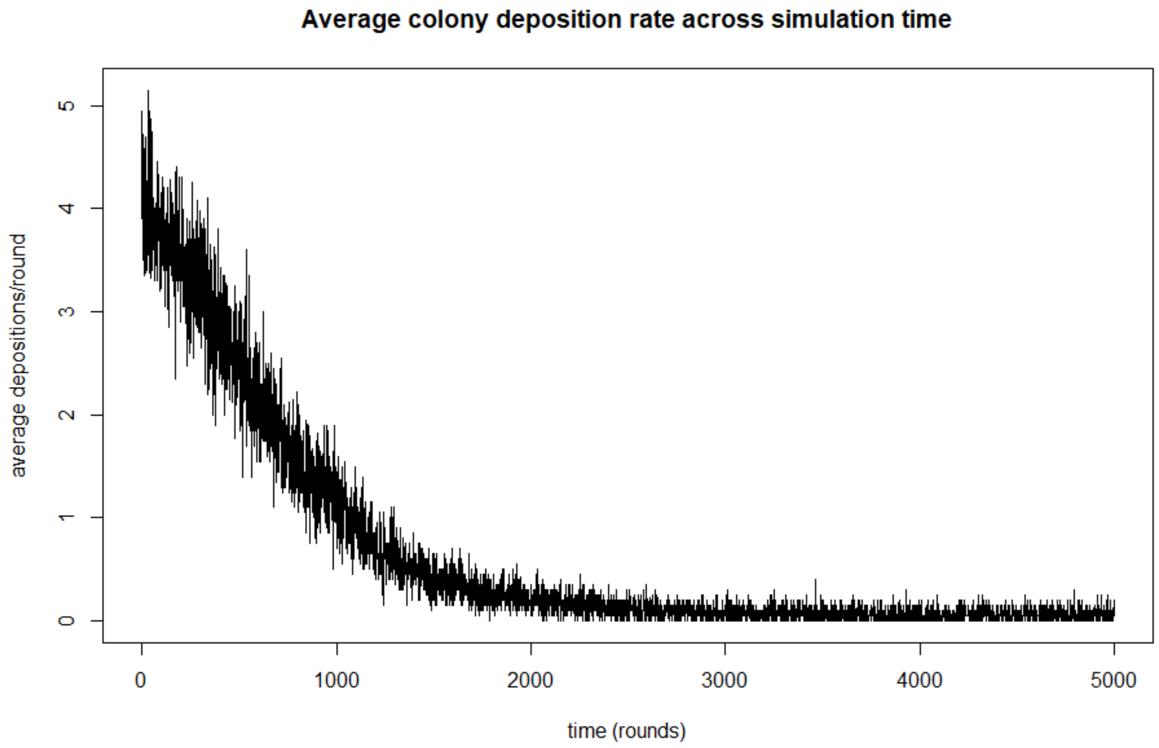

Figure 2.4. Average colony deposition rate across simulation time.

The figure shows the estimated number of depositions per round occurring in the simulations, averaged across 20 runs. Simulations are run with 3000 stones, for 5000 rounds.

2.1.3 Is the Franks and Deneubourg model a useful model of *Temnothorax* nest wall building? A discussion with suggestions for experimental and modelling follow-up studies.

I have shown here that the model presented in Camazine *et al.* (2001) and based on Franks *et al.* (1992) and Franks and Deneubourg (1997) successfully reproduces two key characteristics of *T. albipennis* nest wall building: shape of the wall and hysteresis. I also find the results of this agent-based model representation of the system to be in agreement with the analytical description contained in Franks and Deneubourg (1997). Additionally, I highlight two model predictions missing from the original work: 1. when building material is scarce, the wall remains incomplete rather than simply becoming thinner; and 2. over time, nest size always adjusts to current colony size, even under hysteresis conditions.

The failure of the behavioural model to create a functional wall at low total stone availability does not necessarily conflict with observation. Crevice nesting *Temnothorax* species do not build walls if pre-existing barriers are available, but rather use wall building to fill gaps (*T. albipennis*, Franks et al. 1992; *Temnothorax rugatulus*, personal observation). This behaviour fits the hypothesis of an adaptive solution to avoid wasting building material. Alternatively, the deleterious consequences of a strong positive feedback can be avoided if the direction of stone deposition is guided (in combination with or instead of the use of the brood cluster as a template) by an environmental template such as the presence of air currents (as the authors of the original work suggest; Franks et al., 1992), or a light gradient. Such a template should decrease deposition in areas where a partial wall is already present and encourage stone redistribution to form a complete circular wall. Finally, a third possibility is that this is an incomplete behavioural model: the authors of the original work highlight that the model is based on worker behaviour observed at the *beginning* of the activity (Camazine et al., 2001; Franks et al., 1992). It is possible that additional behavioural rules are used later on in the process and lead to wall gaps being filled. Experimental investigations are necessary to resolve this point.

The original paper (Franks and Deneubourg, 1997) reporting the observation of the hysteresis effect does not mention over what time scales the experiments were run, but it is likely that observations were made over a short-term period of one or two days at most. There are no known data on change in colony size in the long term. Repeating the experiment and maintaining colony size stable for several weeks after the sudden increase (by removing newly developed ants unless they replace dead workers, for example) would enable testing the predictions of the model. In natural settings (in the absence of colony-size control), the tendency of the wall to expand over time only becomes relevant if the colony growth rate is slow compared to the building rate at equilibrium. If the colony grows quickly, then a bout of intense reconstruction is initiated to accommodate nest size.

2.2 Building the behavioural model: Evaluating the evidence

In order to evaluate whether an extension to the Franks and Deneubourg model is necessary, it is important to understand the line of thought that led the authors to present a model with two components, template and stigmergy, and with this specific mathematical representation of the relationship between the change in worker behaviour and the change in cues causing it. The assumptions underlying the behavioural model (Camazine et al., 2001; Franks & Deneubourg, 1997; Pitonakova & Bullock, 2013) were inferred from observational data in Franks *et al.* (1992) and are a plausible hypothesis, but they have never been put to the experimental test. To support their model, Franks and collaborators present the following six observations:

1. The building behaviour of *T. albipennis* is characterised by initial carrying of the stone from outside the nest to a within-nest location close to the brood cluster. The stone is then bulldozed outwards.
2. There is a tendency by individual workers to leave the stone at “one or two of its own body-length from the cluster of its nestmates” (Franks and Deneubourg, 1997, p.782) at the end of the initial transport to the nest.

Points 1 and 2 are in agreement with the use of the cluster as the reference point for a distance template.

3. In some species, workers release a pheromone template as they move within the nest: a *walking pheromone* (Aron et al., 1986; Hölldobler & Wilson, 1978, 1986; Maschwitz et al., 1986). Because worker density in *Temnothorax* is higher at the centre of the nest and becomes lower as ants approach the outer nest area, the concentration of this pheromone (released by workers during their daily activities) would provide the spatial distribution required by a distance template. The lowest deposition tendency and the highest pick-up should be observed at the highest concentration of walking pheromone (close to the brood and in the nest area most used by workers), while the propensity to deposit should increase with decreasing pheromone concentration. The optimal distance for deposition would be situated where pheromone levels are low, but not absent: otherwise, the stone would simply be taken out of the nest. In support of this hypothesis, Camazine *et al.* (2001) note that in winter, when colony metabolism is lower and workers are less active, the ratio between nest size and colony size is smaller (this observation could be also explained by changes in other external environmental cues that might influence building: for example, a smaller nest might help maintain the necessary nest temperature in colder conditions).

Point 3 provides the basis for the hypothesis of a pheromone -based distance template.

4. "(…) the behavior of the first building workers who make contact with the cluster of their nestmates and then seem to 'pace out' a relatively short distance before depositing their building material strongly suggests that the cluster itself acts as a template" (Camazine *et al.*, 2001, p.355). This observation suggests that a mechanical interaction between worker and cluster is necessary.

Point 4 provides the basis for a mechanical-template hypothesis, instead of or in addition to a pheromone-based one. Note that here I use *mechanical* in the same sense as Deneubourg *et al.* (2001) to refer to a physical interaction where no chemical or odour cues are transmitted.

5. There appears to be a statistically significant linear relationship between the number of stones dropped and the number of stones encountered by workers (Franks and Deneubourg, 1997). This correlation, the authors claim, supports the role of deposited stones in triggering further deposition behaviour, either working as a behavioural cue for the worker or as mechanical obstacles inducing deposition upon contact with a laden ant.
6. There also appears to be a statistically significant linear relationship between the number of stones moved and the number of ants that have passed by each stone (Franks and Deneubourg, 1997). This suggests a constant worker pick-up probability over time.

Points 5 and 6 would suggest a stigmergic effect of stones on ant behaviour. It has to be noted that in both cases the stigmergic response does not require recognition by the worker of a specific architectural conformation. In the case of pick-up, it is a consequence of the physical ease and difficulty of removing stones, while in the case of deposition only a physical response to a strong mechanical stimulus is necessary. Neither require advanced cognitive abilities.

Because none of the behavioural mechanisms used in the model has been experimentally proven, the match between the output of the simulations reproduced in this chapter and the observed biological structure and dynamics has the value of supporting evidence – in this case, the only evidence available. It follows that any other behavioural model performing equally well in a simulated setting is a valid alternative to the one proposed by Franks and Deneubourg. I will propose one of such models in **Section 2.4**.

In the following section, I criticise point 5 in the above list, showing that the analysis made by Franks and Deneubourg is not evidence of a causal relationship between number of stones encountered and deposition. The point I wish to make is that there is so far no evidence that stone piles induce deposition in workers. In **Chapter 3**, I will investigate the type of relationship between deposition and stone density using an approach that avoids the problem of time correlation highlighted here.

2.2.1 Criticism of linear regression as evidence of a causal relationship when studying time-correlated variables

The reasoning of Franks and Deneubourg in estimating the relationship between stone number and worker deposition probability is as follows (1997, p. 782). The authors record the behaviour of 50 randomly chosen ants from the moment they pick up a stone to the moment they deposit. Of these, they exclude the seven ants that deposit without encountering any other stones and study the relationship between the number of stones encountered and the number of ants that keep on carrying after having encountered that many stones. The result (**Figure 2.5A**) shows good statistical fitting with a linear regression model where the number of stones encountered is the independent variable. The authors report r^2= 0.92.

The regression analysis in this case is not valid evidence of a causal relationship between the two variables, because both variables are correlated with time (as the authors note). This relationship, alone, is enough to explain the high r^2 value, as I show next.

It is possible to prove that, for any biologically meaningful length of time, there is always a set of independent values of the two probabilities, the probability of deposition and the probability of encountering a stone, that reproduces the observed data.

Let us define d as the probability of depositing a stone and p as the probability of encountering a stone. The number of ants that deposit, over time *T*, before having encountered a stone is

$$D_{0,T} = \sum_{t=1}^{T} Nd(1-p)(1-d)^{t-1}(1-p)^{t-1},$$

where N is the total number of ants. From the data collected by Franks and Deneubourg, we know that in their set of data $N = 50$ and $D_{0,T} = 7$. This yields

$$\sum_{t=1}^{T} 50d(1-p)(1-d)^{t-1}(1-p)^{t-1} = 7$$

from which we get that

$$d(1-p)\sum_{t=0}^{T-1}(1-d)^{t}(1-p)^{t} = 0.14\,.$$

The sum on the left-hand side (LHS) is a geometric series and can be reformulated as

$$d(1-p)\frac{1-[(1-d)(1-p)]^{T-1}}{1-(1-d)(1-p)} = 0.14\ . \qquad \text{eq. 2.2}$$

Since T is time, we know that $T \geq 0$. Looking at **Equation 2.2**, we can conclude that, as long as $0 < (1-d)(1-p) < 1$, and $0 < d < 1$ and $p < 1$, there always exists a set p, d and T for which the equation can be satisfied. We do not know the length of time for which Franks and Deneubourg observed the ants, so let us set *T* to a biologically realistic value: if we consider $1t = 5s$ (5 seconds), setting $T = 100$ means that we are considering approximately 8 minutes of building activity. Arbitrarily setting $p = 0.05$ and solving the equation, we obtain, among the possible solutions, $d \approx 0.030$. We can plug this set of probability values, which fits the biological scenario, into a short simulation (conducted in R, v. 4.0.3) where deposition and encounters with stones occur over time but are completely independent. In pseudocode, the simulation is as follows:

```
s0 = 50 # number of stones being carried at the start of the sim
p = 0.05 # per-round probability of encountering a stone
d = 0.030 # per-round probability of depositing the carried stone
antv = rep(1,s0) # a vector tracking which of the initial s0 laden
                   ants are still carrying (1) and which are not (0)
stonev = rep(0,s0) # a vector recording how many stones each ant has
                   encountered, until it deposits

start;
```

```
until all stones have been deposited:
      for each of the starting s0 ants:
            check if it is still laden;
            if it is:
                 check if it deposits (with probability d) and record
                 any change in antv;
            else:
                 check if it encounters a stone (with probability p)
                 and record any change in stonev;
end;
```

We can then calculate the number of ants depositing after encountering exactly *n* stones from `antv` and `stonev` and study their relationship with the same approach as Franks and Deneubourg.
The relationship obtained in this way between the two variables is very similar to the one obtained by the two authors (**Figure 2.5**), even if in my simulation there is no causal effect between *d* and *p*. Out of 100 random simulation runs, 44% produce $r^2 \geq 0.92$ when a linear model is fitted – equal to or larger than the value reported in the original article.

I wish to stress that the fact that the conclusions drawn by Franks and Deneubourg are unsupported does not affect the validity of the output generated by the Deneubourg *et al.* agent-based model. The agent-based model puts the ability of the proposed behavioural model to the test of replicating the properties of the biological structure and is in itself an experiment, albeit a computational one. However, the criticism presented above has two implications. Firstly, as said above, it means that, in the absence of additional empirical evidence, there is no confirmation that the proposed behavioural model reflects the one found in nature. More than one behavioural model may exist that generates this outcome. Alternatively, the dynamics modelled may work as a proxy for the real dynamics (*i.e.*, they represent an environmental change that parallels the change in the cue that the workers are following). Secondly, the parameter values for the probability of deposition occurring in the presence and in the absence of other stones were estimated in Camazine *et al.* (2001) from the linear regression above. This means that these values may be incorrect. I will address this issue in the following section.

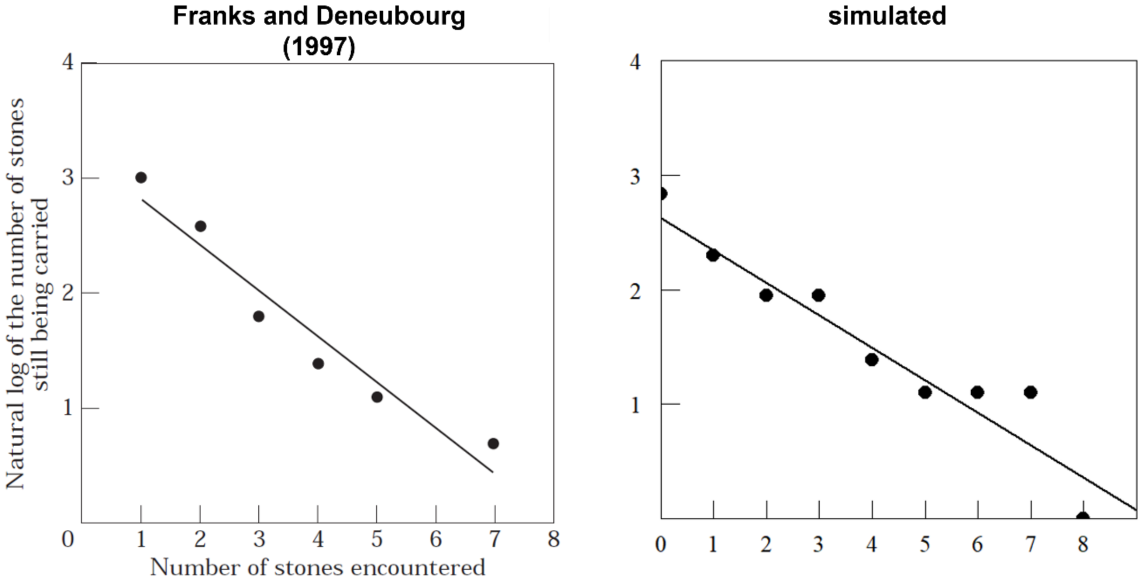

Figure 2.5. Empirical versus simulated relationship between number of stones still carried and number of stones encountered.

Panel A. Figure 3 in Franks and Deneubourg (1997), showing the change in number of stones carried (logged, y-axis) with number of stones encountered (x-axis). (Figure from "Self-organising nest construction in ants: individual worker behaviour and the nest's dynamics," by N. R. Franks and J-L. Deneubourg, 1997, Animal Behaviour, 54(4), 779-796. Elsevier. Reproduced with permission.) **Panel B.** The relationship between the same two variables as measured on a sample (*i.e.*, the 100th replicate obtained by initialising the random number generator with seed 0) of simulated data with 50 laden ants. Note that the replicate obtained from this seed was chosen for display before running the numerical simulation; *i.e.*, it was chosen blindly and is not necessarily the best match between simulated and real data across all simulations. Stone depositions and stone encounters occurred independently in the simulation model. Note that the plot of simulated data also includes deposition events occurring before ants encounter any stones (point 0 on the x-axis).

2.3 Model sensitivity analysis: robustness in the face of measurement error

Because the estimate of the parameters governing the relationship between number of encountered stones and the number of stones deposited is confused by the effect of time on both variables, we cannot be sure that the estimate is close to the real value – assuming, as we do in our behavioural model, that this relationship does exist. The study leading to the estimate (Franks & Deneubourg, 1997) was, moreover, based on a small-sample study of 50 workers from one colony and thus cannot be trusted to represent the inter-colony variation that is likely to exist in nature. How robust is the model's outcome to this estimate error? A similar question, and a more interesting one, can be asked when thinking of this variation under the light of evolutionary ecology: how much can we step away from the behavioural parameters estimated for this colony and species before the predictions of the behavioural model change substantially? That is, how stable is this collective phenotype, evolutionarily? The first problem is important because different parameter values may result in a poorer match between the outcome of simulations and the real phenomenon. The extent to which the behavioural model is sensitive to these changes must be estimated and is a compulsory step in the theoretical modelling approach. The second problem is evolutionary and links to the type of analysis that I conduct in **Chapter 4**: while in the next section I look at variation between colonies, in Chapter 4 I focus on variation within colonies, and how it affects the stability of the collective phenotype. In this chapter, I apply the same analysis to both models, showing that sensitivity analysis done for model evaluation also contains valuable evolutionary and ecological information.

Sensitivity analysis was done by applying a variance decomposition method. This approach, the Sobol's method, estimates the contribution of each parameter to the value of the outcome variable. I explain the details of this method in the following section. For the purpose of assessing the robustness of the model, I am interested in how the two measures of wall quality introduced in **Section 2.1** (distance spread and circular spread) change with varying parameter values – it is therefore their variances that were decomposed as described in **Section 2.3.2-4**.

2.3.1 Sobol's variance decomposition – a brief overview

The Sobol's method (Sobol', 1993; Cariboni *et al.,* 2007) enables us to identify the contribution of variation in each parameter to the total output variance, both on its own and in combination with other parameters. I briefly describe the method here.

The variance in our measured output, $V(Y)$, is decomposed as:

$$V(Y) = V_1 + V_2 + \cdots + V_M + K$$

where V_i is the contribution of parameter X_i to the variance, M is the number of parameters and K is the residual. From this decomposition, it is possible to calculate the proportion of output variance contributed by each parameter without accounting for its interactions with other parameters, its *first order effect* or S_i:

$$S_i = \frac{V_i}{V(Y)}$$

Through decomposition, it is also possible to calculate the proportion of output variance attributable to the interaction between two or more parameters. These variance components are particularly important for systems, such as the behavioural model examined here, where there are non-linear interactions between parameters. In the analyses described in this chapter, the only higher-order component studied, beside the total effect index described below, is the component attributable to the interaction between two parameters. This is the *second order effect*, or S_{ij}, defined as:

$$S_{ij} = \frac{V_{ij}}{V(Y)}$$

where X_i and $X_j$are two distinct parameters.

The total contribution of a parameter, including all its interaction components, is defined as its *total effect index*, ST_i, and can be used to evaluate whether higher order interactions (*i.e.*, interactions between more than two parameters) contribute heavily to the variance.

The output variance of a model is estimated by running the model across multiple values of each parameter, taken so as to cover the entire parameter distribution, and creating, from these parameter values, sets of M-parameter combinations in which all values are kept constant except for one, selected in turn. A computational optimisation of the Sobol's method that also effectively generates parameter sets was proposed by Saltelli (Saltelli, 2002; Saltelli, 2010). Saltelli's sampling method starts from the given sample size to generate $Z = N \times (2M + 2)$ parameter combinations, where N is sample size and M the number of parameters.

2.3.2 Methods

The analysis in this section was run on samples generated using Saltelli's sampling scheme (Saltelli, 2002; Saltelli, 2010). The parameter values were taken from uniform distributions with boundaries shown in **Table 2.1**. 1000 samples were used in this analysis, resulting in 16000 unique parameter combinations.

For each parameter combination, I ran the model once and measured distance and circular spread over the last 1000 time steps. Note that running a single simulation per combination causes some simulation noise to be incorporated into the parameter effect estimates. This is taken into account by reporting the confidence interval for the effect value. Simulations were run with 3000 stones and for a number of rounds T= 5000, which are the parameters used in the simulations described in **Section 2.1**.

The SALib library was used for the analysis (run in Python 3.7.9).

Parameter	Description	Boundaries
P_{M}	maximum possible value of pick-up probability	[0.01,1]
D_{M}	maximum possible value of deposition probability	[0.01,1]
F_{M}	effect of number of stones on pick-up probability below a critical number of nearby stones S_{c}	[0.11,1]
G_{M}	effect of number of stones on deposition probability above the critical number of nearby stones S_{c}	[0.11,1]
F_{m}	effect of number of stones on pick-up probability above a critical number of nearby stones S_{c}	[0.001,0.1]
G_{m}	effect of number of stones on deposition probability below a critical number of nearby stones S_{c}	[0.001,0.1]
τ	parameter regulating the slope of the decrease in deposition probability and increase in pick-up probability as we move away from the optimal building distance	[0.01,0.03]

Table 2.1. Parameter ranges used for the sensitivity analysis. The range for P_{M} and D_{M} are chosen to span the behavioural range from absence of pick-up or deposition propensity to very high propensity (before the interaction with F_{M} and G_{M}). The range of τ reflects a realistic range for wall thickness. $S_{\mathrm{c}} = 6$ in all simulations.

2.3.3 Results: quality of the wall across the parameter space (Deneubourg *et al.*, 2001, model)

In **Table 2.2 and Table 2.3**, I report the proportion of variance in distance spread and in circular spread, respectively, estimated to be generated by each parameter, with or without interactions.

P_{M} and F_{M} (the parameters defining pick-up activity) explain the near-totality of variance in distance spread (**Table 2.2**). I remind the reader that distance spread is a measure of how much variation there is in the distance of deposition sites from the centre of the brood cluster: the lower the value, the more compact the wall, given the same number of stones in the simulations that are being compared. Both for P_{M} and F_{M}, the difference between S_i and ST_i is small, indicating that change in these two parameters, one at a time, generates almost all the variance seen in distance of stones from the brood centre, independently from the values of the other parameters. **Figure 2.6** shows how the distance spread (our output) varies across P_{M}values (left panel) and F_{M} values (right panel). For very low values of both parameters, wall compactness is poor, but it improves rapidly as the values of each parameter increases. There is also little interaction between the two parameters: **Figure 2.7** shows how distance spread changes across the $P_{\mathrm{M}} - F_{\mathrm{M}}$ parameter space. The outcome is stable as long as we avoid very low values in either dimension. Accordingly, the second order effect estimate for the $P_{\mathrm{M}}F_{\mathrm{M}}$ interaction indicates little effect (S_{ij}= 0.061, CI = 0.010).

When examining the variance in circular spread (**Table 2.3**), we see that G_{m} seems to account for much of the existing variance independently from other parameters (S_i), albeit with a fairly large confidence interval. Plotting the change in circular spread across G_{m} (**Figure 2.8 A**) and across G_{m} and all other parameters (**Figure 2.8 B-E**), we realise that this is because there is very little variance in this measure of wall quality across simulations and it is all caused by very low values of G_{m}. The large confidence intervals of the second order effects (**Table 2.4**) confirm that there is likely no contribution from other parameters.

Parameter	$\boldsymbol{S_i}$	$\boldsymbol{S_i}$ **confidence interval**	$\boldsymbol{ST_i}$	$\boldsymbol{ST_i}$ **confidence interval**
$\boldsymbol{P_{\mathrm{M}}}$	0.590	0.071	0.648	0.058
$\boldsymbol{D_{\mathrm{M}}}$	0	0	0	0
$\boldsymbol{F_{\mathrm{M}}}$	0.280	0.053	0.352	0.038
$\boldsymbol{G_{\mathrm{M}}}$	0.021	0.016	0.032	0.005
$\boldsymbol{F_{\mathrm{m}}}$	0	0.01	0.008	0.003
$\boldsymbol{G_{\mathrm{m}}}$	0.024	0.017	0.035	0.006
$\boldsymbol{\tau}$	0.017	0.013	0.022	0.003

Table 2.2. First order effect and total effect of each parameter on distance spread. The 95% confidence interval was used. Note that the sum of $\boldsymbol{ST_i}$ across parameters is higher than one because interaction effects contribute to the total effect of multiple parameters.

Parameter	S_i	S_i confidence interval	ST_i	ST_i confidence interval
P_{M}	0	0.25	0.170	0.072
D_{M}	0	0	0	0
F_{M}	0	0.027	0.146	0.044
G_{M}	0.025	0.031	0.194	0.061
F_{m}	0.005	0.0162	0.037	0.011
G_{m}	0.532	0.214	0.978	0.252
τ	0.010	0.020	0.107	0.034

Table 2.3. First order effect and total effect of each parameter on variance in circular spread. The 95% confidence interval was used. Note that the sum of ST_i across parameters is higher than one because interaction effects contribute to the total effect of multiple parameters.

Parameter 1	Parameter 2	S_{ij}	S_{ij} confidence interval
G_{m}	P_{M}	0.138	0.139
G_{m}	D_{M}	0	0
G_{m}	F_{M}	0.110	0.144
G_{m}	G_{M}	0	0.085
G_{m}	F_{m}	0	0.031
G_{m}	τ	0.259	0.459

Table 2.4. Second order effect of G_{m} with every other parameter on variance in circular spread. The 95% confidence interval was used.

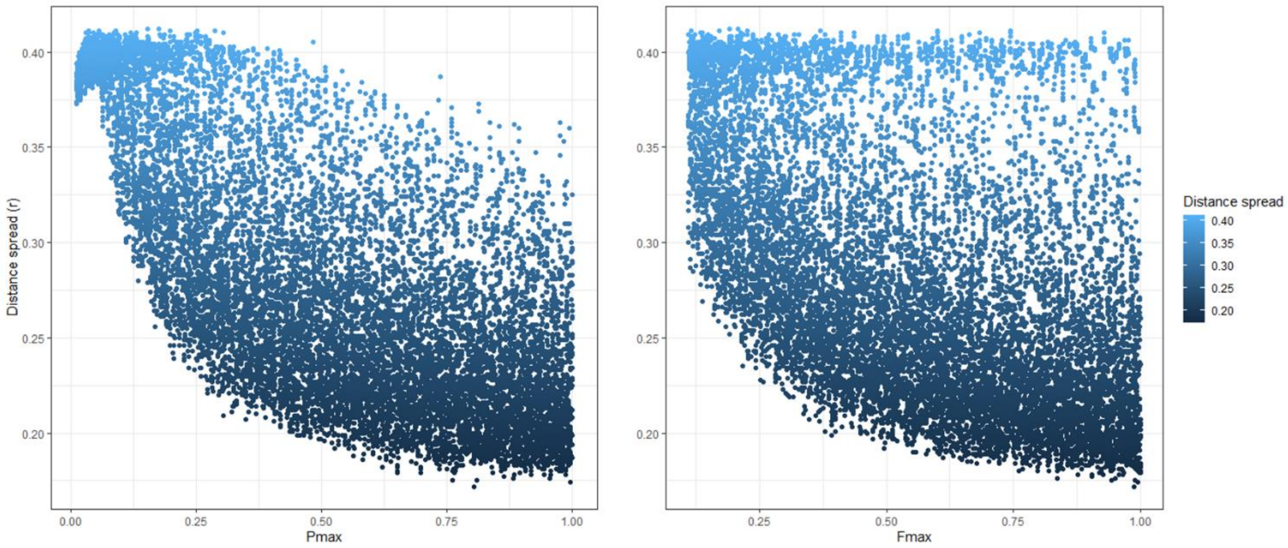

Figure 2.6. Distance spread values across P_{M} and F_{M} values in the Franks and Deneubourg model. The plots show how distance spread (y-axis and *colour-coded*) changes with P_{M} (*left panel*) and F_{M} (*right panel*). Simulations were run for 5000 rounds, with 3000 stone items.

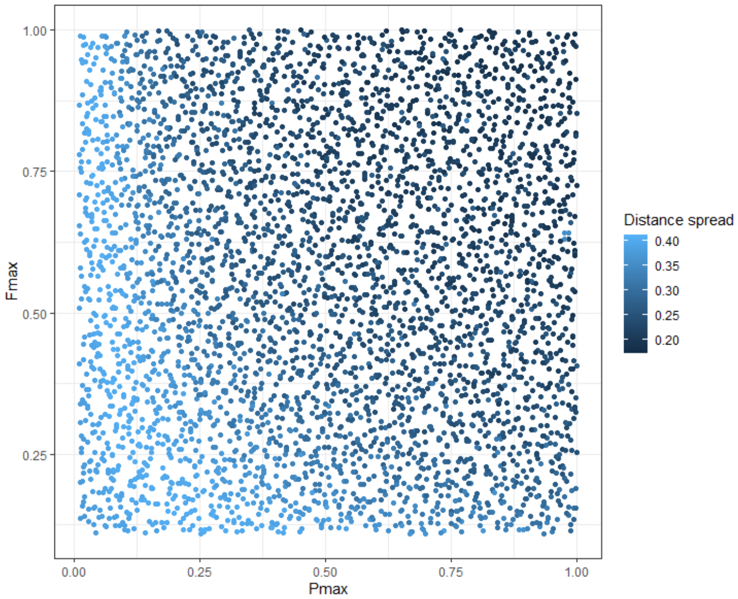

Figure 2.7. Distance spread values in the $P_{\mathrm{M}} - F_{\mathrm{M}}$ parameter space in the Franks and Deneubourg model. Distance spread values (*colour-coded*) are shown for the corresponding $F_{\mathrm{M}} - P_{\mathrm{M}}$ parameter combination. Simulations as for **Figure 2.6**.

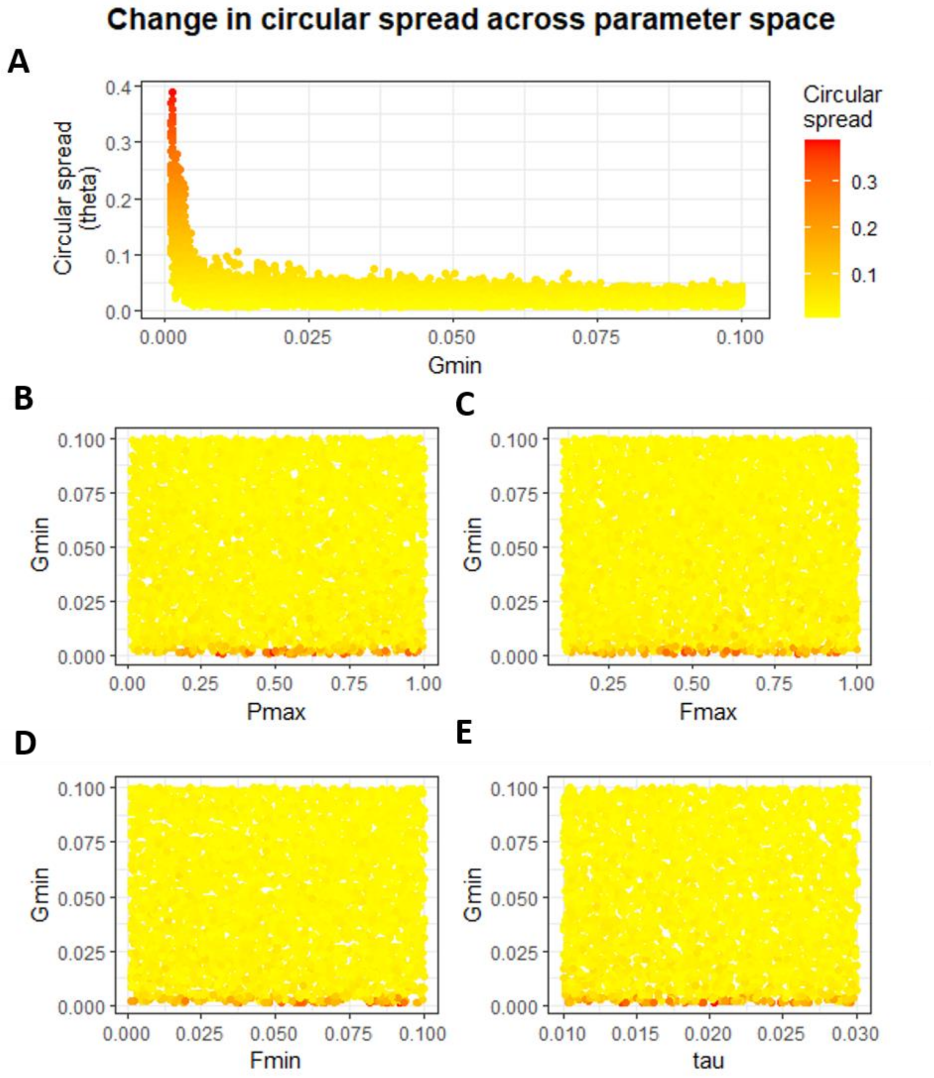

Figure 2.8. Change in circular spread across G_m and across its interaction with other parameters.

Panel A shows how circular spread (y-axis and *colour-coded*) varies across values of G_m. **Panels B-E** display the plots of circular dispersal (*colour-coded*) across the parameter space defined by G_m and P_M, F_M, F_m and τ, respectively. Simulations as for **Figure 2.6**.

2.3.4 Discussion: the behavioural model is robust

The results of the sensitivity analysis show some sensitivity of wall compactness (distance spread) to the minimum frequency of pick-up and a general robustness of wall cohesion (circular spread) across parameter space. There are some caveats to the interpretations of these statistics. Importantly, in our simulation, the speed of building is entirely dependent on pick-up probability, as deposition is forced to occur in the same time step as the stone is picked up (a modelling simplification of real-life dynamics that reduces computation time and memory requirements). It is therefore likely that the low compactness at low P_M and F_M values might disappear given longer simulation time. The apparent lack of contribution of D_{M} to the variance is also, with strong likelihood, an artefact of the same detail of simulation architecture. The trade-off between modelling simplification and accuracy in this case causes us to have an incomplete insight into the interaction of pick-up and deposition parameters when it comes to determining the distance spread outcome.

Wall cohesion seems largely independent of parameter values. Given how the choice between a template-only behavioural model and a self-organised one greatly affects the presence of gaps in the structure, especially at low stone availability (**Figure 2.2**), it seems that the main contributor to this aspect of wall quality is the behavioural model itself, specifically whether the model favours an even distribution of stones at equal distance from the reference point (template-only model) or the aggregation of stones (stigmergy + template model). The only parameter that has an effect on circular dispersal in the self-organisation model is the minimum value for the effect of number of stones on deposition probability, G_{m}. An educated guess would lead us to say that, when G_{m} is very small, gaps in the wall tend not to get filled in time before larger stone piles attract the remaining available building material, leading to separate stone aggregates.

Given the above caveats, Franks and Deneubourg's is a robust behavioural model: it can accommodate changes in parameter values that are expected as naturally-occurring variation among populations and environments, without substantially altering its predictions. This kind of robustness should be a sought-after characteristic of collective behavioural models. The fact that it is found here validate the current behavioural model as a hypothesis of how wall building in *Temnothorax* might be regulated – that is, the hypothesis that this behavioural model can be used by a species in the presence of inter-colony variation is not falsified.

In the next section, I tested whether the issues encountered with the Franks and Deneubourg behavioural model (*i.e.*, the poor performance when little building material is available and the presence of gaps in the wall) can be solved if we use an alternative behavioural model that makes slightly different assumptions of ant behaviour.

2.4 The *gradual* model

In this section, I propose a change to the model presented by Franks and colleagues. My formulation changes the behavioural rule encoding the relationship between stone density and deposition probability. Rather than a threshold system, in which the baseline deposition probability abruptly switches to a maximum value when a critical number of stones is encountered, I suggest a two-component model: 1. a gradual increase of deposition probability following number of stones already present at the site, caused by either a mechanical feedback or recognition of another sensory cue, such as wall conformation (positive feedback); 2. a gradual decrease in deposition, once a critical number of stones at the site is reached, also caused by cue recognition. The decrease in deposition probability following stone number, which represents a key difference compared to the original model, enables behavioural termination and the return to baseline deposition rates once the wall has reached a sufficient size.

Physiologically, this *gradual model* has a different interpretation than the threshold model of Franks and Deneubourg. Their formulation was based on the idea that workers use the hindrance effect of stone piles to decide where to deposit (beside the use of the brood template). This is consistent with the bulldozing behaviour observed by the authors in *T. albipennis*. The *gradual* model instead assumes that workers perceive and respond either to the local amount of stones or to an environmental template that is modified through deposition (such as the intensity of air currents or of light) to decrease their deposition activity in the later stages of building. This assumption can co-exist side-by-side with the mechanical effect observed by Franks and colleagues: the physical interaction between stones can underlie (alone or in combination with non-mechanical perception) the positive feedback effect. Termination of activity, on the other hand, necessitates an explanation other than mechanical interaction.

In **Chapter 3**, I show that the correlation between stone density at building locations and termination of building activity is supported by empirical data. Here, I have encoded this behavioural model through a mathematical equation and tested it in the same agent-based simulation setting as above. The following sections contain methods and results of this *in silico* study.

2.4.1 Extended model details (methods)

Behavioural model and simulations

The simulations were run using the same agent-based model and method described in **Section 2.1.1**. The function describing pick-up behaviour was kept unaltered; we are therefore assuming that pick-up behaviour reaches minimum values once the building site has reached a critical number of stones. The deposition probability function was instead changed to reflect the hypothesis described above. This becomes, in mathematical terms:

$$D(r) = D_{\mathrm{M}} \frac{1}{1 + \tau (r - r_{\mathrm{O}})^2} (G_{\mathrm{m}} + (G_{\mathrm{M}} - G_{\mathrm{m}}) \times e^{-\frac{(S_{\mathrm{c}} - S)^2}{2}}) Q.$$

This formulation represents a symmetric bell curve in local stone number S, with highest point at $S = S_{\mathrm{c}}$. As in the original model, $G_{\mathrm{M}} = 0.55$ and $G_{\mathrm{m}} = 0.01$. All other assumptions, including parameter values, remained unchanged.

Sensitivity analysis

To investigate model robustness in parameter space, I repeated the same variance decomposition used earlier in this chapter for the Franks and Deneubourg model, across the same parameter range

(**Table 2.1**). As for the decomposition of the original model, 1000 samples were used in this analysis and all simulations were run with 3000 stones and with T = 5000.

2.4.2 The *gradual* model reproduces wall structures similar to the ones observed in nature and performs well even when building material is scarce

The *gradual* model produces a compact and circular stone wall even when few building resources are available (**Figure 2.9A**). Compared to the original model, the resulting structure has the same average compactness, but fewer gaps (**Figure 2.9B**). As also seen for the threshold model, moreover, nest size adjusts to colony size over a long time scale (**Figure 2.10**), because stochastic noise overcomes hysteresis.

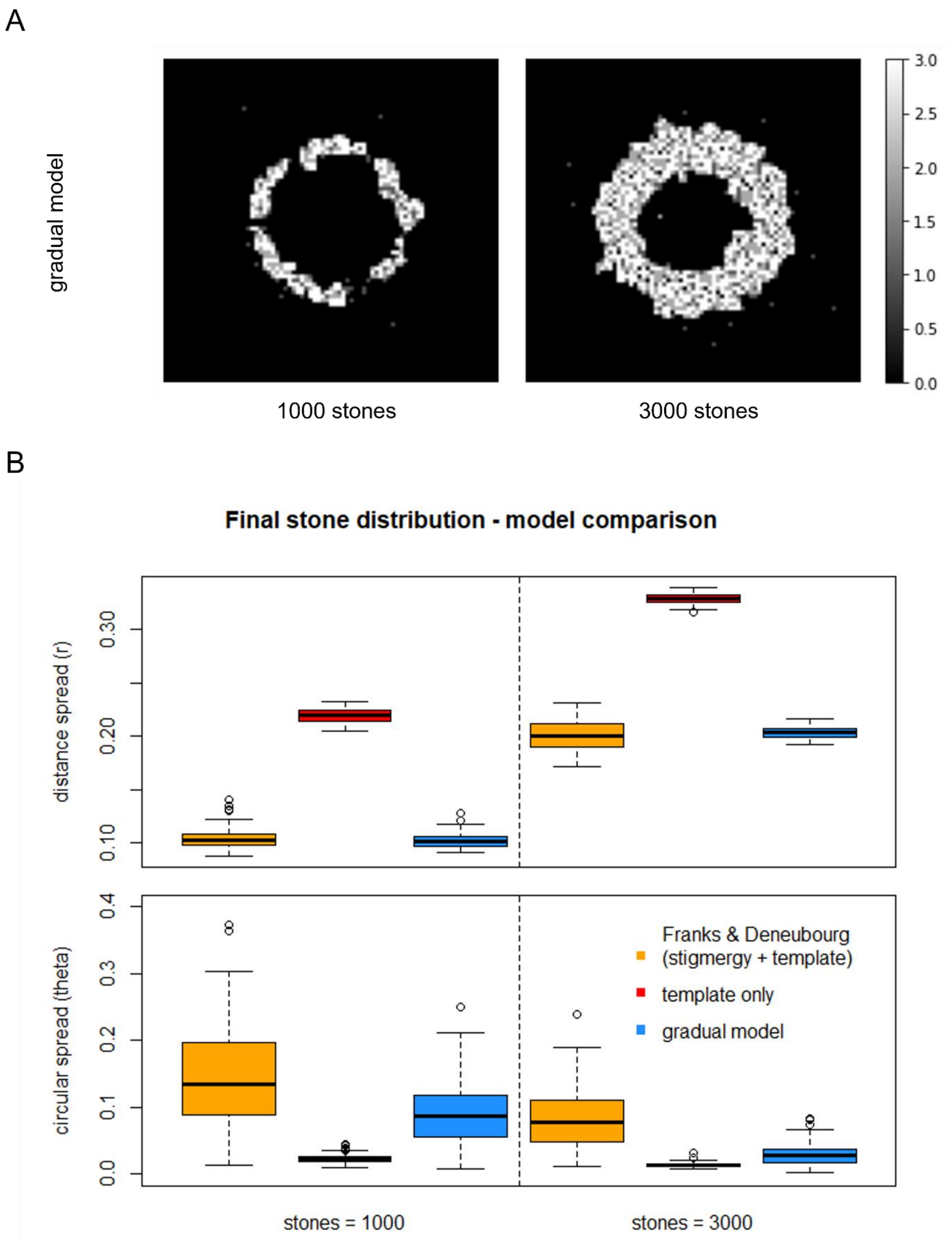

Figure 2.9. Model output measured across stone availability.

Panel A shows the wall structure created by the *gradual* model under low (*left*) and intermediate (*right*) stone availability. The grayscale represents the number of stones at each node. **Panel B** shows a comparison of distance (*top*) and circular (*bottom*) spread value across stone availability and under each of the three behavioural models (the original proposed by Franks and Deneubourg, the model containing just the template effect and the *gradual* model including behavioural termination). The plotted distributions refer to 100 independent simulation rounds. Outliers are shown.

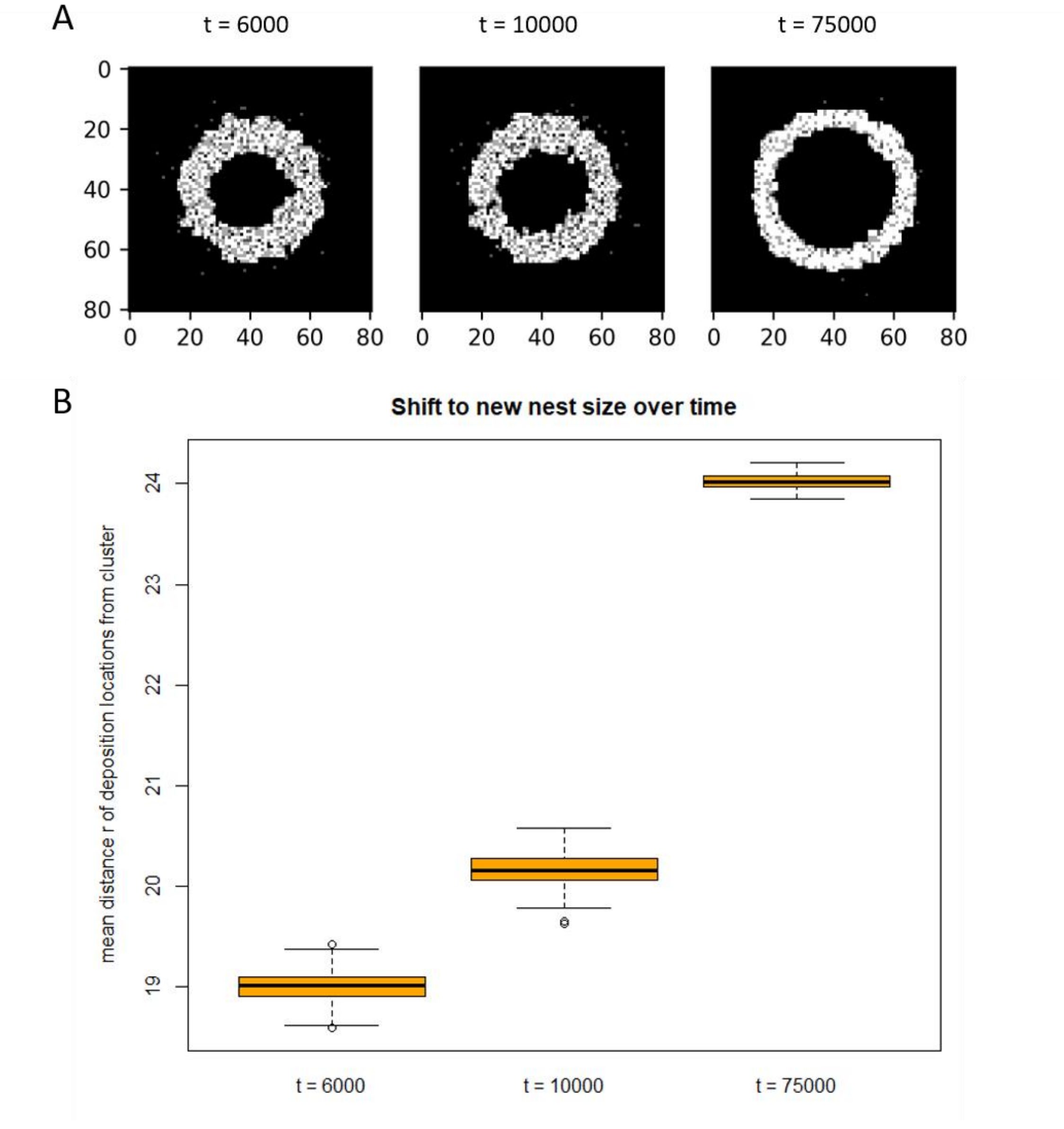

Figure 2.10. Shift to new nest size over time in the *gradual* model.

The figure shows the change of wall location over time if colony size doubles after wall completion (*i.e.*, for all workers, $r_o = 18$ if $t < 5000$ and $r_o = 24$ if $t \geq 5000$). Note that the shown snapshot values are not chosen at equidistant time points. **Panel A** shows the wall at the corresponding time point and **Panel B** shows the distribution, over 100 simulations, of the average distance from the centre at which stones are located.

2.4.3 Sensitivity analysis of the *gradual* model

As I did for the Franks and Deneubourg model, I decomposed here the circular and distance spread variances in their components associated with each model parameter. The model very efficiently maintains the circular structure of the wall across parameter space, so that the resulting variance in circular spread is almost non-existent (**Figure 2.11**). Consequently, I focus here on the decomposition of distance spread variance.

Distance spread shows a similar level of stability to that displayed by the Franks & Deneubourg model, and very similar dispersal values. P_{M} and F_{M} have the largest total effect, through first order effect (**Table 2.6**) and through interactions of order higher than second (as visible in **Table 2.7**, second order effects contribute very little to variance), indicating that the remaining variance is due to complex, non-linear interactions among parameters. The effect of P_{M} and F_{M} is caused by the fact that pick-up probability must reach a minimum value for the wall to form (**Figure 2.12**), as in the original model. Overall, model choice has limited effect on distance spread (**Figures 2.7** and **2.12**).

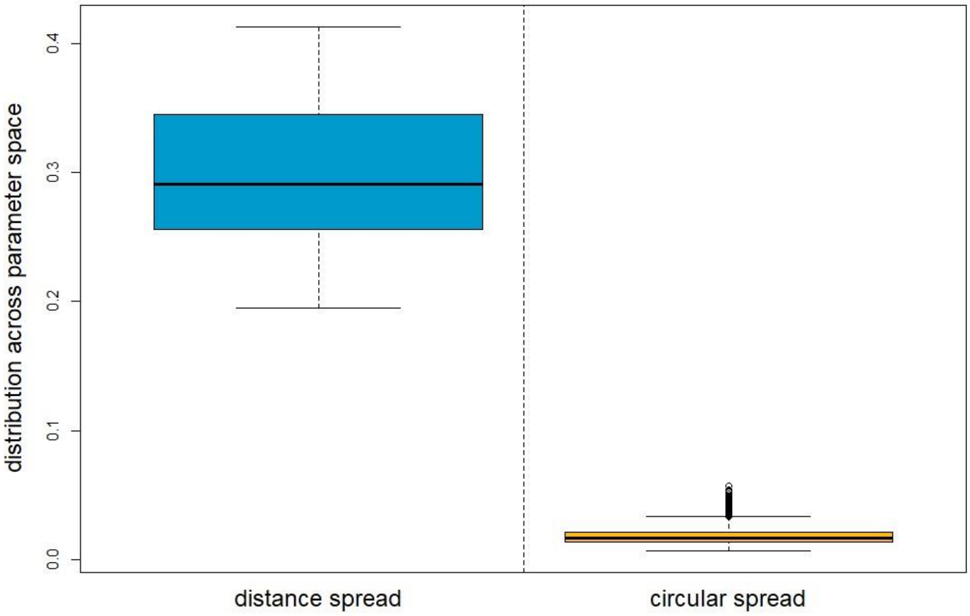

Figure 2.11. Distribution of distance spread and circular spread values obtained from simulations run across the full parameter space.

Distribution of distance and circular spread across 16000 simulations with parameter value combinations sampled across the parameter space defined in **Table 2.1**. There is little variance in circular spread, indicating that the model robustly produces a circular wall shape under all parameter conditions. Outliers are shown.

Parameter	S_i	S_i confidence interval	ST_i	ST_i confidence interval
P_{M}	0.571	0.072	0.642	0.064
D_{M}	0.003	0.004	0.003	0.000
F_{M}	0.297	0.058	0.367	0.033
G_{M}	0.018	0.014	0.022	0.003
F_{m}	0.004	0.008	0.009	0.002
G_{m}	0.021	0.017	0.026	0.003
τ	0.009	0.011	0.016	0.002

Table 2.6. First order effect and total effect of each parameter on distance spread in the *gradual* model. The 95% confidence interval was used. Note that the sum of ST_i across parameters is higher than one because interaction effects contribute to the total effect of multiple parameters. The corresponding values for the Franks and Deneubourg model are found in **Table 2.2**.

Parameter 1	Parameter 2	S_{ij}	S_{ij} confidence interval
P_{M}	D_{M}	0.001	0.075
P_{M}	F_{M}	0.062	0.094
P_{M}	G_{M}	0.004	0.074
P_{M}	F_{m}	0.008	0.075
P_{M}	G_{m}	0.003	0.074
P_{M}	τ	0.006	0.073
D_{M}	F_{M}	0	0.007
D_{M}	G_{M}	0	0.007
D_{M}	F_{m}	0	0.007
D_{M}	G_{m}	0	0.008
D_{M}	τ	0	0.007
F_{M}	G_{M}	0.003	0.057
F_{M}	F_{m}	0.004	0.058
F_{M}	G_{m}	0.006	0.059
F_{M}	τ	0.009	0.058
G_{M}	F_{m}	0	0.019
G_{M}	G_{m}	0	0.019
G_{M}	τ	0	0.019
F_{m}	G_{m}	0.002	0.013
F_{m}	τ	0.002	0.013
G_{m}	τ	0.001	0.023

Table 2.7. Second order effect of each parameter combination on distance spread in the *gradual* model. The 95% confidence interval was used.

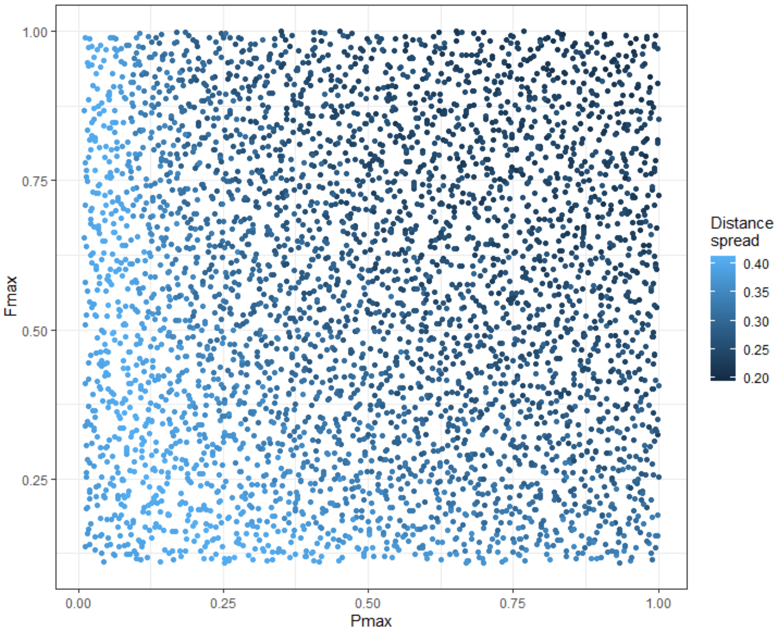

Figure 2.12. Distance spread values in the $P_{\mathrm{M}} - F_{\mathrm{M}}$ parameter space in the *gradual* model.

Note that the values of P_{M} and F_{M} generated by Saltelli's sampling are the same as in **Figure 2.7**, but distance spread values differ, albeit only slightly. This indicates that, overall, the choice of model has limited impact on distance spread.

2.5 Chapter conclusions

This chapter was dedicated to assessing the Franks and Deneubourg behavioural model of *Temnothorax* self-organised collective building. Their model, as I hope I have shown, is quite successful in reproducing the patterns of nest structure seen in nature (**Section 2.1**) and is robust to variation in parameter value (**Section 2.3**). Overall, it can be assumed that this model captures some of the essential dynamics of nest building in this species. Is this *the* conclusive model of *Temnothorax* wall building? With strong likelihood, no: the cue-response mechanisms assumed to trigger and modulate worker building behaviour have so far not been investigated empirically and are only hypotheses (**Section 2.2**). We should expect new research to highlight more and more details that fine-tune this model.

The *gradual* model (**Section 2.4**) is a first step in this direction. This alternative model results in a well-defined wall with fewer and smaller gaps even under scarcity of building material (compare **Figure 2.1** and **Figure 2.9**), with a lower circular dispersal value (**Figure 2.9B**), and displays equal robustness to measurement error and to evolutionary changes in parameter value. Similarly to the Franks and Deneubourg model, the *gradual* model assumes (slightly different) cue-response mechanisms – specifically, of the relationship between deposited stones and deposition rate – that have so far not been tested on empirical data. In the next chapter, I will perform a statistical analysis on laboratory data of *T. rugatulus* wall building, to evaluate how well each of these two alternative hypotheses (the Franks and Deneubourg and the *gradual* model) explains real activity patterns.

Chapter 3: Hidden Markov models of ant activity

In the previous chapter, I proposed an alternative model of *T. albipennis* wall building behaviour, the *gradual* model, that predicts behavioural termination of wall-building triggered in part by a gaussian-shaped response between worker deposition rates and the amount of stones at the building site. *In silico*, the *gradual* model appears to perform well, reproducing a structure that seems very similar to the one built by *T. albipennis* in the laboratory, even when building material is scarce. In this chapter, I present the results of laboratory experiments that were aimed at further investigating whether this model matches the activity data patterns of *Temnothorax* wall building and represents an improvement on Franks and Deneubourg's threshold model.

The work described in this chapter was conducted on *T. rugatulus*, a species found across the Southern United States of America and belonging to the same genus as *T. albipennis*. The Sasaki research group (University of Georgia), with whom I collaborated, uses *T. rugatulus* as a model system for collective decision-making. I have introduced *T. albipennis* and *T. rugatulus* in **Chapter 1**, **Section 1.3**. Similarly to *T. albipennis*, *T. rugatulus* is also crevice-nesting and builds nearly-2D walls identical to those built by *T. albipennis*.

As it will become apparent, the data, once extracted, presented an unanticipated pattern (**Figure 3.3**). Rather than a decrease, increase, or scattered arrangement of values, the time series of colony-wide material deposition rates appears as a sequence of high-rate peaks and low-rate valleys. I hypothesise that the measurements were affected by background changes in overall worker activity levels caused by at least one of two sources: workers switching between tasks, and colony activity cycles (*i.e.*, fluctuations in the fraction of workers active at any one time). Statistically accounting for these two sources of variance in rate values is problematic. Here, I choose to apply a statistical method widely used in animal movement ecology, Hidden Markov models (HMMs). As I will show, this approach allows me to separate the state of the colony at different timepoints of data collection into one of two separate states with associated data distributions: high activity, corresponding to the peaks in our time series, and which we identify with periods in which many workers are engaged in the task or/and work at a higher rate, and low activity, corresponding to the valleys, which we attribute to a low number of builders or/and a lower work rate.

The chapter is organised as follows:

- **Sections 3.1-2** summarise the background literature on task specialisation and on colony-level oscillatory activity patterns in ant colonies, which provides the reader with the necessary knowledge to appreciate the justification of my assumptions of colony states and choice of analysis method.

- **Section 3.3** contains the data analysis methods, results and discussion.
- In **Section 3.4**, I briefly discuss if we can use the same approach to verify that the relationship we measured is generated by responses to local cues. That is, while the analysis presented in the main manuscript used colony-level activity data and analysed their relationship with stone density as measured across multiple building sites, here I discuss the possibility of using a hidden Markov model approach to analyse the relationship between activity at each building site and site-specific stone density. This level of analysis requires incorporating a mixed effects regression approach into the hidden Markov model framework, which presents some challenges. The approach discussed here will be the subject of future work.
- The analysis presented focuses on the relationship between worker building activity and stone density. In **Section 3.5**, I consider if my results and analyses shed light on the functional form of the other important component of the *Temnothorax* behavioural model: distance from the brood. I find that building activity seems to start at locations close to the brood.
- The walls built by *T. albipennis* and *T. rugatulus* do indeed seem identical, but can the same be said of their building behaviour? **Section 3.6** offers a point-by-point comparison between the behaviour of the two species during building activity, using the description of *T. albipennis* behaviour made by Franks and colleagues and my own observations on *T. rugatulus*.
- **Section 3.7** briefly concludes the chapter.

The data used for this analysis were collected during a stay in Dr T. Sasaki's laboratory at the University of Georgia (UGA) in the summer of 2019, with the help of Dr Kazutaka Soji (University of Tokyo) and Ben Taylor (B.Sc. Biology at UGA) for filming setup and video processing. Behavioural data and colony measurements were extracted from the videos by me and N. Ng, A. Rouviere, E. Macqueen, M. Wright, R. Gorgon and L. Gray (B.Sc. students at the University of St Andrews). The applications of HMMs to ant colony activity was achieved with the help and collaboration of Dr Théo Michelot (Centre for Research in Environmental and Ecological Modelling, University of St Andrews).

3.1 The organisation of colony tasks in eusocial species

Division of labour among workers of eusocial species can be defined as the subdivision of the colony workforce into subgroups whose members display behavioural persistence in distinct tasks, each task contributing to colony survival and fitness. Division of labour has historically been hypothesised to be one of the characteristics behind the great ecological success of eusocial species (*e.g.*, Wilson, 1971). The rationale behind this hypothesis is often given with reference to the literature behind division of labour in human factories, which stresses two advantages of increased specialisation that result in an increase in productivity: the equation of specialisation with efficiency, based on the ability of an individual to learn a task at the best of their capacities if they do not also need to learn other tasks, and the decrease in task-switching costs (A. Smith, 1776, cited by, *e.g.*, Bourke & Franks, 1995; Dornhaus, 2008). For eusocial species, increased productivity means improved colony maintenance resulting in higher reproductive fitness. The specific relationship of this latter measure with increased specialisation and division of labour is still the subject of study, mostly complicated by the problem that division of labour is ubiquitous among eusocial species and that a comparison between species with and without this form of organisation is therefore impossible. The problem is further complicated by the fact that, as I discuss below, the individuals of most eusocial species are known to specialise not in a single but in a subset of related tasks (Pinter-Wollman et al., 2012), independently of colony size (Dornhaus et al., 2009), which should both decrease the attainable degree of efficiency and increase the task-switching cost compared to single-task specialisation. Moreover, the relationship between individual specialisation and efficiency is still the subject of discussion (Dornhaus, 2008). In this thesis I will limit myself to the description of colony organisation with respect to division of labour, without reviewing the literature on the evolution of this feature, because the evolutionary process leading to its emergence is irrelevant for the purpose of my study.

The tasks that are most ubiquitously found in eusocial species, and that can be thought of as essential for the colony, are three: brood care, foraging and nest maintenance (nest cleaning and repair). The first recorded observations that workers of eusocial species attend to different tasks depending on their characteristics were made by Aristotle, who noticed how nurse honeybees are hairier than foragers and deduced that foragers must be younger than nest workers (Bourke & Franks, 1995). Today, with a more complete social insect observation record available, we know that the opposite is true and foragers of most eusocial species, including honeybees, are most commonly older workers, while the nursing role is taken up by new workers soon after their own eclosion. The hypothesis that workers change their task behaviour with age, or *temporal polyethism*, has instead stood the test of time and is now supported by observations from many eusocial species, albeit more flexibly than earlier studies seemed to indicate. Studies on honeybees show that, at least in this species, age-dependent changes in task preference are caused by background changes in gene expression occurring during the lifetime of a worker. Two genes have so far been identified, via genetic manipulation, to have a causative effect on age-dependent division of labour: the *foraging* (*for*) gene, whose changes in expression are associated with the transition from nurse to forager (Ben-Shahar, 2005; Ben-Shahar et al., 2002); and the *malvolio* (*mvl*) gene, which causes a precocious transition from nursing to foraging and an increased responsiveness to sucrose (Ben-Shahar et al., 2004). Such genetic changes can be modulated (that is, delayed and reversed) by social cues that carry information on colony status and ongoing activity levels, a mechanism which underlies the self-organised response of the colony to its current needs. A perfect example of how gene expression can be manipulated as part of the self-organisation process is the *for* gene and it will thus be briefly discussed here. The involvement of *for* is thought to act through induction of positive phototaxis in older workers, who increasingly spend time in lighter areas such as the nest entrance and the outside (Ben-Shahar et al., 2003). Changes in the expression of *for* and therefore development into forager are regulated by the transmission of three separate chemical compounds: ethyl oleate, which is transmitted from forager to nurse; brood pheromone, from brood to nurse; and queen pheromone, from queen to nurse. Ethyl oleate is produced in the foragers' crop and transmitted via trophallaxis, seemingly hindering the natural increase in *for* expression (Leoncini et al., 2004). Inhibitory RNAs may also be involved in forager-mediated *for* suppression (Greenberg et al., 2012). Inhibition of nurse development by foragers, however mediated, is a negative feedback loop mechanism. To stabilise the forager population, the development of new foragers is delayed through ethyl-oleate transmission. Once the number of foragers has decreased to the point that not enough ethyl-oleate is transferred to delay development, the foraging workforce is quickly replenished, satisfying the colony's energy needs. Brood pheromone exercises a duplex effect on worker activity: on some workers, it induces a prolonged and increased tendency to rear brood, while in others it induces a precocious transition to foraging. This results in a positive feedback loop on brood production, caused both by extra brood care and additional resources (Pankiw et al., 2004). The absence of nurses causes reversal of some foragers to nursing activity (*e.g.*, Bloch & Robinson, 2001), which may be triggered by brood pheromone. Finally, queen mandibular pheromone is also known to hinder nurse development into foragers (Pankiw et al., 1998).

In ants, studies have focused more on the neuroanatomical and neurophysiological rather than genetic differences between workers of different ages and castes, using the hyper-diverse genus *Pheidole* (Giraldo et al., 2013; Muscedere & Traniello, 2012). Such neurological changes can be epigenetically caused by experience and differential use of sensory organs depending on task, without a genetic underpinning. Indeed, young minor workers of *P. dentata* are not specialised nurses and a brood survives better when cared for by older individuals, which suggests that learning and experience play an important role in nursing efficiency (Muscedere et al., 2009). However, at least one gene – again, *for* – has been found to differ in its expression patterns between nurses and foragers. Differently from the pattern in honeybees, inhibition of *for* expression in *P. pallida* reduces foraging and increases defence behaviour in major workers (Lucas & Sokolowski, 2009).

In ants, the re-allocation of workers in response to colony needs seems to involve repertoire expansion in addition to (or perhaps, in some species, instead of) role reversal (Leitner & Dornhaus, 2019; Seid & Traniello, 2006). Moreover, there is evidence that the extent of role flexibility varies from species to species: in *Pogonomyrmex badius*, the transition to foraging is seemingly irreversible and removal of the nurse force remains uncompensated (Kwapich & Tschinkel, 2013, 2016). It is possible that a more important role in defining individual engagement in tasks is played, in some ant species and in contrast to honeybees, by individual variation in ant response thresholds to environmental stimuli. The idea that workers differ in their thresholds for different task-related stimuli and that workers with lower thresholds engage first in a task, thus causing division of labour, is referred to as the *threshold model* of division of labour. Even if computational simulations show that the existence of thresholds is sufficient to generate division of labour (Beshers & Fewell, 2001), it is more likely that this mechanism interacts with genetic and epigenetic changes in worker preferences to determine colony organisation. Evidence for the role of thresholds in task allocation is found, for example, in honeybees, where different response thresholds to sucrose levels are associated with forager type (*i.e.*, water, nectar or pollen forager; Pankiw & Page, 2000).

Contrary to our original understanding of division of labour in eusocial species, we now know that task groups are not static and well-defined subunits within the colony (Beshers & Fewell, 2001; Gordon, 2016a). Much of this research has been done in *Temnothorax* and therefore offers relevant information for my study. Analysis of colony task groups by worker tracking in *T. albipennis* and in *T. rugatulus* has revealed that workers have weak specialisation (*i.e.*, preference for one single task) and high segregation (*i.e.*, there is positive correlation between worker preference for some tasks and negative correlation for others; Charbonneau & Dornhaus, 2015a), being organised in 'situation'-specific groups so that workers engage most commonly in many or all of the tasks related to, *e.g.*, nest maintenance, but rarely in those related to, *e.g.*, foraging, either for food or material (Pinter-Wollman et al., 2012). Importantly for our study of nest building, foraging and building are often performed by the same individuals in *T. rugatulus* (Charbonneau & Dornhaus, 2015a). These categories partially overlap with those associated with older workers in age-polyethism-drive division of labour. Individual workers differ in their propensity to engage in each task and in their overall activity levels, with a minority of workers (2.6% in *T. rugatulus*) being always active, the majority (72%) being active most of the time and a non-negligible proportion (25%) being always inactive (Charbonneau et al., 2017a). The origin and role of these inactive individuals has been subject to several studies, none of which have yet provided a conclusive answer. "Specialised" inactive – as their inactivity is so consistent in time that these individuals can be defined as such – have the smallest spatial fidelity zones, the smallest behavioural repertoire, increased corpulence, they more often have oocytes, and are found at the periphery of the colony information network when network centrality is measured, disproving the hypotheses that they are static information hubs (Charbonneau et al., 2017a). Their presence is also not an artifact of being lab-reared (Charbonneau et al., 2015). If removed, these workers are not replaced (Charbonneau et al., 2017b). In fact, these workers do not seem to perform any function but might, to some degree, be a reserve force for nurses (Leitner & Dornhaus, 2019). A possibility, already proposed by Corbara *et al.* (1989), is that they are simply immature workers with no task experience, a hypothesis which would be in agreement with their physiology. Testing this hypothesis would require tracking the development of these workers from their identification to their death or old age – however, studies (*e.g.*, Charbonneau & Dornhaus, 2015a; Ishii & Hasegawa, 2012) have shown that inactive workers observed for a period of 3-weeks (expected to be sufficient for behavioural maturation) show no change in their (in)activity.

Of the summary of division of labour in eusocial colonies that I have just provided, a few pieces of information are particularly relevant to the methodological approach used in my study and are worth re-emphasizing here. Firstly, division of labour in many ant species, including *Temnothorax*, does not entail the existence of task-specific subgroups that become active as task-specific needs increase and are inactive if these needs are low. On the contrary, most ants engage in multiple tasks (albeit with segregation among specific task groups, so that there is positive correlation among preference for certain tasks and negative correlation among others), and workers are likely to switch between tasks even when the stimulus levels of the original task are still high, depending on their response thresholds and on the relative stimulus levels of each task. In *T. rugatulus*, building and foraging are performed by the same individuals, so that time series of activity levels of either of these tasks (taken separately) are likely to be affected by the between-task switch events of some workers. Secondly, ants differ in their level of activity, so that the switch of a particularly active ant (termed an *élite*) may cause a large decrease of overall activity rates for that task, unless this is compensated by other workers increasing their activity – this is so far not well studied.

In this section, I have focused on switching between different activities. In the next section, I will discuss a different activity-related phenomenon that occurs at colony-level and is observed in many ant species: synchronised worker switching between activity and inactivity.

3.2 Activity cycles in ant colonies

Several studies looking at the overall activity patterns of colonies have identified a rhythmic component in almost all ant species in which it has been looked for. This periodicity is not linked to external day-night cycles – and indeed occurs on a much shorter timescale (tens of minutes) and in constant laboratory conditions. Cole and Trampus (1999) mention *Camponotus plantus*, *Tapinoma* *littorale*, *Pseudomyrmex cubaensis* and *Monomorium floricola* as showing periodic components in their activity and *Leptothorax pastinifer*, *L. isabellae*, *Pseudomyrmex elongatus*, *Zacryptocerus varians*, *Solenopsis picta* and *Xenomyrmex floridanus* as having periodic activity. The distinction between periodic component and periodic activity is unclear in their text, but my educated guess is that a mathematical analysis confirming the component was done for the first species, while periodicity has been observed but not mathematically proved in the second. Cole and Cheshire (1996) add to the list *P. pallidus* and *Crematogaster ashmeadi*. Both these works mention *Tapinoma sessile* as the only species investigated without finding a periodic component. Theraulaz (Bonabeau, Theraulaz, & Deneubourg, 1998) notes also *Messor barbarus*. The most extensively studied species in this respect are, however and without debate, two from the *Leptothorax* genus, sister to *Temnothorax* (Prebus, 2017): *L. allardycei* (Franks et al., 1990; Hemerik et al., 1990) and *L. acervorum* (Cole, 1991a, 1991c, 1991d, 1992; Cole & Hoeg, 1996). The existence of periodic components in these latter studies has been proved beyond doubt through a combination of either manual or automated (through change in pixels detected between short intervals in videos of colonies) activity measures and mathematical analysis (Fourier analysis and autocorrelation function). The rhythmic component has, in both species, a period 15-35 min, varying from colony to colony, and accounts, in *L. allardycei*, for approximately 33% of variation in activity (Cole & Cheshire, 1996; Cole & Hoeg, 1996).

In all the species listed above, the decrease in activity corresponds not to small decreases in the rate individual activity, summing up to a large colony-level decrease, but to an increase in the number of individuals who become completely inactive. That is, the periodic change in activity reflects a switch from a large number of individuals being active to a large number being inactive. What causes these colony-level oscillations between activity states? One might think it the result of individual periodicity, since individual workers also display shifts from activity to complete inactivity when in isolation. However, the activity of *L. allardycei* workers, which we saw to be periodic at colony-level, is not periodic (Cole, 1991a), but proved to be chaotic (Cole, 1991d) when these workers are isolated. Inactive ants have been shown to become active more often after interaction with an active ant in *L. acervorum* (Hemerik et al., 1990) and *L. allardycei* (Cole, 1991d). In a conclusive experiment on the importance of ant-ant interaction in influencing activity, Cole and Trampus inserted a partition in the middle of a *L. allardycei* nest, interrupting the physical contact (but not pheromone signalling) between the two halves of a colony, and in so doing disrupted the synchrony between the two halves, which took on independent periods (Cole & Trampus, 1999). Synchrony was restored by removing the partition. A later cellular automata model (Cole & Cheshire, 1996) suggests that it is the interaction and reinforcement between active workers that generates synchrony, rather than the induction of activity by an active worker to an inactive one through interaction, which has the opposite effect of disrupting synchronisation. Synchrony between worker activity bursts emerges as the number of workers in the aggregate increases, with groups of 5-7 *L. allardycei* workers showing borderline periodicity. As the number of workers increases, the pattern becomes more predictable (Cole, 1991d). Models using chaotic individual activity and activity-triggering worker interactions (autocatalysis) as the key dynamics (Goss & Deneubourg, 1988; Tofts et al., 1992) successfully reproduce the pattern of emergent synchrony and the lack of periodicity at low colony size, suggesting that these two components are essential and sufficient for the generation of rhythm. A refractory period at the beginning of a worker's inactive bout, during which a switch back to activity cannot be induced, is necessary to generate periodicity from individual chaos, a prediction confirmed by empirical data (Cole, 1991c).

Is there an influence of circadian rhythms on colony periodicity? Ants have been shown to have circadian rhythms: in *T. rugatulus*, for example, more workers are inactive and less workers are engaged in foraging, building and walking at night time (Charbonneau & Dornhaus, 2015a). In the harvester ant *Pogonomyrmex barbatus*, different tasks become prominent at different times of day (TOD; Gordon, 1983). However, the relationship between task switching and colony synchrony, if any, is unclear. As mentioned in the previous section, moreover, there is also evidence that inactivity, in some species, can be classified as a task when looked at in terms of worker specialisation, a fact which complicates the interpretation of these studies on circadian rhythms from the perspective of periodicity. The study on *T. albipennis* by Franks *et al.* (1990) covered the period between 9:30 and 19:30 and no pattern related to TOD is apparent from the data (although this was not tested). Cole and Cheshire (1996) find from their experiments on *L. allardycei* that, in the absence of brood, the size of the group affects periodicity production during the day, but not at night. However, the effect of TOD disappears in the presence of brood, when the size of the aggregate has a larger effect, suggesting that any influence of circadian rhythms (probably resulting from differences in the time workers spend inactive) is overridden when other factors come to influence worker activity (such as the important task of taking care of the brood).

The cause of individual chaotic behaviour is unknown, but two proposed hypotheses are that it is a side effect of changes in colony energy levels (Hemerik et al., 1990) or of the neural network architecture underlying the ant locomotory system (Miramontes et al., 2001). Both models reproduce the rhythmic patterns of real colonies. In the energy model, workers perceive the total energy level in the colony, in terms of food both in storage and being digested by workers and brood. The lower the energy level, the more likely a worker is to return to activity at each time step of an inactivity bout. The link between energy level and worker activity is an interesting hypothesis. However, how tasks that are not related to the level of energy (*i.e.*, other than foraging and trophallaxis) are regulated under this model is unclear. It is unlikely that other essential tasks like nest repair and brood care are relegated to a secondary role to foraging. Franks *et al.* (1990) showed changes in foraging activity following a prolonged period of starvation that are similar to the predictions of the energy model, but did not look at other tasks.

In the remainder of this chapter, I will tackle the problem of identifying the relationship between building activity (specifically, as we will see, deposition) and the amount of already-deposited stones during *Temnothorax* nest wall construction, using empirical data. The information presented in the current and previous section (**Sections 3.1-2**) are necessary background for understanding the choice of analysis method: the patterns observed in the collected time series might be caused by either task switching or periodic activity changes (or a combination of the two). The presence of oscillatory or periodic patterns has not yet been tested in *Temnothorax* and will not be tested here, because the statistical method I apply does not require it – hidden Markov models can separate data into multiple distributions (given the assumptions stated below) independently of their origin or possible periodic pattern. Therefore, the presence of colony-level cyclic patterns in *Temnothorax* activity is a possible explanation underlying our data, as is the simpler explanation of shifts in worker task, but neither hypothesis will be supported or refuted here.

3.3 Using hidden Markov models to study ant collective behaviour: self-organised building activity regulated through a feedback loop

3.3.1 Background

3.3.1.1 Self-organisation and its role in ant colonies

Ant colonies are self-organised (Bonabeau et al., 1997). Self-organisation is the set of behavioural mechanisms that lead to the emergence of coordinated group behaviour from independently-acting individuals that respond to their immediate environment only, without awareness of the collective outcome. To successfully produce coordinated behaviour from the activity of individual actors, a self-organised process must be based on a set of behavioural rules with three characteristics: 1. the rules must be the same for all individuals (with some margin for error); 2. some rules must encode information related to the function of the behaviour, for the collective outcome to be *functional*; 3. rules must be non-overlapping, that is, there cannot be alternative responses to the same cue (Camazine et al., 2001; Theraulaz & Bonabeau, 1995). Together, such characteristics enable the colony to dynamically organise their tasks with a high degree of plasticity (*i.e.*, reacting to changing needs and environments), to maintain colony homeostasis, and to achieve mass-action responses where needed.

There is a direct link between self-organised colony maintenance and reproductive fitness in eusocial insects: because of reproductive division of labour in eusocial species, the colony is believed to be the unit of selection in these systems and enhancing colonies maintenance means increasing the reproductive fitness of the workers (Fewell & Harrison, 2016; Hou et al., 2010). There are therefore two reasons for studying colony organisation in detail. Firstly, building accurate behavioural models of colony organisation systems (in relation to a single task or to the switch between or parallel engagement in multiple tasks) enables us to understand how eusocial colonies achieve plasticity and homeostasis. The rules used by eusocial insects have been applied to several human technologies (*e.g.*, Werfel et al., 2014; Zlochin et al., 2004). Secondly, from the perspective of fundamental science research, these behavioural models can be incorporated into evolutionary theoretical studies, to answer questions on the evolution of self-organised systems under natural selection. Building accurate behavioural models requires understanding the cue-response dynamics that govern worker behaviour. However, empirical estimates of these dynamics can be hard to obtain, because workers rarely specialise in single tasks and measurements of their activity levels, when taken at group level, are affected by switches between tasks. When cue and response are locked in a feedback loop, their study presents additional experimental challenges, because feedback conditions are hard to manipulate. In these cases, the relationship can more easily be estimated from observational data. However, working with observational data in many ant species comes with an additional difficulty: the colony displays oscillations in its activity patterns, so that its average activity decreases when the oscillation reaches low activity and increases when the colony is back to a highly active status (Cole & Trampus, 1999). In this study, I show the use of a statistical method, hidden Markov models, to analyse observed time series of collective building activity in *Temnothorax* ants, accounting for changes in colony activity levels (or task priority) over time. In particular, I focus on a cue-response relationship that involves feedback: worker changes in deposition rates in response to changes in the density of deposited stones at building sites. In the next section, I describe the building behaviour and the behavioural models hypothesised to underly it in detail.

3.3.1.2 Nest wall building in *Temnothorax* ants

In this paper, I used the building activity of the ant *T. rugatulus* as a case study. *Temnothorax* ants are crevice-dwelling species that nest in small cavities such as in acorns or rocks, thanks to the small worker size that is characteristic of this genus. After nest migration, in at least two species in this large genus, *T. albipennis* and *T. rugatulus*, workers build a circular wall that delimits and protects the nest, using small stones found in the surroundings, or excavating the existing material. The building of a wall in an empty cavity, rather than excavation, offers an exceptional opportunity to study this behaviour under laboratory conditions, because the colony can be accommodated between two microscope slides and observed building under conditions suitable for filming. The current behavioural model of *Temnothorax* nest wall building is based on data extracted during the initial phase of the behaviour when the wall is far from complete (Franks et al., 1992; Franks & Deneubourg, 1997). The model hypothesises that deposition and pick-up activity are guided by two rules: a template-based rule and a positive feedback effect generated by the density of already-deposited stones. Much like *Macrotermes* termites respond to the pheromonal template emitting from the queen, *Temnothorax* workers are thought to use the cluster of brood and nurse ants positioned at the centre of the nest as a reference point for a distance template. It is unclear what this template consists of: workers have been hypothesised to measure their body length from the edge of the cluster, but also to perceive a pheromone template released by the brood (Camazine et al., 2001). Regardless of the underlying mechanism, the area occupied by the cluster offers a useful reference point for selecting the building location, because it contains information on colony size, with most workers spending their time in close proximity to the brood. As workers start depositing stones at the distance indicated by the template, their tendency to deposit is hypothesised to increase as they physically come across other stones, if the stone pile is large enough. Conversely, isolated stones or small heaps are more likely to be picked up and moved to a location with larger piles. These two dynamics, corresponding to positive and negative feedbacks respectively, would ensure that building activity is co-localised at the most active building site, which is likely to be near the optimal distance defined by the template rule.

This behavioural model satisfactorily reproduces the shape of the wall observed in nature when applied to wall building beyond the initial phase (*i.e.*, until it generates a complete wall). However, in previous work (Invernizzi & Ruxton, 2021), I have shown that, when the building material available to the workers is limited, this behavioural algorithm produces clumps of material, rather than a (even partial) wall. Because the ability of *Temnothorax* to build under low material conditions has not, to my knowledge, been tested, this observation leads to two possibilities: either these species can build a wall that retains some degree of functionality even when faced with scarcity of material, and the behavioural model is incomplete, or they cannot, and the behavioural model is correct but these species use a poor building strategy. I believe that the first hypothesis is more likely. Additionally, this behavioural model does not include behavioural termination: the wall keeps expanding (getting ever thicker) as long as there is material available, because deposition rate never decreases once it has reached its maximum. It follows that this model should be refined.

We hypothesise that a full behavioural model also includes a phase of negative feedback, which is used to decrease and eventually terminate deposition activity. I propose that this feedback effect is also generated by a response to stone density (exercising a negative effect on activity after the wall has reached a certain density), as is the positive feedback characterising the beginning of the activity.

This is a parsimonious hypothesis, because worker activity throughout the full sequence of building is modulated by one single cue, stone density. In this paper, I tested the existence of a stone-density dependent effect of the wall on worker activity rates, using laboratory data of *T. rugatulus* nest building. I compared two model types, using statistical analysis: 1. a linear model of the relationship between stone density and deposition rate representing the behavioural model proposed by Franks and Deneubourg; 2. a quadratic model of the relationship between stone density and deposition rate, representing our hypothesis. I additionally tested the effect of distance from the brood cluster (*i.e.*, the template component of the behavioural model) in each model type.

3.3.1.3 The problem with ant colonies

We have mentioned above that there are two processes in ant colonies that generate noise in group-level measurements of task-specific activity. The first one is synergy between tasks. It is known that perturbations - such as worker removal - to one task decrease the number of workers in other tasks in some ant species. This is because workers are re-allocated from the other tasks to the one that has become high demand (Gordon, 1986, 1987, 1989 in *P. barbatus*, but see Kwapich & Tschinkel, 2013, 2016 for *P. badius*). Inactive workers can also act as reserve labour in a limited fashion: in *Temnothorax rugatulus*, for example, specialised inactive workers can replace brood workers and previously wandering workers (which are not engaged in any particular task) replace foragers and nest maintenance workers (Leitner & Dornhaus, 2019). Mostly, however, inactive workers do not seem to perform any activity and already-active workers compensate increased task demand by switching task or by taking on more work (Leitner & Dornhaus, 2019; Pinter-Wollman et al., 2012). Accordingly, *T. rugatulus and Temnothorax albipennis* workers exhibit weak specialisation but high segregation: that is, they perform more than one task, but are consistently separated into wider task groups (Charbonneau & Dornhaus, 2015b; Pinter-Wollman et al., 2012). For example, builders are also foragers, but not brood workers, in *T. rugatulus* (Charbonneau & Dornhaus, 2015a). These workers are likely to switch to other tasks if demand increases, decreasing activity level for the previous task even if other conditions are unchanged (Gordon, 1986, 1987).

The second noise-causing process is the generation of periodic shifts in activity levels that are typical of some ant species (Cole & Trampus, 1999). We know that many ant species (of which the most studied is *Leptothorax acervorum*; Cole, 1991e, 1991b, 1991d, 1992; Cole & Hoeg, 1996; Franks et al., 1990) display oscillations in the total activity of their colonies, from phases during which the majority of workers is active to phases in which the majority is inactive. This periodicity is unrelated to circadian rhythms and indeed occurs on much shorter time scales. Measurements of colony activity, accordingly, are affected by the overall colony activity state. This phenomenon begins at the individual level. Workers display chaotic behavioural switches between a state of activity, when they are engaged in a task or walking around the nest (Leitner & Dornhaus, 2019), and one of inactivity, throughout which they remain immobile (Cole, 1991b, 1991e). The reason behind these shifts is unclear: some models assume chaotic locomotory activity generated by the underlying neural network (Miramontes et al., 2001), while others model ant activity as a function of the energy levels of the colony, in relation to food storage and digestion (Hemerik et al., 1990), and they can both reproduce the observed patterns. In colonies, these individual worker activity phases become synchronised through the physical interaction of workers (Cole, 1991c, 1991b; Cole & Trampus, 1999; Franks et al., 1990), and the colony acquires an activity pattern with a periodic component.

In the next section, I will introduce a statistical method that can be used to account for the changes in average task activity rates just described.

3.3.1.4 Hidden Markov models

To analyse long time series of ant activity, I needed to account for differences in rates along the time series that are caused by the oscillating overarching activity state of the colony. There are no studies investigating the existence of periodic phases in *Temnothorax* colony activity. However, whether these states are caused by workers switching to a different task or by the colony switching to inactivity was irrelevant for my intent – I only needed to know whether workers were highly engaged in building behaviour or not. To distinguish between these two alternative colony states (high and low overall building activity) in my data, I implemented a hidden Markov model (HMM) statistical framework. HMMs (McClintock & Michelot, 2018; Rabiner, 1989) are an established method in animal movement ecology. They assume the existence of an unobserved (hidden) process, which switches between a finite number of states through time, each associated with a different probability distribution for the observed variable(s). This is ideal for our situation where the hidden process is overall colony-level building activity, which switches between low and high rates, and where I want to study our observed behaviours (building material uplift and deposition), and to make allowance for these behaviours being affected by the colony-level state of activity.

There are three components to estimate in an HMM: 1. the transition probabilities between the states, 2. the probability of the time series to start in each state, and 3. the state-dependent parameters of the observation distributions (**Figure 3.1**). From a fitted HMM, it is also possible to predict the most likely state sequence given the data, as it is often of great interest to infer which observations were generated by each state. The HMM methodology has been developed so that it is possible to specify any of the parameters as functions of covariates, using an approach similar to the one used in (generalised) linear models.

HMMs have been used, for example, to identify different behavioural states, such as foraging and resting, from movement data, and to estimate the effect of covariates such as group status, season and environment on movement parameters or on behavioural transition probabilities (McKellar et al., 2015; van Beest et al., 2019). In my study, I have assumed the existence of two hidden colony states, corresponding to whether building activity is prioritised (*high activity state*) or workers are instead mostly engaged in other tasks or inactive (*low activity state*). I studied deposition rate (the observed variable), that is, the number of stones per minute brought to each building site by workers. I compared alternative models for the relationships between the mean deposition rate and a series of colony-level predictors, among which the most important is the density of previously deposited stones.

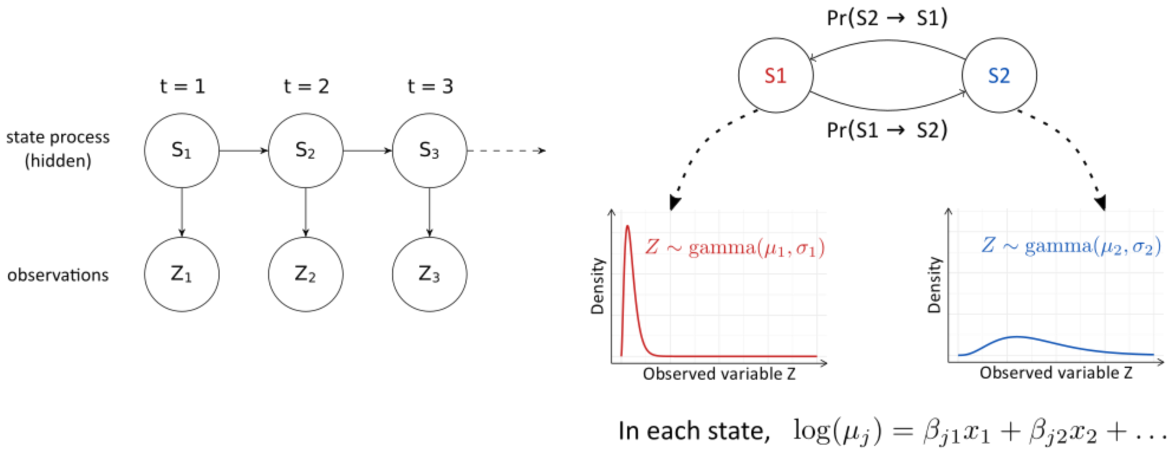

Figure 3.1. Schematic representation of HMM estimation.

The figure is a simplified representation of how a HMM is fitted. HMMs (*right*) consist in 1. a set of hidden states (here, two: S_1 and S_2) with 2. associated probability of a time series to transition to a different state ($Pr(S_i \rightarrow S_j)$), and 3. probability distribution of the data associated with that state (Z), described by some parameters (here, the mean μ_i and the standard deviation σ_i). In the bottom right, I give an example of a linear model that incorporates the influence of some predictors x on the data distribution. The HMM algorithm uses the observations in one or more time series (*left*) to estimate the three components above.

3.3.1.5 The role of feedback loops in self-organised activity

An important dynamic in self-organisation is feedback loops, in which the change produced by past activity is used to regulate current activity. The classic example is that of ant foraging trails, in which workers deposit pheromone (the cue, although technically a signal) on their way to the food source (Camazine et al., 2001). Because other foragers are attracted by the trail, the more and longer-lasting the resource, the more foragers will walk on the trail and deposit their pheromone, in turn attracting more foragers (positive feedback). When the resource runs out, fewer foragers will return to it and the trail will lose intensity as the old pheromone decays without being replaced. No new foragers will be attracted to the trail and foraging at that location will eventually stop (negative feedback). While positive feedback leads to pattern amplification, increasing the occurrence of the behaviour (in the case above, of walking the trail leading to the resource), negative feedback induces the end of activity (behavioural termination) without additional information being needed (Camazine et al., 2001). That is the same process, deposition of a pheromone with a limited lifespan, underlies both the initial concentration of activity on the best food sources, but also the termination of interest in those sources when they are exhausted. Feedback loops can therefore be considered as a mechanism through which the full bout of activity can be regulated using only one cue.

Our results provide strong empirical support for a feedback loop between stones already-deposited at building locations and deposition rate, guiding the full extent of the building activity. My findings extend the previous behavioural model that described nest wall building initiation in *Temnothorax* (Franks et al., 1992), now incorporating wall completion. My data show that stone density is sufficient, as a single cue, to guide the full arc of the deposition behaviour at sites that are at sufficient distance from the brood. That is, in addition to the effect of the template, no other cue but stone density is needed to complete the wall-building activity.

3.3.2 Methods

3.3.2.1 Colonies

Four colonies of *Temnothorax rugatulus* were used for the experiments (for details on colony origin and keeping see *Ethical note* section). Colony sizes and approximate number of brood items are reported in **Table 3.1**. All colonies used were monogynous with no alates present. Each colony was used once and experiments were conducted during the summer of collection, over the course of two months. Each colony was tested on a separate day.

Colony	Workers	Brood items (approx.)
R29	89	20
R34	108	15
R5	68	15
R54	120	30

Table 3.1. Size and approximate number of brood items in each colony. Column 1 contains the colony identifier with which the colony videos are tagged.

3.3.2.2. Filming setup

Video recordings of building behaviour were taken in a setup optimised to guarantee uniform light conditions (**Figure 3.2**). Three lights, right, left and rear, were placed adjacent to a white diffuser containing the arena. Filming was done with a Sony FDR-AX100E camera placed approximately 30 cm above the arena floor. Camera height was controlled for in the data by converting pixels to mm using the microscope slide as a reference. The videos were shot at 25 fps in 4K resolution.

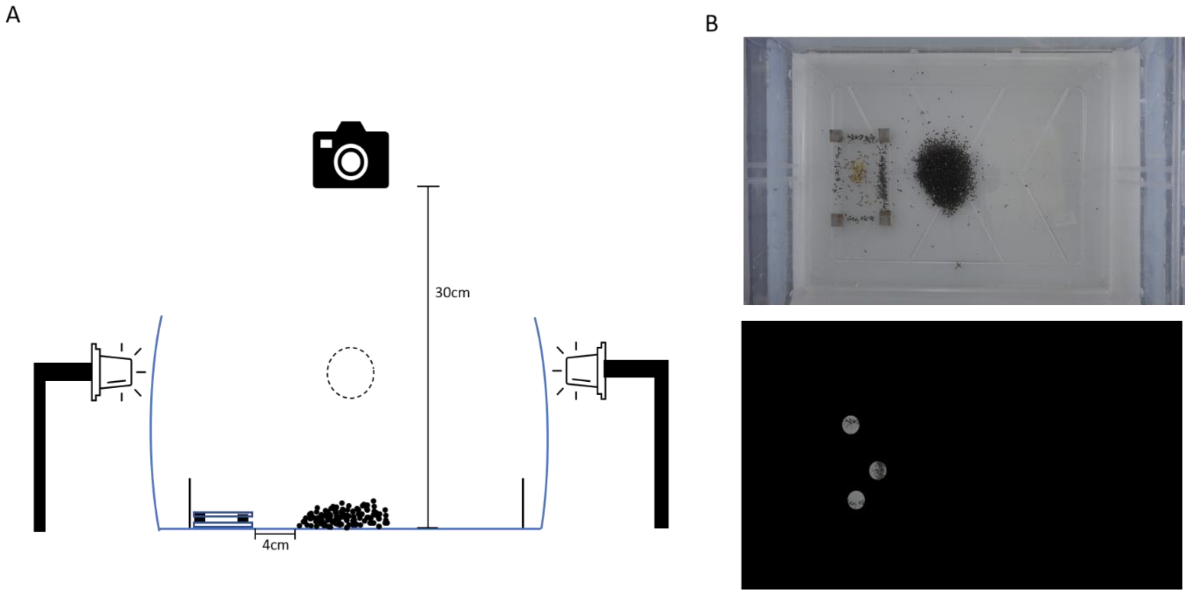

Figure 3.2. Experimental setup and image processing for data collection.

Panel A shows a simplified drawing of the setup as seen from the front. The *blue lines* represent the sides of the white diffuser fixture and the *black lines* the building arena. The artificial cavity was placed against either the left wall or the back wall of the arena, with building stones piled approximately 4 cm from it. The *dotted circle* represents the third (back) light. **Panel B** shows a video frame extracted from the video of one of the colonies before (*top*) and after (*bottom*) a black mask was applied to the video leaving only the three selected building sites visible.

3.3.2.3 Experimental setup

The ants were placed in a filming arena where they were free to move and where the new nest and the stones were also placed (**Figure 3.2**). The arena consisted of a 20x27.5x9.75 cm open plastic box with Fluon©-coated walls that prevented escape from the arena. A clean, empty artificial cavity consisting of two 75x50 mm microscope slides separated by cardboard corners (Franks et al., 1992) and a pile of 4 g black 0.4-0.6 mm aquarium gravel were placed to one side. The top slide was opaque to mimic the darker nesting environments typical of these species. The stones were located approximately. 4 cm away from the side of the artificial cavity facing the arena. The entire set up, as well as the stones and the slides used to build the new nest, was routinely cleaned with 90% Ethanol the day before the experiment. No food or water were provided during the experiment.

Experiments started in the early morning, between 6:30 and 8:30 am. Immediately after the start of the recording, the colony was removed from the housing box and the original nest opened by cutting the tape that holds the slides together. The bottom slide (where the queen, brood and majority of workers are found) was placed in the arena and the remaining ants gently brushed off from the top slide or removed from the housing box with a pair of tweezers. The colony was then left to spontaneously migrate to the new nest, where the new wall would be built. Filming continued for a total of 39 hours.

3.3.2.4 Data collection

Using the final video frame of each colony's recordings, three distinct building sites corresponding to sections of the final wall were selected as observation areas. This number of colony sites is large enough for collecting data on events occurring at multiple sites across the wall, but small enough that these sites are sufficiently distant for events that moved stones from one to another of the chosen sites to be rare. The sites were chosen haphazardly, but purposely on three different sides of the wall, to control for any biases in ant preferred building side. At each chosen section, the final wall width was measured and its midpoint coordinates extracted using FIJI software (Schneider et al., 2012). All colony videos were then blackened out, leaving only three 75-pixel-radius circular areas (approximately 2 ant-body-lengths), centred on the extracted coordinates. These isolated areas (*building sites*) were used as independent (*i.e.*, a site whose activity is unlikely to be affected by the activity at the other sites within the same colony) areas of observations in which to collect building data (**Figure 3.2B**).

Our criterion for determining the duration of data collection was as follows. For all colonies, behavioural recording started at the start of the experiment and the start of building activity was defined as the first deposition event (see below for definition) at any on the three building sites. To determine the end point of behavioural recording, I first collected observations until the number of stones in each site was at least 49 (or 0.36 stones/mm). This corresponds to a stone density value at which the visual count of stones becomes unreliable (manual counting is needed to cross-check that no events have been missed and that no stones were accidentally pushed in or out of the area by the moving ants). Because building proceeds with independent speed at each site, this approach to data collection resulted in time series of varying length. I then extended the two shorter time series in each colony to match the length of the longest time series, so as to obtain, for each colony, three time series covering the same time period.

Only two types of behavioural events are most germane for building activity, and were recorded in my data collection: stone deposition and stone pick-up. *Stone deposition* was defined as the positioning of a carried stone by an ant at any location within the building site. This could happen by lifting (the worker lifts the stone over the ground during transport, using its front legs), by bulldozing (as described by Franks *et al.*, 1992: the worker pushes the stone in front of itself on the ground), or by dragging (the ant pulls the stone through the nest using the front legs while moving backwards towards the chosen deposition site). Lifting and dragging are the most common stone carrying behaviours in *T. rugatulus*, in contrast to the use of bulldozing by *T. albipennis* reported by Franks and colleagues. This might be due to the larger size of *T. rugatulus* workers, which enables them to lift stones of similar weight whereas *T. albipennis* have to push them. Accordingly, dragging was observed most often with larger stones.

Stone pick-up was defined as the removal of a previously deposited stone either for *a.* deposition at a location distant more than 1 ant-length (approximately) within the same building site or for *b.* carrying out of the site (presumably for deposition at a different location; the worker was out of sight when it left the circular area).

For each observed event, the time of occurrence and number of stones in the site at the time of the event (the latter used to calculate stone density) were recorded.

The event rate was calculated by counting events over 15 min intervals, starting from the first deposition event in the colony (the beginning of building activity). The per-minute rate, calculated as $rate = \frac{\text{number of events}}{15}$, was used in the analysis. Only deposition rate was analysed (pick-up events were too few to guarantee stable convergence of my statistical model), but pick-up rate was also calculated and used, visually, to estimate whether colony activity shows the same sequence of high-activity and low-activity periods in all nest building activities.

Brood cluster data were obtained from frame shots taken at 15 min intervals from the start of building activity. I defined ants as belonging to the central cluster if they were within 1.5 ant lengths of a brood item (egg, larva, pupa or queen) or of another ant belonging to the cluster. At each time point, the number of ants in the cluster was manually counted and the coordinates of the centroid of the cluster (in pixels) extracted using FIJI. The distance from brood cluster to the building site was calculated as the distance between the centre of the site and the centroid of the cluster. The average distance across the three building sites was used in the analysis.

All measurements taken in pixels (*i.e.*, distance and building site area used to calculate stone density) were converted to mm. The pixel-to-mm ratio was obtained for each video by using the known length of a side of the microscope slide as reference.

Because colonies vary in size, the number of ants in the cluster was standardised relative to the colony mean for the analysis. This makes the values comparable between colonies. The standardisation corresponds to the change in number of ants in the cluster expressed as number of standard deviations away from the colony mean, that is:

$$\text{standardised number of ants} = \frac{\text{number of ants} - \text{mean number of ants}}{\text{standard deviation(number of ants)}}$$

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3.3.2.5 HMM fitting and model comparison

Because building rates showed sudden changes in mean, likely due to the workers switching to a different task, I used an HMM approach for the analysis. The HMM method enabled us to treat each data point in the time series as generated from either of two statistical distributions, each one corresponding to a different colony-level behavioural state. In my study, the two behavioural states corresponded to whether the workers were primarily engaged in building or not. I called the state where most workers were building (or where the ones that were building did so at higher frequency; my set up did not enable us to distinguish between these two cases) *high activity* and the one where few or no workers were building *low activity*. I assumed that the deposition rate followed a gamma distribution in each state, parametrised in terms of mean and standard deviation parameters. The choice of gamma distribution was made based on the observable skew in the frequency distribution of rate values (visible in **Figure 3.5D**).

Data from the different colonies were treated as independent time series, and I assumed that all model parameters differed between colonies because they depend on colony-level properties such as other ongoing tasks and colony size. The Viterbi algorithm (Viterbi, 2006) was used to estimate the most likely state sequence.

In each fitted HMM model, a different linear model of the relationship between mean deposition rate and predictors was assumed to have generated the data. The full list of models is found in **Table 3.2**. We fitted the models using a numerical optimisation of the likelihood function (McClintock & Michelot, 2018). This procedure is sensitive to the starting parameter values given to the optimiser, and so I fitted each model 100 times with different (randomly-generated) sets of starting values, to ensure that I identified the global maximum of the likelihood function. Only if the best fit of a model was reached from at least 50% of these sets, was the model included in the rest of the analysis. This threshold approach ensures that the estimated best fit is not just one of many alternatives that depend on the starting point of parameter search: that is, there is a single best fit for the model.

The goodness-of-fit was evaluated using the pseudo-residuals of the fitted HMMs (analogous to linear model residuals). All fitted models passed the residual check (*i.e.*, the pseudo-residuals appeared normally distributed and their autocorrelation coefficient was centred around zero) and were included in an information criterion-based comparison, using Akaike's method (Akaike's Information Criterion or AIC; (Akaike, 1974)). I then used posterior predictive checking on the best models, that is, I generated simulated data from each, to check if the simulated patterns displayed a good match to the observed data. Both the simulated data and the simulated state sequence showed a pattern similar to real data: the simulated data points reproduced the pattern of distribution of the real data values when plotted against the variable values that generated them; the simulated state sequences displayed long periods of low activity, inter-dispersed with shorter periods of high activity.

3.3.2.6 Software

Individual building sites were isolated from the rest of the video using ffmpeg software (version N-94383-g3883c9d147; Bellard & FFmpeg team, 2000) and the opencv (Bradski, 2000) and numpy (Oliphant, 2006) libraries for Python 3.6 (Van Rossum & Drake, 2009). Measurements (*i.e.*, brood cluster centroid coordinates and number of ants in the cluster) from video frames were taken using FIJI (Schneider et al., 2012). Data processing and statistical analyses were conducted in R, version 3.6.3 (R Core Team, 2018), using the momentuHMM package (McClintock & Michelot, 2018)

3.3.2.7 Ethical note

The data were collected under the University of Georgia's ethics guideline on animal handling and welfare. Ants are not covered by the Animal Scientific Procedures Act (ASPA) guidelines.

Colonies were collected from the wild (Pinal Mountains near Globe, Arizona; N 33°19.00' N 110°52.56' W) during the summer of 2019. After collection, they were kept at 25° C in a temperature-controlled laboratory room at the University of Georgia, housed in 12x12 cm plastic boxes, pierced to ensure air flow. For nesting, they were given an artificial cavity made of two 75x50 mm microscope slides separated by cardboard corners (Franks et al., 1992). The top slide was opaque. In nature, this species nests in rock crevices with minimal natural light access and this set up adequately reproduces the natural environment. Each box included a water tube and a tray of agar-based diet that were refreshed (Bhatkar & Whitcomb, 1970). The experiments required the colonies to perform nest migration following disruption of the old nest. This type of event poses temporary stress to the colony, but is not believed to have long-term effects on its wellbeing or survival. After the experiment, the colony was placed back in the housing box together with the new nest cavity. Colonies were kept for use in other experiments.

3.3.3 Results

Building activity displays a mountain-range-like pattern (**Figure 3.3**), with alternating periods of gradual increase and slow decline in rate. The similarity between the patterns observed in deposition (**Figure 3.3A**) and pick-up (**Figure 3.3B**) activity suggests that these changes reflect changes in the overall activity of the colony. *T. rugatulus* displays high segregation in worker task groups and partial specialisation within task, and workers that take care of nest building and repair are also foragers (Charbonneau & Dornhaus, 2015a). The activity pattern is not affected by the time of day (TOD; **Figure 3.4**). An alternative explanation is that *T. rugatulus* features periodic activity shifts between high and low activity phases. To my knowledge, the existence of periodic activity has not yet been studied in this species. The trend that I observe does not show regular oscillations, but there might be a hidden periodic component that only becomes evident when the appropriate mathematical analysis is applied (*e.g.*, the Fourier method, or autocorrelation analysis). This was not tested in my analysis – as it is irrelevant to my method, and not the focus of my interest in this study.

Independently from the cause behind activity shifts, I can assume the resulting time sequence of rates to be the product of two combined distributions: one corresponding to the phase of high building activity and one to the phase of low activity. I have no reason to think that building is regulated by different environmental and colony variables during the two phases, so that I can consider these distributions as produced by the same type of worker behavioural responses. The only expected difference is the distributions of the values of overall worker activity rates. I use HMMs to account for these two separate distributions generating the data.

We expect that *Temnothorax* wall building behaviour is guided by two sources of information: distance of the chosen site from the brood cluster and the current density of stones within the site. Because the building sites used in the analysis were chosen to correspond to the final location of the wall, they are expected to be at a distance from the central cluster that is appropriate to colony size and favoured by workers. However, because cluster position shows small changes during the period of building activity analysed, I control for this effect in the analysis. Distance from the cluster was measured as distance from the building site to the centroid of the cluster, for simplicity, but because ants might be measuring the distance from the edges of the cluster, rather than its centre, a standardised measure of the number of ants in the cluster (roughly, a measure of cluster size relative to colony size) was also included in the analysis. In **Table 3.2**, I list the statistical models fitted based on my hypothesis. Some models displayed poor convergence to a best solution when fitting was iterated over different values of starting parameters (see Methods) and were excluded from the analysis. In **Table 3.3**, I show the models with robust convergence and their comparison using Akaike's Information Criterion (AIC; Akaike, 1974). The model including a quadratic (non-linear) relationship between stone density and deposition rate displays a far better fit than models where only the first-degree linear term of stone density is present, supporting my hypothesis that stone density is a stigmergic cue involved in both increased building rate at active sites, for low to intermediate values of density, and in behavioural termination, for high density values. All models perform better than the intercept-only model, indicating that the change in deposition rate cannot be attributed to the change in colony activity states only.

Figure 3.5A-B summarises the hidden Markov chain generating the state sequence. Colonies tend to be engaged in other tasks (likely, brood transfer and brood care) when building is initiated (**Figure 3.5B**). Overall, most of the building occurs while in the low activity state (**Figure 3.5A**). The deposition probability distributions associated with the two states have distinct mean and limited overlap (**Figure 3.5C-D**), indicating that the data support the division of colony building activity observations into two separate colony-level states. **Figure 3.6** shows the state sequence estimated for each colony. Because the distributions are partially overlapping, observations with intermediate values of deposition rate can be generated in either state. However, an observed sequence of intermediate-to-high rate values is likely to belong to a period of high activity and a sequence containing low-to-intermediate values to low activity.

Figure 3.7 shows effect sizes of the best model and the plotted relationship between each predictor and the mean value of deposition rate. There is clear support for a quadratic relationship between deposition rate and stone density, in accordance with a feedback effect, with small confidence intervals. Deposition should stop (*i.e.*, the deposition rate becomes approximately 0) when density reaches around 1 stones/mm. There is no clear relationship between deposition rate and the other two predictors – distance from the brood and number of ants in the brood cluster – , once the large confidence intervals are considered. At high activity, there might be a decrease in deposition rate as cluster size increases, but only if the highest rate values are taken into account.

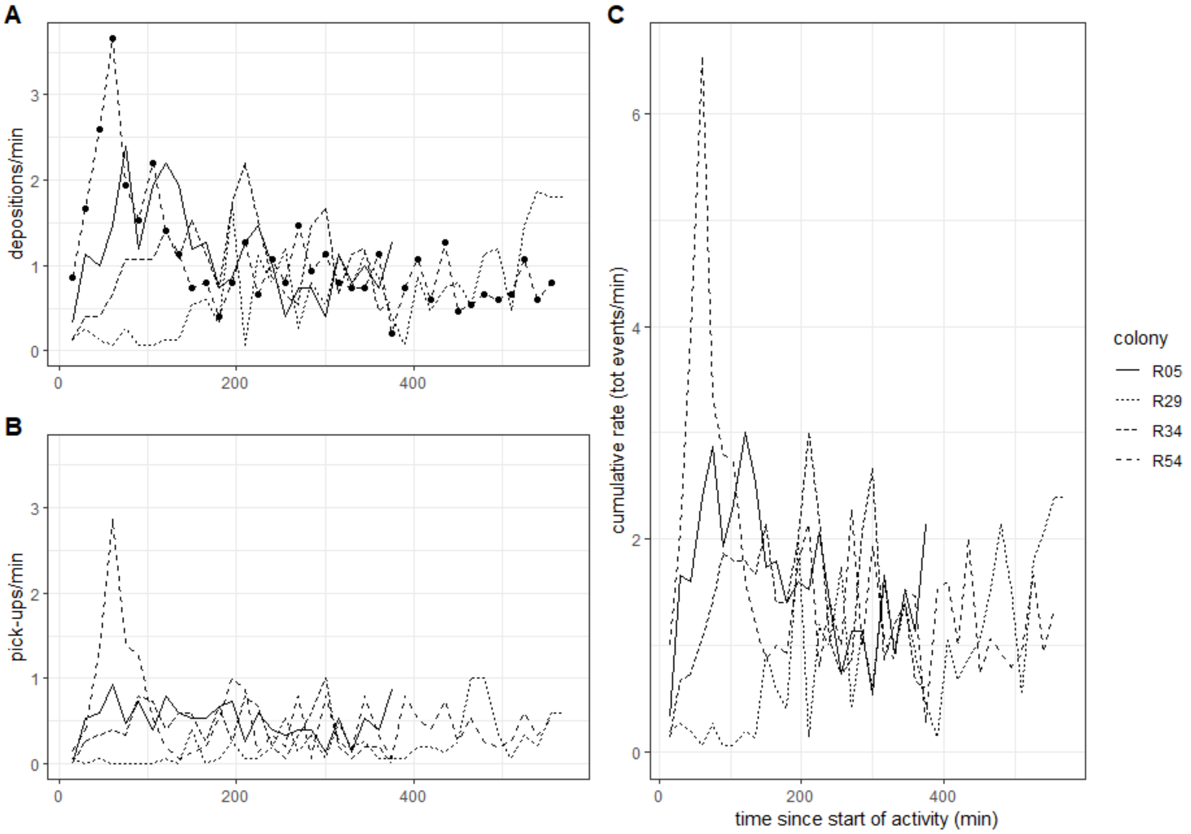

Figure 3.3. Building activity over time.

The figure shows the activity rates of each colony plotted from the start of building activity to the end of the data collection period: deposition rate (**A**), pick-up rate (**B**) and cumulative rate (**C**). The cumulative rate is calculated as deposition rate + pick-up rate. The start of building is defined as the first deposition event in the colony and observation ends for a colony when at least 60 stones have been deposited in each of the three building sites under observation. The *black dots* in panel A correspond to individual timepoints (same for each time series). Rates are calculated over 15 min periods and plotted at the period's last minute timepoint. The first timepoint is therefore plotted at +15 min from the start of building.

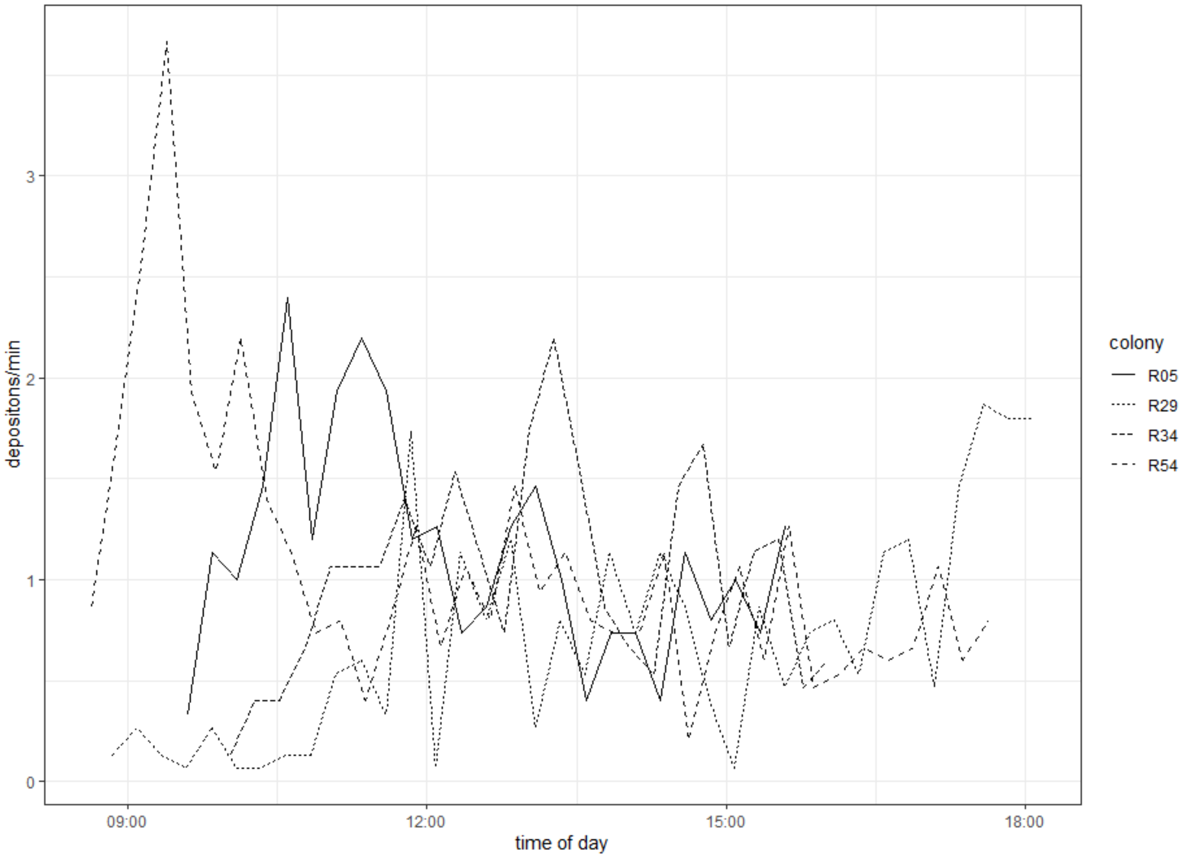

Figure 3.4. Pattern of deposition rate scaled to time of day.

The figure shows the deposition rate for each colony plotted against the corresponding time of day. The first timepoint for each time series corresponds to 15 min from the start of building activity, as in **Figure 3.3**. All experiments started in the early morning and building activity started after a length of time lasting between 7 min and 2 hours, during which the ants were initiating migration to the new nest.

model	
μ_D	SD_D
intercept model:$\mu_D = exp\ (c)$	for all models: $SD_D = exp\ (SD(c))$
$\mu_D = exp\ (\text{stone density} + c)$	
$\mu_D = exp\ (\text{stone density} + \text{stone density}^2 + c)$	
$\mu_D = exp\ (\text{distance} + c)$	
$\mu_D = exp\ (\text{stand n ants} + c)$	
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + c)$	
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + \text{stone density}^2 + c)$	
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + \text{stand n ants} \times \text{distance} + c)$	

Table 3.2. Fitted models. List of all statistical models fitted to the time series. The datapoints in each state are said to be drawn from a gamma distribution, defined by the mean of deposition rate D, μ_D, and by its standard deviation SD_D. Only the mean is assumed to depend on covariates. Models were formulated based on the parameters thought to guide deposition behaviour, from existing literature (see main text). The constant c is the intercept. Each model was fitted 100 times using different starting parameters. Only models that converged to the same best solution at least 50% of the time were included in model comparison. The shortening "stand n of ants" stands for "standardised number of ants".

model	AIC	ΔAIC
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + \text{stone density}^2 + c)$	157.4302	0
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + \text{stand n ants} \times \text{distance} + c)$	166.3841	8.9539
$\mu_D = exp\ (\text{stone density} + c)$	167.7685	10.3383
$\mu_D = exp\ (\text{stand n ants} + \text{distance} + \text{stone density} + c)$	169.2941	11.8639
intercept model:$\mu_D = exp\ (c)$	173.1953	15.7651

Table 3.3. Weighted models. Comparison of high-convergence models using Akaike's Information Criterion. The ΔAIC statistics was calculated as the difference between the model and the best-ranking model. In the table, μ_D indicates mean deposition rate and c indicates the intercept. The shortening "stand n of ants" stands for "standardised number of ants".

A

	low activity	high activity
low activity	0.95	0.05
high activity	0.15	0.85

B

	low activity	high activity
starting probability	0.77	0.23

C

	mean D	SD(D)
low activity	0.83	0.36
high activity	2.04	0.41

D

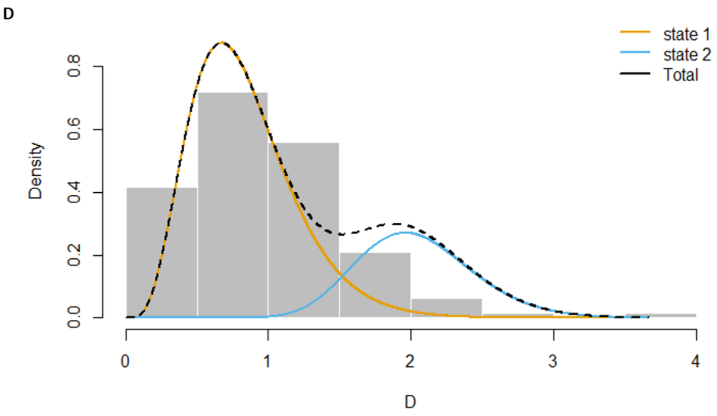

Figure 3.5. Hidden Markov Chain parameters and hidden state distributions as estimated based on the best model. Panels A-B show the estimated probabilities that define the hidden Markov chain. **A** shows the transition probabilities between states, that is, the probability for a colony to be in each state at timepoint t given its state at timepoint $t-1$. **B** shows the probability for a colony to start building during each activity state. Panels C-D describe the estimated gamma distribution of deposition rate (D) that is associated with each activity state. **C** shows mean and standard deviation of the distributions, assuming that all predictors are at their mean value. **D** shows the plot of the two distributions (*orange* indicates low activity or state 1; *blue* indicates high activity or state 2) and of the cumulative distribution (*dashed line*) against the histogram of the observed distribution (*grey*). Mean values of predictors: the mean stone density is 0.38 stones/mm^2; the mean standardised number of ants is 0.01; and the mean distance is 29.83 mm.

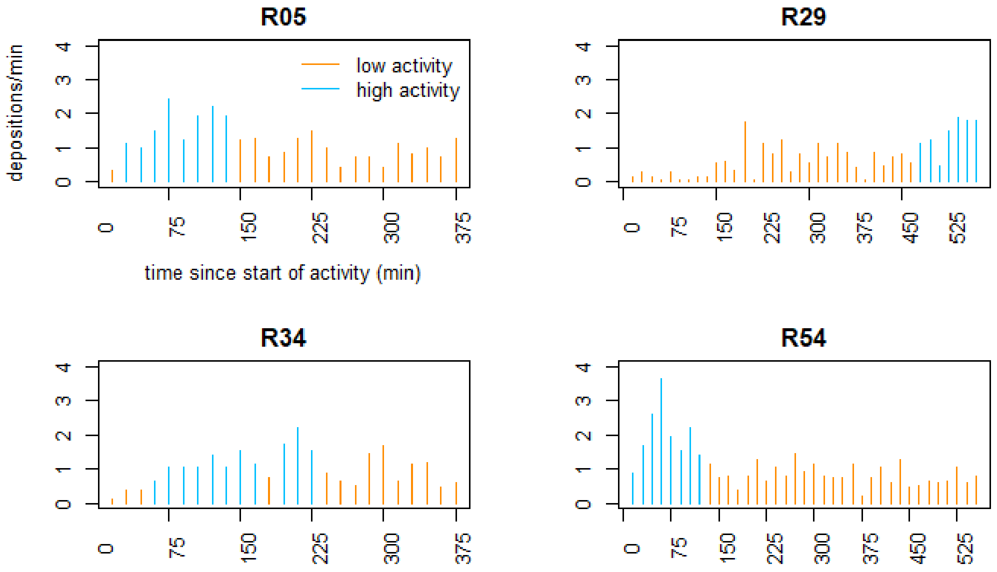

Figure 3.6. Estimated activity state sequence by colony

Each panel in the figure shows the most-likely state sequence estimated from the best model (deposition rate ~ distance + n of ants + stone density + (stone density)^2; **Table 3.3**), plotted over the observed time series of deposition rates, for each colony. The model assumes the existence of two behavioural states, high activity and low activity, generating distinct deposition rate probability distributions. The two distributions are partially overlapping but clearly separate (**Figure 3.5**), so that an observed sequence of higher rate values is likely to belong to a period of high activity (*blue*) and a sequence containing both high and low values to low activity (*orange*).

A

	intercept	intercept (SD)	stone density	(stone density)^2	distance	standardised n ants
low activity	-0.237	-1.016	3.265	-3.264	-0.024	-0.002
high activity	-0.180	-0.896	4.236	-5.518	0.003	-0.261

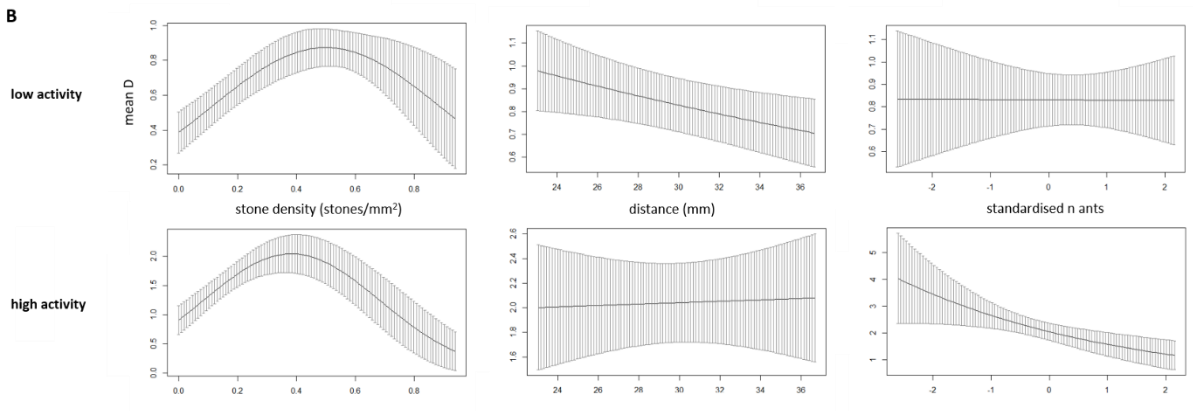

Figure 3.7. Parameter effect sizes and plotted relationship with deposition rate in the best model.
Panel A shows all parameter effect sizes for each activity state in the best model. **Panel B** shows the relationship of each predictor with the mean of the deposition rate distribution for each state, plotted assuming mean values of the other predictors. Mean values of predictors: the mean stone density is 0.38 stones/mm^2; the mean standardised number of ants is 0.01; and the mean distance is 29.83 mm.

3.3.4 Interpreting the results: potential pitfalls and general considerations on *Temnothorax* building behaviour as emerging from this study

3.3.4.1 Self-regulation of deposition activity through stone density

In this study, I have used an HMM approach to distinguish between ant task-specific activity measurements taken during periods when the task is prioritised and measurements taken when the task is performed at low activity levels. The results of the analysis show that *T. rugatulus* nest wall building activity following nest migration can be decomposed into periods of high activity, during which building is prioritised, and periods of low activity, when building proceeds slowly. Building activity does therefore not occur at a constant pace until the wall is completed, but depends on other colony-level factors and the observed trend differs from colony to colony.

There are three criticisms that could be moved to my study, and I wish to address them here. Firstly, my sample size was small (four colonies). Sample size does not affect state-sequence inference, which occurs at the level of the individual (here, colony) time series in HMMs. It does affect the confidence level in the extrapolated relationship among parameters, but in my case the confidence intervals of the extrapolated relationship between stone density and deposition activity are narrow. I believe the confidence interval of the quadratic relationship is consistent across all four colonies and it is thereby reasonable to infer that the same relationship might underlie wall building in *T. rugatulus* generally. I do not draw any general patterns from the relationship between building activity and distance from the deposition site to the brood cluster, for which the confidence intervals are much larger; this variable was not the focus of my study and was therefore also not fully explored in my approach (*i.e.*, all sites were chosen as sites where the wall was known to be built and are thus likely to be close to a worker's optimal distance for building, if such a distance exists). Secondly, it can be suggested that my choice of observation sites was not blind and was somehow informative of previous building activity. The sites were chosen, in each colony, from the last video frame showing the complete wall, and before the rest of the video was investigated. I do not think that the final shape of a wall section contains information on the cue-response relationship that underlies its construction. Finally, the shifts in activity levels measured in my analysis might be caused by changes in the energy level of workers. I did not provide food during my experiments, but colonies were moved to the arena directly from the housing box, where food was available *ad libitum*, and the activity data analysed correspond to the first 10h of the experiment only. Starvation has been shown to increase forager activity, but only after several days (Franks et al., 1990); consequently, I believe that my colonies were unlikely to be affected. Some species of ants preferentially perform certain activities depending on TOD (Gordon, 1983), but building activity in *T. rugatulus* does not seem to be one such case.

The HMM method can be more generally applied to the study of colony organisation. HMMs also enable fitting transition probability models (Patterson et al., 2009), estimating the effect of cues on the switch between two colony states, such as the engagement of a high worker number in two different tasks. The parameter values estimated from HMMs can be plugged into both spatially explicit and non-spatial mathematical simulations to improve the match between real and simulated behaviour. Such simulations can be applied to study plasticity (*i.e.*, how well the system re-organises in response to changing conditions) and robustness (*i.e.*, to what extent the system can respond to changes in conditions, before collapse) in colony organisation and provide a data-based insight into evolutionary and ecological models.

3.3.4.2 A case of stigmergy

The response of workers to stone density that I observe is a case of stigmergic feedback. Stigmergy (Grassé, 1939) is a well-known concept in social insect building, and can be defined as the effects that changes in the architecture created by past building activity have on current worker building behaviour. For example, in the Formosan subterranean termite, the use of depressions in the ground as stigmergic cues for excavation suggests that this cue is sufficient for both tunnel initiation and continuation (Bardunias & Su, 2009b).

T. rugatulus workers may be responding directly, to stone pile conformation, or indirectly, through changes in an environmental cue that is affected by stone deposition. For example, workers may be responding to the change in intensity of air currents or to the amount of light penetrating the nest from the sides. Air currents have been shown to modulate ant behaviour in a similar deposition and pick-up context: that of corpse disposal (Jost et al., 2007). Experimental manipulation is necessary to determine what cues the ants respond to in this case of nest building. I observe, however, that laden ants that carry a stone by dragging seem to pull their load against the pre-existing heap, stopping when the stone encounters a lot of resistance. Thus, at least when dragging, *T. rugatulus* builders actively use stone density to guide their activity, through mechanical contact. Franks *et al.* (1992) report that *T. albipennis* workers bulldoze the stone they are carrying into existing piles. Both species, therefore, appear to be using contact with stone piles for building, even if in slightly differing ways.

Our results support the existence of a feedback loop between the stones deposited at building sites and the deposition activity of the colony. This curve is consistent with a local amplification process driven by the stigmergic effect of building sites, similar to that underlying nest excavation in other ant species (Buhl et al., 2005; Halley et al., 2005; Rasse & Deneubourg, 2001). In the other species, *Messor sancta*, *Linepithema humile* and *Lasius niger*, the coupling of a positive and a negative feedback effect is caused by building site density, which decreases as nest area increases while colony size remains identical. This leads to behavioural termination. In *T. albipennis* and *T. rugatulus*, the phenomenon is not nest expansion (although we might find the same mechanism if we look at the cases where these species excavate their nest – digging behaviour in *Temnothorax* remains so far unstudied). The wall, or, rather, the template component of the building algorithm, limits nest expansion. However, a negative effect similar to that caused by low building site density might be obtained if the ants use air currents as a cue modulating building activity. If, instead, the mechanical interaction with the wall and the associated (indirect) evaluation of stone density is what causes the degree in activity, then the negative effect is caused by negative feedback.

3.3.4.3 Effect of TOD on building activity

Finally, my data do not show any relationship between building activity following nest migration and TOD. It is possible that TOD instead affects regular nest repair activity, as it does, in other species, with other colony maintenance tasks (Gordon, 1983).

3.4 Hidden Markov models and mixed effects

I have identified the relationship between colony average deposition rate and average stone density values across building sites. Is it possible to test if this relationship emerges at the local level, using the same method? Applying HMMs to the same problem, but with local-level resolution, is not straightforward. The issue is caused by some parameters depending on the colony-level (*i.e.*, they are the same for all building sites in the same colony) and others being site-specific, namely:

- The state sequence is identical for all sites in a colony. This is because the task the workers are primarily engaged in at a specific time-point is a colony-level property.
- The transition probabilities are identical for all sites in a colony. As presented in **Section 3.3**, transitions are assumed to occur independently from predictors. In reality, it is possible that stone density affects how many workers are engaged in building – building activity might become a lower priority once the walls have reached a minimum size and workers might relocate to other tasks, such as foraging. This independence assumption provides a simpler model.
- The state distribution parameters vary from site to site. We expect them to depend on our local variables, which are, precisely, *local*, different from site to site at any given time point. Even if we assume the intercept-only null model, we expect differences in the distributions simply due to chance or location: for example, the section of the wall that faces the stone pile is usually built faster and wider.

Trying to apply the method without making the underlying state sequence and transition probabilities the same across the colony results in very different state sequence estimates from site to site, which violates the biological assumption that shifts between high and low activity occur at colony-level (**Figure 3.8**). Incorporating two levels into the HMM approach, on the other hand, requires advanced statistical expertise and will be the focus of future collaborations. Producing one case study containing a baseline discrete-level mixed modelling HMM approach would introduce this methodology to the collective behaviour research community and enable its application to other ant tasks. Developing and publishing such a case study will be the aim of a short postdoctoral project.

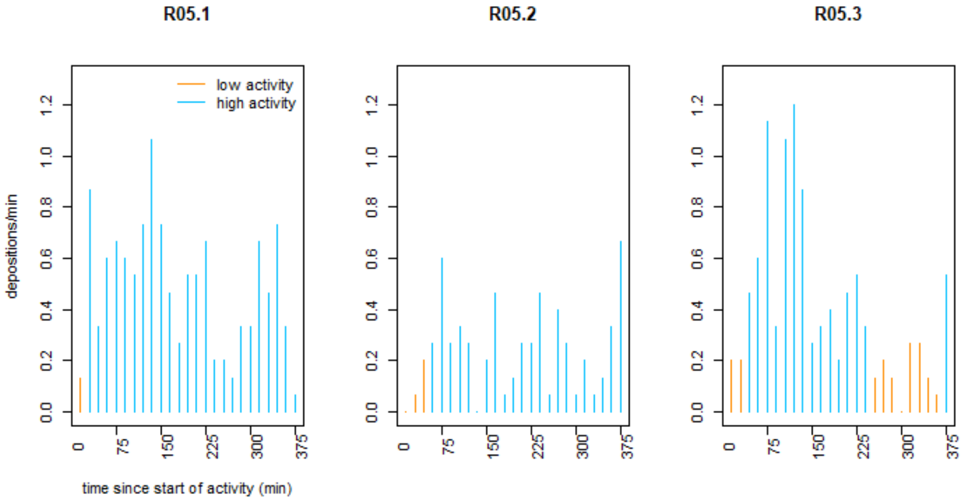

Figure 3.8 Estimated activity state sequence by ROI.

The figure shows an example of the estimated state sequence by ROI when the sequence (and the transition probability matrix) is allowed to vary independently from site to site. The three sequences shown belong to colony R05. Note that a time point in the time series of a site corresponds to the same time point at a different site. Without the unifying assumptions above, the state estimate differs from site to site. Biologically, states are expected to depend on the colony and their sequence should be the same for all sites within the colony. The difference is caused by part of the variance due to local predictor values or to stochastic differences in activity between sites (at the same time point) being conflated by the algorithm into the state estimate.

3.5 The relationship between building activity and distance from the brood cluster

Because building activity was not studied at the local (*i.e.*, building site) level in the analysis presented in this chapter, it was impossible to investigate the exact relationship between parameters that change locally and deposition activity – importantly, the distance between deposition site and brood cluster. Even in the case of a mixed-level analysis, keeping fixed observation sites means that changes in distance are caused by the movements of the brood cluster, which are small. A better designed approach to experimentally test the template component of the *Temnothorax* behavioural model would be to select observation sites at different distances from the location that is occupied on average by the cluster over the full length of the time series. It would then be possible to fit a mixed model using the relationship between deposition rate at each location and distance of the location to the cluster as the individual level. While this could not be done in the current study, there is at least one observation that can be made on the effect of brood distance on building activity.

Figure 3.9 shows the distance of each building site (coded by colony) from the brood cluster when the first stone is deposited at that location. The x-axis shows the time of the event, as calculated from the beginning of the recorded building activity in that colony. As we can see (highlighted by the red square), all recorded colony first events (*i.e.*, time equals zero) occur at a short distance to the cluster (20-25 mm), while deposition at other sites seems to be initiated independently from the distance. This observation only has anecdotal value at this stage: the first *recorded* deposition event is not the first *occurred* deposition event and four is a small sample size, so that it is not unlikely that this correlation is caused by chance. However, it leads to the hypothesis – to be tested using occurred deposition events and, if possible, on a larger sample size – that building activity is initiated at locations that are closer to the brood cluster than the final midpoint of the wall.

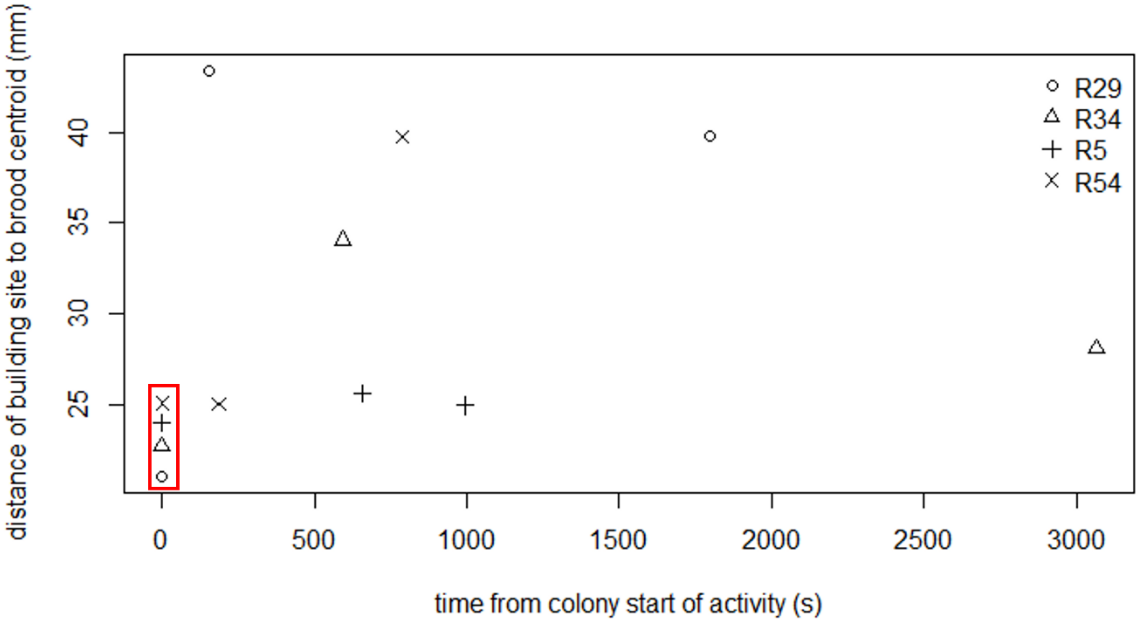

Figure 3.9. Distance of building location to brood cluster at first deposition event for each building location. The plot shows the distance from the brood cluster of each building location analysed in the study in **Section 3.3** at the time of first deposition at that location, plotted against the time of the event calculated from the beginning of building activity in that colony. That is, events occurring at time 0 s are the first building events of each colony. Data points are coded by colony.

3.6 Nest wall building in *Temnothorax*: *T. albipennis* versus *T. rugatulus*

The striking similarity between the characteristics of the nest walls built by *T. albipennis* and *T. rugatulus* would lead to the hypothesis that the two species use a similar, if not identical, building algorithm. This is indeed a crucial assumption of this chapter. Can we find support for this assumption when we compare building behaviours and structural characteristics of the wall in fine detail? In **Table 3.4**, I present a table summary of some of these features and their presence or absence in each species, which I also discuss below.

Let us start with the features of the wall. Under laboratory conditions, *T. albipennis* builds a circular wall (**Figure 1.2**). This fact lends itself to the assumption that they use a circular template to guide deposition to an appropriate building location. *T. rugatulus* does not, at a first glance, do the same: in my experiments, their walls were built parallel to the square edges of the microscope slide, filling in the gaps between the corners (**Figure 1.7**). However, *T. rugatulus* is approximately two times larger than *T. albipennis* in worker body size, with colonies of similar numbers of individuals, while I used the same slide size for *T. rugatulus* as Franks *et al.* (1992) for *T. albipennis,* and similar sized stones. It is therefore possible that, given more space, *T. rugatulus* could also build round walls. Notably, a circle is an optimal solution to the problem of enclosing any given surface within a minimum perimeter – that is, building a circular wall would minimise effort in the absence of pre-existing walls.

Franks and colleagues also report that *T. albipennis* nests usually have only one entrance. I did not observe clear nest entrances in *T. rugatulus* walls. Instead, workers that leave the nest usually make their way through wall sections with lower stone density, pushing their way through and squeezing their body between wall and nest roof. Workers of *T. rugatulus* might not need to leave an entrance open in this setting because the size of the stones is small relatively to their body size.

How similar are the details of the two building behaviours? *T. albipennis* is reported to use bulldozing as their primary way of moving stones. In *T. rugatulus*, workers bulldoze only 2% of the time. Carrying and dragging are instead the most common methods, again possibly because of size (we estimated dragging in *T. rugatulus* to occur in approximately 5% of all events, *i.e.*, pick-ups and depositions combined). A high frequency of bulldozing and/or dragging, however, are a key assumption if we believe that the effect of stone density on ant deposition is the product of a mechanical interaction, so that the more friction is encountered, the more likely the workers are to drop the stone.

Franks and co-workers also describe the presence of task partitioning in *T. albipennis* building behaviour: external workers bring the stones in and leave it near the brood cluster, from where it is bulldozed outwards by internal workers (Franks *et al.*, 1992). I did not focus on this behaviour and I therefore do not know whether *T. rugatulus* shows a similar partitioning of activity.

The building process seems to unfold similarly for both species. The very first depositions usually occur against the cardboard corners that hold up the upper microscope slide, which suggests that the ants exploit structures that they find in the environment. Building then occurs simultaneously at multiple locations across the colony, but it is not synchronous: different sites grow at different speeds and a section of the wall may take from one to several hours to be completed. Once the wall is finished, building slows down but never completely terminates, with stones being re-organised, or removed and deposited elsewhere. This indicates that there are baseline pick-up and deposition rates higher than zero, consistent with the fact that the probabilistic nature of behaviour is important for behavioural initiation in self-organised systems. Baseline activity is also likely to be important for the long-term maintenance of the nest wall: holes forming over time through the natural process of degradation are filled in by workers acting on their baseline deposition rate and the same set of rules used for building the full wall structure.

Finally, both species display a “gap-filling” tendency that guides their building. For example, in *T. rugatulus* wall sections are not usually completed just by progressive side-ways expansion of the neighbouring stone piles. Workers are often seen to deposit a stone at an equal distance between the two piles to start the joining section.

Overall, there is support for the assumption that *T. albipennis* and *T. rugatulus* share the same underlying behavioural model of nest wall building. The two behavioural models are very similar, once some adjustments due to species size are granted. In *T. rugatulus*, the hypothesis of a mechanical interaction with stones might not be sufficient to explain the effect of stone density on deposition and workers might be using additional information: if not the configuration of the pile, then the intensity of air currents or of incoming light. The same hypothesis was raised for *T. albipennis* by Franks (Franks *et al.*, 1992) and this might also be a feature of the behavioural model rather than a difference between the two species.

feature or behaviour	***T. albipennis***	***T. rugatulus***
one nest entrance	Y	N
circular wall	Y	unknown
bulldozing	Y	rare, replaced by dragging
task partitioning: external vs internal workers	Y	unknown
brood cluster as template	unknown	unknown
building activity slowing down but never ending	Y	Y
simultaneous building at multiple sites	Y	Y
site-specific speed and timing of building	Y	Y
often first depositions occur against cardboard corners	Y	Y
gap-filling	Y	Y

Table 3.4. Comparison between *T. albipennis* and *T. rugatulus* building behaviour.

Observations for *T. albipennis* are based on Franks *et al.* (1992) and Franks and Deneubourg (1997).

3.7 Chapter conclusions

I have shown that deposition activity in *Temnothorax rugatulus* responds to stone density at building sites in a way that is consistent with the existence of a positive stigmergic building feedback loop generated at low stone density, followed by a negative stigmergic effect of high stone density on building activity. The estimated relationship matches the one described by the *gradual* model and indicates that this behavioural model is at least a good proxy of real dynamics. In the next chapter, I will use the *gradual* model to test some hypotheses on the role of self-organisation in maintaining a high-quality collective phenotype during colony life history phases or under ecological conditions that involve a high level of individual variation and are potentially disrupting of the architectural outcome.

Chapter 4: Rule mutation, genetic differences and individual variation: testing model behaviour over different colony backgrounds

In Chapters 2-3, I have developed and refined the existing behavioural model of *Temnothorax* wall nest building. I started from the model describing the first phase of building activity that Franks and colleagues (1992) proposed, and I demonstrated, both through simulation modelling (**Chapter 2**) and empirical data (**Chapter 3**), the likely existence of a stigmergic feedback mechanism that regulates deposition for the full length of building activity. The new behavioural model more closely reproduces the building dynamics of *Temnothorax* and it can be used to test how this particular form of nest building is altered by short-term and long-term changes in colony genetic structure. One stimulating question arising from this is how we expect the collective phenotype to change with the colony frequencies of the alleles underlying building activity. Evolutionarily, potentially beneficial mutations in genes linked to group behaviours are at risk of being counter selected and removed from the population pool before they are expressed at the collective level. This should happen every time the intermediate collective phenotype, generated by genetically mixed groups, causes a decrease in colony fitness. In the current chapter, I will use the model of *Temnothorax* wall building to test how self-organised collective nest building responds to different distributions of phenotypic variation. Based on this case study, I identify two mechanisms of self-organisation that are necessary for generating a functional intermediate collective phenotype: *buffer zones*, that is, spatial or temporal zones in which building activity of the same type occurs with high frequency under every variant in the population, and *convergence*, that is, a behavioural rule type responsible for moving the building activity away from the zones specific to each variant and towards the buffer zones.

Note that in this chapter I use the term *zone* to indicate a finite length of time or amount of space when referring to the use of or overlap of building rules. In relation to this model of *Temnothorax* building behaviour, the term specifically refers to a spatial zone, because environment, and not time, determines the change in individual building (pick-up and deposition) probabilities. Temporal zones are expected in algorithms where time plays a role in behaviour, for example if a volatile pheromone is important in guiding activity. In such a case, if there is individual variation in pheromone duration or in the amount of pheromone deposited, overlap between the temporal zones of activity of pheromone laid by different ants is likely to play an important role in convergence.

The breakdown of the chapter is as follows:

- In **Section 4.1**, I present the core question of this chapter in more detail.
- In **Sections 4.2-4.4**, I discuss the colony foundation strategies of ants in more detail and the types of genetic and individual variation we expect to find within colonies, and why this variation is an important component of eusocial groups that cannot be ignored in evolutionary models.
- In **Section 4.5**, I describe the agent-based models used. These models are all adaptations of the models described in **Chapter 2**, changed to incorporate within-colony variation.
- In **Section 4.6**, I present all results.
- In **Section 4.7**, a brief discussion of the findings is offered.
- **Section 4.8** summarises my conclusions from the chapter.

4.1 The matter of transition between building phenotypes

In **Chapter 1**, I discussed how self-organisation may contribute to multiple ecological and evolutionary aspects of social insect nest building, namely transition between substructures, flexible responses to a changing and heterogeneous environment, and robustness to disrupting levels of individual variation affecting the shape of the architecture. This chapter is concerned with this latter concept. I suggested (**Section 1.3.5**) that nest building rule sets include rules that enable phenotypes produced by mixed colonies to either reflect an average of all phenotypic variants or align to one (dominant, in some aspect) single variant. Note that the outcome is expected to depend on the relative strength and frequency of each variant and on the existence of a zone of overlap between them (*e.g.*, when templates and fixed rule sequencies are applied). Evolution may tinker with these parameters, selecting for robustness and evolvability.

Enabling transitions between alternative variants of a collective phenotype is a problem of organisms (or, in the case of eusocial colonies, of superorganisms) that do not have a bottleneck stage of reproduction – that is, when the daughter organism originates as a collective and not from the reproduction of one single unit. In eusocial colonies, bottleneck reproduction occurs with independent colony foundation (ICF; *see* **Box 4.1** *for terminology*), but this strategy is often abandoned (both facultatively, within species, and obligately, for a whole species) because of the high mortality risk. The alternative strategy of dependent colony foundation (DCF) has evolved multiple times in all eusocial insect groups, including numerous instances of independent evolution in ants (Cronin et al., 2013). In DCF, mutations in the new queen(s)'s eggs or in the sperm she carries post-mating generate a mutant workforce that works side-by-side with workers expressing the resident variant. Moreover, we can envisage at least one case applicable to ICF where multiple variants are co-resident: the presence of a multiply-mated queen (Boomsma & Ratnieks, 1996; Strassmann, 2001). Phenotypic variation not underpinned by genotypic differences is also likely to be common, following developmental idiosyncrasies. In all the above cases, incompatibility between variants can be expected to result in a non-functional intermediate structure, even if all variants would be viable when expressed unanimously. Advantageous genetic mutations emerging under DCF or multiple paternity, moreover, must cross the intermediate phase to be expressed at colony level and allow the collective phenotype to be exposed to selection. Transition between collective building phenotypes is therefore a problem that most eusocial nest-building species must have successfully solved. My suggestion is that self-organised rule sets contain the ingredients to enable both this transition and the existence of worker phenotypic variance more generally. A possible mechanism for this robustness to variation is the existence of what I will call *convergence rules*: rules that cause individual workers to incorporate information that, either directly or indirectly, is related to nestmate activity. As seen in **Chapter 1**, convergence rules in the form of positive feedback are a staple of all self-organised rule sets for collective behaviour. Convergence rules are especially well-defined in the literature of self-organised group decision-making and collective intelligence (*e.g.*, Camazine et al., 2001; Franks et al., 2006). Collective nest building is, effectively, a case of decision-making, where what needs to be decided is (most commonly) the location of building or excavation, so that workers co-localise their efforts in time and space, avoiding the initiation of the same architectural feature in parallel at separate locations. In the case of large structures, such as walls, convergence is necessary to ensure structural continuity: workers responding to environmental heterogeneities will initiate sections of the same structures at positions that are at some degree of offset from each other, and these partial structures need to be joined. When we apply genetically-driven variation in the preferred building distance to this scenario, we also expect an offset in section alignment, caused, for example, by workers perceiving the same distance template with different intensities because of the presence of receptor-gene allelic variants within the colony.

In this chapter, we will see that convergence rules are indeed important in conferring robustness to genetic and phenotypic variation in *Temnothorax* wall building. The expectation is that the parameters of the building algorithm (*sensu* Gordon, 2016b) governing convergence and the size of the buffer zone are subject to optimisation under selection for robustness. As mentioned in **Chapters 1-2**, the wall is thought to fulfil a nest defence purpose and have a homeostatic function for internal temperature and humidity. A functional wall has no gaps, can be built with limited material and leaves sufficient space for worker movement, qualities that I used to develop two measures of stone dispersal in **Chapter 2**. I use these measures to assess the quality of the wall emerging in the presence of genetic diversity. I test the robustness of the phenotype (*i.e.*, the architecture) in the presence of different types and degrees of genetic variation among builders, and in the presence of widespread time-consistent phenotypic variation (*worker personalities*). Specifically, the following four scenarios are examined.

1. Replacement of the old queen in monogynous colonies introduces a mutation in building behaviour that is carried by all workers born to the new queen. This results in gradual replacement of the workforce, from resident to mutant phenotype (this is the same scenario as depicted in **Figure 1.5**). In this scenario, the degree of conflict between rule variants depends on the frequency of the mutants in the colony and on the difference between the original and mutant rule (*i.e.*, the size of the mutation step).
2. Co-existence of multiple patrilines among workers, each carrying and expressing a different genetic variant of the building rule. This scenario is equivalent to one of polygyny with singly-mated queens, in which case both genetic and maternal effects can underlie the phenotypic difference among

matrilines. The degree of conflict among variants depends on the relative proportion of workers of different lines and on the size of the mutation step.

3. Genetic variation among patrilines (or matrilines; from here on, the patriline example will be used) and patriline-specific building-site fidelity. The degree of conflict in this scenario is equivalent to that in the absence of building-site fidelity, but convergence rules here have a limited effect as the buffer zone is reduced by the limited overlap between the building sites of different variants.
4. Widespread phenotypic variation among workers (caused by epigenetic factors such as learning, experience, and development). The degree of conflict depends on the standard deviation in rule variant values.

I test the quality of the phenotype emerging in each of these scenarios under two model variants: with the presence of stigmergy (*stigmergy + template model*) and without it (*template-only model*). This stratagem enables me to break down the individual effects of these two building rules, template and stigmergy, on the robustness of the collective phenotype.

Spatial fidelity (scenario 4) is unknown for *Temnothorax albipennis* and *rugatulus*. However, individual within-nest spatial fidelity extending to division of labour has been found in other *Temnothorax* species, for example in *T. unifasciatus* (Sendova-Franks & Franks, 1995). The use of the model for this scenario represents a simple proof of concept of the proposal that self-organised nest building can confer robustness in the presence of this type of variation.

From the analysis contained in this chapter, it emerges that the functionality of the wall is largely robust to most frequencies, distributions and strengths of genotypic and phenotypic variation. Even a high degree of conflict only slightly reduces the quality of the wall. In the *Temnothorax* wall building rule set, the convergence rule is the stigmergic effect, which here guarantees wall compactness. The parameter controlling wall thickness (τ), on the other hand, controls the size of the buffer zone. The buffer zone ensures that individual phenotypes can converge to a single solution. Both components of the building algorithm are necessary to guarantee robustness to variation.

In the following sections and before presenting the modelling study, I offer a more detailed overview of the biology behind genetic and phenotypic variation in behaviour that is expected in eusocial colonies, particularly in ants. This should allow the reader to better appreciate the ecological and evolutionary context of the study.4.2 Colony foundation in ants

The mode of colony reproduction ancestral to all eusocial insects is ICF. In ICF, the new queen lives solitarily in the new nest for several weeks (Bourke & Franks, 1995), a period necessary to establish the first generation of workers. The mortality risk (to her, and thus to the whole colony) at this stage is high, although variations on the solitary strategy have evolved that reduce this risk. In the current section, I mostly review the ant family, preferring to offer a more detailed overview of the case at hand rather than a less-detailed but wider perspective on all eusocial insects.

In many higher ants (*i.e.*, *Formicinae*, *Dolichoderinae*, and *Myrmicinae*), the queen uses her thorax as a metabolic reserve, feeding the first batch of brood which then become foragers (*claustral foundation*; a similar strategy is found in termites, which utilise the cellulose extracted from the incipient nest as a nutritional source). This reduces risks associated with the queen foraging outside her new nest-site. In other species, multiple and usually unrelated queens co-found a nest and then compete for the position of reproductive (*pleometrosis*). This means that the loss of one queen does not immediately lead to colony failure and that the burden of nest maintenance and foraging can be shared. Social parasitism of a colony of the same or of a different species is also used. Here the queen trades off the risks associated with attempting to usurp an established colony with the benefits (in terms of immediately available workers and resources) if that usurpation is successful. These alternative strategies vary from an intermediate to a high risk level (with social parasitism becoming low risk once the host colony has been successfully infiltrated; Peeters & Ito, 2003).

DCF has evolved multiple times in ants and has been found in almost all lineages even when its most strict definition is applied in the count (Cronin et al., 2013; it is also common in eusocial wasps and bees, while it has been reported in termites but its frequency in this genus is unclear). Strictly, DCF refers to cases in which a new colony is established, with queens migrating to a new location together with a large number of workers from the parent colony (a *propagule*). I will, here, use the wider definition, including re-adoption of a newly-mated queen by the colony of origin (*e.g.*, Heinze & Keller, 2000; Peeters & Ito, 2003), because the consequences of the two scenarios are identical for the phenomenon I am studying (that is, the co-existence of resident and mutant building rule variants).

The reproductive that detaches from the original colony in the propagule may have various origins: in some families, such as *Ponerinae*, workers and inter-castes (*i.e.*, workers with an intermediate developmental pathway between queen and worker) retain spermatheca and may reproduce sexually, leading to the formation of a propagule (Peeters & Ito, 2003).

With the exception of species without flying queens, for which DCF is an obligate choice, in most ants ICF and DCF co-exist as dispersal tactics used alternatively depending on ecology: DCF is thought to be more common is harsher environments where survival is harder and in ones with limited availability of suitable nesting locations. If multiple queen morphs co-exist within one species, they might also be associated with different colony foundation strategies, although the question is still open as to whether these morphs have evolved to fill different environmental niches and the association with the colony-foundation mode has followed (or paralleled this specialisation) or whether the morphs have become adapted to the colony-founding strategy. In *Temnothorax*, for example, macrogynes (larger queens) independently establish a new colony, while microgynes (worker-size queens) are re-adopted into the colony of origin, leading to polygynous colony formation. The association between microgynes and enhanced survival at higher altitudes is controversial, at least in *T. rugatulus* (Catarineu et al., 2017; Choppin et al., 2021; Rüppell et al., 1998).

DCF leads to genetic variation between old and new workers, but there can be additional sources of genotypic and phenotypic variation within a colony. We explore these in the next section.

4.3 Sources of inter-worker phenotypic diversity in Hymenoptera

Eusocial colonies, whatever their origin, contain variation in the phenotype of their workers. Recently, indeed, the concepts of *personality* and of *behavioural syndrome* have been found to be as applicable to eusocial insects as they are to vertebrates (Jandt et al., 2014; Jeanson & Weidenmüller, 2014; Leboeuf & Grozinger, 2014; Pinter-Wollman, 2012). This behavioural diversity has been shown to be underpinned by both genetic and non-genetic factors.

I will first review the sources of genetic diversity within Hymenoptera more at large, because some key insights in this area come from the study of eusocial species other than ants, and from honeybees in particular. Specifically, I ask: how much genetic variation do we expect to find in eusocial colonies and what are its origins?

On the maternal side, two main mechanisms lead to the co-existence of multiple matrilines among workers and they have already been described in **Section 4.2**: they are queen turnover and queen adoption. Queen turnover (*i.e.*, **Scenario 1** in my modelling study) is a frequent occurrence in ant colonies, as ant queen lifespan is much shorter than colony lifespan (Bourke & Franks, 1995; Heinze & Keller, 2000; Keller & Genoud, 1997). Workers are expected to welcome an additional newly-mated queen (**Scenarios 2-3** if all queens are singly-mated) to the nest, under the two conditions: that she is highly related to them and that the probability that the resident queen dies is high (Keller, 1995).

Re-adoption of newly-mated daughter queens when their mother is still alive and fertile avoids the costs of ICF and can increase the colony's reproductive fitness (Heinze & Keller, 2000; Peeters & Ito, 2003). Surprisingly, however, genetic data have shown low queen-to-queen relatedness in many instances of polygynous nests. This has been suggested to be caused by the accumulation of the effects of queen turnover over time (Keller, 1995). The acceptance of unrelated (or more distantly related) queens might also be facilitated if workers can sense that the current queen is failing, if replacement queen arrival is a rare event or if the benefit of additional workers to the colony surpasses the cost of rearing unrelated brood.

On the paternal side, variation is created by multiple paternity of a queen's offspring (also **Scenarios 2-3**). Hymenoptera show varying rates of *effective paternity*. Rates in ants are low, but, even in predominantly single-mating ant species, queens with multiple effective mates are often found. Across ant species, the average number of males contributing to any one queen's sexual reproduction is 1.43 (Strassmann, 2001). The only exceptions with consistently high multiple paternity, within the ant family, are two genera of higher attines, the advanced fungus-growers *Acromyrmex* and *Atta*, and one species of *Myrmicinae*, *Pogonomyrmex occidentalis*, the latter reaching the ant record rate value of 6.76 (Boomsma & Ratnieks, 1996; Cole & Wiernasz, 1999; Strassmann, 2001). In social bees, the very low rates measured in many stingless species and in all bumblebees are matched by rate values that are similar to the ant average, within the same stingless group, and by the extremely high values found in honeybees (9.1 – 25.65). In wasps, *Polistinae* have rates close to 1, but the rates of *Vespinae* range to much higher values (1-7.14; Strassmann, 2001). Notably, all rates cited have been obtained through genetic sequencing of microsatellites and correspond to a much reduced and more accurate estimate than the values historically measured from observations of multiple mating (Boomsma & Ratnieks, 1996).

In addition to multiple co-existing parental lines, the parental genetic material in the offspring of the same gyne and male is re-organised by recombination, creating offspring with different allele combinations across loci (**Scenario 4** can be interpreted as this case if the building rule is a quantitative trait underpinned by so many loci that the resulting phenotypic variation is approximately normally distributed). Recombination rates are very high in many Hymenoptera and it has been hypothesised that they are under selection to generate additional diversity (Gadau et al., 2000; Wilfert et al., 2007). However, some modelling work indicates that *polyandry* plays the main role in creating unique genotypes (Rüppell et al., 1998).

There is evidence that the parental line is associated with differences in worker activity. The association between genotype and task specialisation is well-known in honeybees (Oldroyd et al., 1992, 1993, 1994). More recently, studies have found a relationship between parental lineage and task specialisation in many ant species, even when accounting for differences in worker size independently from the parental line (Holbrook et al., 2013; Julian & Fewell, 2004; Saar et al., 2018; Schwander et al., 2005): in *Acromyrmex versicolor, Pogonomyrmex californicus* and *Formica selysi*, parental lineage is correlated with task allocation (Holbrook et al., 2013; Julian & Fewell, 2004; Schwander et al., 2005). In the case of *F. selysi*, the degree of within-colony worker polymorphism has also been studied and found to have no association with genetic diversity, suggesting that the influence of this latter on task allocation in this species is not exercised through worker morphology (Schwander et al., 2005). Two other important traits in division of labour are also affected by genetic diversity. In *A. versicolor*, the age of switch between internal and external worker task is correlated to the matriline (Julian & Fewell, 2004) and in *Messor arenarius* (but not a local generic *M. sp.*), colony performance in foraging and nest maintenance is directly correlated to the degree of genetic diversity, contrary to what would be the expectation under an inclusive fitness perspective (Saar et al., 2018). These associations may be due to parental-line specific timing in the expression patterns of the genes involved in division of labour, such as the *foraging* gene (Ingram et al., 2005).

Beside genetic variation, developmental, environmental and social factors contribute to differences in worker behaviour (**Scenario 4**, in which it is assumed that the resulting phenotypic variation is normally distributed). Hard evidence of the importance of non-genetic factors is offered by parthenogenic ants: the genetically identical workers of *Strumigenys membranifera* nevertheless display variation in sucrose response thresholds (Hasegawa et al., 2018). Developmental experiences, including learning, can affect sensitivity to stimuli and individual task preference and performance (*e.g.*, Franklin et al., 2012; Ravary et al., 2007; Richardson et al., 2018; Weidenmüller et al., 2009). As mentioned in **Chapter 3**, the effect of these experiences is closely related to colony performance: workers are known to adjust their task preference and their workload depending on external conditions such as the number of workers dedicated to that task that are already present in the colony and such preferences can persist over time, indicating a long-term effect (Kwapich & Tschinkel, 2016). Physiologically, phenotypic variation, and task specialisation in particular, have been linked to neurological differences that are as likely to be caused by development as by genes (Giraldo et al., 2013; Kuebler et al., 2010; Muscedere & Traniello, 2012). Finally, the social context can influence individual behaviour (Webster & Ward, 2011): for example, in *Apis mellifera*, more explorative foragers become more likely to use familiar over novel sites when in a mixed group with non-explorative foragers (Cook et al., 2020).

4.4 Importance of inter-worker phenotypic diversity in eusocial colonies

Why should collectives that rely on inclusive fitness for their functioning have mating strategies that decrease within-group relatedness? Are there advantages to within-group phenotypic diversity? Several experimental and theoretical studies suggest that indeed there are.

Mainly, worker phenotypic variation seems to be beneficial in the context of self-organised labour division. Modelling work suggests that the varying threshold model, in which workers subfamilies (*i.e.*, parental lines) differ in their response thresholds, results in increased colony performance in task allocation and in response to a changing environment (Myerscough & Oldroyd, 2004). This is in spite of the fact that a uniform-threshold model, where workers have identical thresholds for the same task, is equally able to give rise to organised task allocation (Bonabeau et al., 1996). The difference is that colonies where workers vary in their thresholds make a faster switch in the number of workers allocated to a task, following an increase in task stimulus intensity (*e.g.*, availability of a foraging source, or damage to a nest area that needs fast repairing), and they also better match workforce allocation to task needs. Empirical studies indicate that there are task-specific and even species-specific differences in this advantage. For example, the number of aggressive individuals is positively correlated with nest relocation efficiency and defence in the ant *Temnothorax longispinosus*, but not in *T. curvispinosus* (Modlmeier et al., 2014), while in *Linepithema humile* the level of genetic diversity has no influence on task performance (Rosset et al., 2005), but the proportion of phenotypically highly explorative individuals has, at least on nest choice (Hui & Pinter-Wollman, 2014). The utility of individual phenotypic variation to collective performance may depend on the environment: for example, increased diversity might be advantageous under changing conditions, but its costs might be too high in more stable ecological niches. In addition, the type of dynamics underlying self-organised behaviour may also influence the impact of individual differences: some mechanisms might enhance and others reduce the contribution of the individual to the collective phenotype. This consideration may be relevant when considering why the advantages of individual variation differ from task to task. Additional theoretical and empirical studies are necessary to clarify if this is the case.

When considering the interaction between individual variation and self-organised dynamics, it is interesting to analyse how the contributions of individual personalities sum up to colony-level personality and to collective performance. Pinter-Wollman (2012) proposes two ways in which individual traits can generate the collective phenotype: *i.* individual personalities linearly add up to create colony personality, so that the latter equals the mean of the individual level (additive model); and *ii.* collective personality is determined by the distribution of individual personalities, so that colonies with the same average worker trait but different underlying worker trait distributions have different personalities (synergistic model). Empirical evidence points to both effects existing in nature. In *L. humile* (Hui & Pinter-Wollman, 2014) and *Formica fusca* (Carere et al., 2018), colony performance and colony personality, respectively, are simply explained by correlation with worker average phenotype. In *T. longispinosus*, variance, and not average worker trait, correlates with per worker offspring production, although, in this study, a causal effect of the environment on both variables cannot be excluded (Modlmeier & Foitzik, 2011). Pinter-Wollman (2012) notices how the within-task distribution of work effort among workers in many species (where a few élite workers undertake the majority of the load; *e.g.*, Pinter-Wollman et al., 2012) would suggest that worker trait distribution has important consequences for colony performance.

Outside division of labour, individual variation may have beneficial consequences for one more colony feature that also belongs to the realm of complex systems: network structure. Worker-worker networks in eusocial colonies contain a few highly connected individuals (that is, they are likely to follow a power law distribution of degree connectivity; Fewell, 2003; Newman, 2003). The degree of connectivity of a worker has been linked to its spatial position, its age and task and its movement pattern within the nest (*e.g.*, Mersch et al., 2013; Naug, 2008; Pinter-Wollman et al., 2011). The distribution of, for example, exploratory personalities or task preferences among workers can therefore have substantial consequences on the resulting network. A simulation model based on the interaction pattern of *Pogonomyrmex barbatus* in the nest entrance chamber, where external workers are recruited, shows that individual variation in connectivity results in information flow as fast as that generated by uniformly distributed high connectivity rates (Pinter-Wollman et al., 2011). Since high connectivity has a metabolic cost, the network structure with the lowest number of highly connected individuals should be preferred.

Finally, genetic diversity in eusocial colonies has been linked to a range of potential benefits other than the performance of collective behaviour (Crozier & Fjerdingstad, 2001; Crozier & Page, 1985). To conclude this section, I will briefly summarise recent empirical findings relative to one of these hypotheses: resistance to parasites (Hamilton, 1987).

Genetic diversity confers resistance in at least some eusocial species. This hypothesis has been met with conflicting results in *A. mellifera* (Neumann & Moritz, 2000; Tarpy, 2003) but was confirmed and is well-documented in *Bombus terrestris* (Baer & Schmid-Hempel, 1999, 2001; Liersch & Schmid-Hempel, 1998). Among ants, it has been studied in leaf-cutter species: when exposed to a fungal parasite, *Acromyrmex echinatior* shows differences in resistance among patrilines and genetically-diverse groups generally fare better (Hughes & Boomsma, 2004; however, Crozier & Fjerdingstad, 2001 report an unpublished null result for a similar study in *Lasius niger*).

It is unclear what traits confer resistance. Genetic variation seems to provide a benefit over time, by hindering the parasite's ability to adapt to its host: an experiment using the same leaf-cutter ant and pathogen species as above found that the latter is more likely to go extinct when passed through several generations of genetically diverse individuals (Hughes & Boomsma, 2006). Importantly, the relationship between relatedness and colony fitness in the presence of a parasite might not be straightforward, but instead follow a U-curve, where intermediate levels of genetic heterogeneity suffer from lower fitness (Baer & Schmid-Hempel, 2001). Indeed, Hughes and Boomsma (2004) declare that the degree of relatedness is not associated with worker survival in *A. echinatior*, which may indicate a complex relationship between genetic diversity and fitness (however, note that they used a generalist ant parasite in their experiment and consequently that pathogen's ability to thrive on different genetic backgrounds is to be expected).

Overall, the evolutionary causes behind genetic diversity in eusocial species need to be evaluated bearing in mind the species-specific ecological and evolutionary context, as well as possible drift effects.

To my knowledge, individual variation in the specific context of nest building behaviour is poorly studied. We know from Pinter-Wollman *et al.* (2012) that workers performing nest maintenance (including, presumably, repairs to the nest structure) in *T. rugatulus* and *T. albipennis* vary in how much work they take on, with a few élite members of the group performing most of it. Presumably, workers therefore differ in their response thresholds for this task. However, the underlying mechanism is unclear (Leitner & Dornhaus, 2019). The presence of phenotypic variation in response to building stimuli might be selected for, but it might also be an inevitable side product of selection for genotypic variation or for threshold variance in other tasks. I can envisage some scenarios where variation is advantageous also in the building context, such as when workers differ in their tendency to repair a gap in the wall. If the colony contains a mixed worker population where some builders are triggered by a small decrease in stone density at a wall section and others by a larger decrease, the wall structure may more easily reach a balanced state where some air flow is enabled (because there are not enough builders with high sensitivity to make the wall uniformly tight), but not so much that the structure is weakened and susceptible to invasion by other colonies.

Having stated that phenotypic variation in building behaviour in eusocial colonies is to be expected, and what kinds, this chapter is now concerned with analysing its effects on the collective phenotype, *i.e.*, on the nest architecture. I now proceed to illustrate the model I use for this purpose.

4.5 Methods

The models used for this chapter are based on the agent-based model described in **Chapter 2** (**Section 2.1.1**). The complete behavioural model (*stigmergy + template*) assumed in these simulations consists of two components: a distance template, whereby the worker's choice of optimal building location and its stone pick-up activity are both functions of the distance from the cluster of brood and nurses; and a stigmergic feedback system, causing worker deposition to increase with stone density until a critical density threshold, after which deposition decreases towards activity termination and pick-up (which decreases with increasing stone density) reaches a minimum value equal to minimum deposition after termination, guaranteeing the stability of the finished wall. To better understand the effect of model components on robustness, I run all simulations also under the *template only* model, in which behaviour is only affected by distance from the central cluster. This model is the same as the *stigmergy + feedback*, but the part of the behavioural functions modelling stigmergy is not incorporated (**Chapter 2**, **Section 2.1.1**). The exact parameters used in the two behavioural models are summarised in **Table 4.1**. I used the parameter defining the optimal distance between building site and template, r_0, as the rule undergoing mutation or for which individual variation is present in the population. A mutation in the optimal distance rule causes the worker to prefer a different distance from the brood cluster as the position at which to build the wall. The difference between rule variants can therefore be expressed as a difference in distance units: for example (using the 1 node = 1 mm equivalence applied in the spatial architecture of the model), a rule variant $R_1 = 18\ \mathrm{mm}$ and a variant $R_2 = 15\ \mathrm{mm}$ have a difference of $R_1 - R_2 = 3\ \mathrm{mm}$. Biologically, differences in this optimal distance rule can be thought of as genetic differences affecting the receptor responsible for template perception, so that mutations cause an altered perception of its intensity.

I analysed four models: one model simulating building as it occurs at progressive snapshots in time, in colonies where the worker population is gradually replaced by mutants in distance rule following queen replacement (**Scenario 1**), and three models simulating building by colonies under different distributions of phenotypic variation in distance rule (**Scenarios 2-4**). Because these scenarios are only meant to simulate the effect of individual variation on collective phenotype and not its evolutionary change over colony generations, through sexual reproduction, the genetic architecture underlying the phenotype is irrelevant. Therefore, when considering the model, it was sufficient to assume that the mutations caused a change in rule expression of the given degree (the mutation step, or difference between mutant and resident). For example, when interpreting the model, it can be assumed that the rule is a quantitative trait, and differences (*e.g.*, between patrilines or between the old workforce and the offspring of the replacement queen) are the results of genetic variation at multiple loci.

4.5.1 Model architecture

The structure of the simulation models is the same as described in **Chapter 2**, **Section 2.1.1**. I summarise it below.

The models consisted of agent-based spatially explicit simulations of a worker group (30 workers) moving, picking up and depositing stones within an 80x80 node space matrix, corresponding to 8 cm x 8 cm real-life space. Deposition and pick-up behaviour followed the *gradual* model (**Chapter 2**, **Section 2.4.1**) and were a function of distance from the centre of the space matrix (corresponding to the centre of the nest, where the cluster of brood and nurse workers is located) and of the total number of stones at the current and adjacent locations. Each simulation was initialised with 3000 stones randomly (as drawn from a uniform distribution) scattered across the matrix (the number of available stones remained the same throughout the simulation as stones could not be removed from the matrix) and lasted 5000 rounds (unless otherwise stated). Each node of the matrix had a maximum carrying capacity of three stones. Workers could pick up but not deposit at nodes that had reached their carrying capacity. At each round, the following steps were repeated for each worker. The worker randomly selected a position at any point in the matrix, irrespective of its previous location (which was not tracked). If the location contained at least one stone, it had a chance to pick one up (based on the pick-up function). If no stone was present, the simulation skipped to the next worker, otherwise the present worker moved from location to location (again, randomly and with no relation to its previous position) until it deposited what it was carrying (based on the deposition function). The simulation then moved to the next worker. Only the positions of the stones were tracked from round to round.

Parameter	Value	Description
P_{M}	0.35	maximum possible value of pick-up probability
D_{M}	0.5	maximum possible value of deposition probability
F_{M}[1]	0.55	effect of number of stones on pick-up probability below the threshold number of nearby stones
G_{M}[1]	0.55	maximum value of the effect of number of stones on deposition probability, reached at the threshold number of nearby stones
F_{m}[1]	0.01	effect of number of stones on pick-up probability above the threshold number of nearby stones
G_{m}[1]	0.01	minimum value of the effect of number of stones on deposition probability, reached when the number of nearby stones is either 0 or very large
r_{o}	scenario-specific	optimal wall building distance for the worker
τ	0.025*	parameter regulating the slope of the decrease in deposition probability and increase in pick-up probability as we move away from r_{o}
S_{c}	6	threshold number of nearby stones

Table 4.1. Model parameters. Summary of the parameters of the equations describing pick-up and deposition probability under the *gradual* model. The values used are the same as in the simulations **Chapter 2** and are reported here for reference only.

[1] The parameter is absent in the *template only* model.

* The value of τ is modified in order to study the effect of buffer zone size in the corresponding simulations.

4.5.2 Data collection

Measures of stone dispersal were averaged over iterations as described in **Chapter 2.1.3**, with the same initialising values of the random number generator repeated across conditions.

4.5.3 Scenarios

Below are the modelling details used to reproduce the four phenotypic variation scenarios studied in this chapter. In all scenarios where variants were drawn from a normal distribution, the mean of the distribution was 18 mm.

4.5.3.2 Mutation in worker germline (Scenario 1)

In this scenario, the case of workforce replacement was considered. I studied how the quality of the architecture changes over time as the mutant proportion of the workforce increases and the resident proportion decreases. This scenario occurs in monogynous colonies with a singly-mated queen. The queen must be newly-mated and either have replaced the resident queen in an established colony with a resident worker population or have founded a new colony through DCF. The workers born from the new queen carry a mutation in distance rule, that is present either in her eggs or in the sperm of her mate. I assumed that old workers die gradually and new workers (carrying the mutation) replaced them (if workers are born, mature and die seasonally, there is no overlap between rule variants). The mutant workforce expressed a distance rule r_0 with value R_2, as opposed to the resident variant value R_1. I simulated progressive snapshots in time in the workforce turnover process. Each snapshot was defined by model parameter q, corresponding to the proportion of mutant workers in the total colony building workforce. I analysed the time points in the colony turnover process corresponding to q values [0.1,0.3,0.5]. The value of the resident rule variant R_1 was kept constant at 18 mm in all simulations, while I explored the effect of the mutant rule variant R_2 values [8 mm, 12 mm, 16 mm, 20 mm, 24 mm]. Each (q,R_2) combination was iterated 100 times over different initialising values of the random number generator.

4.5.3.2 Existence of multiple patrilines (Scenario 2)

In this scenario, the existence of four genetically distinct patrilines within a colony, each carrying a unique rule variant, was modelled. The choice of four patrilines was motivated by the ease with which the space matrix can be split into four quadrants, when spatial specialisation is added to this scenario (**4.5.3.3** or **Scenario 3**). Three cases were considered: the case where patrilines are present at equal frequency ($f_1 = f_2 = f_3 = f_4 = 0.25$) and two cases where either one or two patrilines are numerically predominant ($f_1 = 0.5, f_2 = 0.2, f_3 = 0.2, f_4 = 0.1$ and $f_1 = 0.5, f_2 = 0.4, f_3 = f_4 = 0.05$). Each variant is drawn from a normal distribution with mean 18 mm. The effect of the standard deviation of the distribution was studied ($SD = 2\,mm$ or $SD = 5\,mm$ used). For each case of standard deviation, 10 simulations were run, each with independently drawn rule values, and the effect of standard deviation and rule value and frequency studied.

4.5.3.3 Multiple patrilines with building-site fidelity (Scenario 3)

This scenario was identical to the one described in **Section 4.3.2.2**, with the exception that patrilines displayed building-site fidelity. The space matrix was divided into four quadrants and each patriline was assigned a single quadrant to build in, with a 10 mm overlap with the two adjacent quadrants. For example: patriline 1 was assigned the first quadrant but could also build at the first 10 nodes of each of the second and fourth quadrant (coordinates [*x*: 0-50; *y*: 0-50]). Patriline 2 was assigned the second quadrant and the adjacent 10 nodes in both directions (coordinates [*x*: 30-80; *y*: 0-50]), *etc.* Workers could not move to any location in the matrix that was not part of their quadrant and overlap area.

Wall sections built by patrilines that are very low frequency in the colony take longer to complete. Therefore, these simulations were run for 10000 time steps, enough for all sections to reach their equilibrium state.

4.5.3.4 Individual variation or individual personalities (Scenario 4)

In this scenario, I examined the case where all workers within a colony potentially differ from each other (phenotypically) in the distance rule used. This variation can be assumed to be caused by epigenetic factors as well as a high number of loci with small individual effects underlying the trait, enough that the resulting distribution of phenotypic values approximates a Gaussian curve.

All rules in a colony were drawn from a normal distribution of mean 18 mm. The effect of the size of standard deviation in worker rule value was studied ($SD = 2\,mm$ or $SD = 5\,mm$ used). Each standard deviation case was iterated 100 times over different initialising values of the random number generator.

4.5.4 Role of tau in phenotypic robustness

Outcome robustness in the presence of rule variation may depend on the size of the “buffer zone” around the optimal distance expressed under a variant. In the buffer zone, building activity still occurs with high probability, even if we are spatially slightly removed from the optimal distance. If the buffer zones of two or more variants are wide enough that the activity peak of those variants overlap, this may facilitate phenotypic convergence. In my model, the buffer zone is regulated by the wall-thickness parameter τ.

I visually inspected the role of the buffer zone by re-running a single set of simulations of the mutation-in-germline condition and of the individual-personalities condition with an increased value of the wall-thickness parameter $\tau = 0.075$ (from $\tau = 0.025$; a higher value results in a shorter buffer zone diameter).

4.6 Results

In this section I present the results of agent-based simulations that reproduce the wall building behaviour of *Temnothorax* ant colonies under four different scenarios of individual phenotypic variation. Variation manifests in the template distance rule that is a component of this species' building behavioural model. Rule sets generating self-organised collective building are likely to contain components that counteract the effect of individual variations in the rule used, by enabling phenotypic convergence and the emergence of consensus in the group-level phenotype (in this case, the architecture; that is, some rules may generate robustness of the collective phenotype to individual variation). In this study, I try to identify the rules that do so.

Results are organised by scenario. These are, briefly (described in more detail in **Section 4.1**): 1. workforce turnover following queen replacement, when the new queen's offspring carries a mutation in building behaviour; 2. co-existence of four patrilines, each expressing a different rule variant; 3. co-existence of four patriline-specific variants with the addition of patriline-specific spatial specialisation in building activity; 4. normally distributed individual phenotypic variation caused by epigenetic (*e.g.*, developmental or environmental) effects.

The expected degree of conflict among individual variants building the collective architecture depends on the difference between variants (here, measurable as the difference between individual preferences in the building distance in mm) and the relative frequency of variants among builders. Both factors are taken into account in this analysis. To measure the impact of individual variation on wall structural quality, I rely on the two measures introduced in **Chapter 2** (**Section 2.1.1**): circular dispersal (how dispersed stones are on the θ axis of a circular plane centred on the brood, at the end of construction; this measure is indicative, approximately, of the presence of asymmetric gaps in the circular wall) and distance dispersal (how dispersed stones are on the radial axis r that indicates distance from the brood cluster; this measure can be used to tell whether collective building activity has converged to a single building distance). The closer these measures are to zero, the better the quality of the wall.

4.6.1 Scenario 1: Effect of worker turnover following queen replacement

In this scenario, a mutation in building rule emerges in the worker germline of a colony with a resident population of workers. The proportion of the workers carrying the mutation, q, increases over time as old workers are replaced by mutant ones after their death. The strongest conflict between the resident and mutant building rule is expected when the two variants are equally represented in the workforce

($q = 0.5$). The degree of conflict depends also on the mutation step that separates the variants, which in this case is the distance difference in mm.

In **Figure 4.1**, we can see how stone dispersal changes depending on the distance difference between resident and mutant (x-axis), at increasing mutant frequencies (**panels left to right**). Circular dispersal remains almost unchanged in all conditions, meaning that the wall keeps its structural continuity at all stages (**Figure 4.2**). As the two variants approach equal frequency, given constant difference between variants, distance dispersal increases (**Figure 4.1**). Part of this measured change in distance dispersal has to be discounted as a side effect of measuring change as difference in distance without considering the change in area: when the mean optimal building distance is shifted inwards, the corresponding area in which workers prefer to build shrinks and the excess of stones after area capacity is reached is deposited at less preferred locations at higher or lower distance, increasing the distance spread value (conversely, the area increases as the mean preferred distance is shifted outwards, and distance dispersal decreases when the mutant distance is greater than the resident). If we look at the change in structure, we see that the wall remains compact under all conditions except with the largest negative difference between resident and mutant and when the mutant has at least a frequency of 0.3. In these cases, the wall increases in diameter (**Figure 4.2, leftmost panels**). Wall compactness is maintained by the stigmergic feedback, causing stones to be clustered tightly into the wall, and in its absence the wall always increases in diameter and decreases in density, losing defence and homeostatic functionality (**Figure 4.3**). Stone dispersion in the absence of stigmergy does not improve over longer periods of simulation time (data not shown).

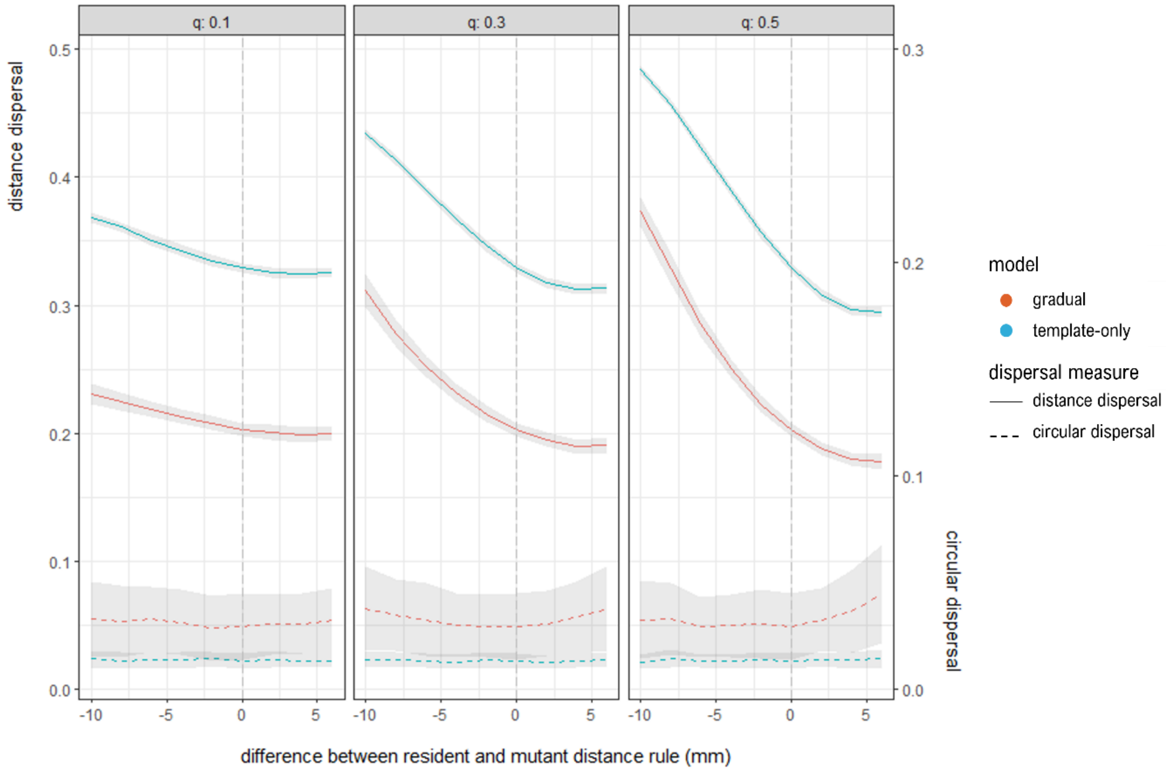

Figure 4.1. Effect of mutant frequency and of difference between resident and mutant rule on stone dispersal measures.

Change in distance dispersal (*solid line* and *left y-axis*) and circular dispersal (*dashed line* and *right y-axis*) across resident-mutant difference values (*x-axis*). Three frequencies of mutant workers (q) are compared. The coloured lines show the mean values and the grey shading highlights the area within one standard deviation of the mean. The figure shows both the outcome of the full model, including stigmergy (*gradual* model, *red*), and of the model with only the template effect (*template only*, *blue*). The grey vertical dashed line intersects the dispersal-mean lines at the point in parameter space where there is no mutant in the population. Values are averaged over 100 simulations.

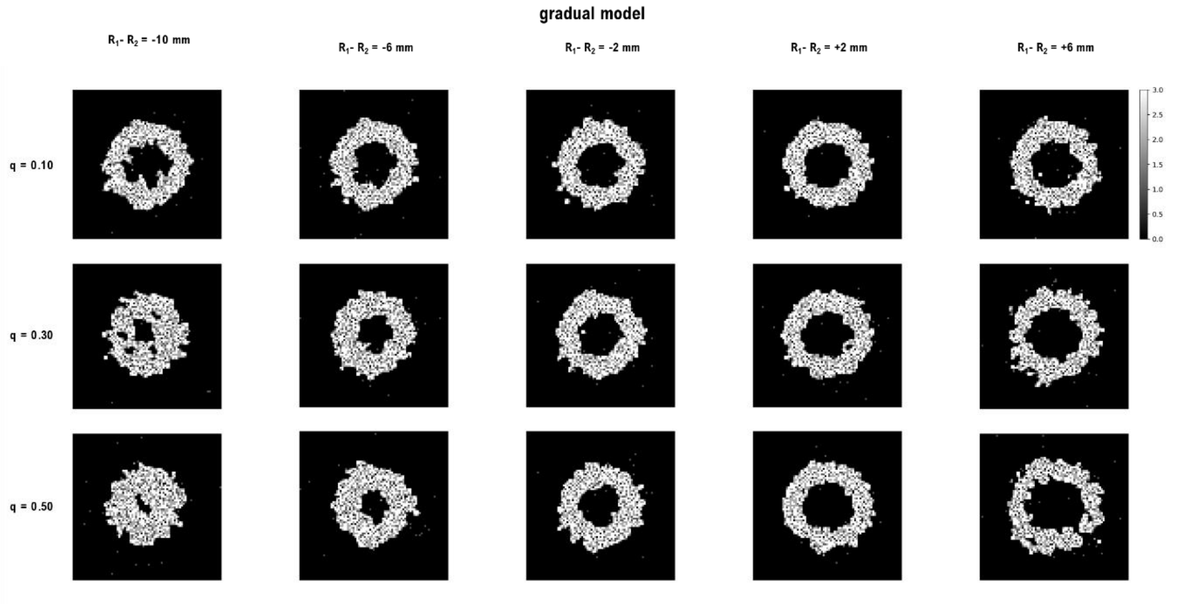

Figure 4.2. Wall change across parameter space (*stigmergy + template* effect).

The figure shows the same wall built by the full model (*gradual* model) under different mutant frequencies (q) and resident-mutant difference values ($R_1 - R_2$). The scale on the top right shows the number of stones at each location.

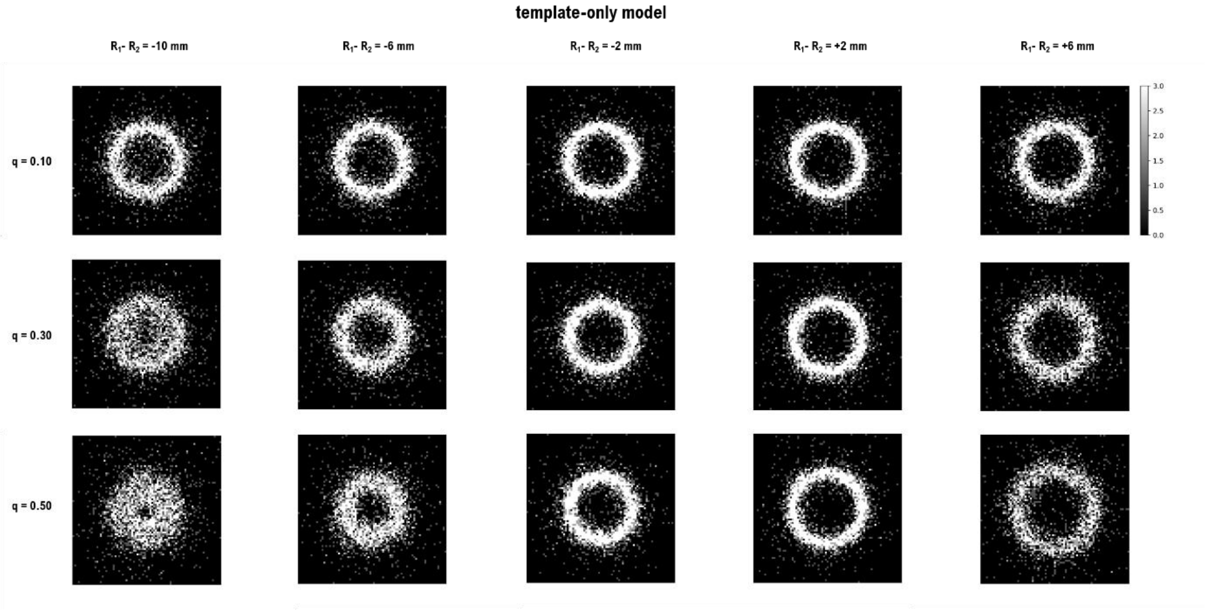

Figure 4.3. Wall change across parameter space (*template only* effect).

The figure shows the same wall built in the absence of stigmergy (*template only* model) under different mutant frequencies and resident-mutant difference values.

4.6.2 Scenario 2: Effect of multiple patrilines

In this scenario, the workforce of a colony consists of four genetically distinct patrilines, each expressing a different rule variant. The structural quality of the wall is only affected if there are large differences among variants (that is, when the variants are drawn from a distribution with a standard deviation of 5 mm; **Figure 4.4**). I considered three combinations of variant frequencies. In the first, the workforce was equally split among patrilines. This balanced condition poses the most strain on wall structural quality. In **Figure 4.5A**, on the **top left**, the outlier with the highest distance dispersal under this condition is shown. Because two of the variants have opposite trends (a large and a small distance value), the wall closes in onto the brood cluster (note that the cluster is represented as a point at the centre of the space matrix in the model, so a true representation of the wall still has space at its centre). The phenotype is rescued (that is, the wall regains a functional form; in this case, the nest area is larger compared to the previous condition) under the second combination of variant frequencies tested, where one of the patrilines is predominant (**Figure 4.5A**, **top right**). When one of the variants pushes building even further away from the centre, and away from other variants, the integrity of the wall structure is damaged and an outer ring corresponding to the largest building distance starts to appear (**Figure 4.5A**, **bottom left**). This effect is likely to disappear if a greater supply of building material is available. Under the limited resource assumption made here, the phenotype is nevertheless and once again rescued when one of the patrilines predominates (**Figure 4.5A**, **bottom right**). However, not all cases where two patrilines have largely contrasting rules result in structural failure: **Figure 4.5B** shows one of such high-quality cases.

Overall, most structures maintain compactness and continuity, under these two frequency combinations, even if gaps become more common. If there are two majority patrilines in the colony, with the other two relegated to a minority role, structural quality returns (this condition resemble that of a germline mutation). The circular shape of the wall is again not affected by variation (**Figure 4.4**). In the absence of stigmergy, stones are left dispersed both outside and, more importantly, in the area inside the nest, as in the previous scenario (**Figure 4.6**).

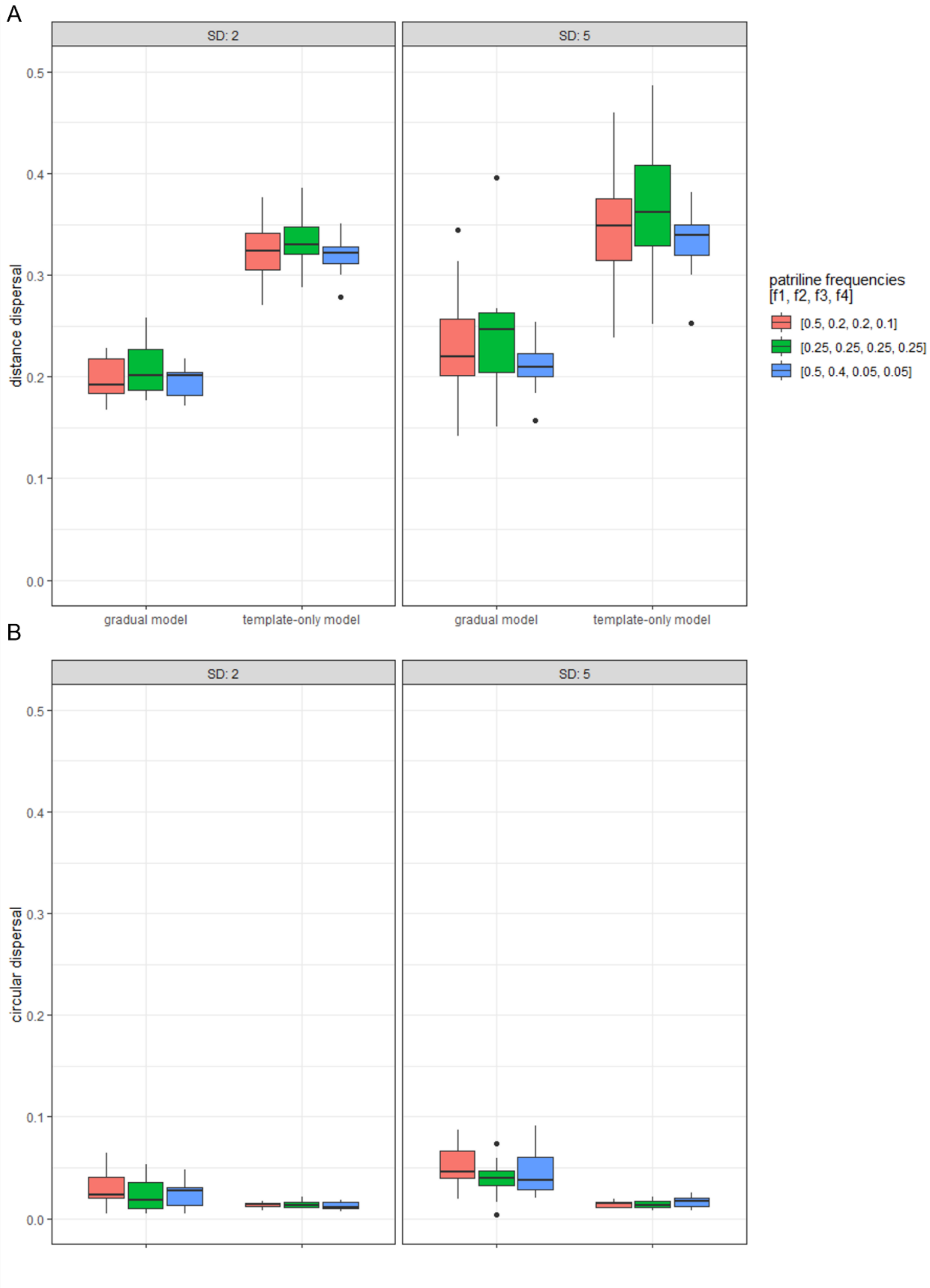

Figure 4.4. Effect of relative patriline frequency and of the standard deviation in rule distribution on distance spread.

The boxplots show the distribution of distance spread (**Panel A**) and circular spread (**Panel B**) values across 10 simulations of the same condition. Patriline variant values in each simulation are drawn from a normal distribution of mean 18 mm and of the standard deviation indicated (*left and right*). The model with (*gradual* model) and without (*template-only* model) stigmergy are compared. Colours indicate the patriline frequency condition (where f_x is the frequency of workers of patriline x in the colony). The horizontal line in the box represents the median value, and the bottom and top border the limits of the first and third quantile, respectively. Outliers are shown. For circular dispersal, values remain low in all conditions, indicating that the wall never deviates from a circular shape.

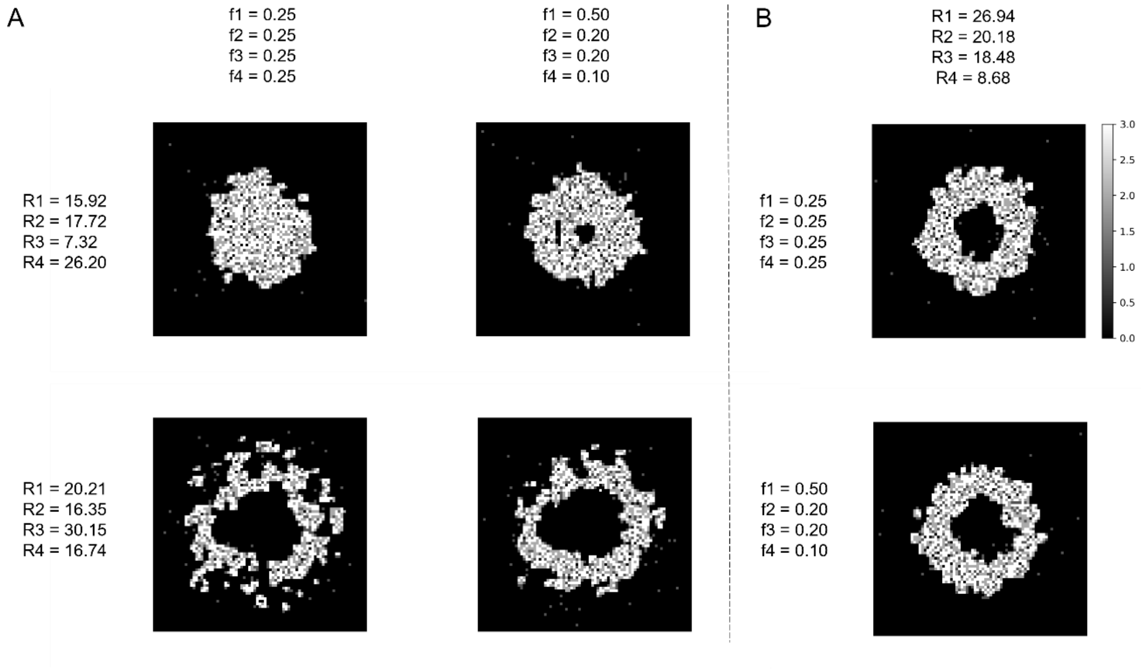

Figure 4.5. Change in wall shape caused by change in relative patriline frequencies, under large differences in patriline-specific rules.

Examples of walls built in the four-patriline scenario when variants are drawn from a normal distribution with a mean of 18 mm and a standard deviation of 5 mm. **Panel A** shows two combinations of distance rules (R_x) that cause the phenotype to collapse if present at equal frequencies (f_x; *left column*). Both conditions are rescued if one variant is predominant in the colony (*right column*). The top-left figure corresponds to the outlier in **Figure 4.4**. **Panel B** shows a case where the four variants are very different from each other, but the wall remains functional even when they are equally represented.

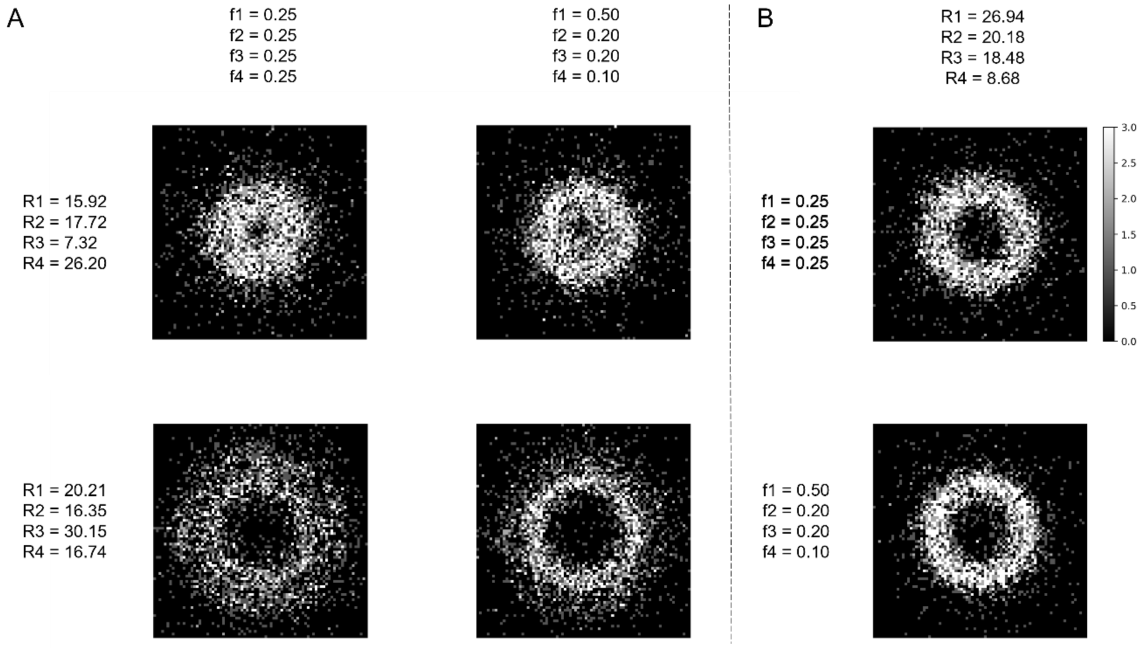

Figure 4.6. Wall structure under different patriline frequencies in the absence of stigmergy, under large differences in patriline-specific rules.

Final wall structure in the absence of stigmergy, under the four-patriline condition. The simulations correspond to the ones in **Figure 4.5**, run with the *template only* model.

4.6.3 Scenario 3: Effect of spatially specialised patrilines

Wall coherence holds even in the case of patriline-specific building-site fidelity, until the differences in rule among patrilines become too large (*i.e.*, when they are drawn from a distribution with a standard deviation of 5 mm). **Figure 4.8** shows what walls correspond to the labelled points in **Figure 4.7**, which attain very different values of distance and circular dispersal. If the variants present in the colony pull the construction in the opposite direction, as it was the case in **Figure 4.5A**, **top row**, stones are amassed to the edges of the brood cluster (**Figure 4.8A**, **point 1 and 2**). The opposite case, where one patriline pulls one section of the wall outwards, causes the wall to remain incomplete (**Figure 4.8A**, **point 3**; note that this point scores low in both distance and circular dispersal, indicating that these measures cannot be used to detect some low-quality phenotypes – **Figure 4.7**). However, once again some combinations of variants still produce a good quality wall (**Figure 4.8A**, **point 4**; here, all variants are within one standard deviation of the mean). If rules are drawn from a distribution with a smaller standard deviation, the wall is always high quality (**Figure 4.7** and **Figure 4.8B**).

4.6.4 Scenario 4: Effect of widespread phenotypic variation (worker personalities)

In this final scenario, each worker expresses an individual rule, assumed to be the product of learning and experience acting in combination with the genotype. This wall building rule set is extremely robust to diffused phenotypic variation: wall functionality is maintained even when the colony standard deviation in rule values is large (**Figures 4.9-10**).

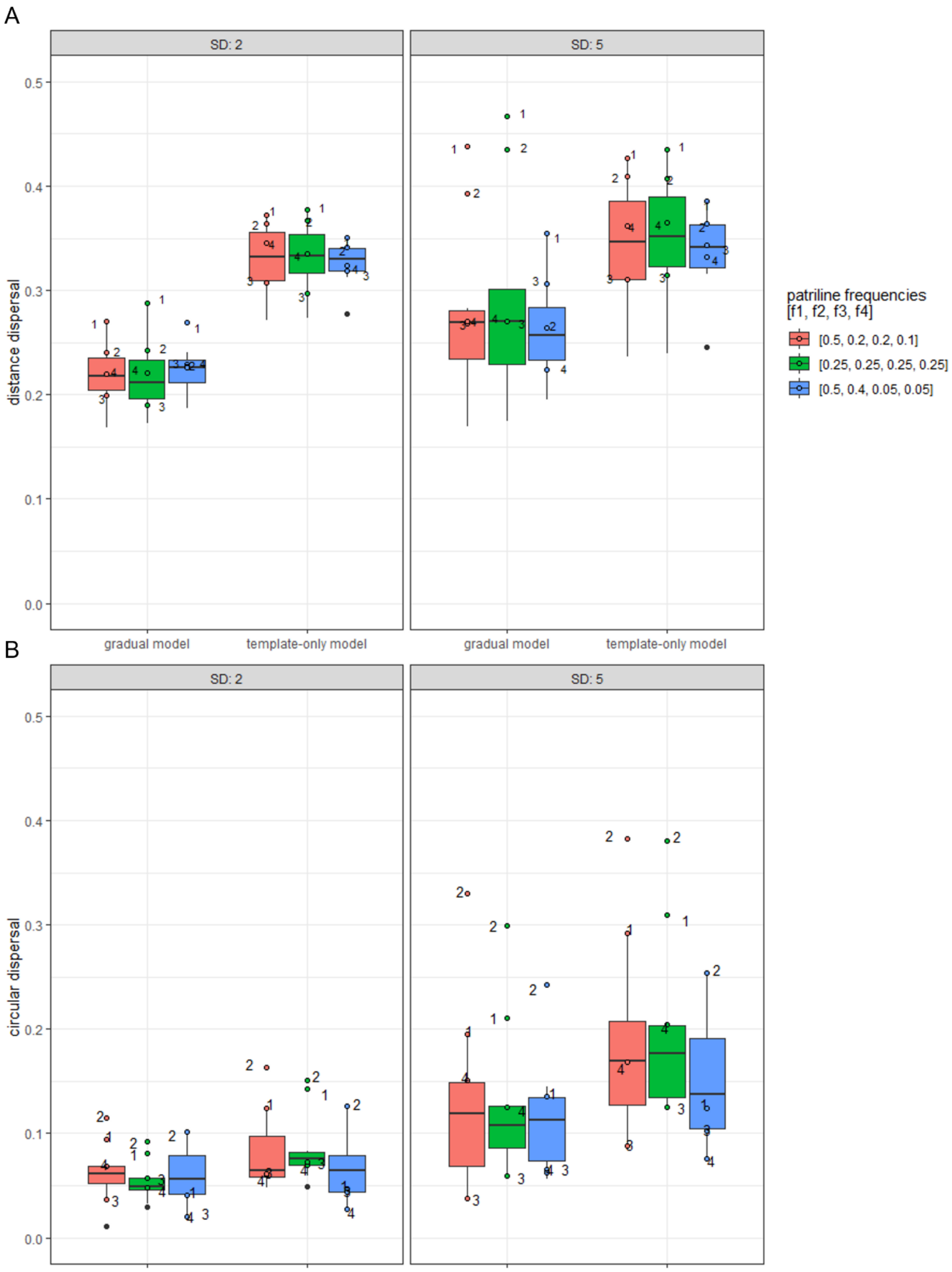

Figure 4.7. Effect of relative patriline frequency and of standard deviation on distance and circular spread when patrilines are spatially specialised.

The figure shows the distribution of distance dispersal (**Panel A**) and circular dispersal (**Panel B**) of 10 iterations of wall building run with different variant combinations. Simulations are run with variants drawn from a normal distribution with a mean of 18 mm and a standard deviation of either 2 mm (*left*) or 5 mm (*right*). Three patriline frequency combinations are tested (*colour-coded*). Labels correspond to the four wall structures shown in **Figure 4.8**. Points **1** and **2** are outliers in most cases. Box plot details as in **Figure 4.4**.

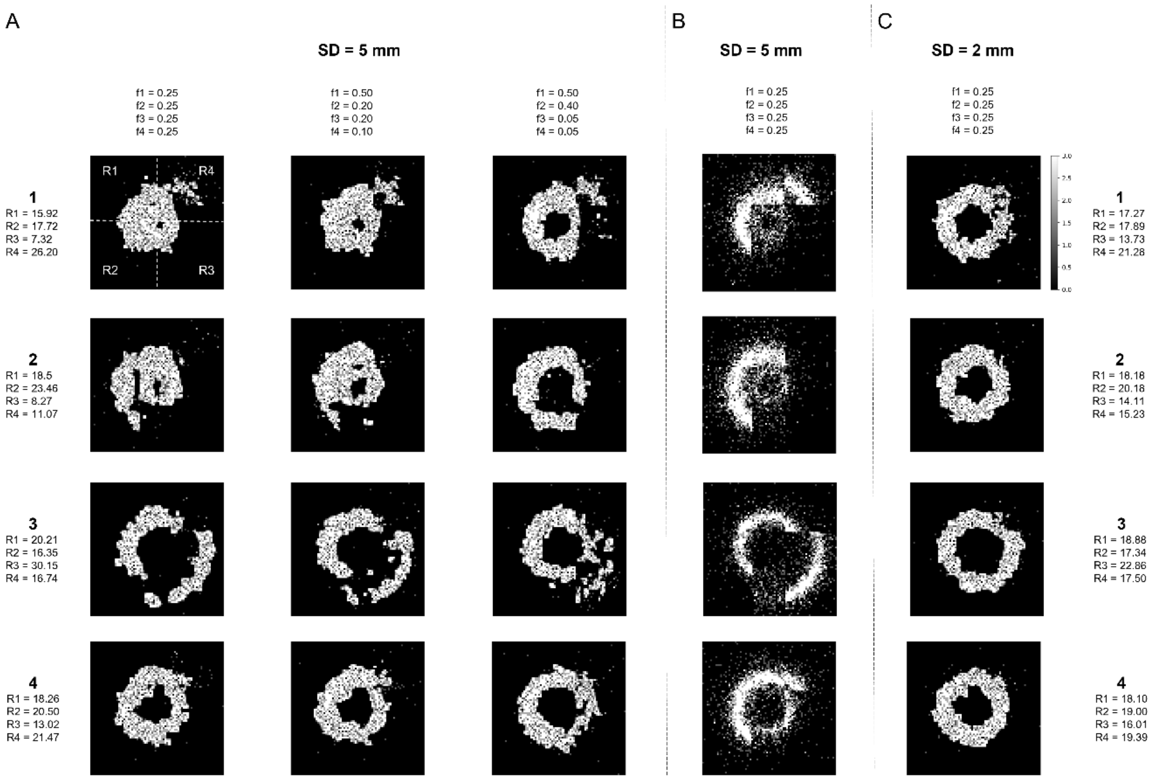

Figure 4.8. Walls built by spatially specialised patrilines carrying a patriline-specific rule variant.

The figure shows the structures corresponding to the points labelled in **Figure 4.7**. Point label and rule variant values for each patriline are shown to the sides. Patriline frequencies are found at the top. In the top leftmost panel, dashed lines show which quadrant was built by each patriline (with a small overlap to allow joining of the sections). **Panel A** shows the walls built when the variants present in the colony are drawn from a normal distribution with mean 18 mm and standard deviation 5 mm, under different combinations of patriline frequencies (*left to right*). **Panel B** shows the same wall built in the absence of stigmergic effect. **Panel C** shows the wall built when variants are drawn from a normal distribution with the same mean and a standard deviation of 2 mm, under the condition where all patrilines are represented at equal frequency in the colony. This condition poses the most strain on structural integrity. All variables except variant values, frequency, and standard deviation are identical for all structures in the same row (including the same initialising value of the random number generator).

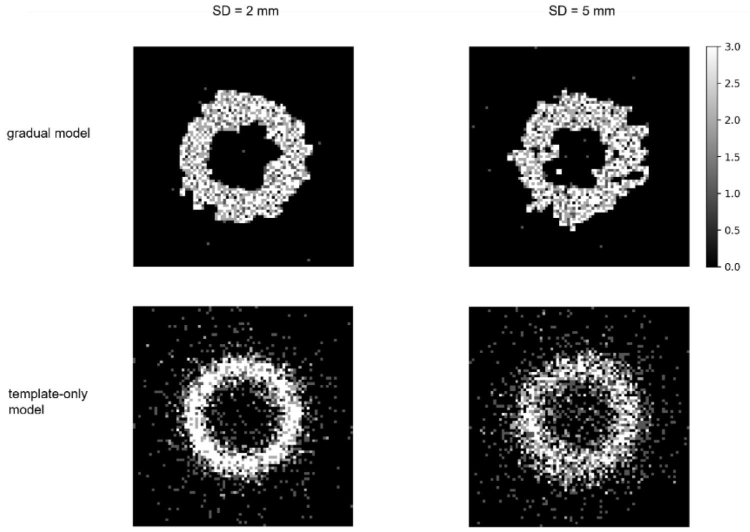

Figure 4.9. Wall structure resulting from workers expressing individual rules.

Examples of walls built under conditions of widespread phenotypic variation. All individual rule variants are drawn from a normal distribution with mean 18 mm and a standard deviation of 2 mm (*left*) or 5 mm (*right*). The model with (*top*) and without (*bottom*) stigmergy are compared.

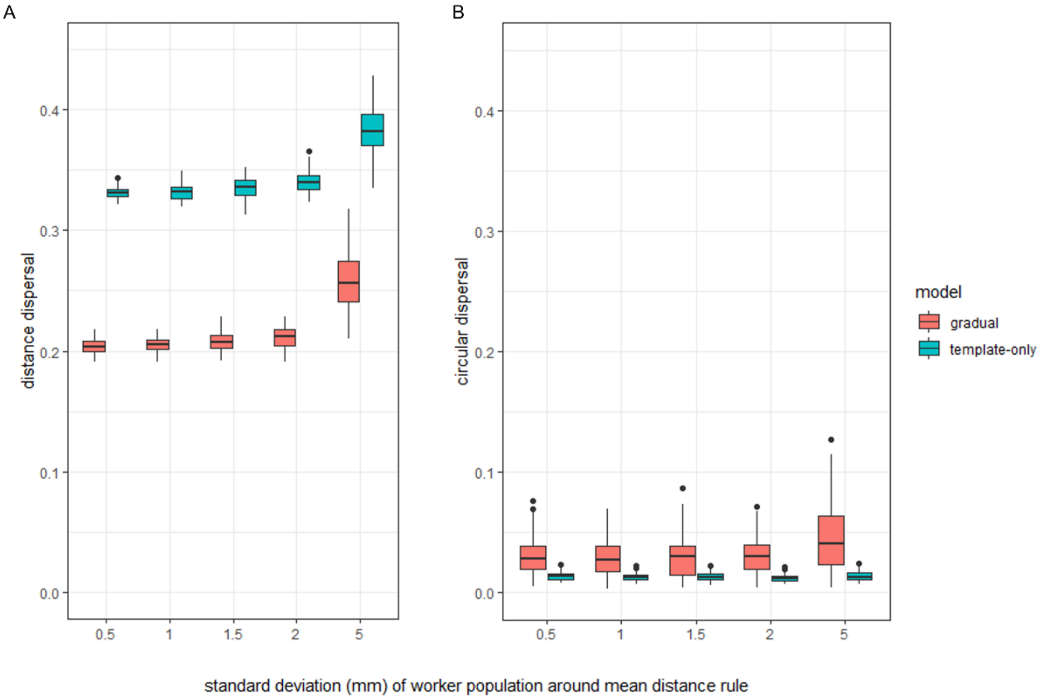

Figure 4.10. Effect of the standard deviation in worker rule value on stone dispersal measures.

The plot shows how the distribution of distance (**Panel A**) and circular (**Panel B**) dispersal values changes with the standard deviation of worker phenotypic variants (in a normal distribution of mean 18 mm). The model with or without stigmergy are compared (*colour-coded*). The boxplots summarised the outcome of 100 iterations per condition.

4.6.5 Role of wall thickness in maintaining wall functionality

What mechanism leads to phenotypic convergence when more than one rule exists in the population? I hypothesise that one key factor is the effect of overlapping “buffer zones”, where building activity occurs with high probability under both variants. The buffer zone of a variant corresponds to the area on either side of the optimal building distance line in which the probability of building remains high. If there is overlap between the buffer zones of two variants, then the stigmergic effect (the convergence rule) is likely to lead to the two structures being joined (and possibly the centre of mass of the building material to shift towards the area of overlap, depending on the relative strength of the stigmergic to the template effect). For example, a rule variant resulting in an optimal building distance of 10 mm and a variant of distance 15 mm may produce a single continuous wall because a high number of deposition events occur at an intermediate distance 11-14 mm under both and stigmergy subsequently shifts the centre of activity to this intermediate area, towards which the wall is compacted.

The size of the buffer zone is controlled by the parameter describing the relationship between the difference between current and optimal position and the effect on deposition and pick-up probability. In our model, this is the parameter τ. In biological terms, this parameter controls wall thickness. In **Figure 4.11A**, I show the outcome of a mutation in germline that leads to a favoured building location 10 mm closer to the brood than the resident, with τ modified to decrease the size of the buffer zone. At the shown timepoint in worker turnover, the mutant has already reached high frequency ($q \geq 0.30$) in the colony. In the absence of stigmergy (**Figure 4.11A, bottom**), we can see that there is sufficient building activity around the building distance favoured under each variant to lead to the formation of two distinct walls: an inner and an outer. The addition of stigmergy (**Figure 4.11A, top**) causes them to start merging. For comparison, modifying τ in the presence of individual phenotypic variation does not affect the structure, because the buffer zones of many normally distributed rule variants overlap extensively.

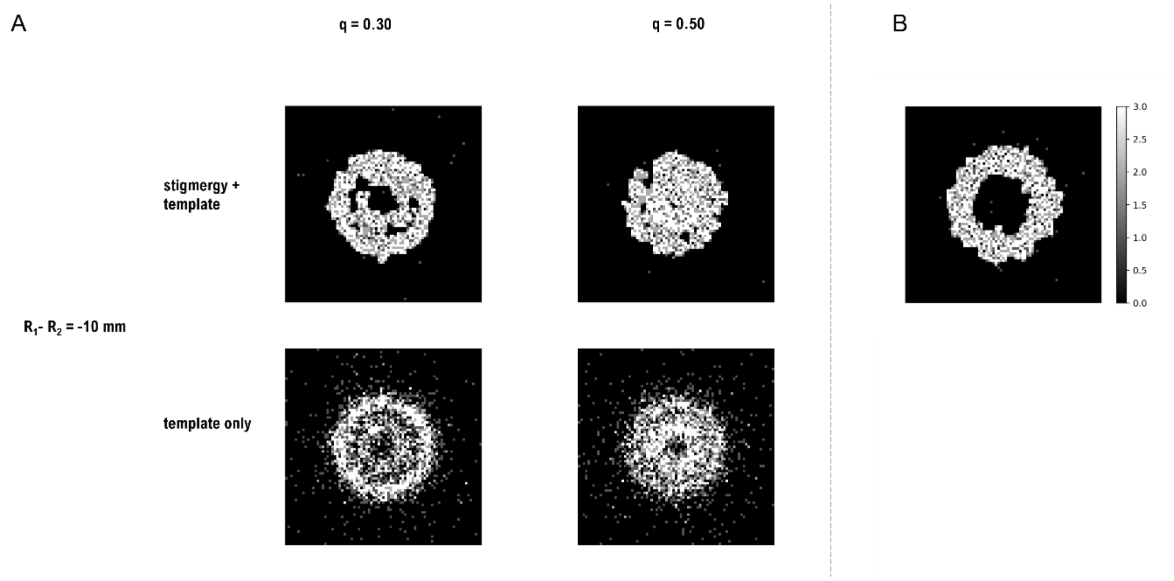

Figure 4.11. Effect of a reduced wall thickness on convergence between variants.

Effect of reducing wall-thickness in the presence of variation. **Panel A** shows the effect of a mutant in worker germline with a difference of 10 mm from the resident and that has reached high frequency in the colony (left to right: $q = 0.30$ and $q = 0.50$). The model with (*top*) and without (*bottom*) stigmergy are compared. **Panel B** shows the outcome of a simulation with the same wall-thickness parameter value, but in the presence of widespread phenotypic variation (worker rules are drawn from a normal distribution with mean 18 mm and a standard deviation of 5 mm).

4.7 Discussion

It has previously been suggested that processes leading to variation in worker phenotype might disrupt the coordination needed for collective building (Hansell, 2006). While this suggestion is logically plausible, it is in contrast with what we observe in nature: functional nests built by eusocial insect colonies are the norm, despite these societies being now recognised as containing individual variation in the worker caste. Therefore, collective building behaviour must contain the necessary ingredients to deal with this variation. The nature of these ingredients has not yet been rigorously tested within the body of literature related to models of collective building. Using the model developed in earlier chapters, I offered a first testbed for this phenomenon. I explored different colony life history scenarios that lead to different scenarios in terms of the distribution of phenotypes. The results of these simulations allow us to begin to understand how evolution can progress in collective building systems.

Had my modelling suggested that phenotypic diversity causes disruption of the integrity of the collectively built structure under the current *Temnothorax* model, then this would suggest that the model is incomplete: there would, that is, be the need to explore additional mechanisms or restrictions not included in my (and other) models of collective building to try to explain how evolution could occur. However, as it transpires, the current model is robust to phenotypic variation, thereby passing an additional test of its status as a valid model of *Temnothorax* wall building behaviour.

The fact that the wall built by *Temnothorax* is robust does not mean that the structure remains unchanged. In the turnover process between an old workforce and a new mutant worker generation, the building phenotype changes following the relative frequencies of each variant. Unless the conflict between the old and new variant is particularly pronounced (*i.e.*, when the difference between the old and the new building distance phenotype is very large), all intermediate stages between the colony-level expression of each variant are functional. From the analysis of multiple-patriline simulated colonies, we learn that variants can co-exist in a colony at most relative frequencies without resulting in a non-functional structure, unless the patrilines are spatially specialised. Therefore, the prediction of the model is that spatial specialisation should be absent in building, unless there is no correlation between variation in building rules and subdivision between spatial groups. We can derive a general rule that states that, as long as the total number of variants approaches the number of workers engaged in building, the collective outcome will be functional - accordingly, the presence of multiple (normally-distributed) phenotypic variants produces a high-quality outcome. In the intermediate case in which workers are divided into a few (*i.e.*, more than two and much less than the total number of workers) variant groups, such as patrilines, we see that the phenotype is destabilised if all variants are present at equal frequency in the colony and if the difference among variants is large.

How functional are the products of the activity of mixed colonies? Do the colonies building them suffer from lower fitness compared to colonies where all builders apply the same rule variant? There is currently not a good empirical understanding of how features of the nest and of the building process (*e.g.*, time to structure completion) map onto measures of reproductive fitness. In this thesis, I have made the plausible assumption that two indexes of wall quality, the presence of gaps and the compactness of the structure, are important for the survival and the adaptation of the colony. Even in the case where this assumption is correct, the relationship between these wall traits and colony reproductive fitness remains an open question. Experimental studies will help determine what features of the wall are important for colony survival and how much so. In this study, I have therefore been unable to track the changes in colony fitness resulting from changes in colony allele frequencies in the building rule genes and, more generally, under varying degrees of within-colony phenotypic variation. In the case of transition between workforce phenotypes that is expected following the queen replacement with introduction of a mutant worker line, given the short time scale, we are looking at a decrease in colony survival chances rather than reproductive fitness directly, because the transition might be completed in less than one reproductive cycle. The expected fitness under transition structures may be intermediate to the one under the start- and end-point collective phenotypes. If the relationship between individual and collective phenotype is additive, then selection on the group phenotype in genetically mixed colonies always leads to the group phenotype optimum. On the contrary, non-linear relationships are expected to lead to local optima or even fix the phenotype in a stable suboptimal evolutionary point. Convergence rules and buffer zones can avoid extreme non-linearity resulting in low-fitness intermediate phenotypes, but the intermediate stage may still suffer from lower fitness compared to the expectation under an additive relationship between individual and collective phenotype.

I have identified two elements that are linked to phenotypic robustness in the case of *Temnothorax* walls: stigmergic feedback and wall thickness. Stigmergy acts as a convergence rule in *Temnothorax* wall building and is responsible for the merging of structures that would otherwise be spatially separated (compare **Figure 4.11A top** and **bottom**). Worker preference for optimal wall thickness is associated with changes in the size of the buffer zone, because the larger the desired wall, the larger the area in which stone deposition should occur with high probability. When the convergence rule is absent but the buffer zone present, all variants are expressed in the collective phenotype, creating a dispersed wall. When even the buffer zone is removed, they are expressed separately in space, resulting in a discontinuous structure. The size of the buffer zone makes convergence possible by allowing the two templates to overlap. Under evolution for evolvability, we expect selection on the strength of the stigmergic effect, which should be sufficiently strong to counteract the template effect and lead to convergence, but not enough to lead building activity astray towards the formation of incipient structures in areas not favoured under the template. This is important as the template contains information on structure function. We also expect selection on the size of the buffer zone, which should be optimised to enable overlap between different variants. My expectations are that constraints that might limit the size of the buffer zone should be rare and caused by selective pressure for a thinner wall: for example, in hard-soil environments it might be harder to find sufficient building material, in which case a thinner wall with no gaps may be preferable (*i.e.*, more defensible or better at preserving the right nest conditions) to a thicker wall with a few gaps. In more dangerous environments with more predators, or in hotter climates where the colony needs to isolate itself from the outside as fast as possible, there might be a stronger selective pressure on time needed to complete the wall, which again may result in preference for thinner walls. These circumstances might result in preference for thin walls even more easily if within-colony phenotypic and genotypic variation affecting building behaviour is low in the colony population and there is little selective pressure for this type of robustness. The first step towards better understanding the selective pressures at play is, again, to experimentally identify how individual characteristics of the wall are related to its function, and this latter to colony reproductive fitness. Eusocial nest building species for which nest functionality is clearer, such as the termite *Macrotermes* or the leaf-cutter ants *Acromyrmex*, might offer an alternative testbed to *Temnothorax*. An interesting question would be to test the theoretical limits of the buffer zone while shedding some light on wall functionality. How thin can walls get before they are unable to fulfil their homeostatic function? At what point does it become undefendable from invaders? Long-term evolution experiments conducted in the laboratory might also shed light on how evolvable buffer zone and convergence rules are.

The *Temnothorax* wall is a simple structure that can be modelled two-dimensionally. 3D nest structures might result in more complex interactions between variants of the same building rule, or between one rule's variants and the other rules of the same set. Other cases of nest building may therefore result in more rugged phenotypic landscapes of the changes in architecture following changes in colony phenotypic composition. However, my expectation is that, in cases featuring a template, buffer zone and convergence rules will play a similar role to the one identified in this study. Additional studies on more complex nest building rule sets will verify whether these findings can be generalised and if additional rules, beside the one modulating convergence and buffer zone, are necessary under specific scenarios.

Simultaneous variation in multiple building rules is also a scenario where more complex dynamics of interaction are likely. The effect on the emerging structure will depend on the nature of the rules in which variation is present. More modelling work is necessary to understand whether we should expect more complex interaction landscapes and how different rule types interact with each other. Some of this work could be undertaken using the *Temnothorax* model; however, it offers a limited range of rules (for example, simultaneous variation in the distance rule and in τ could be studied using this model). A more in-depth understanding will be achieved by analysing and comparing multiple building algorithms. Studying multiple scenarios of this type, and in multiple nest building species, can inform us on the real degree of robustness of eusocial building systems.

From an ecological perspective, my findings indicate that the adaptive landscape of single collective building rules in eusocial colonies can be smooth, and that ICF is not a necessary step for mutations to survive until their phenotype is expressed at colony level.

4.8 Chapter conclusions

The components of the *Temnothorax* model that achieve robustness of the collective phenotype to individual variation are common ingredients of collective behaviour algorithms. Collective behaviour algorithms as we know them are the product of a long process of species survival and evolution in which nest building plays an important brood care role. One possibility, in regard to the evolution of the algorithm robustness components, is that their existence is the result of selection for a smooth transition of individual-level phenotypes into a group-level one (in the case of eusociality, a major transition), and for the evolvability of such group phenotypes. Alternatively, their initial emergence might have nothing to do with their robustness (to individual variation) properties. For example, they might emerge from basic requirements for nest architectures, such as the structural or defensive need for thick walls (which creates buffer zones), or follow on from rules in the algorithms for individual self-organised building, such as individual estimates of local stone density, which in individual behaviour might be used to avoid cognitive requirements of conformation recognition or of recipes and in collective behaviour result in the indirect transmission of information among nestmates, through stigmergy. In this case, the evolution for robustness to individual variation and for evolvability of the collective phenotype is a matter of fine-tuning pre-existing rules rather than the creation of new ones. That evolution of self-organised behaviour might involve fine-tuning more than step-by-step initial emergence has previously been suggested in relation to other features of eusocial colonies, such as division of labour (Page & Mitchell, 1998). In this case, the authors suggest that division of labour might follow on from the behavioural and neurological mechanisms underlying responses to cues in insects (*i.e.*, the existence of individual variation in response threshold and the use of stimulus-response mechanisms to regulate task activity), even in solitary behaviour. I will discuss some ideas about the evolution of self-organised collective building in the concluding chapter, **Chapter 5**.

Chapter 5: Discussion and conclusions

In this thesis, I have so far evaluated, revised and further developed a behavioural model of self-organised social insect nest building, in order to study one specific evolutionary question: how collective building algorithms transition from one rule variant to another, without the colony suffering from a major loss of fitness during the intermediate phase (in which multiple rules are expressed at the same time). In particular, my goal has been that of identifying the role played, if any, by building algorithm components in this process of evolution of the algorithm itself. The literature on the evolution of self-organised algorithms is increasing steadily, albeit slowly, partially because of methodological difficulties, partially because knowledge of many algorithms is still lacking or incomplete. In this chapter, I present an overview of the current state of knowledge and main hypotheses on the subject, and fit my findings in this framework. In particular:

- In **Section 5.1**, I discuss the evolutionary origin of individual algorithm components, and how evolution can result in a set of compatible rules that coordinate the activity of multiple individuals.
- In **Section 5.2**, I move on to addressing adaptation in building algorithms, introducing the concept of parameter fine-tuning. I distinguish between two alternative modes of adaptation that result in interspecific differences but that rely on different mechanisms. They are genetic hardwiring and environmental plasticity. Researchers need further experimental studies to identify the components of social insect nests that emerge from the one and the other process. I conclude this section by addressing the problem of distinguishing common evolutionary origin from convergence, and by identifying some potential case studies.
- In **Section 5.3**, the final section of this discussion, I propose a hypothesis for the evolutionary trajectory taken by *Temnothorax* nest wall building. This time-line can be tested by addressing a specific set of research questions, which I list, together with the proposed research approaches.
- In **Section 5.4**, I summarise the major emerging themes from this chapter and from the thesis as a whole.

5.1 Evolution of self-organised social insect nest building: from solitary to collective

There are two conceptual challenges that need resolving to understand the emergence of self-organised collective building algorithms:

1. *How do sets of compatible rules emerge?* Self-organised algorithms are complex systems and, as such, characterised by the non-linear interaction of their components. In a complex context, subsets of components might have lower fitness compared to a different subset containing one less unit. Therefore, it can be argued that step-by-step evolution of these algorithms requires either

the existence of a linear evolutionary trajectory in which each additional component results in a new subset with equal or higher fitness than the previous subset or the emergence of multiple components at the same time.

2. *How do sets of rules come to ensure coordination when used by multiple individuals?* Algorithms that produce a coherent and efficient structure when used by a single individual might not be effective when applied in parallel by many individuals. They might result, in a collective building context for example, in the same structure being redundantly built multiple times by different individuals, or in inefficient time allocation due to the lack of coordinated traffic at or between building sites (see **Section 1.2.3** for a more detailed discussion of the problem of applying solitary rules as a group).

Both issues are problematic independently from the point along the phylogeny from solitary to group living at which the building algorithms have evolved. For example, if the building behaviour under study has evolved at the solitary stage, it is possible that the original algorithm might have resulted in a lack of coordination after the transition to group living, with a consequent, expected decrease in architectural functionality or increase in time needed for construction, and thus with a decrease in reproductive fitness. Evolution of the behaviour after the transition to group living, on the other hand, means that new rules appear in a context in which the majority of workers lack the new behaviour, resulting in potential incompatibility between rules followed by colony-mates. Time of evolution in relation to the timeline of social evolution needs to be taken into consideration when attempting to reconstruct the evolutionary history of an algorithm.

Below, I will discuss the two problems separately.

5.1.1 How do sets of compatible rules emerge?

One simple answer to this question is that of the already-mentioned step-by-step trajectory. In this scenario, the first rule to evolve must be adaptative in the absence of other rules, while subsequent components are adaptive either on their own or in combination with pre-existing rules. A complex system that can be decomposed in this fashion is called *reducible* (Suzuki, 2017).

A second solution is the emergence of multiple rules within the same evolutionary time step. However, this route so far lacks empirical support: studies of several complex traits indicate that, for all cases for which a most likely evolutionary trajectory has been proposed based on data analysis, this trajectory is always one of step-by-step evolution, and does not require large evolutionary jumps involving multiple simultaneous innovations (Suzuki, 2017). In particular, such studies indicate that initial or intermediate steps were often adaptive towards a function different than the current one. For example, in the case of mammalian lactation, the milk gland is believed to have emerged from a skin gland through co-option. In the case of self-organised building algorithms, some rules might initially have emerged to fulfil a function other than nest building. One potential case are the pick-up and deposition responses to material density seen in *Temnothorax*. These can be ascribed to a more general class of local-activation-long-range-inhibition mechanisms (Camazine et al., 2001) involving material quantity as a cue, and that are observed across ant species in a wider range of behaviours than those involved in building, particularly the formation of corpse piles (Theraulaz et al., 2002). This resemblance between the mechanisms underlying two key colony behaviours might be due to chance: the same, very simple dynamic has evolved twice to underlie both. Alternatively, modulation of pick-up and deposition activity might have initially evolved as a hygienic response, leading to corpse removal, and been subsequently co-opted for nest construction (in this case, assumed to evolve after colony living). Improving the range of available data on the cues modulating necrophoric pick-up and deposition activity across ant species will enable the application of phylogenetic approaches to distinguish between convergence and single evolutionary origin of wall and corpse pile formations in *Temnothorax*.

A third solution is that the ancestral form of the algorithm is a direct consequence of system properties: the physical properties of, for example, the construction material, or the neurophysiology of worker behaviour. In the context of self-organised behaviour, Page and Mitchell (Page, 1997; Page & Mitchell, 1990, 1998) show that division of labour can emerge from the basic assumptions of cue-response mechanisms (underlying colony tasks) and inter-worker variation in the response threshold, two system properties. Miramontes and colleagues (Miramontes et al., 2001) show, with an agent-based model, that neural network structure is sufficient to explain the alternation of activity and inactivity in ant workers, which is then turned, through simple individual interactions, into periodic collective activity. A detailed understanding of the neural architecture of social insects is needed in order to enable researchers to identify any potential origin of behavioural traits as a by-product of the pre-existing cognitive architecture – perhaps even the coupling of multiple behaviours through this architecture, which may underlie the first link between building algorithm rules. Cavagna and colleagues (2018) prove the importance of theoretical models for understanding which components of a behavioural algorithm are the result of the logical and physical constraints of the system and which ones arise through adaptation. Studying collective motion in bird flocks, the authors show that the scale-free correlation of the group's velocity change is a consequence of the system's symmetry properties. Conversely, the observed scale-free correlation of speed can only emerge through adaptation: this property comes into being only when one of the parameters sits at its critical-point value.

In the case of social insect nest building, a characteristic that could emerge from system properties (in this case, material properties) is structure shape. Thompson (Thompson & Thompson, 1942) made the argument that the optimal shape and structure of honeybee combs could emerge from physics-driven adjustment of molten wax on a tubular cell (through tension of the adjacent film), if the wax were to be applied in a liquid state. Experiments have shown that, although the temperature needed to maintain the wax liquid is higher than that observed in honeybee nests (Bauer & Bienefeld, 2012), cells are indeed created circular and become hexagonal through progressive fusion, possibly (but not definitely) through worker manipulation, following the predicted physical mechanism (Karihaloo et al., 2013). These findings do not, then, exclude the need for specialised behaviour to evolve to perfect cell shape, but they do reduce the number of evolutionary steps needed, and therefore this process remains a useful example of how similarly advanced features of an algorithm (in this case, of a self-assembly algorithm) could emerge through physics rather than biological adaptation.

5.1.2 How do sets of rules come to ensure coordination when used by multiple individuals?

Whether the building algorithm originated in a solitary species, as the set of rules for building a solitary nest, or in an already colony-living species, the use of the algorithm by multiple individuals requires additional components to coordinate parallel activity – for example, in order to avoid crowding issues, such as workers queuing for access at the same building site while other sites remain unattended to. Some research using evolutionary algorithms and robot swarms (Fujisawa et al., 2019) has shown that regulatory mechanisms enabling collective use need to evolve before self-organised components: in the case presented in the study by Fujisawa and colleagues, a traffic-avoidance rule needs to evolve in advance of pheromone trail-following behaviour. If this evolutionary sequence turns out to be a rule-of-thumb in the evolution of collective (building) algorithms – and, again, more case studies are needed –, then we should expect co-option (*i.e.*, the regulatory mechanism evolved under selection for a different function) or/and cryptic variation (*i.e.*, the regulatory mechanism was present but not expressed or constituted neutral variation) to play an important role in the evolution of these algorithms.

There is also an alternative possibility: some solitary species rule sets might be coordinated or nearly-coordinated when used by multiple individuals at the same time. That is, combinations of building rules that are efficient (or result in little loss in fitness) also when used by a collective are more common than can be intuitively expected. Testing this hypothesis requires simulations (such as evolutionary algorithm approaches) to explore the evolutionary landscape of solitary building algorithms when used collectively. Theraulaz and Bonabeau (1995) and Bonabeau *et al.* (2000b) conducted a similar investigation, probing the landscape of self-assembly algorithms that give rise to wasp nest-like structures in the search for algorithms that produce coherent (*i.e.*, reliably emerging across multiple simulation repeats as well as structurally organised) architectures. Their findings contradict the idea that coordination is a common property: at least for self-assembly rules, the subset of landscapes containing coordinated algorithms is small and localised. To the point, in fact, that Theraulaz and Bonabeau had to adopt a backward approach, starting from a coherent final structure to identify one set of coordinated rules, because a random search of the landscape could not return any algorithm with these properties.

Once a coordinated algorithm has emerged, however, it benefits from robustness to rule variation (Theraulaz & Bonabeau, 1995). This finding is equivalent to the outcome of the investigation I presented in **Chapter 4** for a self-organised algorithm, albeit robustness refers to two different processes in the two studies. In the case of Theraulaz and Bonabeau's study, the coherence of the phenotype appears robust to small-step mutations in algorithm structure, that is, to transition between distinct neighbouring algorithms; in the case of *Temnothorax* wall building behaviour, robustness refers to quantitative variation in parameter values, without alterations in the algorithm used. In my study, I have shown that the convergence rule and the buffer zone are the key elements of the algorithm that enable the translation of individual variation in parameter value to a common collective phenotype. That is, if a functional form of these two elements existed in the archaic form of the algorithm that was first used at collective level, coordination would have ensued even in the presence of phenotypic variation (albeit, perhaps, with a lesser degree of efficiency than the final form of the algorithm). Both the tendency to build a thick wall (the buffer area) and stigmergy (the convergence rule) should be expected to be ancestral elements of the building algorithm, because they are essential for nest defence (the first) and to assemble a higher quality wall through gap-filling behaviour (the second). Therefore, once this ancestral algorithm evolved, multiple individuals expressing variation in the parameter values of other building rules might have still been able to build in a coordinated manner – that is, successive forms of the algorithm might have emerged after the evolution of, for example, DCF. The only requirement is that the range of parameter space of these two key components that allows for robustness to individual variation (not explored in **Chapter 4**) be sufficiently large that many combinations of parameter values result in sufficiently coordinated activity. Later selection (parameter fine-tuning; see **Section 5.2**) is expected to move the convergence rule and buffer zone parameter values closer to the optimum for this type of robustness. Notably, this proposed trajectory does not take into account interference in traffic flow: my model uses random movement from any location of the nest to any other, meaning that issues with traffic control could not be identified. It is possible that *Temnothorax* also uses avoidance mechanisms, and in this case regulatory mechanisms might also be part of (and perhaps pre-date other components of) the ancestral algorithm, as in the case study of Fujisawa and colleagues.

Detangling the problem of the initial evolution of collective building algorithms requires widening the variety of case studies to include new species, and improving the resolution and accuracy of the existing behavioural models using a mix of empirical data and theoretical studies. Using this background knowledge, evolutionary algorithms can be applied to identify the most likely evolutionary trajectory that leads to the formation of a functional rule set. In the next section, I discuss how the same range of knowledge will enable us to understand evolutionary change on already-emerged algorithms.

5.2 Evolution of self-organised social insect nest building: adaptation, phylogeny and convergence

Central to the processes of adaptation and convergence is the concept of parameter fine-tuning. Parameter fine-tuning is the quantitative change (either short-term and in response to the environment, or evolutionarily hard-wired through selection and drift) in the behaviour of workers, such as in the intensity or in the probability with which their response occurs. This corresponds, in the mathematical representation of a behavioural model, to a change in the value of model parameters, and not in the alteration of the algorithm through the gain or the loss of rules (that is, the structure of the algorithm remains unaltered). Several theoretical models created in the past three decades (combined with phylogenetic work; Franks et al., 1991; Karsai & Penzes, 1998; Khuong et al., 2011; Mizumoto et al., 2015; Mizumoto & Bourguignon, 2020; Pratt & Sumpter, 2006) point to the fact that it is parameter fine-tuning and not differences in the algorithm that underlie observable between- and within-species differences in collective behaviour outcome, such as nest shape or foraging trails (*e.g.*, Franks et al., 1991; Khuong et al., 2011).

We know – from a ground-breaking long-term study of desert harvester ants by Gordon (2013) – that variation in parameter values (in this case, in worker probability to forage under harsh environmental conditions) is directly linked to colony reproductive fitness and thus under natural selection. One question arising from this is to what extent and where have fitness-enhancing values of parameters become genetically encoded (from here on, *hard parameter fine-tuning*) and to what extent do they remain environmentally modulated (that is, parameter value changes in response to conditions; *soft parameter fine-tuning*) and provide a dynamic response to varying environments and challenges. In the context of nest building, the question translates as whether differences in nest features are a case of hard fine-tuning, and therefore often species-specific, or are the result of different environments, and moving the same species to a new environment will cause it to build a nest with features similar to those of indigenous closely-related species. A study of shelter tube building in lower termites (Mizumoto & Bourguignon, 2020) found that most modern termites, including species that do not build these structures in the wild, are capable of creating them if triggered by nest damage and starvation. This case study seems, therefore, to coincide with soft parameter fine-tuning. A study by the same research group seems to suggest that there is also a colony-specific component to shelter tube building: in the termite *Reticulitermes speratus*, shelter tubes built by workers of the same colony, in small and isolated groups and in a neutral environment, are more similar than shelter tubes built by workers from a different colony (Mizumoto et al., 2015). Based on this study, it cannot be excluded that these behavioural differences are transmitted through, for example, experience, rather than genetics: the colonies used in this study were collected from the wild, and this species is known to differ from most termites for having a high proportion (46.6%) of “mixed families” (colonies where workers are the offspring of multiple unrelated reproductives; Hayashi et al., 2005). This fact implies that randomly selected workers might share a low degree of relatedness and therefore similarities in the structures they build must be attributed to epigenetic rather than genetic effects. Unfortunately, the authors did not perform a genetic analysis that could support either hypothesis.

Are there species where differences in nest shape and structure are known to be species-specific without being environment-specific? East-Australian stingless bees are one such case: the brood nest morphology of four, molecularly identified species, inhabiting close geographic locations and displaying similar worker morphological variation with latitude, varies, without being correlated to environmental characteristics (Brito et al., 2014; Franck et al., 2004; Oldroyd & Pratt, 2015).

Of features, including flexible response mechanisms, that are common to multiple species, it is necessary to distinguish between those conserved across closely related species and the convergent products, created by different rule sets, of similar environments or requirements. Gordon (2016b) discusses convergence and divergence in ant foraging strategies and highlights some points of interest. Gordon makes the argument that, while on the one hand similar foraging strategies of different ant species might be a convergent response to similar pressures, including risk of foraging disruption, on the other hand the intermediate stages that arise from disruption are also a source of material for selection and adaptation that might result in divergence. A similar point can be raised in relation to nest building.

The work done by Mizumoto and Bourguignon (2020), described above, is one of the few cases where phylogenetics has been applied to social insect nest building to address the problem of separating convergence from phylogeny. A different, potential test case for this method is the reliance on temperature and sun exposure by *Odontotermes obsesus* and *Macrotermes michaelseni*, two species of higher termites. In both species, the diurnal oscillations in heating and insolation drive the convective flow of air within the nest, driving out stratified gases and ventilating the colony (King et al., 2015; Ocko et al., 2017). Despite inhabiting very different environments (the forests of South Asia versus the grasslands of South Africa) and building nests with distinct shapes, it has been shown that both species may relay on the same mechanism to control ventilation and nest expansion (Ocko et al., 2019). This model of mound mechanics also explains the diverging mound shapes of the two species. Does this similarity in mechanism have a single evolutionary origin or is it a case of independent 'discovery' at different points along the lineage? By breaking down the rules used by multiple termite species for mound building, we can obtain data on which to apply a phylogenetic approach to distinguish common origin from convergence.

As the literature on social insect nests increases, we are starting to see patterns whereby the same templates or the same mechanisms are used by different species, sometimes distantly related ones. Phylogenetic analysis will help us identify convergence and common origin for many of them. For example, both *Formica lemani* and *Myrmica ruginodis*, but not *Lasius niger*, use their position in relation to the vertical as a reference for the digging angle (Sudd, 1972). Modelling work has also shown that a stigmergic effect of surface curvature is sufficient to generate structures similar to the nests of *M. michaelseni* and of two species of arboreal *Nasutitermes*: *N. ephratae* and *N. walkeri* (Facchini et al., 2020). For *M. michaelseni*, this mechanism is supported by empirical data (Calovi et al., 2019). But perhaps the mechanism that is most strikingly similar across species is the stigmergic feedback regulating excavation and underlying nest enlargement in ants. As mentioned in **Chapter 3**, in the nest-digging species *Linepithema humile*, *Messor sancta* and *L. niger*, nest expansion is modulated by the density of building sites along the nest perimeter: the smaller the nest, the higher the density of ants initiating digging at its edges, the higher the recruitment at these building sites, which exercise an attractive effect on nestmates (Buhl et al., 2005; Halley et al., 2005; Rasse & Deneubourg, 2001; Toffin et al., 2009). This positive feedback mechanism slows down and eventually ends as the nest (and its perimeter) grows, causing a decrease in the density of excavation sites. Digging eventually stops, until colony size increases sufficiently to produce a new increase in the density of building sites. The correlation between colony size and nest size in ants is thereby explained. In some species, a pheromone might be emitted during digging that reinforces the positive stigmergic effect of active excavation sites. This might be a component of later evolution, emerged to reinforce and limit in time the pre-existing stimulating effect of digging site configuration. Similarly, the relationship between stone density and deposition activity in *Temnothorax* might have the same origin as this stigmergic local amplification process, with the difference that the use of a template limits nest expansion – so that a substitutive mechanism for the decrease of building site density is needed for behavioural termination. As, again, previously discussed in **Chapter 3**, this alternative could be provided by a stigmergic response to high stone density that occurs through the mechanical interaction with a compact wall, or through the use of air currents as a cue. The template component of the *Temnothorax* algorithm might initially have evolved to optimise nest size for the temperature and humidity homeostasis needed for brood rearing, or as an alternative mechanism to link nest size to colony size, or both.

I started this section discussing parameter fine-tuning and how it is key to evolutionary adaptation and divergence. An additional consideration should be made on how algorithm parameters evolve. Notably, a study by Page and Fondrk (1995) involving artificial selection on the proportion of pollen foragers in honeybee colonies suggests that fine-tuning of some parameters might involve trade-offs between features, either because the parameters controlling them are interconnected or because they are under the control of one single parameter. In their study, the authors show that an increase in the proportion of pollen foragers corresponds to a decrease in the proportion of nectar foragers, and vice versa.

The picture emerging from the literature is one where algorithm parameter values contain both hard-wired components and flexible response mechanisms that trigger soft fine-tuning in response to local conditions. This is not unlike the combination of genetically encoded and plastic responses characterising most other animal traits. Of the nest characteristics and algorithm structures shared by social insects, much similarity might be caused by conservation, some by adaptation to similar environments, and perhaps a little by fortuitous independent evolution of the same, simple mechanism to meet different needs. Of the features not shared, some might be cryptically present in closely-related species, as is the case for shelter tubes in lower termites, and re-emerge when exposed to the right stimuli. More experimental and computational studies are needed to appreciate the true range of mechanisms used in social insect nest building, after which we will be able to apply phylogenetic methods to identify the most likely evolutionary path.

In the next section, I will summarise the points I have made so far on the possible evolutionary events that gave rise to *Temnothorax* wall building and propose a few testable hypotheses for the evolutionary trajectory taken.

5.3 Evolution of *Temnothorax* nest wall building: proposed evolutionary path

I have mentioned the importance of widening the variety of species used in studies of social insect nest building, for a more complete understanding of the existing collective building behavioural algorithms. These data and the associated mathematical and computational models will provide the specimens we need to reconstruct the evolutionary history of these algorithms through phylogenetics. As an illustrative example, based on the material presented in this thesis, I propose here a putative path that the evolution of nest building might have taken in *T. rugatulus* and *T. albipennis* – with no claim that this path is likely to be the one that actually occurred, but accompanied by a suggested research programme to put its correctness to the test.

Below, **Figure 5.1** summarises the trajectory. Given that the same nest enlargement mechanism seems common among distantly related ant species, it is not unreasonable to hypothesise that digging is the ancestral form of nest building in ants. This conjecture is testable with a phylogenetic approach, once we have identified the nest building strategy of a larger number of species. Both *T. albipennis* and *T. rugatulus* are capable of both excavation and wall building using material found outside the cavity, and it can be suggested that the emergence of wall building behaviour increased colony reproductive success by widening their ecological niche – making previously unsuitable, because unprotected, cavities inhabitable – and by reducing nest building time and/or effort. Building with foreign material might have emerged as an exaptation of the same mechanisms underlying necrophoresis, which relies on local activation followed by termination via substrate depletion (Theraulaz et al., 2002). Based on our understanding of midden pile formation mechanisms in *Messor sanctus* (Jost et al., 2007), we can hypothesise that co-option of building behaviour from midden work implies that building termination is based on the use of air currents as a cue. The mechanics of necrophoresis in *Temnothorax* are currently unknown and so is the involvement of air currents in wall building behaviour – two issues that can be resolved through experimental manipulation of air flow in laboratory-housed colonies. Under the ancestral-excavation hypothesis, nest size would be originally modulated only by colony size. Reliance on air currents only, as a building cue, breaks this link: the wall emerges close to the cavity entrance (entailing a waste of building effort if the cavity is large) or wherever response to air currents is stronger, and nest conditions may be suboptimal for worker movement and/or for brood care, if wall location is important for temperature and humidity homeostasis. The final component of the building algorithm, a pheromone- or a mechanic-based template relying on worker-brood or worker-worker interactions, may thus have been positively selected for. The type of template mechanism used can be identified by manipulating the presence and quantity of brood and walking pheromone in laboratory colonies. Worker-worker encounter rates at the edges of the nest might also be important in linking nest size to colony size, if high encounter rates are used as a cue for crowding and consequently trigger nest enlargement. The correlation between encounter rates and building rates can be measured experimentally and the role of the mechanism tested computationally.

Already at the stage of co-option of necrophoric mechanisms, we find the presence of a convergence rule. What about the buffer zone? Depending on the thickness of the wall produced by the original algorithm, the size of the buffer zone might have been sufficient to generate a homogeneous wall in the presence of individual variation. Selection for robustness to variation is expected to have driven parameter fine-tuning of both components to the values that we observe today. Phylogenetic reconstruction of the parallel evolution of building behaviour and queen re-adoption may inform us as to how early on this type of selective pressure might have been present – assuming, of course, that genetic rather than epigenetic factors underlie worker variation. Current *Temnothorax* colonies might have preserved plasticity in their buffer zone and convergent rule parameter values, using soft fine-tuning to match them to their environment.

Whatever the exact nature of the components of the *Temnothorax* wall building algorithm, once these have been identified with sufficient accuracy, we can rely on an evolutionary algorithm approach (particularly, genetic algorithms) to infer the most likely evolutionary sequence of this rule set.

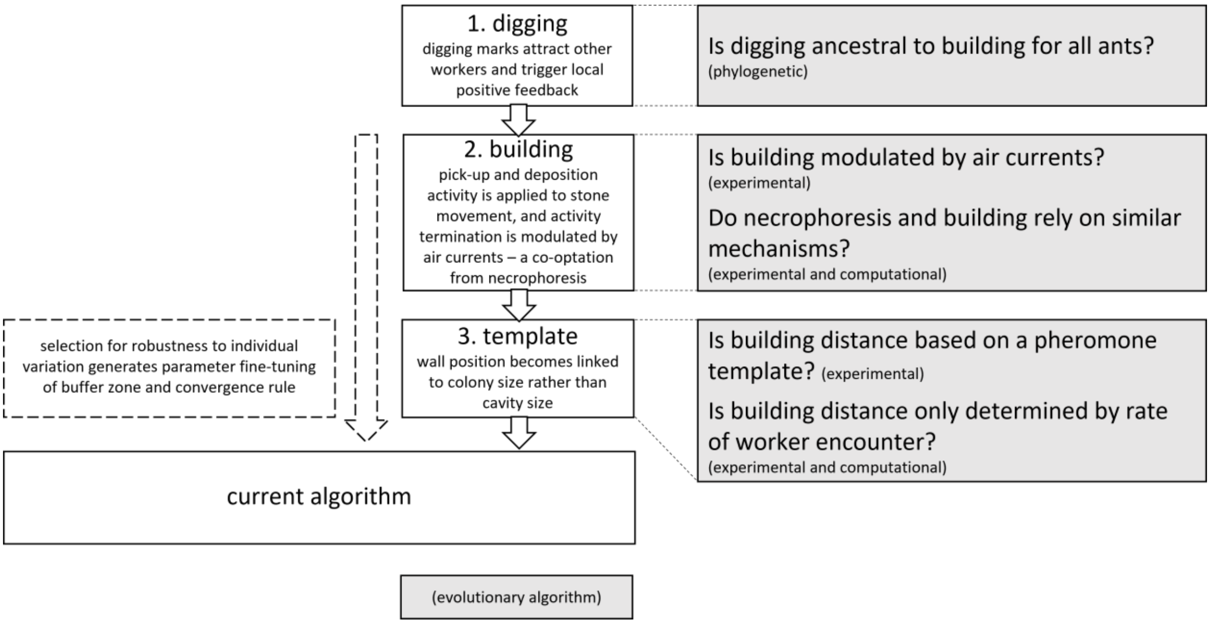

Figure 5.1. A proposed path for the evolution of *Temnothorax* nest building and questions to be resolved. The figure shows a proposed evolutionary sequence for the evolution of *Temnothorax* nest building behaviour. The transition from digging to building is assumed to emerge after the transition to social living, which is in agreement with digging being ancestral to ants. *Solid box outlines* indicate that the event results in the addition of an algorithm component. *Dashed box outlines* indicate a parameter fine-tuning process. *Grey shaded boxes* contain outstanding research questions (*large font*) and the research methods needed to address them (*small font, in brackets*).

5.4 Conclusions

Our knowledge of the *Temnothorax* behavioural model has now gained two pieces: a termination mechanism and the identification of the components that grant it evolutionary robustness to variation in individual parameter values. Stigmergy and other feedback mechanisms that generate convergence of individual phenotypes – and that are usually associated to decision-making – play a role in evolutionary robustness. I hope I have reinforced, in this thesis, the notion that developing accurate and data-based theoretical and simulation models of collective nest building allows us to compare and identify the algorithms used by different species and relate them to those used in different tasks, thereby collecting the data necessary to reconstruct the evolutionary trajectory of these complex traits. Understanding what parameters and rules have evolutionary implications, such as the buffer zone and the convergence rule, is important for building motivated hypotheses: for example, understanding how much the strength of positive stigmergic feedback and the thickness of the wall can vary before the structure breaks down in the presence of variation, in *Temnothorax*, allows us to put the evolution of these components in the right position on the time line of the evolution of DCF.

Our current understanding of the evolution of these algorithms tells us that there is likely a combination of mechanisms at play: full algorithms emerge, most likely from step-by-step evolution, while parameter fine-tuning (soft and hard) acts on existing rules to optimise the mechanism towards the current environment. In addition, we have so far not eliminated the hypothesis that, in a subset of cases, some properties (especially those regarding shape) may emerge in their most ancestral form from properties of the system, including the physical properties of the material.

The nests of eusocial colonies are complex architectures. The full algorithms producing the architectures that we observe in nature are much larger than the ones simulated in single studies: they connect and account for many more structures and scenarios than what has so far been modelled. For example, in many ant species, nest construction occurs both through building and through digging, and the two behaviours produce very different structural elements (*e.g.*, in *L. niger*; Khuong et al., 2016; Rasse & Deneubourg, 2001; Sudd, 1972). Current research is (with a few exceptions; *e.g.*, Toffin et al., 2009) rightly focusing on the structural units that can be identified as fairly independent components of the full architecture. Future research will need to switch the focus to the mechanisms that generate structural transition and reconstruct an evolutionary sequence that encompasses the full nest.

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