SYNCHRONIZED ACTIVITY RHYTHMS AND COLLECTIVE MOTION IN ANT COLONIES

SYNCHRONIZED ACTIVITY RHYTHMS AND COLLECTIVE MOTION IN ANT COLONIES.

By GRANT NAVID DOERING, B.S.E.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the
Requirements for the Degree Doctor of Philosophy

McMaster University

McMaster University DOCTOR OF PHILOSOPHY (2021) Hamilton, Ontario.
Department of Psychology, Neuroscience & Behaviour

TITLE: Synchronized activity rhythms and collective motion in ant colonies.
AUTHOR: Grant Navid Doering, B.S.E (Arizona State University)
SUPERVISOR: Paul A. Faure
NUMBER OF PAGES: xi, 155

THESIS ABSTRACT

The ability to synchronize both the movements and activity rhythms of many interacting individuals is a major component of the repertoire of collective behaviour in social species. Among the many social animals on Earth, ants have some of the most spectacular forms of synchronized behaviour because of their eusociality. One example of this impressive behavioral synchronization in ants is the so-called *short-term activity cycle*, where the workers inside a nest will move together in rhythmic pulses of activity that are separated by moments of collective rest and inactivity. There are many aspects of short-term activity cycles that are poorly understood. The first chapter of this thesis makes the case for why ants are a useful taxonomic group for studying social synchronization and introduces the phenomenon of ant short-term activity cycles. In chapter 2, I study intraspecific variation in short-term activity cycles and show that colonies' queens, number of brood items, and number of workers all influence aspects of colony-level activity oscillations. In chapter 3, I show that colonies are capable of synchronizing despite noise in the behaviour of individual ants, and that colonies can modulate between multiple collective rhythms. In the same chapter, I also devised a model to study the role of noise more generally in excitable systems that have properties similar to those in ant colonies. Chapter 4 examined the effect of 24-hour light cycles on circadian activity, and I found that colonies kept in conditions with constant light or darkness had weaker circadian rhythms than colonies in an alternating light/dark regime. However, colonies' short-term activity cycles were not affected by the external light regime. In chapter 5, I provide evidence that having synchronized rhythms of rest and activity provides a functional benefit for colonies: workers can, on average, more fully inspect the inside of the nest when activity is synchronized because inactive ants aggregate into piles that active ants have difficulty penetrating. In chapter 6, I recount a history of the research into synchronized and rhythmic activity patterns inside ant nests and consolidate the new results from the previous chapters with the existing literature to create an up-to-date review of what we currently know about short-term activity cycles. The work compiled here thereby sheds new light on an enigmatic form of synchronized behaviour in ants, one of the most ecologically important groups of animals on the planet.

ACKNOWLEDGEMENTS

I would like to thank my advisor Paul Faure for his kind support. Although circumstances made it so that he could not remain my supervisor, I would also like to thank my former advisor Jonathan Pruitt for his outstanding encouragement and support during my degree. I am also grateful for the guidance provided to me by all the other members of my thesis committee: Stephen C. Pratt, Laurel Trainor, and Kari Dalnoki-Veress. I am very appreciative of all the help Nancy Ridell, Milica Pavlica, Judith Shedden, Sally Presutti, and Wendy Selbie provided me in understanding the administrative processes of both McMaster and the department of Psychology, Neuroscience & Behaviour. Many undergraduate students were essential to the success of this thesis. I especially thank Greg Thung, Martina Bravo, Amy Kalbfleisch, Amy Cameron, Madeleine Matthews, Paul Ostlund, Tricia Skelton, Avani Pathak, and Haolin Ye for assistance with ant feeding and maintenance. I thank John Bentley at McMaster along with Nancy Ruth and Brenda Connelly of the CFIA for help navigating the processes of importing of ants into Canada. I am grateful to the Ghent University Library for providing me with a scanned copy of a 1944 French article by R. Chauvin that was not otherwise available online. I would also like to acknowledge the input and feedback that I received on my research and the support from numerous collaborators, mentors, family members, and acquaintances: Corrie Moreau, Matthew Prebus, David Earn, Michael Guevara, Leon Glass, Holly Moeller, Linda Petzold, Brian Drawert, David Fisher, Jim Barnett, James Lichtenstein, Jonathan Dushoff, Sigal Balshine, Andrew Burchill, Yohan Cho, Takao Sasaki, Ted Pavlic, Kirsten Sheehy, Janice Yan, Tovah Kashetsky, Brendan McEwen, Hannah Anderson, Raul Costa-Pereira, Pat Bennet, Elva Robinson, Adam Cronin, Słwavomir Mitrus, Fariba Zarghami, Shidan Dadfar, Richard Doering, and Mahvash Doering. Finally, I would like to thank Reuven Dukas for allowing me to use the dissecting scopes in his laboratory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

DECLARATION OF ACADEMIC ACHIEVEMENT

This dissertation, which contains 6 chapters, belongs to the "sandwich thesis" category defined by McMaster University. **Chapter 1** serves as an introduction to the overall content in the thesis, summarises the contents of each subsequent chapter, and contains some additional data that was not presented in any of the subsequent chapters. **Chapter 2** is a published manuscript, **Chapter 3** is a submitted manuscript that is under revision, **Chapters 4** and **Chapter 5** are manuscripts that are being prepared for submission to peer-reviewed journals. Lastly, **Chapter 6** reviews the findings of the preceding chapters and explains how they contribute to our understanding of short-term activity cycles in ants.

CHAPTER 1 – Introduction

Author: Grant Navid Doering

Comments: Data on the miscellaneous time series was collected and processed by G.N.D. Data on the activity of *Temnothorax crassispinus*, *Temnothorax albipennis*, and *Temnothorax congruus* were kindly provided by Sławomir Mitrus, Elva Robinson, and Adam Cronin respectively. Matthew M. Prebus provided colonies of *T. tricarninatus*, *T. emmae*, *T. caguatan*, *T. quercicola*, and *T. nitens*. Tricia Skelton and Avani Pathak helped set up recordings for some of the miscellaneous time series.

CHAPTER 2 – Sources of intraspecific variation in the collective tempo and synchrony of ant societies

Authors: Grant Navid Doering, Kirsten A. Sheehy, James L. L. Lichtenstein, Brian Drawert, Linda R. Petzold, and Jonathan N. Pruitt

Publication: Behavioral Ecology (2019), 30(6), 1682–1690. doi:10.1093/beheco/arz135

Comments: G.N.D designed the study, performed all analyses, and wrote the first draft of the manuscript. G.N.D and K.A.S collected the data. JNP provided laboratory space and suggestions of interpreting the results. J. L. L. L., B. D., and L. R. P. provided guidance on the data analysis methods used in the paper. All authors contributed to the writing of the final version of the manuscript.

CHAPTER 3 – Noise resistant synchronization and collective rhythm switching in a model of animal group locomotion

Authors: Grant Navid Doering, Brian Drawert, Carmen Lee, Jonathan N. Pruitt, Linda R. Petzold, Kari Dalnoki-Veress

Comments: As stated in the main text of the paper, G. N. D conceived the study, collected all empirical data, performed all analyses, wrote the first draft of the manuscript, and built the initial agent-based model. K. D, C. L, B. D, and L. R. P were involved in revising and further developing the agent-based model. J. N. P provided laboratory space and cameras for the empirical portion of the study. All authors contributed to the writing of the final version of the manuscript.

CHAPTER 4 – Dissimilar effects of light on short versus long period collective rhythms in ant colonies

Authors: Grant Navid Doering, Matthew M. Prebus, Jonathan N. Pruitt

Comments: G.N.D designed the study, collected all the data, performed all analyses, and wrote the first draft of the paper. M. M. P helped collect colonies for the experiment and assisted with species identification. J. N. P. provided funding and helped interpret the results of the study.

CHAPTER 5 – Synchronized locomotion improves spatial accessibility in ant colonies and in oscillating active particles
Authors: Grant Navid Doering, Carmen Lee, Kari Dalnoki-Veress
Comments: G.N.D designed the study, collected the empirical observations, carried out the analyses, contributed to the agent-based model, and wrote the first draft of the manuscript. C. L built the initial agent-based model and helped develop the accessibility metrics. K. D provided guidance on the analysis of both the empirical data and the model simulations. All authors contributed to the writing of the paper.

CHAPTER 6 – Discussion
Author: Grant Navid Doering

CHAPTER 1 – INTRODUCTION

1.1 Rhythms and synchronization in animals

Rhythmic phenomena are a conspicuous feature of life and are crucial for many fundamental biological processes, such as the circadian cycles of rest and activity (Yerushalmi and Green 2009), seasonal patterns of hibernation and reproduction (Geiser 2013; Williams et al. 2017), and the periodicity of respiration (Glass 2001). In many instances, multiple rhythms can also synchronize, which, at a basic level, is when two or more oscillations become coupled either through mutual influence or through a common exogenous force, and their activity patterns consequently resemble each other (Pikovsky et al. 2003). Because it necessarily requires multiple oscillators or individuals, synchronization is a form of *collective* behaviour, and it occurs in a rich variety of situations involving interacting agents (Strogatz 2000; Glass 2001; Pikovsky et al. 2003; Boccaletti et al. 2018; Couzin 2018). Because many animal species live with conspecifics in groups, it is not surprising that synchronization is also prevalent in social contexts. Simultaneous claw waving displays in fiddler crab congregations (Backwell 2019), the chorusing vocalizations in groups of bush crickets (Greenfield et al. 2017), and coordinated motion in swarms of insects and various vertebrates (King and Sumpter 2012; Attanasi et al. 2014) are all examples of individual animals synchronizing their actions.

Rhythmic behaviour and synchronization both have adaptive value (Ravignani et al. 2014; Duranton and Gaunet 2016). Rhythmical behaviour can be beneficial if animals need to adjust their activity level to coincide with predictable changes in the environment (e.g., circadian rhythms) (Yerushalmi and Green 2009). Animal physiology often also requires rhythmic behaviour; consider how an irregular heartbeat can be fatal for instance (Glass 2001). The synchronization of multiple rhythms carries its own set of advantages. Some major benefits of synchronization for social animals are predator avoidance (Hoogland and Sherman 1976; Ioannou 2017), superior reproduction outcomes (Schiml et al. 1996; Grafe 1999; Moiseff and Copeland 2010), and better cooperation and communication among conspecifics (Chen 1937; Couzin 2018; Behrens et al. 2020). Research on rhythms and synchronization in animals is a rapidly developing field (Ravignani et al. 2014; Couzin 2018; Ravignani 2019; Klibaite and Shaevitz 2020), and it is now becoming easier to perform more comprehensive studies. The mathematical tools needed to quantify and analyse synchrony and rhythm in the often noisy time series characteristic of animal behaviours are relatively recent developments (Eckmann et al. 1987; Tass et al. 1998; Rosenblum et al. 2001; Quian Quiroga et al. 2002; Cazelles and Stone 2003; Kreuz 2011a; Kreuz 2011b; Schwabedal and Pikovsky 2013). New technologies that automatically track the activity of multiple interacting animals are enabling more detailed measurements of behavioural rhythms (Crall et al. 2015; Alarcón-Nieto et al. 2018; Romero-Ferrero et al. 2019; Leoni et al. 2020). Studying a wide variety of rhythmic behaviours in different taxa and determining if and how these rhythms synchronize will further elucidate the importance of these twin phenomena to the biology of social species (Bulla et al. 2016).

1.2 Ants as a model system for social synchronization

Ants are insects belonging to the family Formicidae in the order Hymenoptera. There are over ten thousand described ant species, all of which are social (Hölldobler and Wilson 1990; Bolton 2021). The nature of sociality in ants is highly advanced, and they are the definitive example of *eusociality*. This entails a reproductive caste system where males and queens create offspring while subordinate (sometimes sterile) workers care for the young and are responsible for colony upkeep and maintenance (Hölldobler and Wilson 1990). Many species of ants also exhibit additional forms of labour specialisation within the worker caste, and it is hypothesised that this helps optimize a colony's efficiency when completing tasks (Oster and Wilson 1978). Division of labour among worker ants is often achieved through divergent morphologies. For example, members of the "soldier" caste from the genera *Cephalotes* and *Colobopsis* possess modified head shapes that are used to plug nest entrances as fortification against predators (Wheeler 1904; Powell 2008). Division of labour can also occur in species who only have a single morphological worker caste if some workers focus on foraging while others primarily engage in brood care or other tasks inside the nest (Stuart and Page 1991; Beshers and Fewell 2001; Schwander et al. 2005; Dornhaus 2008; Pinter-Wollman et al. 2012). In some species, worker roles can also shift as they age; younger workers will typically nurse the brood and older individuals engage in the risker tasks of foraging and scouting that must take place outside the nest (Calabi et al. 1983; Franks et al. 1997; Traniello and Rosengaus 1997; Mersch et al. 2013).

The sophisticated cohesiveness of an ant colony has inspired the label "superorganism" to describe them. The analogy is that the integrated actions of individual workers in a colony are akin to the cooperative interactions of the cells that make a solitary animal (Wheeler 1911; Hölldobler and Wilson 2009; Canciani et al. 2019). The eusociality and "superorganism" status of ants is one of the chief factors believed to have led to the group's immense ecological success (Wilson 1987). The combination of ants' ecological dominance, their abundance, their eusociality, and their diversity means that studying ants has proved vital for advancing our understanding of numerous concepts in evolutionary biology (Sundström et al. 1996; Bourke and Franks 2019). The fact that ant colonies are "superorganisms" also makes ants ideal candidates as research subjects on many aspects of collective behaviour, including synchronization. Ant colonies have evolved a wide array of collective behaviours. For example, ants are capable of swiftly reaching consensus on emigrating to the best available nest site (Sasaki and Pratt 2018), synchronizing the nuptial flights of reproductives from multiple nests (Staab and Kleineidam 2014), excavating and constructing intricate nest structures (Pinter-Wollman 2015; Tschinkel 2021), forming teams that transport prey back to the nest (Buffin et al. 2018; Feinerman et al. 2018), linking bodies to assemble into living rafts and bridges (Wheeler 1910; Mlot et al. 2011), and regulating the colony's intake of food to meet nutritional targets (Dussutour and Simpson 2009). Due to their impressive adaptations, studying collective behaviour in ants not only delivers insights into fundamental aspects of social insect biology, but it also has implications for other disciplines. In fact, the behavioural rules that individual ants follow have already found applications and led to discoveries in mathematical optimization (Dorigo and Blum 2005), traffic organization (Fourcassié et al. 2010), robotics (Kube and Zhang 1993; Hsieh et al. 2008), and soft matter physics (Gravish et al. 2015; Tennenbaum et al. 2016; Aguilar et al. 2018).

One type of behaviour in ants that has received less attention than the other forms of collective behaviour previously mentioned is the phenomenon of short-term activity cycles (Cole and Trampus 1999). Short-term activity cycles are a form of ultradian (i.e., a periodicity less than 24-hours) oscillations that occur within the nest cavities of some ant species. During short-term activity cycles, the majority of workers inside the nest will be inactive (i.e., remain motionless) at the same time, but they will activate and begin moving together in bursts of activity. Collective activity bursts occur rhythmically with a period of approximately 10-170 minutes depending on the species and particular colony (Figure 1.1; Video S1.1 Chapters 2-5 of this thesis; Cole, 1991; Hatcher, 1992; Richardson et al., 2017). Trophallaxis and other physical interactions between nestmates primarily occur during the bursts of colony movement (Cole 1991a; Richardson et al. 2017).

Previous work on short-term activity cycles has primarily relied on the genera *Temnothorax* and *Leptothorax*. These two genera are closely related, and prior to 2003 they were regarded as belonging to a single genus (Bolton 2003). They are grouped together in what is referred to as the *Formicoxenus* genus group, which currently includes the genera *Temnothorax*, *Leptothorax*, *Vombisidris, Gauromyrmex, Harpagoxenus*, and *Formicoxenus* (Blaimer et al. 2018). *Harpagoxenus* and *Formicoxenus* only contain socially parasitic species, but the free-living members of *Temnothorax* and *Leptothorax* share several natural history traits. Colonies of *Temnothorax* and *Leptothorax* are often smaller than those of other ants, being populated by just a few dozen to a few hundred workers (Wheeler 1903). Colonies also tend to inhabit pre-formed cavities (rock crevices, rotting sticks, rotting acorns, hollow oak galls, etc.) instead of excavating or building their own nests (for those interested in an extraordinary outlier see *T. sallei* (Wheeler and Mann 1914)). These characteristics make *Temnothorax* and *Leptothorax* ants useful models for laboratory study because entire colonies can inhabit simple artificial nests where all individuals can be monitored simultaneously (Wilson 1975; Herbers and Cunningham 1983; Cole 1991a; Sendova-Franks and Franks 1995). Research using these genera has contributed to multiple areas of behavioral ecology, including the mechanisms underlying collective decision making (Mallon et al. 2001; Franks et al. 2002; Pratt 2005; Sasaki and Pratt 2018), the formation of dominance hierarchies and reproductive conflict (Cole 1981; Bourke 1991; Heinze et al. 1997), animal cognition (Langridge et al. 2004; Franks and Richardson 2006), alternative reproductive tactics (Rüppell et al. 2001; Howard and Kennedy 2007), nestmate recognition (Stuart 1992), chemical and tactile communication (Möglich 1979; Valentini et al. 2020), the evolution of social parasitism (Foitzik et al. 2001; Heinze 1995), and animal personality (Modlmeier and Foitzik 2011; Bengston and Dornhaus 2014; Lichtenstein et al. 2016).

Prior to the 2020's, data from two species (*Temnothorax allardycei* and *Leptothorax acervorum*) constituted the sole source of information on the mechanistic basis of short-term activity cycles. Work with these two species uncovered several details about the process by which collective cycles emerge. Activity spreads like a wave through physical contact, with active ants stimulating inactive ants (Cole 1991a; Cole 1991b; Hatcher 1992; Boi et al. 1999). Neither pheromonal cues nor any kind of external signal appear to be involved in generating the collective cycles (Cole and Trampus 1999). Isolated individual ants of *T. allardycei* do not have a precise periodicity like colonies (Cole 1991a; Cole 1991c; Cole 1991b), and they become less active as they age (Cole 1992). Although isolated workers oscillate erratically, in increasingly larger groups of workers, interactions between individuals cause the ants to eventually synchronize their locomotor activity into highly coherent rhythms (Cole and Hoeg 1996). It has been argued that the locomotor activity traces of isolated *T. allardycei* workers are actually chaotic; individual activity may be deterministic yet highly unpredictable (Cole 1991c). The evidence for this claim is however not conclusive. The analyses used to support deterministic chaos in isolated ants could have been confounded by the inherent noisiness and brevity of the underlying time-series (Barahona and Poon 1996). A handful of other species that also have relatively small colony sizes have anecdotally been reported to exhibit short-term activity cycles as well (Cole and Trampus 1999). However, synchronized pulses of activity have more recently been observed in the invasive fire ant *Solenopsis invicta* (Tennenbaum and Fernandez-Nieves 2017), which typically have much larger colonies than either *Temnothorax* or *Leptothorax*, being made up of thousands of workers (Tschinkel 1993). Several mathematical and computational models have been formulated that replicate some features of short-term activity cycles in ants. Notably, models based on either random excitable oscillators or mobile cellular automata can both generate coherent population-level rhythms even when starting with erratically oscillating individuals (Goss and Deneubourg 1988; Solé et al. 1993).

To provide a general sense of the type of periodicities that are possible in the *Formicoxenus* group, I share here a catalogue of various collective activity time series I obtained through exploratory observations of colonies from 19 different species. None of these species apart from *T. albipennis* have previously had any data about their activity cycles published. The periodicities that are present in the time series of collective activity that I collected (Figure 1.1) are more diverse than the routinely 20 to 30 minute oscillation periods found in prior studies of *Temnothorax allardycei* and *Leptothorax acervorum*. Periods as short as approximately 11 minutes can be seen in *T. quercicola*, and long cycles of about 1.5-hours are present in *T. rudis* colonies (Figure 1.1). The activity patterns depicted in Figure 1.1 were determined using an image analysis technique that I employed throughout my thesis research. The method was pioneered in the 1990's by Blaine Cole, Tofts, and Hatcher during their work on *T. allardycei* and *L. acervorum* (Cole 1991a; Tofts et al. 1992). Briefly, colonies were filmed using a camcorder (Canon VIXIA) and frames were extracted from the resulting videos in intervals of 30 seconds. These frames were binarized using adaptive thresholding in MATLAB to segment the ants inside each nest from the background. The proportion of segmented pixels that changed from 0 and 1 between successive frames was used as an estimate of the proportion of the individuals in a colony that had moved positions in the corresponding 30 second interval. This algorithm makes it possible to track the approximate amount of colony-level active inside the nests over time (see Chapters 2-5 for additional details on this method).

Because it is easy to observe activity levels in both isolated individuals and entire colonies and because of the vast behavioral diversity present in ants, short-term activity cycles can serve as a paradigmatic object of study that can teach us about general aspects of social synchronization. However, there are still major gaps in the scientific understanding of short-term activity cycles. This thesis erodes some of this ignorance by tackling three broad questions surrounding collective activity cycles. The first question asks what biotic and abiotic factors influence the rhythms and strength of synchrony in short-term activity cycles. The effect of many factors, such as external light cues or the potential influence of queens have not yet been evaluated, nor is it known if different colonies from the same species have repeatable differences in how they oscillate. The second question asks what mechanisms cause short-term activity cycles to emerge. Current mathematical models of short-term activity cycles do not explicitly consider the behavioural noise (unpredictability) present in individual ants. The existing models also do not consider potential difference between species in cycle traits. The third question asks what the ultimate reasons for activity cycles are. Do they confer any adaptive or functional benefit to colonies? Each data chapter in this thesis seeks to advance our understanding of one of these three questions.

1.3 Structure of this thesis

In the second chapter of my thesis, I describe exploratory work that studied factors that might be associated with intraspecific variation in short-term activity cycle patterns in *Temnothorax* ants. I first recorded and automatically tracked the activity of multiple colonies of *T. rugatulus* several times over the course of 6 weeks to assess whether different colonies were consistent in three primary metrics: 1) their typical period of collective oscillation, 2) their level of worker synchrony, and 3) the level of rhythmicity (predictability) of their activity cycles. I then looked to see if these three collective activity metrics were correlated with either the number of workers in a colony, the number of brood, or the presence of a queen. My data uncovered repeatable differences between colonies in all three activity metrics. I found that both the number of workers and brood in a colony were negatively correlated with both the rhythmicity and synchrony of short-term activity cycles. Notably, I also found that colonies that lost their queen experienced a reduction in the strength of worker synchronization.

The third chapter of my thesis aimed to quantify the amount of noise or unpredictability in individual-ant behaviour and investigate whether the collective oscillations of *Leptothorax* colonies are capable of multirhythmicity, where colonies switch between different oscillation frequencies. I also present an agent-based model of short-term activity cycles in ants that is inspired by empirical-observations from two species: *Leptothorax crassipilis* and *L*. sp W. I found that the most prominent collective oscillations in *L*. sp W colonies did not greatly deviate from approximately 20 minutes. However, it was not uncommon for colonies of the related *L. crassipilis* to have dominant periods greater than 40 minutes. Colonies of both species are also capable of transiently exhibiting periods greater than 2 hours. These longer periodicities coexist with the faster oscillations. At the individual level, isolated ants show less rhythmic activity patterns than whole colonies. I show that individual ants also have imperfect refractive states where the length of time they are inactive is correlated with the likelihood that the ant will start moving if an active ant runs into it. In addition to the information I contribute to the biology of short-term activity cycles, the results from my model simulations contribute to our knowledge of multirhythmicity and noise in excitable systems, which are both active areas of research (Muratov et al. 2007; Rozenblit and Copelli 2011; Biswas et al. 2017).

For chapter four, I conducted an experiment that examined the potential link between short-term activity cycles and circadian collective activity within ant nests. Endogenous circadian rhythms are widespread in animals. Many species will entrain their activity to the alternating cycle of day and night in the wild, and individuals can maintain these rhythms even if placed in an environment with constant lighting or temperature conditions (Aschoff 1981). Diurnal, nocturnal, and irregular activity patterns have long been recorded in the foraging patterns of ants (Talbot 1946; Urbani 1965; Fellers 1989). However, less is known about the nature of circadian oscillations of collective activity *within* the nest chambers of ants (Fujioka et al. 2021). Even less is known about the potential interaction between short-term activity cycles and collective 24-hour rhythmicity inside the nest (Hatcher 1992; Richardson et al. 2017). To determine if North American ant species that form small colonies have endogenous circadian rhythms of within-nest collective activity, I tested whether signals of 24-hour rhythms were stronger for colonies when they were exposed to an alternating light/dark cycle versus constant darkness or constant light. I also tested whether colonies exposed to a light/dark cycle adjust the frequency of their short-term activity cycles based on the photoperiod (e.g., oscillating slower at night than during the day for diurnal species).

Chapter five of my thesis examines a potential functional advantage that synchronized activity might provide colonies. It has been previously noted that *Temnothorax* and *Leptothorax* ants will aggregate around their brood, which they gather into a pile inside the nest (Franks and Sendova-Franks 1992; Sendova-Franks and Franks 1995; Sendova-franks and Franks 1995; Heinze et al. 1996). It is also known that ants inside a nest appear to maneuver around one another to avoid collisions and possibly dominance interactions as well (Cole 1981; Sendova-Franks and Franks 1995; Gravish et al. 2015). If inactive ants also congregate near one another, such dense clusters could act as locally jammed regions where active ants would have greater difficulty walking through. I therefore tested the hypothesis that synchronized activity cycles improve spatial accessibility within the nest by reducing the likelihood that workers will be active when there are dense clusters of inactive ants. I present evidence for this hypothesis using real colonies of

Leptothorax, and I build a simple computational model of mobile oscillators that reproduces the same qualitative trends observed in actual colonies. I then used this model to explore the question more generally by finding the conditions where synchronized active-rest rhythms are most beneficial to the spatial accessibility of confined active particles.

I conclude the thesis with chapter six, which gives a historical overview of research into the within-nest activity patterns of ants and summarizes the contributions of the other chapters of my thesis. I explain the current state of our knowledge regarding 1) the level of intraspecific and interspecific variation in short-term activity cycles in the *Formicoxenus* group, 2) the different biotic and abiotic factors that influence short-term activity cycles, 3) the mechanisms that lead to collective rhythms of activity within nests, and 3) the potential adaptive value and functional implications of short-term activity cycles.

1.4 References

Aguilar J, Monaenkova D, Linevich V, Savoie W, Dutta B, Kuan H-S, Betterton MD, Goodisman M a. D, Goldman DI. 2018. Collective clog control: Optimizing traffic flow in confined biological and robophysical excavation. Science. 361:672–677.

Alarcón-Nieto G, Graving JM, Klarevas-Irby JA, Maldonado-Chaparro AA, Mueller I, Farine DR. 2018. An automated barcode tracking system for behavioural studies in birds. Methods Ecol Evol. 9:1536–1547.

Aschoff J. 1981. Freerunning and Entrained Circadian Rhythms. In: Aschoff J, editor. Biological Rhythms. Boston, MA: Springer US. p. 81–93. [accessed 2021 Sep 15]. https://doi.org/10.1007/978-1-4615-6552-9_6.

Attanasi A, Cavagna A, Castello LD, Giardina I, Melillo S, Parisi L, Pohl O, Rossaro B, Shen E, Silvestri E, et al. 2014. Collective Behaviour without Collective Order in Wild Swarms of Midges. PLOS Comput Biol. 10:e1003697.

Backwell PRY. 2019. Synchronous waving in fiddler crabs: a review. Curr Zool. 65:83–88.

Barahona M, Poon C-S. 1996. Detection of nonlinear dynamics in short, noisy time series. Nature. 381:215–217.

Behrens F, Snijdewint JA, Moulder RG, Prochazkova E, Sjak-Shie EE, Boker SM, Kret ME. 2020. Physiological synchrony is associated with cooperative success in real-life interactions. Sci Rep. 10:19609.

Bengston SE, Dornhaus A. 2014. Be meek or be bold? A colony-level behavioural syndrome in ants. Proc R Soc B. 281:20140518.

Beshers SN, Fewell JH. 2001. Models of Division of Labor in Social Insects. Annu Rev Entomol. 46:413–440.

Biswas D, Banerjee T, Kurths J. 2017. Control of birhythmicity: A self-feedback approach. Chaos Interdiscip J Nonlinear Sci. 27:063110.

Blaimer BB, Ward PS, Schultz TR, Fisher BL, Brady SG. 2018. Paleotropical Diversification Dominates the Evolution of the Hyperdiverse Ant Tribe Crematogastrini (Hymenoptera: Formicidae). Insect Syst Divers. 2. [accessed 2021 Jun 9]. https://doi.org/10.1093/isd/ixy013.

Boccaletti S, Pisarchik AN, Genio CI del, Amann A. 2018. Synchronization: From Coupled Systems to Complex Networks. Cambridge University Press.

Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF. 1999. Coupled Oscillators and Activity Waves in Ant Colonies. Proc Biol Sci. 266:371–378.

Bolton B. 2003. Synopsis and classification of Formicidae. Mem Am Entomol Inst. 71:1–370.

Bolton B. 2021. AntCat. Online Cat Ants World. [accessed 2021 Jul 22]. https://www.antcat.org/.

Bourke AFG. 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. Anim Behav. 42:295–310.

Bourke AFG, Franks NR. 2019. Social Evolution in Ants. Princeton University Press. [accessed 2021 Jul 24]. https://www.degruyter.com/document/doi/10.1515/9780691206899/html.

Buffin A, Sasaki T, Pratt SC. 2018. Scaling of speed with group size in cooperative transport by the ant *Novomessor cockerelli*. PLOS ONE. 13:e0205400.

Bulla M, Valcu M, Dokter AM, Dondua AG, Kosztolányi A, Rutten AL, Helm B, Sandercock BK, Casler B, Ens BJ, et al. 2016. Unexpected diversity in socially synchronized rhythms of shorebirds. Nature. 540:109–113.

Calabi P, Traniello JFA, Werner MH. 1983. Age Polyethism: Its Occurrence in the Ant *Pheidole hortensis*, and Some General Considerations. Psyche (Stuttg). 90:395–412.

Canciani M, Arnellos A, Moreno A. 2019. Revising the Superorganism: An Organizational Approach to Complex Eusociality. Front Psychol. 10. [accessed 2020 Sep 20]. https://www.frontiersin.org/articles/10.3389/fpsyg.2019.02653/full#ref67.

Cazelles B, Stone L. 2003. Detection of imperfect population synchrony in an uncertain world. J Anim Ecol. 72:953–968.

Chen SC. 1937. The Leaders and Followers among the Ants in Nest-Building. Physiol Zool. 10:437–455.

Cole BJ. 1981. Dominance Hierarchies in *Leptothorax* Ants. Science. 212:83–84.

Cole BJ. 1991a. Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction. Am Nat. 137:244–259.

Cole BJ. 1991b. Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav. 4:129–137.

Cole BJ. 1991c. Is Animal Behaviour Chaotic? Evidence from the Activity of Ants. Proc Biol Sci. 244:253–259.

Cole BJ. 1992. Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. Behav Ecol Sociobiol. 31:181–187.

Cole BJ, Hoeg L. 1996. The influence of brood type on activity cycles in *Leptothorax allardycei* (Hymenoptera: Faruicidae). J Insect Behav. 9:539–547.

Cole BJ, Trampus FI. 1999. Activity cycles in ant colonies: worker interactions and decentralized control. In: Detrain C, Deneubourg JL, Pasteels JM, editors. Information Processing in Social Insects. Basel: Birkhäuser Basel. p. 289–307. [accessed 2018 Dec 24]. https://doi.org/10.1007/978-3-0348-8739-7_16.

Couzin ID. 2018. Synchronization: The Key to Effective Communication in Animal Collectives. Trends Cogn Sci. 22:844–846.

Crall JD, Gravish N, Mountcastle AM, Combes SA. 2015. BEEtag: A Low-Cost, Image-Based Tracking System for the Study of Animal Behavior and Locomotion. PLOS ONE. 10:e0136487.

Dorigo M, Blum C. 2005. Ant colony optimization theory: A survey. Theor Comput Sci. 344:243–278.

Dornhaus A. 2008. Specialization Does Not Predict Individual Efficiency in an Ant. PLOS Biol. 6:e285.

Duranton C, Gaunet F. 2016. Behavioural synchronization from an ethological perspective: Overview of its adaptive value. Adapt Behav. 24:181–191.

Dussutour A, Simpson SJ. 2009. Communal Nutrition in Ants. Curr Biol. 19:740–744.

Eckmann J-P, Kamphorst SO, Ruelle D. 1987. Recurrence Plots of Dynamical Systems. Europhys Lett EPL. 4:973–977.

Feinerman O, Pinkoviezky I, Gelblum A, Fonio E, Gov NS. 2018. The physics of cooperative transport in groups of ants. Nat Phys. 14:683–693.

Fellers JH. 1989. Daily and seasonal activity in woodland ants. Oecologia. 78:69–76.

Foitzik S, DeHeer CJ, Hunjan DN, Herbers JM. 2001. Coevolution in host–parasite systems: behavioural strategies of slave–making ants and their hosts. Proc R Soc Lond B Biol Sci. 268:1139–1146.

Fourcassié V, Dussutour A, Deneubourg J-L. 2010. Ant traffic rules. J Exp Biol. 213:2357–2363.

Franks NR, Pratt SC, Mallon EB, Britton NF, Sumpter DJT. 2002. Information flow, opinion polling and collective intelligence in house-hunting social insects. Philos Trans R Soc Lond B Biol Sci. 357:1567–1583.

Franks NR, Richardson T. 2006. Teaching in tandem-running ants. Nature. 439:153–153.

Franks NR, Sendova-Franks AB. 1992. Brood sorting by ants: distributing the workload over the work-surface. Behav Ecol Sociobiol. 30:109–123.

Franks NR, Tofts C, Sendova-franks AB. 1997. Studies of the division of labour: neither physics nor stamp collecting. Anim Behav. 53:219–224.

Fujioka H, Abe MS, Okada Y. 2021. Individual Ants Do Not Show Activity-Rest Rhythms in Nest Conditions. J Biol Rhythms. 36:297–310.

Geiser F. 2013. Hibernation. Curr Biol. 23:R188–R193.

Glass L. 2001. Synchronization and rhythmic processes in physiology. Nature. 410:277–284.

Goss S, Deneubourg JL. 1988. Autocatalysis as a source of synchronised rhythmical activity in social insects. Insectes Sociaux. 35:310–315.

Grafe TU. 1999. A function of synchronous chorusing and a novel female preference shift in an anuran. Proc R Soc Lond B Biol Sci. 266:2331–2336.

Gravish N, Gold G, Zangwill A, Goodisman MAD, Goldman DI. 2015. Glass-like dynamics in confined and congested ant traffic. Soft Matter. 11:6552–6561.

Greenfield MD, Marin-Cudraz T, Party V. 2017. Evolution of synchronies in insect choruses. Biol J Linn Soc. 122:487–504.

Hatcher MJ. 1992. Activity patterns and organization within ant nests [PhD Thesis]. University of Bath.

Heinze J. 1995. The Origin of Workerless Parasites in *Leptothorax* (S. Str.) (Hymenoptera: Formicidae). Psyche (Stuttg). 102:195–214. [accessed 2021 Jan 21]. https://www.hindawi.com/journals/psyche/1995/069812/.

Heinze J, Puchinger W, Hölldobler B. 1997. Worker reproduction and social hierarchies in *Leptothorax* ants. Anim Behav. 54:849–864.

Heinze J, Stahl M, Hölldobler B. 1996. Ecophysiology of hibernation in boreal *Leptothorax* ants (Hymenoptera: Formicidae). Écoscience. 3:429–435.

Herbers JM, Cunningham M. 1983. Social organization in *Leptothorax longispinosus* Mayr. Anim Behav. 31:759–771.

Hölldobler B, Wilson EO. 1990. The Ants. Harvard University Press.

Hölldobler B, Wilson EO. 2009. The superorganism: the beauty, elegance, and strangeness of insect societies. WW Norton & Company.

Hoogland JL, Sherman PW. 1976. Advantages and Disadvantages of Bank Swallow (Riparia riparia) Coloniality. Ecol Monogr. 46:33–58.

Howard KJ, Kennedy D. 2007. Alternative mating behaviors of the queen polymorphic ant *Temnothorax longispinosus*. Naturwissenschaften. 94:945–950.

Hsieh MA, Halász Á, Berman S, Kumar V. 2008. Biologically inspired redistribution of a swarm of robots among multiple sites. Swarm Intell. 2:121–141.

Ioannou CC. 2017. Grouping and predation.

King AJ, Sumpter DJ. 2012. Murmurations. Curr Biol. 22:R112–R114.

Klibaite U, Shaevitz JW. 2020. Paired fruit flies synchronize behavior: Uncovering social interactions in *Drosophila melanogaster*. PLOS Comput Biol. 16:e1008230.

Kreuz T. 2011a. Measures of spike train synchrony. Scholarpedia. 6:11934.

Kreuz T. 2011b. Measures of neuronal signal synchrony. Scholarpedia. 6:11922.

Kube CR, Zhang H. 1993. Collective Robotics: From Social Insects to Robots. Adapt Behav. 2:189–218.

Langridge EA, Franks NR, Sendova-Franks AB. 2004. Improvement in collective performance with experience in ants. Behav Ecol Sociobiol. 56:523–529.

Leoni J, Tanelli M, Strada SC, Berger-Wolf T. 2020. Ethogram-based automatic wild animal monitoring through inertial sensors and GPS data. Ecol Inform. 59:101112.

Lichtenstein JLL, Pruitt JN, Modlmeier AP. 2016. Intraspecific variation in collective behaviors drives interspecific contests in acorn ants. Behav Ecol. 27:553–559.

Mallon EB, Pratt SC, Franks NR. 2001. Individual and Collective Decision-Making during Nest Site Selection by the Ant *Leptothorax albipennis*. Behav Ecol Sociobiol. 50:352–359.

Mersch DP, Crespi A, Keller L. 2013. Tracking Individuals Shows Spatial Fidelity Is a Key Regulator of Ant Social Organization. Science. 340:1090–1093.

Mlot NJ, Tovey CA, Hu DL. 2011. Fire ants self-assemble into waterproof rafts to survive floods. Proc Natl Acad Sci. 108:7669–7673.

Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. Behav Ecol. 22:1026–1032.

Möglich M. 1979. Tandem calling pheromone in the genus *Leptothorax* (Hymenoptera: Formicidae): Behavioral analysis of specificity. J Chem Ecol. 5:35–52.

Moiseff A, Copeland J. 2010. Firefly Synchrony: A Behavioral Strategy to Minimize Visual Clutter. Science. 329:181–181.

Muratov CB, Vanden-Eijnden E, E W. 2007. Noise can play an organizing role for the recurrent dynamics in excitable media. Proc Natl Acad Sci. 104:702–707.

Oster GF, Wilson EO. 1978. Caste and Ecology in the Social Insects. Princeton University Press.

Pikovsky A, Kurths Jurgen, Rosenblum M, Kurths Jürgen. 2003. Synchronization: A Universal Concept in Nonlinear Sciences. Cambridge University Press.

Pinter-Wollman N. 2015. Nest architecture shapes the collective behaviour of harvester ants. Biol Lett. 11:20150695.

Pinter-Wollman N, Hubler J, Holley J-A, Franks NR, Dornhaus A. 2012. How is activity distributed among and within tasks in *Temnothorax* ants? Behav Ecol Sociobiol. 66:1407–1420.

Powell S. 2008. Ecological specialization and the evolution of a specialized caste in *Cephalotes* ants. Funct Ecol. 22:902–911.

Pratt SC. 2005. Behavioral mechanisms of collective nest-site choice by the ant *Temnothorax curvispinosus*. Insectes Sociaux. 52:383–392.

Quian Quiroga R, Kraskov A, Kreuz T, Grassberger P. 2002. Performance of different synchronization measures in real data: A case study on electroencephalographic signals. Phys Rev E. 65:041903.

Ravignani A. 2019. Rhythm and synchrony in animal movement and communication. Curr Zool. 65:77–81.

Ravignani A, Bowling DL, Fitch WT. 2014. Chorusing, synchrony, and the evolutionary functions of rhythm. Front Psychol. 0. [accessed 2021 Aug 3]. https://www.frontiersin.org/articles/10.3389/fpsyg.2014.01118/full.

Richardson TO, Liechti JI, Stroeymeyt N, Bonhoeffer S, Keller L. 2017. Short-term activity cycles impede information transmission in ant colonies. PLOS Comput Biol. 13:e1005527.

Romero-Ferrero F, Bergomi MG, Hinz RC, Heras FJH, de Polavieja GG. 2019. idtracker.ai: tracking all individuals in small or large collectives of unmarked animals. Nat Methods. 16:179–182.

Rosenblum M, Pikovsky A, Kurths J, Schäfer C, Tass PA. 2001. Phase synchronization: From theory to data analysis. In: Moss F, Gielen S, editors. Handbook of Biological Physics. Vol. 4. North-Holland. (Neuro-Informatics and Neural Modelling). p. 279–321. [accessed 2020 Nov 23]. http://www.sciencedirect.com/science/article/pii/S1383812101800129.

Rozenblit F, Copelli M. 2011. Collective oscillations of excitable elements: order parameters, bistability and the role of stochasticity. J Stat Mech Theory Exp. 2011:P01012.

Rüppell O, Heinze J, Hölldobler B. 2001. Alternative reproductive tactics in the queen-size-dimorphic ant *Leptothorax rugatulus* (Emery) and their consequences for genetic population structure. Behav Ecol Sociobiol. 50:189–197.

Sasaki T, Pratt SC. 2018. The Psychology of Superorganisms: Collective Decision Making by Insect Societies. Annu Rev Entomol. 63:259–275.

Schiml PA, Mendoza SP, Saltzman W, Lyons DM, Mason WA. 1996. Seasonality in squirrel monkeys (*Saimiri sciureus*): social facilitation by females. Physiol Behav. 60:1105–1113.

Schwabedal JTC, Pikovsky A. 2013. Phase Description of Stochastic Oscillations. Phys Rev Lett. 110:204102.

Schwander T, Rosset H, Chapuisat M. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. Behav Ecol Sociobiol. 59:215–221.

Sendova-Franks AB, Franks NR. 1995. Demonstrating new social interactions in ant colonies through randomization tests: separating seeing from believing. Anim Behav. 50:1683–1696.

Sendova-franks AB, Franks NR. 1995. Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. Anim Behav. 50:121–136.

Solé RV, Miramontes O, Goodwin BC. 1993. Oscillations and Chaos in Ant Societies. J Theor Biol. 161:343–357.

Staab M, Kleineidam CJ. 2014. Initiation of swarming behavior and synchronization of mating flights in the leaf-cutting ant *Atta vollenweideri* FOREL, 1893 (Hymenoptera: Formicidae). Myrmecol News. 19:93–102.

Strogatz SH. 2000. From Kuramoto to Crawford: exploring the onset of synchronization in populations of coupled oscillators. Phys Nonlinear Phenom. 143:1–20.

Stuart RJ. 1992. Nestmate recognition and the ontogeny of acceptability in the ant, *Leptothorax curvispinosus*. Behav Ecol Sociobiol. 30:403–408.

Stuart RJ, Page RE. 1991. Genetic component to division of labor among workers of a leptothoracine ant. Naturwissenschaften. 78:375–377.

Sundström L, Chapuisat M, Keller L. 1996. Conditional Manipulation of Sex Ratios by Ant Workers: A Test of Kin Selection Theory. Science. 274:993–995.

Talbot M. 1946. Daily Fluctuations in Aboveground Activity of Three Species of Ants. Ecology. 27:65–70.

Tass P, Rosenblum MG, Weule J, Kurths J, Pikovsky A, Volkmann J, Schnitzler A, Freund H-J. 1998. Detection of *n*:*m* Phase Locking from Noisy Data: Application to Magnetoencephalography. Phys Rev Lett. 81:3291–3294.

Tennenbaum M, Fernandez-Nieves A. 2017. Activity-driven changes in the mechanical properties of fire ant aggregations. Phys Rev E. 96:052601.

Tennenbaum M, Liu Z, Hu D, Fernandez-Nieves A. 2016. Mechanics of fire ant aggregations. Nat Mater. 15:54–59.

Tofts C, Hatcher M, Franks NR. 1992. The autosynchronization of the ant *Leptothorax acervorum* (Fabricius): Theory, testability and experiment. J Theor Biol. 157:71–82.

Traniello JFA, Rosengaus RB. 1997. Ecology, evolution and division of labour in social insects. Anim Behav. 53:209–213.

Tschinkel WR. 1993. Sociometry and Sociogenesis of Colonies of the Fire Ant *Solenopsis Invicta* During One Annual Cycle. Ecol Monogr. 63:425–457.

Tschinkel WR. 2021. Ant Architecture: The Wonder, Beauty, and Science of Underground Nests. Princeton University Press.

Urbani CB. 1965. Sull'attivita' di foraggiamento notturna del *Camponotus nylanderi* Em. Insectes Sociaux. 12:253–263.

Valentini G, Mizumoto N, Pratt SC, Pavlic TP, Walker SI. 2020. Revealing the structure of information flows discriminates similar animal social behaviors. Keller L, Rutz C, Deneubourg J-L, editors. eLife. 9:e55395.

Wheeler WM. 1903. A Revision of the North American Ants of the Genus *Leptothorax* Mayr. Proc Acad Nat Sci Phila. 55:215–260.

Wheeler WM. 1904. The American ants of the subgenus *Colobopsis*. Bull Am Mus Nat Hist. 20:139–158.

Wheeler WM. 1910. Ants; their structure, development and behavior. New York, Columbia university press.

Wheeler WM. 1911. The ant-colony as an organism. J Morphol. 22:307–325.

Wheeler WM, Mann WM. 1914. The ants of Haiti. order of the Trustees, American Museum of Natural History.

Williams CT, Klaassen M, Barnes BM, Buck CL, Arnold W, Giroud S, Vetter SG, Ruf T. 2017. Seasonal reproductive tactics: annual timing and the capital-to-income breeder continuum. Philos Trans R Soc B Biol Sci. 372:20160250.

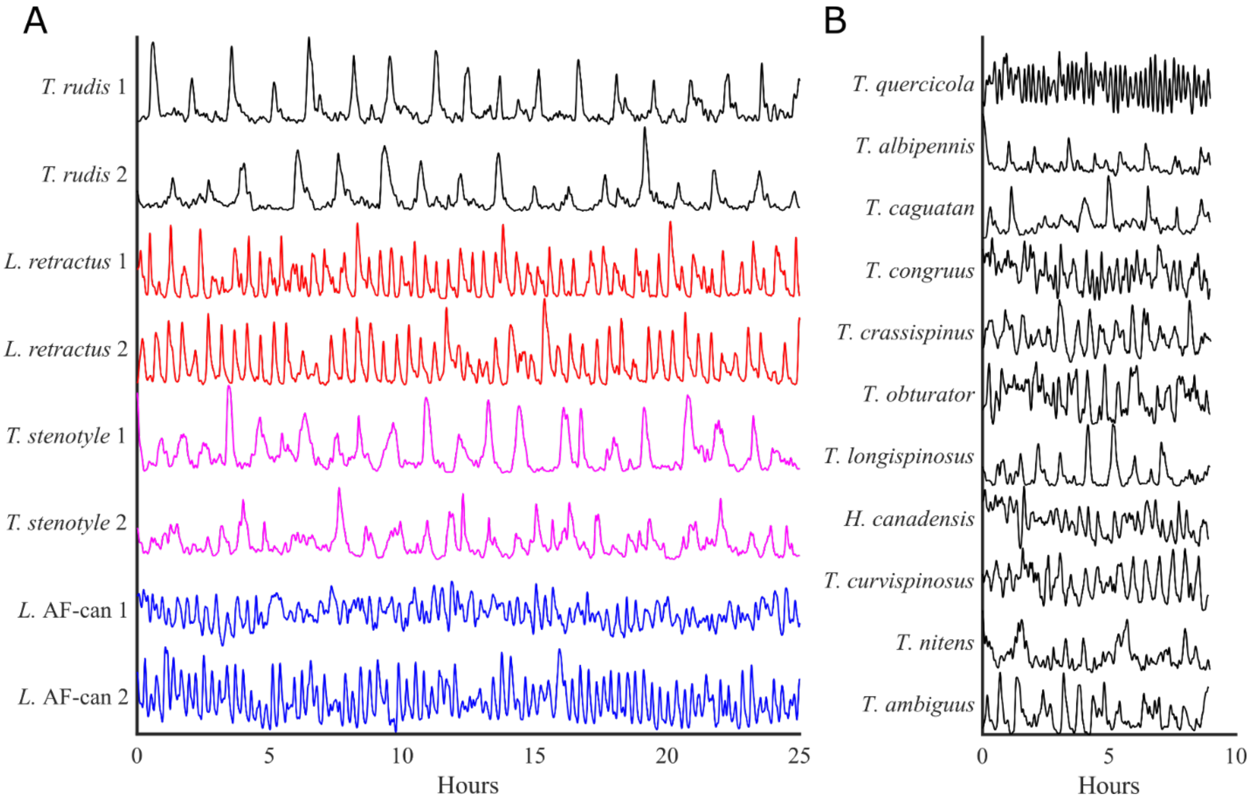
Wilson EO. 1975. *Leptothorax duloticus* and the Beginnings of Slavery in Ants. Evolution. 29:108–119.

Wilson EO. 1987. Causes of Ecological Success: The Case of the Ants. J Anim Ecol. 56:1–9.

Yerushalmi S, Green RM. 2009. Evidence for the adaptive significance of circadian rhythms. Ecol Lett. 12:970–981.

Figure 1.1

Example time series of within-nest, colony-level locomotor activity from 25-hr segments of longer recordings (a) and 9-hr shorter recordings (b) conducted under constant light and temperature showing substantial variation in the oscillation frequencies of short-term activity cycles in *Temnothorax* and *Leptothorax* ants. The time series have been rescaled to fall between 0 and 1 and were smoothed with a Gaussian moving average filter with a window of 15 points (i.e., 7.5 minutes). Each time series comes from a different colony, and each color in panel (a) corresponds to a different species.



CHAPTER 2 – SOURCES OF INTRASPECIFIC VARIATION IN THE COLLECTIVE TEMPO AND SYNCHRONY OF ANT SOCIETIES

Doering GN, Sheehy KA, Lichtenstein JLL, et al (2019) Sources of intraspecific variation in the collective tempo and synchrony of ant societies. *Behav Ecol* 30:1682–1690.
https://doi.org/10.1093/beheco/arz135

2.1 Abstract

Populations of independently oscillating agents can sometimes synchronize. In the context of animal societies, conspicuous synchronization of activity is known in some social insects. However, the causes of variation in synchrony within and between species has received little attention. We repeatedly assessed the short-term activity cycle of ant colonies (*Temnothorax rugatulus*) and monitored the movements of individual workers and queens within nests. We detected persistent differences between colonies in the waveform properties of their collective activity oscillations, with some colonies consistently oscillating much more erratically than others. We further demonstrate that colony crowding reduces the rhythmicity (i.e., the consistent timing) of oscillations. Workers in both erratic and rhythmic colonies spend less time active than completely isolated workers, but workers in erratic colonies oscillate out of phase with one another. We further show that queen absence can impair the ability of colonies to synchronize worker activity, and that behavioral differences between queens are linked with the waveform properties of their societies.

2.2 Introduction

Rhythms are widespread in animal behavior (Winfree 2001). Most species possess at least one periodically repeating behavior or state, such as the circadian cycles of wakefulness and sleep (Paranjpe and Sharma 2005). Groups of animals can also exhibit collective rhythms, a consequence of coupled interactions between individuals. Aggregations of certain fireflies, for example, can blink together in precisely timed flashes (Buck and Buck 1968). Entrainment of groups need not even involve inherently periodic constituents; groups of chaotic oscillators are also capable of attaining a global rhythm in some systems (Rosenblum et al. 1996). A charismatic example of this kind of synchronization of chaos in biology is the collective activity cycles seen in some ant species (Cole and Trampus 1999). No individual ant exhibits a rhythmic cycle in its movements when alone (Cole 1991a), but interactions with conspecifics alter the rate at which individuals become active, leading to highly predictable bursts of colony-wide activity (Cole and Cheshire 1996).

If there is any functional benefit of activity cycles in social insects to colony performance, it is unknown (Cole and Trampus 1999; Couzin 2018). Beginning with early studies (Barnes 1941), several behavioral mechanisms underlying synchrony have been proposed (Hemerik et al. 1990; Cole 1991b; Cole 1991c; Cole and Cheshire 1996; Cole and Trampus 1999; Richardson et al. 2017). Several species of ant exhibit collective bouts of activity approximately every 15-30 min inside the nest (Cole 1991b; Richardson et al. 2017). In the time between moments of universal activity, ants remain nearly completely motionless. Other species lack such regular oscillations (Cole 1992; Hatcher 1992; Cole and Cheshire 1996). Yet, the consequences of these between-species differences and the mechanisms underlying them remain unknown. These mechanisms could easily vary between species. Separate colonies of even a single species could also conceivably vary in their activity patterns. Differences in the activity patterns of individuals (Cole 1992; Hatcher 1992; Hayashi et al. 2012), the outcomes of interactions between workers (Fujioka et al. 2019), the quantity and developmental stages of brood (Cole and Hoeg 1996; Fujioka et al. 2017), and the ratio of castes within a colony (Sharma et al. 2004) could all conceivably alter the emergence of group-level activity cycles.

The structuring of worker activity in ants is a crucial element of their social organization (Herbers 1983; Cole 1986; Charbonneau and Dornhaus 2015): brood care, foraging, or nest maintenance all require ants to move. Regularly spaced oscillations of activity and inactivity (and deviations from this pattern) thus demand explanation. To make progress towards accounting for *intraspecific* variation in activity cycles, we investigated here the colony and individual-level activity patterns of the ant *Temnothorax rugatulus*, which lives in pre-formed cavities (often in rock crevices) (Möglich 1978). Species with small colony sizes and simple one-chambered nests, like those belonging to the genus *Temnothorax* (Bolton 2003), are common models for collective behavior research (Pratt et al. 2002; Pratt and Sumpter 2006).

Variation between societies in various collective traits can often be caused by behavioral differences between the individuals that comprise each society (Keiser et al. 2014; Modlmeier et al. 2014) or by demographic differences (Dornhaus et al. 2012). We first evaluated whether any differences in colony activity patterns were random noise or were consistent over time (Bengston and Dornhaus 2014). We then tested whether these differences could be predicted by either colony size (i.e., number of workers and brood items) or by the activity patterns of individual workers.

Another candidate mechanism for causing variation in colony activity waveforms is queen behavior. In ants, queens usually do not oversee or control collective behaviors (Gordon

1995; Detrain and Deneubourg 2006). For species with vast colonies of millions of workers, the scheduling of activity is self-organized; no single ant could conceivably set the entire colony's agenda (e.g., collective nest construction (Franks et al. 1992), forming networks of foraging trails (Latty et al. 2011)). The situation is however noticeably different for smaller colonies. Social insect queens in smaller societies can, in fact, influence colony behavior in numerous ways, including changing the course of collective decision-making (Doering and Pratt 2016), impeding disease transmission (Keiser et al. 2018), physically punishing reproducing workers (Smith et al. 2012), and determining collective personality traits (Wright et al. 2017). In *Polistes fuscatus* wasps and primitively eusocial bees, queens can actually serve as activity pacemakers; their absence can disrupt normal colony rhythms (Breed and Gamboa 1977; Reeve and Gamboa 1983). Likewise, because *Temnothorax* colonies are small compared to other ants, their queens could be important for regulating colony activity patterns. Queens in some species of *Temnothorax* (and the once synonymous genus *Leptothorax*) are known to exert influence over worker behavior, at least in a reproductive context, by suppressing the incidence of egg laying by workers, but this is not universal (Heinze et al. 1997). The presence or absence of a queen appears to be irrelevant to the oscillation dynamics of at least one other species of *Temnothorax* (Cole and Cheshire 1996). However, this might not be true for the entire genus. We therefore also explored the effects of targeted queen removal on *T. rugatulus* activity cycles.

2.3 Materials and methods

Colony collection & maintenance

The 29 colonies of *Temnothorax rugatulus* used in this study were collected in February 2018 on Madera Peak in the Pinal Mountains of Arizona (33.317N 110.876W). All colonies were monogynous and had approx. 100-260 workers and approx. 30-200 brood items. Once in captivity, colonies were housed in nests consisting of a balsa wood slat (2.4 mm thick) with a 38 mm hole drilled through the center. Each slat was sandwiched between two glass microscope slides (50 × 75 mm), and a 2 mm wide slit was cut in one side of the slat that allowed ants to enter the nesting cavity. Sasaki et al. (2015) gives additional information on this type of nest (Sasaki et al. 2015). Each nest was kept in a lidded plastic box (11 × 11 × 3 cm). Colonies were maintained in the laboratory with a diet of protein (freeze-killed mealworms, Fancy Feast salmon pâté) and sugar (maple syrup, honey), which was provided weekly. Colonies always had access to water from cotton-stopped plastic tubes that were kept with colonies in each nest box.

Experiment 1: Variation in activity oscillations

Colony-level activity measurements: 18 colonies had their activity patterns assessed four times. Trials for each colony were separated by 48 hours. 12 hours before starting a 9-hour recording session, colony nest boxes were arrayed on a laboratory bench and placed beneath camcorders (Canon VIXIA) mounted on 18cm tall tripods. The bench surface was covered with white stationary to improve contrast between ants and the video background. The recording area was partially darkened by a curtain of 3-stop light-filter paper (Rosco Cinegel) to mitigate any disturbances caused by ambient light. Colonies were recorded for 9 hours (approx. from 12:00 to 21:00). No colonies were recorded on days that they received food.

Three weeks after filming the fourth trial, colonies were filmed for two additional 9-hour recording sessions to see how their activity patterns changed over a longer interval than the 9-day span needed to complete the first 4 trials. Between the fourth and fifth trials, colonies were made to emigrate into new nests and were run through a series of collective personality assays (data not shown). Brood and worker populations were nearly identical at the time of the first and second recording sessions (Brood: Pearson correlation coefficient [PCC] = 0.974, p ~ 0; Workers: PCC = 0.996, p ~ 0).

Videos of colony activity were processed with a method based on those used in previous studies (Cole 1991b; Boi et al. 1999). First, still frames were extracted from each video at 30 sec intervals. Each frame was sequentially and automatically evaluated using a custom MATLAB (Version 9.4 R2018a, MathWorks) script that relied on adaptive thresholding, converting the frames into binary images where only ants were present. Images were filtered of noise by removing any spuriously detected groups of pixels that were smaller than would be possible for an ant. After binarization, consecutive frames were subtracted from each other, thus giving the number of total pixels that had changed. A higher number of changed pixels indicates a greater amount of worker activity. For each pair of frames, the total number of changed pixels was then divided by the number of pixels detected in the first frame in order to express the colony's activity as the percent difference between each time step. This measure of activity thereby estimates the proportion of workers moving over time (Boi et al. 1999).

Individual-level activity measurements: To assess the relationship between the activity of individual workers and the properties of collective colony oscillations, we tracked the movements of several separate workers in ten recordings from the first run of trials in experiment 1. These ten recordings all came from different colonies. Recordings were selected so that five weakly rhythmic (i.e., erratic) colonies and five highly rhythmic colonies were represented. Ten worker ants per recording were randomly selected in the first frame of each video. These ants were manually tracked by collecting the x-y coordinates at the center of each of their mesosomas every 30 sec for the first two hours of each 9-hour recording. We then computed the pixel displacement of ants between frames. We excluded ants that wandered out of the nest during recording, along with one ant who became occluded from our view while still in her nest. This produced 27 workers from five erratic colonies and 27 workers from five rhythmic colonies. We further manually tracked the sole queen in each colony over the entire 9-hour duration of the recordings, but otherwise used the same protocol. Each of the queens from the 10 chosen colonies also had their movement data collected for all of the first 4 trials of experiment 1, resulting in 9-hour queen activity records from 40 separate trials.

Experiment 2: Queen removal

Colony-level activity measurements: 12 colonies were used in experiment 2. A set of recordings was made for the 12 colonies to assess their group activity patterns prior to queen removal. In order to film all 12 colonies in a single day, two blocks, each containing 6 randomly chosen colonies, were filmed for six hours in the morning (approx. 7:00 to 13:00) and for six hours in the evening (approx. 13:00 to 19:00), respectively.

Immediately after the colonies in a block had finished their recording session, they were forced to emigrate to a new nest. Emigrations were conducted in circular plastic arenas (25 cm diameter, 9 cm height). Ants were induced to relocate by removing the glass roof of the currently occupied nest (Dornhaus et al. 2008) and by placing a new nest (identical in construction to their current one) 6 cm in front of the destroyed nest. Three randomly chosen colonies in each block had their queens removed at the start of the emigrations. The remaining colonies in each block had a single random worker removed as a procedural control. All removed workers and queens, along with five larvae from their respective source colonies, were then imprisoned in separate new nests. These nests were identical to all other nests used in the experiments, except that they had no entrance, which prevented the individually isolated queens and workers from leaving. The day after queens and workers were abducted, every colony was refilmed. The filming of colonies post-removal used the same schedule as the initial round of filming (i.e., with colonies in the morning cohort being filmed from approx. 7:00 to 13:00 and those in the evening cohort being filmed from approx. 13:00 to 19:00).

Since some species of *Temnothorax* will compete to form dominance hierarchies centered around reproductive privileges, it was also necessary to examine the level of worker-worker aggression in queenless and queenright colonies. If *T. rugatulus* also creates hierarchies, the onset of fighting bouts among workers might account for any observed differences between treatments. Previous work has shown that, for species that exhibit them, dominance interactions will dramatically increase within 24hrs of queen removal (Heinze et al. 1997). Thus, we sampled recordings of colonies before and after queen/worker removal and manually scanned for aggressive interactions between workers. The following stereotyped interactions were classified as being "aggressive": biting, mandible spreading, dragging/pulling, and antennal boxing (Heinze et al. 1997; Heinze 2008; Modlmeier and Foitzik 2011).

Isolated individual-level activity measurements: Isolated workers and queens were filmed as well. This enabled us to test how different queens and workers were from each other when barred from any nestmate interactions. Since each nest contained only a single ant, location and movement data could be obtained automatically. Video frames, again spaced in intervals of 30 sec, were converted to binary images and the ant's centroid displacement between frames was stored. Workers and queens were filmed approximately 42 hours after they had been removed.

Thus, depending on the block assignment of their source colony, 6 individuals were filmed in the morning and 6 were filmed in the evening.

Colony-level activity analysis

Wavelet analysis was used to evaluate the rhythmicity of oscillations and the average period between peaks of activity for every time series in experiments 1 and 2 (see Supplementary Information). This allowed us to estimate the most prominent period of oscillation in each time series and quantitively compare the rhythmicity of oscillations between colonies (i.e., more rhythmic colonies would exhibit a higher maximum wavelet magnitude and the timing between their peaks of activity would be more consistent).

Colonies can be synchronized yet still oscillate erratically. For example, if during peaks of colony activity every ant is moving and during depressions of activity every ant is stationary, a colony could be said to be perfectly synchronized, even though the timing between peaks might not occur in regularly spaced intervals. To have a metric for synchrony that is distinct from rhythmicity, we used the index of dispersion:

$$S = \frac{\sigma^2}{\mu}$$

This index takes the variance in the number (percentage) of ants active during a trial and divides it by the average activity level over the same interval. Increasing values of S signify that comparatively more individuals are active together while also having more individuals inactive together. This metric, sometimes called the Fano factor in neuroscience contexts (Stevens and Zador 1998), has also been used in the past to assess variation in the synchronized motion of other insects (Despland and Simpson 2006).

Repeatability estimates for each of these three waveform traits was calculated through general linear mixed-effects models, with colony ID set as a random factor and a Gaussian error distribution. This was carried out using the package *rptR* for R version 3.4 (https://www.r-project.org). Our models' residuals conform to a Gaussian distribution, as determined by q-q plots.

Finally, we also used the MATLAB functions *findpeaks* and *islocalmin* to automatically identify locations of peaks in activity cycles and valleys (low points) in activity cycles. These functions detected peaks and valleys in each time series based on whether they exceeded a pre-set topographical prominence threshold. Prior to local maxima/minima detection, colony time series were smoothed using the Savitzky-Golay filter. We then calculated the average level of highest and lowest activity for each colony replicate. The average time between detected peaks in a trial was also computed to get a secondary measure of cycle period. This alternate measure of period needed to be used for the repeatability calculations (see Supplementary Information).

Individual-level activity analysis

For each individual worker and queen activity pattern from experiments 1 and 2, we classified an ant as being “active” if it had moved more than 1 pixel in 30 sec. and classified ants as “inactive” otherwise. Following (Cole 1992), we quantified differences between individuals by calculating the probability each ant had of switching from an inactive state to an active state (P_a) and the probability of switching from an active state to an inactive state (P_i). We also calculated the proportion of time each ant spent active. We avoided using wavelet analysis for comparing activity time series of individual ants because of the differing lengths of time that individuals were tracked for in experiments 1 & 2.

2.4 Results

Experiment 1: Variation in activity oscillations

Colony-level activity: Looking at all 108 activity records, the period of group oscillations ranged from 16 min to 104 min (mean: 48.4 min ± standard deviation: 16.0 min). There was a substantial degree of inter-colony variability in the shape of group activity oscillations (Figure 1; Supplementary Information, Figures S1 and S2). These differences were highly repeatable across the first 4 trials: synchrony ($r = 0.84$, CI = 0.67–0.92, $p < .0001$), period ($r = 0.59$, CI = 0.33–0.77, $p < .0001$), and rhythmicity ($r = 0.65$, CI = 0.39–0.81, $p < .0001$). When all 6 trials are included, colonies are still repeatable, but their repeatability is reduced: synchrony ($r = 0.68$, CI = 0.46–0.81, $p < .0001$), period ($r = 0.40$, CI = 0.17–0.58, $p < .0001$), and rhythmicity ($r = 0.51$, CI = 0.24–0.68, $p < .0001$). Despite the overall consistency in waveform traits over time, colonies can shift in trait-space. Colony D13, for example, becomes more synchronous in its last two trials, and colony A7, while remaining highly synchronous, elongates its period and reduces its rhythmicity in its final trials (Figure 1). Although differences between colonies persisted throughout the experiment, in aggregate, there was a common downward shift for colonies in average activity level after the fourth trial (Pearson correlation coefficient [PCC]: -0.643, $p < 0.0001$) along with an increase in synchrony (Pearson correlation coefficient [PCC]: 0.319, $p = 0.0008$), but not rhythmicity (Pearson correlation coefficient [PCC]: 0.170, $p = 0.08$).

The three primary waveform traits (i.e., rhythmicity, synchrony, and period) along with the average peak activity height, average valley activity height, and average total activity were averaged across the 6 trials to give each colony a single overall score for each metric. We then evaluated the correlation between these metrics and colony size by computing Pearson correlation coefficients (PCC). Rhythmicity and synchrony were highly correlated (PCC = 0.832,

$p < 0.0001$). Brood number was negatively correlated with both synchrony (Table 1; PCC = -0.646, p = 0.004) and rhythmicity (PCC = -0.660, p = 0.003). Worker number was negatively correlated with both rhythmicity (PCC = -0.572, p = 0.013) and synchrony (PCC = -0.468, p = 0.05), but this latter association was only marginally significant and is driven by two extreme points. Since all colonies inhabited nests of the same size, larger colonies had higher population densities. Thus, larger, denser colonies and those containing more brood were less synchronized and rhythmic in their oscillations. Worker number was negatively correlated with average peak height (PCC = -0.638, p = 0.004), but brood number was not (PCC = -0.404, p = 0.096). Neither worker number nor brood number were significantly correlated with average overall activity (worker number: PCC = -0.363, p = 0.138; brood number: PCC = -0.006, p = 0.981) or average valley height (worker number: PCC = -0.109, p = 0.666; brood number: PCC = -0.210, p = 0.403). Period was only correlated with brood number (PCC = 0.540, p = 0.021). The relation between worker number and brood number in *T. rugatulus* is known to scale allometrically (Cao and Dornhaus 2013). Thus, when our data is log-transformed, we verified that there was a positive relation between worker number and brood number (PCC = 0.301, p = 0.012). The worker to brood ratio was also not significantly correlated with any of the six waveform metrics.

Individual-level activity:

Workers in rhythmic colonies activate in unison. They have segments of rest and start moving at approximately the same time. Workers in erratic colonies also have segments of rest, but their activations are out of phase with each other. Despite the clear contrast between erratic and rhythmic colonies in the composite profiles of individual workers (Figure 2a, b; Supplementary Information, Figures S1a and S2a), workers in erratic colonies are not more active than workers in rhythmic colonies. Specifically, the proportion of time spent in an active state is not significantly different between workers in either type of colony ($t = -1.55$, $df = 50.616$, $p = 0.127$). Nor are there any detectable differences between ants in the probability of switching between behavioral states: P_a ($t = -1.17$, $df = 49.182$, $p = 0.247$) and P_i ($t = 1.24$, $df = 51.18$, $p = 0.222$).

Altogether, queens spent less time active on average than workers ($t = 5.065$, $df = 77.086$, $p < 0.0001$). Queens also had a significantly lower *inactive to active* transition probability P_a ($t = 3.3645$, $df = 68.662$, $p = 0.001$) and a higher *active to inactive* transition probability P_i ($t = -4.844$, $df = 78.44$, $p < 0.0001$) than workers. Unlike workers, queens in rhythmic colonies are significantly less active than their counterparts in erratic colonies (Supplementary Information, Figures S1b and S2b; $t = -2.516$, $df = 37.189$, $p = 0.016$). Thus, colonies containing queens that move more are more erratic, and vice versa. Only P_a, the probability of transitioning from *inactive to active*, was significantly lower in rhythmic colonies' queens ($t = -3.453$, $df = 32.107$, p-value $= 0.002$). The statistically significant differences in queen behavior between erratic and rhythmic colonies is detectable when queens are analyzed over the full 9-hours of their activity (above) *and* when only the first two hours of each queen's activity record is considered: proportion active ($t = -2.161$, $df = 32.147$, $p = 0.038$), P_a ($t = -2.402$, $df = 27.156$, $p = 0.023$). Both erratic and rhythmic queens were highly repeatable in the proportion of time spent active ($r = 0.80$, CI = 0.51–0.92, $p < .0001$), P_a ($r = 0.78$, CI = 0.48–0.91, $p < .0001$) and P_i ($r = 0.48$, CI = 0.08–0.74, $p = .001$). Colony synchrony is also highly negatively correlated with a queen's *inactive to active* transition probability (PCC = -0.535, $p = 0.0004$; Supplementary Information, Figure S3), and this relation follows an exponential decay curve.

Experiment 2: Queen removal

Colony-level activity: Removing queens had no effect on either the period (paired t-test: t = 0.483, df = 5, p = 0.650) or rhythmicity (paired t-test: t = 0.714, df = 5, p = 0.507) of a colony's oscillations, but synchrony was significantly reduced (Figure 3; Supplementary Information, Figure S4; paired t-test: t = 3.356, df = 5, p = 0.020). Colonies where workers were removed experienced no significant change in any of the three primary traits (Figure 3; Supplementary Information, Figure S5; paired t-tests: period, t = -1.189, df = 5, p = 0.288; rhythmicity, t = 0.291, df = 5, p= 0.783; synchrony, paired t-test: t = 1.099, df = 5, p = 0.322). The decrease in synchrony in queenless colonies seems to be a result of fewer ants being inactive together and an increase in average activity overall. Specifically, the average proportion of ants moving during valleys (minimums) of colony activity were substantially raised after queen removal (Figure 3; Supplementary Information, Figure S4; paired t-test: t = -10.253, df = 5, p = 0.0002), but not after worker removal (Figure 3; Supplementary Information, Figure S5; paired t-test: t = -1.5994, df = 5, p = 0.171). We did not observe any instances of aggressive interactions before or after either worker or queen removal.

Isolated individual-level activity: When deprived of social stimulation from nestmates, workers behave very differently (Figures 2a, b and 4). They no longer exhibit intervals of sustained inactivity. Instead, they roam endlessly, presumably in search of a conspecific. The proportion of time spent active by isolated workers is therefore much higher than for individuals in populated nests (t = 6.083, df = 11.123, p < 0.0001). Isolated queens do *not* spend more time active than queens in fully populated nests (t = -1.372, df = 5.503, p-value = 0.223), but isolated queens do spend less time active than isolated workers (t = 7.2, df = 8.261, p < 0.0001).

2.5 Discussion

Understanding the processes that underlie the emergence of synchronization in animal groups remains a perennial goal in the field of collective behavior (Couzin 2018). Here we sought to identify whether there exists stable intraspecific variation in the group activity of an ant model, and to illuminate candidate mechanisms that could underlie such differences. Our experiments confirm the existence of contrasting waveform geometries in *T. rugatulus* activity. Colonies occupy a gradient of possible levels of rhythmicity and synchrony, from predictable and synchronized to disordered, frenzied meanders (Figure 1). The absence of a single individual (the queen) can modify, perhaps temporarily, at least one of these group-level traits (i.e., synchrony).

The effects of colony size on activity cycles has been investigated in ants before (Cole 1991b; Cole and Cheshire 1996). Our findings agree with at least one major trend seen in this past work: there is no association between the number of workers and the period length of colony cycles (Cole 1991b). Yet, the negative correlation observed here between worker/brood number and rhythmicity is the reverse of the pattern observed in a congener (Cole 1991a; Cole and Cheshire 1996; Cole and Hoeg 1996). These differences could be explained by the colony sizes used in each study. Previous empirical tests in *T. allardycei* (formerly *Leptothorax*) used colonies with no more than 15 workers (Cole and Cheshire 1996; Cole and Hoeg 1996), which is comparatively small relative to this species' natural colony sizes. All of our colonies far exceeded that limit and more closely approximate the size of a typical colony. The discrepancy of our findings might also be due to genuine biological differences between the species, but because so few species have had their activity cycles examined, it is unclear what ecological characteristics are linked with interspecific differences in activity cycle properties. Alternatively, there could be some intermediate colony size that maximizes rhythmicity. Additional small colonies would be needed to test this hypothesis. Why large colony sizes and more densely populated nests should beget more erratic behavior is not certain. One speculative possibility is that larger colonies slow the propagation of activity within the nest by either physically impeding worker movement (e.g., piles of brood) or through different topologies of worker interaction networks [10]. It is also plausible that this trend reflects scalar relationships between metabolic rate, hunger level, and population density. Prolonged starvation increases colony activity in some ants (Franks et al. 1990), resulting in disrupted activity rhythms within nests (Hatcher 1992; Boi et al. 1999). Crowding inside the nest also increases metabolic rate in *T. rugatulus* (Cao and Dornhaus 2008). Densely packed nests might therefore be pulled towards erratic oscillations due to increased hunger or metabolic rate. This is in line with some computer simulations of colony activity cycles, which predict that higher densities will decrease colony synchrony (Cole 1992). But, other mathematical models of activity cycles predict the exact opposite (Miramontes et al. 1993). In natural conditions, *Temnothorax* colonies seem to have preferences for certain nest population densities when a queen is present (Mitrus 2015) and will split between multiple nests or expand their current one to maintain their desired density (Franks et al. 1992; Cao 2013). Thus, if activity cycles are adaptive, colonies could conceivably minimize variation in waveform traits through such density preferences. There is also some evidence that starvation in *T. rugatulus* actually *decreases* overall colony activity (Rueppell and Kirkman 2005).

We also detected a trend in how colonies' waveform traits changed over the six trials. The reasons for this trend are unclear, but this may indicate that there are consequences of recent nest emigration on colony activity cycles, or that some other cryptic external cue or experiential effects influence colony activity cycles.

The fact that queens improve the synchrony of intranidal activity cycles implies that, at least in *T. rugatulus*, not all aspects of this phenomena are solely driven by self-organization of behaviorally-equivalent agents. This does not appear to be the case with *T. allardycei*, where queenless colony fragments retain their synchrony and rhythmicity (Cole and Cheshire 1996; Cole and Trampus 1999). The hypothesis that activity desynchronization after queen removal might be due to the initiation of dominance interactions between workers was not supported by our results, but a more detailed study of queenless colonies is needed before some effect of queen fertility signaling can be completely ruled out. The loss of rhythmicity in the locomotor activity of *Camponotus* queens has been tied with phases of egg-laying (Sharma et al. 2004). We did not collect any data on the fecundity of queens in our study, but the possible connection between egg-laying behavior and activity cycles deserves further attention. Experiments on activity rhythms in *Aphaenogaster fula* uncovered special "catalyst" workers (Barnes 1941). Within a nest, certain worker ants spend more of their time active than their sisters, and their movement precipitates waves of motion inside the nest when they collide with others. Queens might take an analogous role in *T. rugatulus.* The queens of *T. curvispinosus* emit a volatile pheromone from a gland in their head, and when workers encounter a moving queen they hastily move out of her way, producing "explosions" of activity (Wilson 1974). Although we found that queens are not more active than workers in this study, queens might still elicit stronger reactions from interacting workers. If queens do trigger waves of worker activity, then this might explain the strong correlation between a queen's activation probability and the degree of her colony's synchrony (Supplementary Information, Figure S3). However, the present study can not conclusively demonstrate that more erratic queens are the cause of more erratic colonies. Queens might instead be reflecting the erratic or synchronous environment in their respective nests.

The increase in the average minima of colony activity levels after queen removal could suggest that, in addition to being a catalyst, queens might also be smothering worker activation. Workers of the related *T. unifasciatus* can be locked in both positive and negative associations with their colony's queen; some workers will follow the queen when she moves, and others will avoid her (Sendova-Franks and Franks 1995). Moreover, workers of some ant species have a tendency to aggregate near their queen (Coglitore and Cammaerts 1981; Cariou-Etienne et al. 1992; Doering and Pratt 2016). If a subset of *T. rugatulus* workers ceases moving in order to surround a stationary queen while a separate set of workers is repelled by her motion, then queen loss could conceivably destabilize normal group activity. The increase in minimum activity we observed is opposite to the trends noted in both wasps and other ants. As far as we are aware, other studies of queen removal on colony activity have found that it either depresses overall colony activity (Wheeler 1921; Breed and Gamboa 1977; Reeve and Gamboa 1983) or has no sensible effect (Jha et al. 2006).

How the activity patterns of individual workers assemble to create collective oscillations varies starkly by species. Unlike *T. rugatulus*, isolated *T. allardycei* workers have much longer intervals of inactivity (Cole 1991a; Cole 1991b). These intervals are normally distributed around a duration characteristic to each worker (Cole 1991c), and individuals spend more time inactive when alone. Interactions between an active worker and an inactive worker leads to a phase advance and onset of activity in the inactive worker in *T. allardycei* (Cole 1991c). This interaction then lengthens the amount of time each worker stays active (Cole and Cheshire 1996), thus creating an "infective" process of spreading activity. These features appear essential for sustaining rhythmicity and synchrony in *T. allardycei* (Cole and Trampus 1999). In *T. rugatulus*, by contrast, isolated workers spend nearly all their time active. It is only in a group that sustained intervals of inactivity appear in workers. Isolated workers of both *T. rugatulus* and *T. allardycei* also behave very differently from singleton *Diacamma* workers, which do show rhythmic bursts of activity even when alone (Hayashi et al. 2012).

It is possible that group activity cycles in *T. rugatulus* uses the inverted rule set of *T. allardycei* (i.e., interactions between pairs of active workers *shorten* the durations of activity). The absolute size of the nest might also contribute to how active each worker is (Christensen et al. 2015). It remains puzzling, however, why erratic *T. rugatulus* colonies lack rhythmicity. Workers in both rhythmic and erratic colonies spend less time active than isolated workers, but the phases of individual activity patterns clash in erratic colonies. The cause of this dissonance is unclear. How can it be that colonies are erratic, yet also have workers that oscillate at similar frequencies to workers in rhythmic colonies? It may be that erratic colonies experience a more limited type of synchronization (Rosenblum et al. 1996).

Multifarious explanations for the function of periodic activity cycles in ants have been proposed, and many questions remain unanswered. Functional explanations of the phenomenon include increased brood care efficiency (Hatcher et al. 1992; Delgado and Solé 2000), a tool for altering patterns of information transfer (Richardson et al. 2017), or that it is nothing more than an epiphenomenon of how workers interact (Cole 1991b). The coexistence of both erratic and rhythmic oscillatory modes in the same species, the evidence of queen influence, and the hyperactivity of isolated workers seen here are all conspicuously different to the traits observed in other close relatives. While these observations help to highlight the diversity of assembly mechanisms that can give rise to synchronized oscillations, they do not yet help us to explain why so many species exhibit this phenomenon. Thus, additional work on the topic of activity cycles in ants is certainly needed, as a unified framework for their purpose and their mechanistic underpinnings remains elusive. Our results here undermine the idea that self-organized processes among workers are solely responsible for the phenomenon, and there remains much to be discovered.

2.6 Funding

This research was supported by the National Institutes of Health (GM115509 to JNP).

2.7 Acknowledgements

We thank Brendan McEwan and Hannah Anderson for help with ant colony maintenance. We are grateful to Yohan Cho for helping to collect wild colonies. Matthew Prebus confirmed the identity of the *Leptothorax crassipilis* used in establishing baseline noise levels in the group activity analysis program. Finally, we thank Roger M. Nisbet, Holly V. Moeller, Stephen C. Pratt, and Corrie S. Moreau for helpful input on the experiments.

2.8 Data Accessibility

Analyses reported in this article can be reproduced using the data provided by Doering et al. (2019): https://doi.org/10.5061/dryad.4b92t21

2.9 References

Barnes TC. 1941. Rhythms of activity in ant colonies. J Gen Psychol. 25:249–255.

Bengston SE, Dornhaus A. 2014. Be meek or be bold? A colony-level behavioural syndrome in ants. Proc R Soc B. 281:20140518.

Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF. 1999. Coupled Oscillators and Activity Waves in Ant Colonies. Proc Biol Sci. 266:371–378.

Bolton B. 2003. Synopsis and Classification of Formicidae. American Entomological Institute.

Breed MD, Gamboa GJ. 1977. Behavioral Control of Workers by Queens in Primitively Eusocial Bees. Science. 195:694–696.

Buck J, Buck E. 1968. Mechanism of Rhythmic Synchronous Flashing of Fireflies: Fireflies of Southeast Asia may use anticipatory time-measuring in synchronizing their flashing. Science. 159:1319–1327.

Cao TT. 2013. High social density increases foraging and scouting rates and induces polydomy in *Temnothorax* ants. Behav Ecol Sociobiol. 67:1799–1807.

Cao TT, Dornhaus A. 2008. Ants under crowded conditions consume more energy. Biol Lett. 4:613–615.

Cao TT, Dornhaus A. 2013. Larger laboratory colonies consume proportionally less energy and have lower per capita brood production in *Temnothorax* ants. Insectes Sociaux. 60:1–5.

Cariou-Etienne A, Aron S, Passera L. 1992. Queen attractivity in the Argentine ant *Iridomyrmex humilis* (Mayr). Behav Processes. 27:179–186.

Charbonneau D, Dornhaus A. 2015. Workers “specialized” on inactivity: Behavioral consistency of inactive workers and their role in task allocation. Behav Ecol Sociobiol. 69:1459–1472.

Christensen K, Papavassiliou D, de Figueiredo A, Franks NR, Sendova-Franks AB. 2015. Universality in ant behaviour. J R Soc Interface. 12:20140985.

Coglitore C, Cammaerts MC. 1981. Étude du Pouvoir Agrégatif des Reines de *Myrmica rubra* L. Insectes Sociaux. 28:353–370.

Cole BJ. 1986. The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. Behav Ecol Sociobiol. 18:165–173.

Cole BJ. 1991a. Is Animal Behaviour Chaotic? Evidence from the Activity of Ants. Proc Biol Sci. 244:253–259.

Cole BJ. 1991b. Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction. Am Nat. 137:244–259.

Cole BJ. 1991c. Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav. 4:129–137.

Cole BJ. 1992. Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. Behav Ecol Sociobiol. 31:181–187.

Cole BJ, Cheshire D. 1996. Mobile Cellular Automata Models of Ant Behavior: Movement Activity of *Leptothorax allardycei*. Am Nat. 148:1–15.

Cole BJ, Hoeg L. 1996. The influence of brood type on activity cycles in *Leptothorax allardycei* (Hymenoptera: Faruicidae). J Insect Behav. 9:539–547.

Cole BJ, Trampus FI. 1999. Activity cycles in ant colonies: worker interactions and decentralized control. In: Detrain C, Deneubourg JL, Pasteels JM, editors. Information Processing in Social Insects. Basel: Birkhäuser Basel. p. 289–307.

Couzin ID. 2018. Synchronization: The Key to Effective Communication in Animal Collectives. Trends Cogn Sci. 22:844–846.

Delgado J, Solé RV. 2000. Self-synchronization and Task Fulfilment in Ant Colonies. J Theor Biol. 205:433–441.

Despland E, Simpson SJ. 2006. Resource distribution mediates synchronization of physiological rhythms in locust groups. Proc Biol Sci. 273:1517–1522.

Detrain C, Deneubourg J-L. 2006. Self-organized structures in a superorganism: do ants “behave” like molecules? Phys Life Rev. 3:162–187.

Doering GN, Pratt SC. 2016. Queen location and nest site preference influence colony reunification by the ant *Temnothorax rugatulus*. Insectes Sociaux. 63:585–591.

Doering G, Sheehy K, Lichtenstein J, Drawert B, Petzold L, Pruitt J. 2019. Data from: Sources of intraspecific variation in the collective tempo and synchrony of ant societies. Dryad Digital Repository. https://doi.org/10.5061/dryad.4b92t21

Dornhaus A, Holley J-A, Pook VG, Worswick G, Franks NR. 2008. Why do not all workers work? Colony size and workload during emigrations in the ant *Temnothorax albipennis*. Behav Ecol Sociobiol. 63:43–51.

Dornhaus A, Powell S, Bengston S. 2012. Group Size and Its Effects on Collective Organization. Annu Rev Entomol. 57:123–141.

Franks NR, Bryant S, Griffiths R, Hemerik L. 1990. Synchronization of the behaviour within nests of the ant *Leptothorax acervorum* (Fabricius)—I. Discovering the phenomenon and its relation to the level of starvation. Bull Math Biol. 52:597–612.

Franks NR, Wilby A, Silverman BW, Tofts C. 1992. Self-organizing nest construction in ants: sophisticated building by blind bulldozing. Anim Behav. 44:357–375.

Fujioka H, Abe MS, Fuchikawa T, Tsuji K, Shimada M, Okada Y. 2017. Ant circadian activity associated with brood care type. Biol Lett. 13:20160743.

Fujioka H, Abe MS, Okada Y. 2019. Ant activity-rest rhythms vary with age and interaction frequencies of workers. Behav Ecol Sociobiol. 73:30.

Gordon DM. 1995. The Development of Organization in an Ant Colony. Am Sci. 83:50–57.

Hatcher MJ. 1992. Activity patterns and organization within ant nests [PhD Thesis]. University of Bath.

Hatcher MJ, Tofts C, Franks NR. 1992. Mutual exclusion as a mechanism for information exchange within ant nests. Naturwissenschaften. 79:32–34.

Hayashi Y, Yuki M, Sugawara K, Kikuchi T, Tsuji K. 2012. Rhythmic behavior of social insects from single to multibody. Robot Auton Syst. 60:714–721.

Heinze J. 2008. Hierarchy length in orphaned colonies of the ant *Temnothorax nylanderi*. Naturwissenschaften. 95:757–760.

Heinze J, Puchinger W, Hölldobler B. 1997. Worker reproduction and social hierarchies in *Leptothorax* ants. Anim Behav. 54:849–864.

Hemerik L, Britton NF, Franks NR. 1990. Synchronization of the behaviour within nests of the ant *Leptothorax acervorum* (Fabricius)—II. Modelling the phenomenon and predictions from the model. Bull Math Biol. 52:613–628.

Herbers JM. 1983. Social Organization in *Leptothorax* Ants: Within-And Between-Species Patterns. Psyche (Stuttg). 90:361–386.

Jha S, Casey-Ford RG, Pedersen JS, Platt TG, Cervo R, Queller DC, Strassmann JE. 2006. The queen is not a pacemaker in the small-colony wasps *Polistes instabilis* and *P. dominulus*. Anim Behav. 71:1197–1203.

Keiser CN, Jones DK, Modlmeier AP, Pruitt JN. 2014. Exploring the effects of individual traits and within-colony variation on task differentiation and collective behavior in a desert social spider. Behav Ecol Sociobiol. 68:839–850.

Keiser CN, Vojvodic S, Butler IO, Sartain E, Rudolf VHW, Saltz JB. 2018. Queen presence mediates the relationship between collective behaviour and disease susceptibility in ant colonies. J Anim Ecol. 87:379–387.

Latty T, Ramsch K, Ito K, Nakagaki T, Sumpter DJT, Middendorf M, Beekman M. 2011. Structure and formation of ant transportation networks. J R Soc Interface. 8:1298–1306.

Miramontes O, Solé RV, Goodwin BC. 1993. Collective behaviour of random-activated mobile cellular automata. Phys Nonlinear Phenom. 63:145–160.

Mitrus S. 2015. The cavity-nest ant *Temnothorax crassispinus* prefers larger nests. Insectes Sociaux. 62:43–49.

Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. Behav Ecol. 22:1026–1032.

Modlmeier AP, Keiser CN, Shearer TA, Pruitt JN. 2014. Species-specific influence of group composition on collective behaviors in ants. Behav Ecol Sociobiol. 68:1929–1937.

Möglich M. 1978. Social-Organization of Nest Emigration in *Leptothorax* (hym Form). Insectes Sociaux. 25:205–225.

Paranjpe DA, Sharma VK. 2005. Evolution of temporal order in living organisms. J Circadian Rhythms. 3:7.

Pratt SC, Mallon EB, Sumpter DJT, Franks NR. 2002. Quorum Sensing, Recruitment, and Collective Decision-Making during Colony Emigration by the Ant *Leptothorax albipennis*. Behav Ecol Sociobiol. 52:117–127.

Pratt SC, Sumpter DJT. 2006. A tunable algorithm for collective decision-making. Proc Natl Acad Sci. 103:15906–15910.

Reeve HK, Gamboa GJ. 1983. Colony activity integration in primitively eusocial wasps: the role of the queen (*Polistes fuscatus*, Hymenoptera: Vespidae). Behav Ecol Sociobiol. 13:63–74.

Richardson TO, Liechti JI, Stroeymeyt N, Bonhoeffer S, Keller L. 2017. Short-term activity cycles impede information transmission in ant colonies. PLOS Comput Biol. 13:e1005527.

Rosenblum MG, Pikovsky AS, Kurths J. 1996. Phase Synchronization of Chaotic Oscillators. Phys Rev Lett. 76:1804–1807.

Rueppell O, Kirkman RW. 2005. Extraordinary starvation resistance in *Temnothorax rugatulus* (Hymenoptera, Formicidae) colonies: Demography and adaptive behavior. Insectes Sociaux. 52:282–290.

Sasaki T, Colling B, Sonnenschein A, Boggess MM, Pratt SC. 2015. Flexibility of collective decision making during house hunting in *Temnothorax* ants. Behav Ecol Sociobiol. 69:707–714.

Sendova-Franks AB, Franks NR. 1995. Demonstrating new social interactions in ant colonies through randomization tests: separating seeing from believing. Anim Behav. 50:1683–1696.

Sharma VK, Lone SR, Goel A, Chandrashekaran MK. 2004. Circadian consequences of social organization in the ant species *Camponotus compressus*. Naturwissenschaften. 91:386–390.

Smith AA, Hölldobler B, Liebig J. 2012. Queen-specific signals and worker punishment in the ant *Aphaenogaster cockerelli*: the role of the Dufour's gland. Anim Behav. 83:587–593.

Stevens CF, Zador AM. 1998. Input synchrony and the irregular firing of cortical neurons. Nat Neurosci. 1:210–217.

Wheeler WM. 1921. Observations on Army Ants in British Guiana. Proc Am Acad Arts Sci. 56:291–328.

Wilson EO. 1974. Aversive Behavior and Competition within Colonies of the Ant *Leptothorax curvispinosus*. Ann Entomol Soc Am. 67:777–780.

Winfree AT. 2001. The Geometry of Biological Time. Springer Science & Business Media.

Wright CM, Skinker VE, Izzo AS, Tibbetts EA, Pruitt JN. 2017. Queen personality type predicts nest-guarding behaviour, colony size and the subsequent collective aggressiveness of the colony. Anim Behav. 124:7–13.

Figure 2.1

Data points of collective activity plotted for 3 selected colonies across 6 trials. Colonies A18 (178 workers, 92 Brood) and A7 (101 workers, 52 Brood) exhibit rhythmic and synchronous oscillations. D13 (262 workers, 131 Brood) is erratic. The bottom two plots in each column represent trials 5 & 6, which were separated from trial 4 by three weeks. Colonies can show consistent differences in rhythmicity, period, and synchrony.

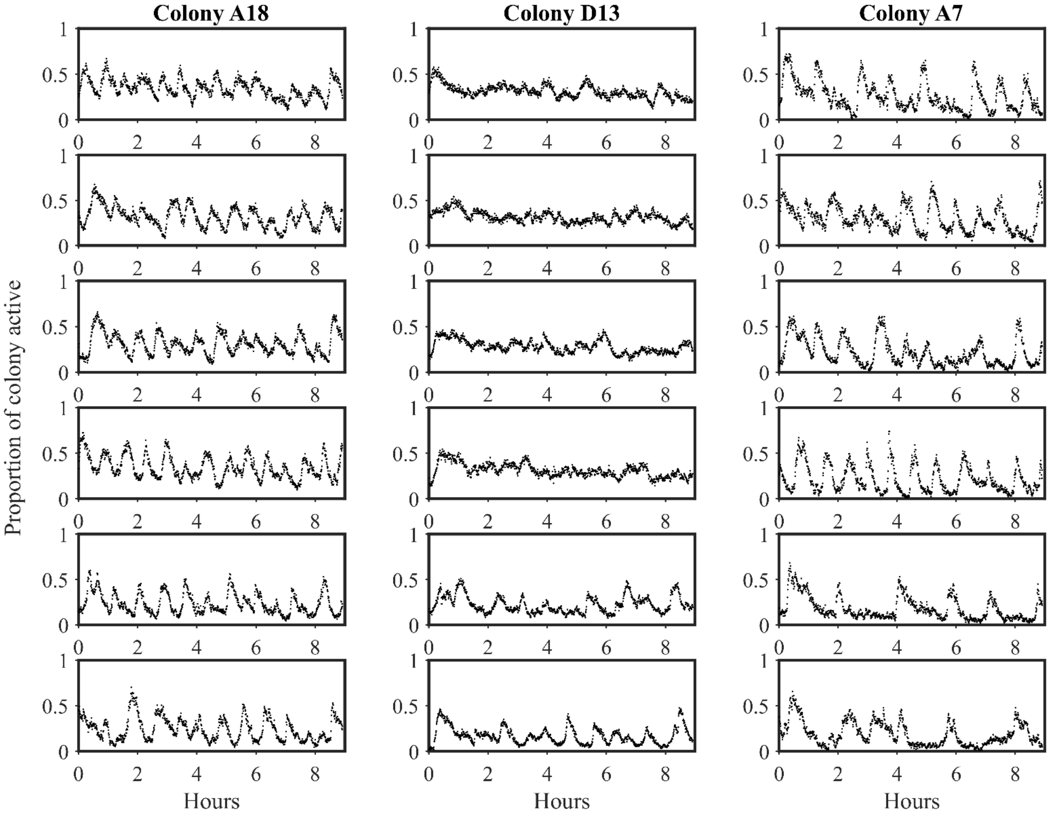


Figure 2.2

Data points of collective activity and movement patterns of individuals for two colonies (A7's fourth trial and A9's third trial). Panels A and B are activity records over the first two hours of a trial, Panels C and D are the activity records over the entire 9-hours of the same trials. Colony A7 (101 workers, 52 Brood) represents a rhythmic colony. A9 (137 workers, 119 Brood) represents an erratically oscillating colony. Each solid colored line in panels A and B depict the movements of individual ants (10 per colony; 5 ants in A9 and 8 ants in A7 span the full 2 hours), and black dots represent colony-level activity. The thicker purple line depicts the movement of the queen. Queens in rhythmic colonies spend less time active than queens in erratic colonies, and workers in erratic colonies oscillate out of phase with each other.

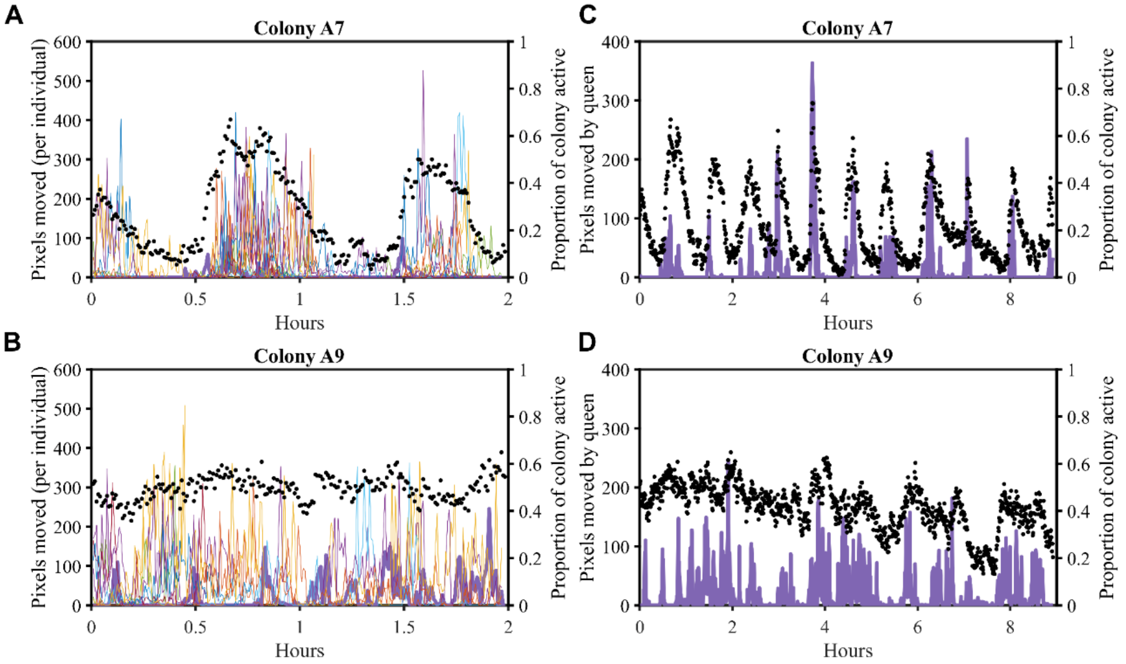


Figure 2.3

The synchrony and average valley heights (point of lowest activity) for colonies before and after the removal of queens/workers. Black dots indicate initial readings from colonies. Red dots indicate readings from colonies after individuals were removed. Lines between dots connect readings from the same colony. Removing Queens (but not workers) reduces colony synchrony and increases the average proportion of ants moving during valleys of activity.

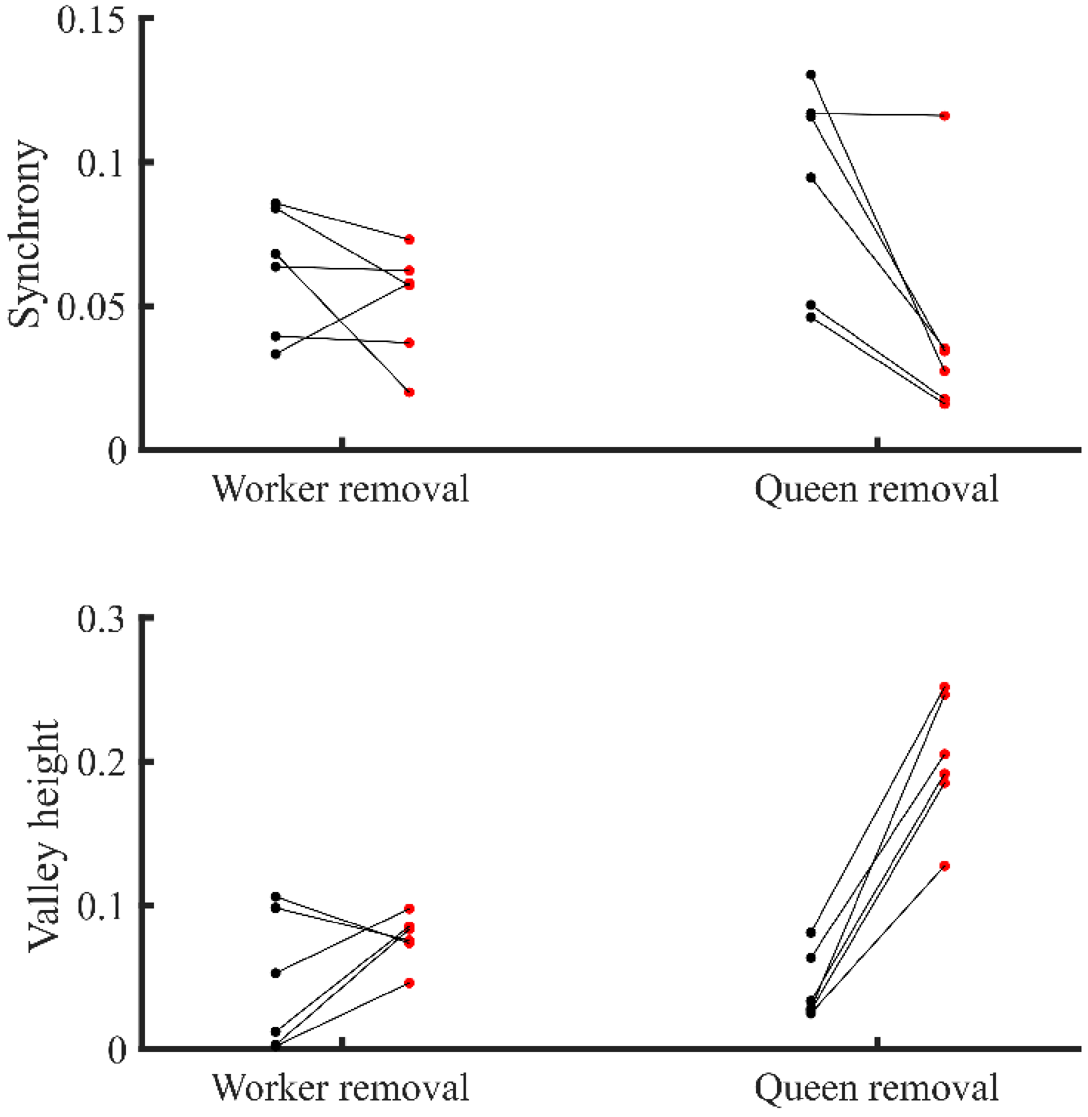
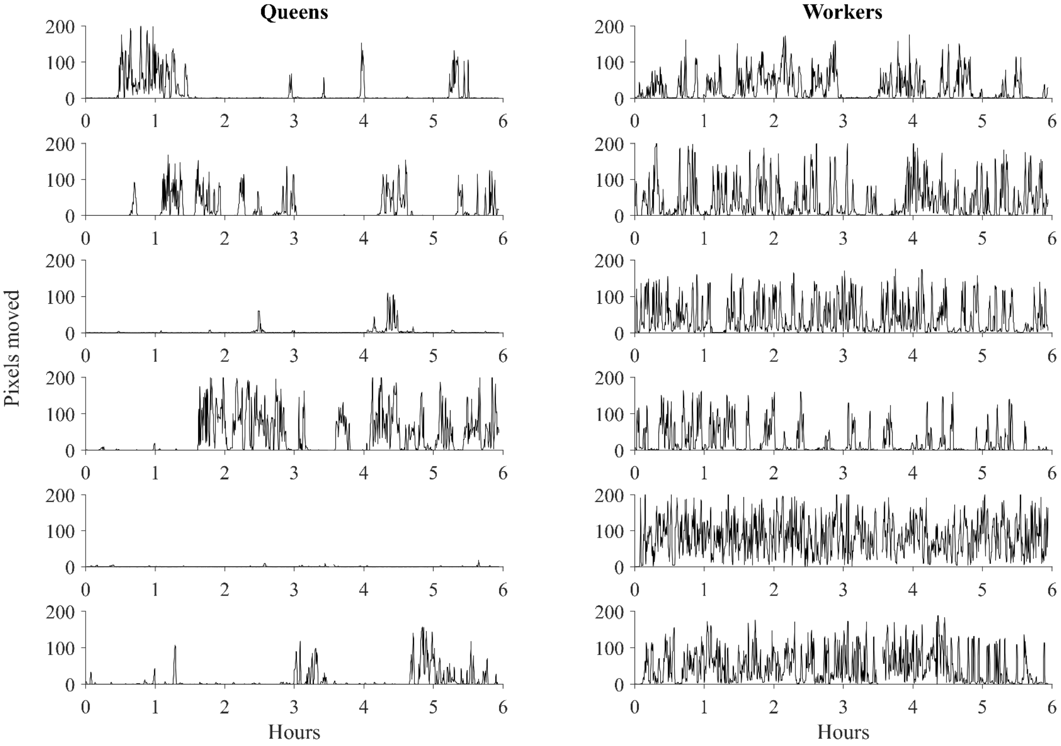


Figure 2.4

The individual activity patterns of 6 isolated workers and 6 isolated queens over a 6-hour interval. Queens exhibit lower levels of activity than workers. Works exhibit more frantic and apparently stochastic activity patterns when isolated.



1

Table 2.1.

A summary of the p values for the relationships between the 6 waveform traits and worker and brood populations.

	Worker number	*Brood number*
Synchrony	0.050	0.004
Average activity	0.138	0.981
Valley Height	0.666	0.403
Peak height	0.004	0.096
Period	0.749	0.021
Rhythmicity	0.013	0.003

CHAPTER 3 – NOISE RESISTANT SYNCHRONIZATION AND COLLECTIVE RHYTHM SWITCHING IN A MODEL OF ANIMAL GROUP LOCOMOTION

3.1 Abstract

Biology is suffused with rhythmic behaviour, and interacting biological oscillators often synchronize their rhythms with one another. Colonies of some ant species are able to synchronize their activity to fall into coherent bursts, but models of this phenomenon have neglected the potential effects of intrinsic noise and interspecific differences in individual-level behaviour. We investigated the individual and collective activity patterns of two *Leptothorax* ant species. We show that in one species (*Leptothorax* sp. W) ants converge onto rhythmic cycles of synchronized collective activity with a period of about 20 min. A second species (*Leptothorax crassipilis*) exhibits more complex collective dynamics, where dominant collective cycle periods range from 16 min to 2.8 hours. Recordings that last 35 hours reveal that, in both species, the same colony can exhibit multiple oscillation frequencies. We observe that workers of both species can be stimulated by nestmates to become active after a refractory resting period, but the durations of refractory periods differ between the species and can be highly variable. We model the emergence of synchronized rhythms using an agent-based model informed by our empirical data. This simple model successfully generates synchronized group oscillations despite the addition of noise to ants' refractory periods. We also find that adding noise reduces the likelihood that the model will spontaneously switch between distinct collective cycle frequencies.

3.2 Introduction

Synchronization is one of the most pervasive examples of collective behaviour, being present throughout numerous biological [1] and physical contexts [2]. An extensive literature exists on the synchronization of coupled oscillators [3], and many fundamental aspects of synchronization are consequently well understood. More recently, however, efforts have shifted towards understanding the generation of rhythms and synchronization in more complex situations. Chief among these are scenarios that involve mobile oscillators [4], heterogeneity [5], and the role of noise in synchronization [6]. These features are especially relevant to the study of synchronized behaviour in animal social groups because they frequently mingle all three elements, having constituents that are mobile, inherently noisy, and heterogenous in their behaviour [7–9].

Insect societies provide an excellent opportunity to experimentally investigate social synchronization because, in some taxa, the entire population of a colony can be observed simultaneously, and the behaviours of separate individuals can be directly assessed [10]. Several species of ants exhibit reliable short-term activity cycles (STACs), where worker ants inside a nest partition their activity into coherent, repeating pulses with periods ranging from 20 to 50 min [11–13]. Colony tasks, like trophallaxis or feeding larvae, are believed to be fulfilled during these activity bursts, as most ants remain motionless during the time separating cycles [14]. Ant STACs are generated endogenously; there is no evidence for any kind of external signal that synchronizes colonies [12], and although the presence of a queen can help to maintain STACs, neither she nor any other specific ant is necessary for these activity cycles to emerge [12,13]. Individual worker ants can move and become active through their own agency in an arrhythmic fashion [15] but can also stimulate nestmates to become active [16]. Activity pulses can therefore propagate through the colony analogous to a wave [17].

Most previous studies on ant STACs have been conducted using colonies from the closely related genera *Temnothorax* and *Leptothorax* [12]. These genera often have simple, single-chambered nests [18] with small colonies (< 200 workers) where all individuals can be monitored continuously. Although some work has been directed at modelling periodic activity waves in ants [19,20], empirical data are scarce. Moreover, several aspects of the physics underlying this phenomenon are not understood [21]. For instance, it is not known how noise in the behaviour of individual ants may alter their synchronization. In this context we define noise as the amount of inherent randomness or unpredictability in the behaviour of individuals. Noise, defined in this way as probabilistic behaviour, is pervasive in biology [22] and can be essential to the spatiotemporal characteristics of coupled oscillators and excitable media [23]. In the phenomenon of coherence resonance, for example, a group of oscillators that share a single external source of noise can experience greater levels of synchronization than they would without noise [6].

There is evidence that worker ants are likely to have refractory periods where they are inactive and less susceptible to activation by nestmates [16,24]. Because many individual-level behaviours in ants are probabilistic and are not rigidly predictable [25,26], the durations of these refractory periods are not expected to be absolute [24]. The lengths of time that workers are refractive are instead likely to fluctuate randomly for each ant within some range. Different species also appear to oscillate in distinct frequency ranges [13,14,17], and it has been argued that colonies appear to be capable of exhibiting multirhythmicity [12], which is defined as a spontaneous switching between different oscillation frequencies [27]. Models of ant STACs have yet to tackle the possible causes of interspecific differences in *cycle frequency*, the potential for STAC multirhythmicity, or the effects of noise in ants' refractory periods. It is plausible that there are interspecific differences in individual-level behaviour that account for the variation seen in STAC frequencies between species. Like other models of excitable media [6,28], it is also conceivable that when intrinsic behavioural noise is added to STAC models, the rhythms of collective oscillation may become more predictable. We sought to address these topics by first conducting a set of exploratory observations with colonies and individuals from two previously unstudied Nearctic species of *Leptothorax*: *Leptothorax crassipilis* (Figure 1A) and the taxonomically undescribed *Leptothorax* sp. W (Figure 1C). Using these empirical observations, we then built an agent-based model of short-term activity cycles and investigated whether 1) collective-level interspecific differences in STAC frequencies could be reclaimed by our model, 2) if collective oscillations can survive in the presence of stochastic refractory periods, and 3) if refractory noise can induce or inhibit multirhythmic oscillations.

3.3 Materials and methods

Colony information

The *Leptothorax crassipilis* Wheeler 1917 colonies used in this *study* were collected from rock crevices in the Pinal Mountains near Globe, Arizona in February and May 2018 and June 2019. The *L.* sp. W colonies were collected from rotting acorns in Fish Creek, Wisconsin in July 2018 and in May and July 2019. *Leptothorax crassipilis* colonies ranged in size from 8-248 individuals, and *L.* sp. W colony sizes spanned 7-61 individuals. Six brood-less *L.* sp. W colony fragments with less than 5 workers each contributed ants for our studies on isolated workers. Colonies were maintained using standard ant husbandry techniques (supplementary material).

Activity measurements

We filmed 23 colonies of *L. crassipilis* and 15 colonies of *L.* sp. W for approximately 9 hours each to characterize the typical patterns of collective movement activity in both species. From this set of colonies, two colonies of *L.* sp. W and four colonies of *L. crassipilis* (plus two additional *L. crassipilis* colonies not from the original 23) were chosen to be filmed for an additional 35 hours to examine how cycles change over a longer observation window.

Time series of collective locomotor activity for entire colonies were obtained using a version [13] of the automated techniques originally developed by Cole [14], and Tofts and Hatcher [24,29]. Colonies' nest boxes (11 × 11 × 3 cm) were placed over pink/white paper to enhance contrast with the ants and recorded with Canon VIXA camcorders. Colony recordings were processed by extracting frames from each video to generate image sequences where each image was separated from the next by 30 seconds. Each image in a sequence was binarized using an adaptive threshold [30] so that all objects other than ants residing in their nest were filtered out of the image. Regions in an image that contain ants can be distinguished from non-ants due to the insects' dark integument appearing over the lighter paper background. Pairs of successive images were then subtracted from each other to determine the number of pixels that had changed from 0 to 1 between frames, and this quantity was divided by the number of pixels in the first frame of each pair to estimate the proportion of ants in a nest that moved every 30 seconds [13,17].

We studied the movements of isolated ants to see if individual-level behavioural patterns differed between the two species and to guide the parameterization our agent-based model. Previous work in *Temnothorax allardycei* using isolated workers and small groups of ants removed from their nests has shown that short-term activity cycles emerge gradually as aggregate size is increased [31]. This result suggests that studying workers in isolation can provide at least some insight into the mechanisms that enable STACs in colonies. Twenty workers from each species were removed from multiple source colonies, and each individual was filmed in isolation for 30.8 hours so that movement patterns could be tracked in the absence of social interactions. Recordings of isolated individual ants were conducted by confining workers to separate plastic petri dishes (45 mm diameter). The cotton tip of a tube of water was available to ants in each dish through a hole drilled in the side of each dish. A damp cotton plug blocked escape through the hole and provided the ants with a constant source of moisture to prevent desiccation over long filming sessions. One *L. crassipilis* worker was injured and perished while it was being isolated, resulting in one fewer individual-level time series for that species. Because recordings of isolated singletons involved only one ant in each video, we automatically tracked the locomotor activity (confined to two-dimensions) of these individuals by calculating the distance the centroid of the focal ant moved in pixels every 30 seconds [13].

Time series analysis

All empirical time series examined in this study were analysed in the same way. Time series were first processed with a Gaussian-weighted moving average filter with a window size of 15 points (i.e., 7.5 min) to reduce noise. Smoothing the time series with this window size prevented the spurious detection of extremely fast oscillations that were merely artifacts of the tracking algorithm (Figure S1). Data was then normalized so that the largest and smallest values in a time series were reassigned to be 1 and 0 respectively, and all intermediate values were rescaled to fall between these two points. The locations of peaks in activity time series were determined using the MATLAB function *findpeaks*. This function was set to detect peaks in the time series that exceeded a prominence of 0.2 units of normalized activity [13]. These automatically detected peak locations were used to compute the mean inter-beat interval (IBI) and coefficient of variation (CV) associated with each time series. The coefficient of variation was defined as the variability (standard deviation) in time between automatically detected activity peaks (T_p) divided by the mean time between peaks (i.e., the mean IBI).

$$CV = \frac{\mathrm{std}(T_p)}{\langle T_p \rangle} \tag{1}$$

For time series of colony-level activity, we calculated the dominant oscillation period of each smoothed and rescaled time series using wavelet analysis, which is well suited to process the often non-stationary activity patterns of ant colonies [13,32]. The wavelet analyses to detect the dominant periods in colony activity time series were conducted in MATLAB using a 1D Morse continuous wavelet transform implemented with the *cwt* function. Briefly, after computing the continuous wavelet transform of each colony time series, we excluded results occurring within the "cone-of-influence" to reduce edge artifacts. We then found the frequency band associated with the highest wavelet magnitude. It should be noted that this method can result in identical estimates of period for different time series. Previous work provides greater detail about using this method on ant activity cycles [13]. Like their empirical counterparts, time series obtained from all model simulation runs were also processed with a 15-point moving average filter before we applied wavelet analysis. Because the long simulation outputs from our agent-based model exhibited stationarity, we also used Lomb-Scargle spectral analysis on these time series. All time series summary data are presented as average ± standard deviation.

In addition to the wavelet analysis described above, the 35-hour recordings of colony activity were also analysed with Lomb-Scargle periodograms to explore whether colonies could exhibit different oscillation frequencies within the same time series. Because the 35-hour time series are somewhat non-stationary, we detrended the 35-hour time series prior to Lomb-Scargle spectral analysis to remove trends in the data that were not part of short-term activity cycles. This was done using the *detrend* function in MATLAB with a 4th degree polynomial. Because these time series are 35 hours long, sustained oscillations from short-term activity cycles should be detectable in the power spectra.

Ant-ant interactions

Physical encounters between individual ants can promote activity in dormant individuals and spread activity throughout *Leptothorax* nests [16,24,33]. Because physical touch spreads activity in these ants, interspecific differences in how ants respond to encounters may also exist between the two *Leptothorax* species, which in turn could affect their collective activity cycles. Acquiring empirical information on how workers in both species react to physical stimulation is also necessary to inform the construction of our agent-based model. We therefore collected data on the activity patterns of ants when they were among their sisters inside their nests. First, we investigated the likelihood that inactive ants would respond to physical interactions with their nestmates. We randomly selected (haphazardly, without the aid of a pseudorandom number generator) video recordings of two colonies of each species and selected 15 focal ants from each video that became active through stimulation during a single, pre-determined cycle of colony activity. We recorded the times at which any ant made tactile contact with the inactive focal ants, and whether contact elicited activity from the focal ants (see supplementary material). To confirm that refractory-like periods are indeed present in both species, we used binomial generalized linear mixed models (GLMM) to test whether there was a relationship between an ant's length of time inactive and its probability of waking from nestmate stimulation. We also included the number of stimulations each ant received before becoming active as a fixed effect in the GLMM models to assess whether "response thresholds" could better explain the activation patterns of individuals. The idea of response thresholds (where workers perform an action only after their perception of a stimulus exceeds an internal threshold) is commonly used to explain division of labour and other aspects of collective behaviour in social insects [34–36]. If a basic kind of response threshold system were at play here, it would mean that an inactive ant's probability of activation would depend on the cumulative number of physical stimulations she receives after becoming inactive. Additionally, we estimated stimulation survival curves relating the probability of an inactive ant ignoring a stimulation event with how long that ant had been inactive.

Although this analysis may provide evidence for differences between the two species in how workers respond to physical contact, the workers were selected for survival analysis based on if they had become active during a *single* colony cycle. This sample may therefore underestimate the variation in refractory periods exhibited by workers in both species. To investigate the range of possible refractory periods in colonies, we also monitored individuals using an additional method. We selected 4 colonies of each species and randomly (i.e., haphazardly) chose 5 ants every 30 min of a colony's recording over 9 hours (resulting in 45 observations per colony), identified the time when each ant became inactive closest to these 30 min intervals, and recorded the duration that each ant spent inactive before either activating spontaneously or through stimulation. We used this set of inactivity durations as a proxy to estimate the range of refractory periods possible in each species. Finally, we also manually gathered data on the typical amount of time workers spend active when they are inside their nests. To do this, we selected 11 focal ants from one recording of each species (colony sizes: *L.* sp. W = 18; *L. crassipilis* = 31). For each focal ant, we recorded all physical interactions as outlined above along with every time the ant became either active or inactive for 3 hours or until the focal ant left the nest to forage.

Model description and simulations

We built a model of collective ant activity cycles by first considering the two known processes that cause an ant to become active: 1) spontaneous activation and 2) nestmate stimulation. We combined these processes into a simple algorithm followed by individual ants (Figure 5A). Individual ants could be in two possible states: active or inactive. When an ant becomes active, it remains so for a fixed duration (A). While active, the ant will roam in a random walk through the simulation arena, where it can potentially awaken inactive ants it encounters (nestmate stimulation). While active, walking ants randomly pick a heading within 45 degrees of their current orientation and move 1 step in that direction. The two-dimensional arena (grid) that simulated ants could explore was bounded, and if an ant reached an edge it would select an integer in the range [0, 180], rotate by that many degrees, and continue moving.

We simulated our model in the NETLOGO language [6] using aggregates on a grid whose size was held constant at 32 x 32 patches (each patch is a square of 1x1 arbitrary units of length). Individual agents (ants) could move on the grid (i.e., between patches) while in their active state. A stimulation event was defined as the moment an active and inactive agent became at least 1 length unit apart. Ants were allowed to freely pass through one another (i.e, more than one ant could occupy the same patch). If two inactive agents occupy the same patch and one of them becomes active, this would therefore also qualify as a stimulation event if the ants were within 1 length unit of each other. The random walk of simulated active ants (moving 1 unit every time step in a direction ± 45 degrees of its current heading) is similar to other models of random ant movement [7]. The relative amount a simulated ant moves in each time step is approximately equal to one second of movement in real ants. Although the precise walking and interaction patterns of *Leptothorax* are not directly relevant to the research questions we are addressing with our model, we also ran simulations where the amount of stochasticity in the random walk of agents was varied to see if this had any impact on our model's results. This was accomplished by having agents determine the direction of their next step in the arena by adding either ± 5 degrees or adding ± 360 degrees to their current heading. Agents in simulations where headings were adjusted by ± 360 degrees at each walking step thus had fully random walks, and agents in simulations that adjusted headings by only ± 5 degrees had straighter and more predictable walking paths.

Every time an ant becomes inactive, two parameters are set: 1) the length of time the ant will remain inactive before activating (S; i.e., spontaneous activation) and 2) the length of time the ant will ignore contacts from other ants (R; i.e., refractory period). These parameters are set by sampling from pre-defined distributions of intrinsic inactivity durations and stimulation refractory periods, respectively. The level of noise (uncertainty) in individual ant behaviour can be controlled by modifying the two underlying distributions from which parameters R and S are sampled. All simulations were run using a colony size of 50 ants, and all simulations consisted of 100,001 time steps (corresponding to roughly 27.8 hours of live ant observation). Although this study was not designed to assess the effect of worker density, the choice of using 50 ants in simulated colonies results in a biologically reasonable population density. Because worker *Leptothorax* ants are approximately 3mm long and agents in the model are essentially 1 unit/patch long, the size of a patch in the model can be thought of as being approximately 3x3 mm. The area of the simulated nests is thus approximately $(32*3)^2 = 9216$ square millimetres, and the area of the artificial circular nests from our empirical observations is $\pi * (19)^2 = 1134$

square millimetres. Since the populations of our *Leptothorax* colonies ranged from 7-248 individuals, 50 simulated ants occupying approximately 9216 square millimetres falls near the kind of densities that the smaller colonies in our artificial nests experienced.

Using the empirical data collected from individuals to parametrize our model, we ran simulations to determine if any of the observed collective level behaviours seen in real colonies of either species could be reproduced by the model. The mean for parameter S was determined for both species by taking the average value (rounded to the nearest integer) of isolated individuals average IBI values. Simulated ants would then set S each time they became inactive by sampling from an exponential distribution with a rate parameter of $\lambda = \frac{1}{\langle S \rangle}$. Parameter R was determined for *L. crassipilis* by taking the mean duration of inactivity of ants inside colonies, and ants would set their R when inactive by sampling from an exponential distribution with a rate parameter of $\lambda = \frac{1}{\langle R \rangle}$. Parameter R was instead determined for *L.* sp. W by having ants sample from a uniform distribution whose limits were the edges of the interquartile range of inactivity durations of ants inside colonies. Because the durations of activity had less variation than the durations of inactivity, we set A as a constant in both species. A was determined for each species using their median durations of activity when in nests with conspecifics.

The parameters for artificial *L.* sp. W colonies were: $R \sim$ Uniform(530 sec, 1415 sec) ; $S \sim$ Exp(3824 sec); and A = 218 sec. The parameters for artificial *L. crassipilis* colonies were: R ~ Exp(1513 sec); S ~ Exp(2385 sec); and A = 138 sec. Simulations of colonies always used aggregates with 50 ants with an initial condition of 25 ants starting in the active state and 25 ants starting in the inactive state.

To understand how the refractory period and its associated noise might modify the tempo of collective oscillations in the model and whether or not these factors can lead to multirhythmic behaviour, we also conducted simulations where we systematically varied the refractive period (*R*) along with the amplitude of refractory noise (Ω). Starting with a fixed value of *R,* we ran simulations where ants could sample their refractory periods from a uniform distribution with a progressively increasing width whose mean remained *R*. For example, if $\Omega = 300$ and $\langle R \rangle = 1100$ sec, every time an ant becomes inactive, it will determine its refractory period by randomly selecting any integer in the range [800 seconds, 1400 seconds] with equal probability. To ensure arrhythmic spontaneous activation of individuals, the values of parameter *S* were sampled from an exponential distribution with a rate parameter of $\lambda = \frac{1}{\langle S \rangle}$.

3.4 Results

Activity patterns of colonies

Although both *Leptothorax* species possess STACs, we found the distributions of colony cycle periods differ significantly between them (Figure 1B; Kolmogorov–Smirnov test: D = 0.734, $p < 0.0001$). *Leptothorax.* sp. W shows little variation between colonies in the dominant period of its STACs; colonies oscillate with a period of 21.2 ± 4.6 min (Figure 1E; supplementary material, Video S5). These period values are similar to those reported for the related species *L. acervorum* [29,32]. In contrast, *L. crassipilis* has an average period of 56.8 ± 39.9 min, and colonies expressed multiple oscillation periodicities ranging from 16.0 to 169.4 min (Figure 1D). The dominant period of the collective oscillations was not correlated with colony size in either species (*L. crassipilis* - Pearson correlation: $r = 0.1009$, $p = 0.6024$; *L.* sp. W - Pearson correlation: $r = -0.0848$, $p = 0.7463$; Figure S2).

An examination of the longer, 35-hour colony time series indicates a potential for multirhythmic collective cycles in *Leptothorax* (Figure 2). In multiple colonies from both species, more than one distinct short-term activity cycle periods co-occur within the same time series. This can be seen in the Lomb-Scargle periodograms of the time series as at least two clear peaks in the power spectra (Figure 2A-C). For instance, in the *L*. sp W colony presented in figure 2A, the dominant oscillation period is approximately 20 min, and this rhythm pervades throughout the 35-hour recording, yet the periodogram reveals a secondary rhythm with a period of about 3 hours. This longer rhythm becomes visually obvious when larger amounts of smoothing are applied to the time series (see green line of figure 2A). *Leptothorax crassipilis* colonies also exhibited multiple rhythms within the same time series (Figure 2B,C). In colonies that had both a "long" and "short" rhythm, the long rhythm occurred simultaneously with the shorter one, but the long rhythms also give the impression that they might sometimes fade out, leaving just the faster rhythm. Not all colonies expressed multiple rhythms. The *L. crassipilis* colony L4, for example, has just one clear peak in its periodogram. This peak occurs at 2.6 hours, and the time series plot shows that the long cycles persist for the entire activity record (Figure 2D). As evidenced by the two tall peaks that emerge when the rescaled Lomb-Scargle power spectra of all 35-hour time series are summed together, several of the "long" periods from different colonies are all very close to 3.8 hours, and several of the "shorter" periods in different *L. crassipilis* colonies are all very close to 1.4 hours (Figure 3).

Activity patterns of isolated individual ants

We found the activity of isolated workers of both species showed sustained intervals of inactivity interspersed with short bursts of movement (Figure 4A,B). Worker activity resembled trains of action potentials in spiking neurons, and were accordingly analysed by calculating the mean time between activity spikes (inter-beat interval - IBI) and the coefficient of variation of inter-beat times (CV), two common metrics used in neuroscience [37]. Processions of activity spikes in workers of *L.* sp. W were largely arrhythmic (CV = 0.97 ± 0.25, Figure 4C), and were often indistinguishable from a Poisson process (i.e. CV = 1). A lower coefficient of variation for *L. crassipilis* spike trains (CV = 0.74 ± 0.16, Figure 4C) reveals that activity bursts are more predictable in this species than in *L.* sp. W (LME: $t_{20} = 3.38$ $p = 0.003$). The average interval between consecutive spikes in *L. crassipilis* individuals (IBI = 39.7 ± 17.3 min, Figure 4D) are also shorter than those of *L.* sp. W (IBI = 63.7 ± 34.6 min, Figure 4D) but not significantly so (LME: $t_{20} = 1.96$, $p = 0.064$). We also observed substantial intraspecific variation in CV and mean IBI values across workers of both species (Figure 4C,D).

Activity propagation through individual physical contact & typical durations of activity

For both species, we found the longer a focal ant was inactive the higher the likelihood that physical stimulation would induce activity (*L.* sp. W - GLMM: $z = 4.677$, $p < 0.0001$; *L. crassipilis* - GLMM: $z = 2.976$, $p = 0.0029$). However, the effect was significantly weaker in *L. crassipilis* than in *L.* sp. W (GLMM species/time interaction: $z = -2.941$, $p = 0.0033$). Furthermore, the number of interactions that an ant received was not significantly associated with becoming active in either species (*L.* sp. W - GLMM: $z = -1.371$, $p = 0.1703$; *L. crassipilis* - GLMM: $z = 1.155$, $p = 0.2482$). The effect of the number of interactions on activation was also not significantly different between species (GLMM species/no. of stimulations interaction: $z = -1.757$, $p = 0.0789$). This is consistent with the idea that workers have a refractory period during which they will tend not to respond to nestmate stimulation [19,29].

An inspection of the survival curves reveals that after 10 mins of inactivity there was a distinct decline in the probability that *L.* sp. W would remain inactive, possibly suggesting a less variable refractory period than that seen in *L. crassipilis* (Figure 4E). We also found that the probability of ignoring the stimulus decreased significantly more quickly for *L.* sp. W than *L. crassipilis* (Figure 4E, Logrank test: $\chi_1^2 = 8.1$, p = 0.005).

The distributions of each species' individual ant inactivity durations are distinct (Kolmogorov–Smirnov test: D = 0.189, p = 0.0033, Figure 4F). The aggregate data from *L.* sp. W is right skewed and unimodal, but the distribution of *L. crassipilis* is more consistent with an exponential distribution. Based on our observations of individuals over 3 hours, the mean duration of activity inside nests is not significantly different between species (LME: $t_{18} = 1.29$, *p* = 0.212).

Model simulations

The appearance of rhythmic oscillations in our model occurs despite noise in individual refractory periods. Specifically, when parameterized to approximate the individual-level data from *L.* sp. W and using a uniform distribution for parameter *R* to introduce refractory noise (see supplementary material), this model generates individuals that are erratic when on their own but who can oscillate rhythmically when other ants are present. These cycles are, qualitatively, like those seen in real colonies (Figure 5B). However, according to our wavelet analysis, the dominant cycle periods of simulated *L.* sp. W colonies (11.92 ± 3.41 min) are shorter than those seen in real colonies (Figure 5C). Although an exponential distribution of refractory periods also generates collective oscillations, when the model's parameters are set to match *L. crassipilis*, the resulting cycles (8.07 ± 1.62 min) do *not* exhibit the large range of cycle periods seen in real colonies of this species (Figure 5C). When the random walk of agents is modified to be less stochastic (next step is their old heading ± 5 degrees), there is no impact on the dominant periods of the model's simulations when using the parameter set of either species (Figure S3). However, making the agents choose the direction of their next step completely randomly (next step is their old heading ± 360 degrees) results in the simulated time series of *L.* sp W colonies having dominant periods that more closely match those of real colonies (Figure S3).

When we examined the effect of refractory period length and refractory noise on the model's rhythmic behaviour, we noticed that the long simulation outputs had stationary means, so we used Lomb-Scargle periodograms to analyse their spectral properties and to find the period with the highest spectral peak in each time series (i.e., the dominant period). Inspection of simulation time series and their periodograms reveals that multirhythmicity is possible in this model (Figure 6). When there is no refractory noise, the dominant collective period increases linearly with the refractory period R (Figure 6A,C). However, once R exceeds a threshold value (in this case R = 900 seconds), birhythmic collective oscillations become common; simulated colonies intermittently switch between a long cycle and a short cycle (Figure 6A,D). Longer collective cycles are thus more susceptible to multirhythmic behaviour. The addition of refractory noise has a nonlinear effect on multirhythmicity (Figure 6B). Small amounts of noise (e.g., $\Omega = 50$) have no effect on the collective oscillations, but larger amounts of noise reduce the birhythmicity associated with larger values of R, causing simulated colonies to favour the longer cycle (Figure 6B,E). Additionally, when the refractory periods of agents are determined by sampling from an exponential distribution, clear evidence for multirhythmicity does not appear in any of the resulting simulations at all (Figure S4).

3.5 Discussion

Our findings show that there are detectable interspecific and intraspecific differences in the activity patterns of singleton workers and whole colonies of *Leptothorax*. We also show that, in both of the studied species, multiple collective oscillation frequencies can be present in the same colony. The collective oscillations and individual-level locomotor patterns of *Leptothorax* ants are therefore more diverse than previously known. Although both of the evaluated species have collective activity cycles, the two species vary in 1) the distributions of dominant colony oscillation frequencies, 2) the predictability of isolated worker activations, and 3) the distributions of worker inactivity durations. Our model simulations corroborate that collective oscillations naturally manifest in ants that move spontaneously and stimulate conspecifics, even when individuals lack a fundamental underlying rhythmicity or possess noise in their refractory periods. For some parameter values, collective cycles may also exhibit switching between different frequency regimes, yet the occurrence of such multirhythmicity is reduced in the model when noise is added to the refractory periods of each worker.

In excitable media and certain network configurations of neurons and coupled oscillators, both noise and heterogeneity can have profound implications for collective behaviour including sometimes destroying or promoting precision and synchronization [5,6,28,38,39]. Behavioural heterogeneity between workers within social insect colonies has long been noted [40,41]. These differences are thought to be crucial to division of labour [35], and are positively linked with reproductive output [29] and swift collective decision-making [42,43]. The contribution of noise to social synchronization in insects has received little attention, but behavioural noise is known to affect ant collective behaviour in other contexts, such as aiding colonies' decision-making in dynamic environments [44] and causing more accurate navigation during cooperative prey retrieval [45]. Models also suggest that colonies can maintain a near-optimal allocation of workers to different tasks even when the ability of ants to sense task demand is imperfect [46]. The data herein extends this idea by showing that collective oscillations in social insects need not be contingent on behavioural uniformity in workers. This result also matches the outcome of work with non-mobile excitable cellular automata that lack spontaneous activation, which similarly found that synchronization can persist despite stochasticity in refractory periods [47].

Our model reveals that multirhythmicity can arise in excitable systems if individuals are also capable of spontaneous individual activation and the stimulation refractory period is sufficiently long. This effect may contribute to the diverse collective-level frequencies of *L. crassipilis* and to the multiple co-occurring rhythms in both species, though this remains uncertain. The reduction in multirhythmicity associated with higher levels of noise in our model is reminiscent of work done on stochastic resonance and coherence resonance in other models of excitable systems, where limited amounts of noise emanating from a common external source improves coherence [6,48]. In our case, instead of an improvement in oscillator coherence we detected less switching between collective rhythm frequencies. It has also recently been shown that adding independent and uncorrelated sources of noise separately to individual oscillators can still improve synchronization [49]. The refractory noise in our model was added independently to each ant and was therefore uncorrelated, not originating form a common source. Our finding thus uncovers a novel impact that uncorrelated noise can have on oscillations in excitable systems.

Multirhythmicity has been documented in a handful of physical systems and in models of biological oscillators such as the mammalian circadian clock [27], but additional research could uncover a wider set of conditions where the phenomenon occurs. The results of our simulations raise the possibility that other natural oscillatory systems or theoretical models with either mobile, non-identical, or excitable elements (like aggregations of microorganisms [50], firefly swarms [51], or biological neuron models [38]) may harbour similar collective frequency switching behaviour under the right conditions, namely wherever there exists sufficient randomness in the intrinsic activations of individual components. Understanding the factors that can lead to and control multirhythmicity is an active area of research, as the phenomenon can be undesirable [52]. Evaluating the functional consequences of behavioural noise and heterogeneity on multirhythmicity in these types of systems could thus be an attractive direction for future study.

The simplicity of our model results in some limitations which should form the subject of future work. We do not know if the multirhythmicity seen in our model is caused by similar processes as those which lead to the multiple rhythms that we observed in the 35-hour recordings of live colonies. Although we have shown that a single colony can possess multiple oscillation frequencies, genuine multirhythmicity involves switching between distinct frequencies. It is not yet clear if this happens reliably in *Leptothorax* or if the multiple periodicities must always occur at the same time. The origin of the long dominant periods in *L. crassipilis* also deserves more attention. Achieving long collective periods in this type of system cannot be trivially accomplished by lengthening the average refractory period of workers because of the spontaneous activation of workers. Either most ants will activate spontaneously before they are susceptible to stimulation by another worker (when $<R>$ is long and $<S>$ is short) or collective cycles will become arrhythmic (when both $<R>$ and $<S>$ are long). The long dominant periods we observed in *L. crassipilis* are inconsistent with earlier cellular automata models of short-term activity cycles as well. These models can produce simulated colonies with long periods, but this results in every agent in the simulated colony being in a near constant state of activity, with periodic dips in the sustained universal activity [20,53]. This is not what happens in actual colonies. It is also worth considering that the ways in which we parameterized the model, calibrated the movement per time step, and estimated the distributions of refractory periods were all simplifying approximations to make the model tractable, which can lead to inaccuracies.

There are likewise factors that we ignored in favour of generality, but which may be relevant to STACs. We did not consider behavioural heterogeneity between workers, and we treated the movements of workers as correlated random walks. However, *Temnothorax* workers in a single colony are known to vary in their average level of total activity when measured over more than a week [54]. Worker movement paths (and interactions with nestmates) can also be influenced in complex non-random ways by the environment inside the nest. For example, workers from species that are closely related to *L. crassipilis* and *L.* sp W are known to spend more time in some regions of the nest than others, which are sometimes referred to as "spatial fidelity zones" [55]. Interactions between individuals in a colony are further complicated by dominance hierarchies and avoidance behaviour [56–58]. Workers and gynes will sometimes alter their walking paths depending on the dominance rankings or identity of nearby individuals [56,57,59]. All of these factors could therefore have consequences for STACs. It is additionally possible that workers may be able to sense the current rhythm of the colony and modify their refractory period to avoid missing a colony cycle, leading to greater coherence. A more detailed exploration of activity patterns in individuals of both species and how colonies achieve synchrony is therefore necessary. Despite the inability of the current model to fully reproduce the intricacies of short-term activity cycles, our model's primary insights still stand: mobile excitable systems can synchronize when agents have noisy refractory states, and birhythmicity can be diminished through the addition of refractory noise.

The present study does not resolve a central enigma surrounding STACs: why do they exist? No experiment has been able to demonstrate any advantage for colonies that possess STACs. Some investigators have suggested that STACs foster more efficient brood care, though others have argued that they might not have any adaptive significance at all [12]. Even if synchronized activity cycles themselves do not confer an inherent functional benefit, the ability to express different dominant cycle frequencies like *L. crassipilis* may still have fitness consequences. Of the six *Temnothorax*/*Leptothorax* species where STAC data are now documented [12,13,24], three species (*T. allardycei, L. acervorum*, and *L.* sp. W) consistently exhibit oscillations of 15-30 min, two species (*T. albipennis* and *T. rugatulus*) exhibit slower oscillations of approx. 50 min, and *L. crassipilis* is notable for its large variability in dominant frequency. Because colony tasks are believed to be completed primarily during times of high activity, the tempo of a colony's oscillations might dictate how rapidly it can respond to changing conditions outside the nest (e.g., detecting and exploiting food resources) or inside the nest (e.g., heightened levels of hunger in larvae). Testing more species will likely help resolve the question of whether activity cycles are adaptive and uncover new types of collective movement behaviours in ants, the most ecologically dominant terrestrial invertebrate on the planet.

3.6 Data availability

Data and relevant code for this research work are stored in GitHub: [https://github.com/naviddio/Leptothorax_cycles] and have been archived within the Zenodo repository: [https://zenodo.org/badge/latestdoi/287580941]

3.7 Acknowledgements

We thank Brendan McEwan, Hannah Anderson, Tricia Skelton, Avani Pathak, Amy Kalbfleisch, Martina Bravo, Haolin Ye, Janice Yan, and Greg Thung for help with ant colony maintenance. We are grateful to Richard T. Doering and James L. L. Lichtenstein for helping collect wild colonies. Matthew Prebus helped with species identification. We also thank Michael Guevara, Jonathan Dushoff, David Earn, Kirsten Sheehy, and Dobromir Dotov for helpful discussions. Patrick Bennett and Stephen Pratt provided valuable feedback on the manuscript. Finally, we thank Reuven Dukas for access to his dissecting microscope. This research was supported by the National Institutes of Health (GM115509 to JNP).

3.8 Author contributions

GND conceived the study, collected all empirical data, performed all analyses, wrote the first draft of the manuscript, and built the initial agent-based model. KD, CL, BD, and LRP were involved in revising and further developing the agent-based model. JNP provided laboratory space and cameras for the empirical portion of the study. All authors contributed to the writing of the final version of the manuscript.

3.9 Competing interests

The authors declare that we have no competing interests.

3.10 References

1. Winfree AT. 2001 *The Geometry of Biological Time*. Springer Science & Business Media.
2. Pikovsky A, Kurths J, Rosenblum M, Kurths J. 2003 *Synchronization: A Universal Concept in Nonlinear Sciences*. Cambridge University Press.
3. Strogatz SH. 2000 From Kuramoto to Crawford: exploring the onset of synchronization in populations of coupled oscillators. *Phys. Nonlinear Phenom.* **143**, 1–20. (doi:10.1016/S0167-2789(00)00094-4)

4. O'Keeffe KP, Hong H, Strogatz SH. 2017 Oscillators that sync and swarm. *Nat. Commun.* **8**, 1–13. (doi:10.1038/s41467-017-01190-3)

5. Nishikawa T, Motter AE. 2016 Symmetric States Requiring System Asymmetry. *Phys. Rev. Lett.* **117**, 114101. (doi:10.1103/PhysRevLett.117.114101)

6. Pikovsky AS, Kurths J. 1997 Coherence Resonance in a Noise-Driven Excitable System. *Phys. Rev. Lett.* **78**, 775–778. (doi:10.1103/PhysRevLett.78.775)

7. Castellano C, Fortunato S, Loreto V. 2009 Statistical physics of social dynamics. *Rev. Mod. Phys.* **81**, 591–646. (doi:10.1103/RevModPhys.81.591)

8. Shaw AK. 2020 Causes and consequences of individual variation in animal movement. *Mov. Ecol.* **8**, 12. (doi:10.1186/s40462-020-0197-x)

9. Schleidt WM. 1974 How 'fixed' is the fixed action pattern? *Z. Tierpsychol.* **36**, 184–211. (doi:10.1111/j.1439-0310.1974.tb02131.x)

10. Kennedy P *et al.* 2017 Deconstructing Superorganisms and Societies to Address Big Questions in Biology. *Trends Ecol. Evol.* **32**, 861–872. (doi:10.1016/j.tree.2017.08.004)

11. Franks NR, Bryant S, Griffiths R, Hemerik L. 1990 Synchronization of the behaviour within nests of the ant *Leptothorax acervorum* (Fabricius)—I. Discovering the phenomenon and its relation to the level of starvation. *Bull. Math. Biol.* **52**, 597–612. (doi:10.1007/BF02462100)

12. Cole BJ, Trampus FI. 1999 Activity cycles in ant colonies: worker interactions and decentralized control. In *Information Processing in Social Insects* (eds C Detrain, JL Deneubourg, JM Pasteels), pp. 289–307. Basel: Birkhäuser Basel. (doi:10.1007/978-3-0348-8739-7_16)

13. Doering GN, Sheehy KA, Lichtenstein JLL, Drawert B, Petzold LR, Pruitt JN. 2019 Sources of intraspecific variation in the collective tempo and synchrony of ant societies. *Behav. Ecol.* **30**, 1682–1690. (doi:10.1093/beheco/arz135)

14. Cole BJ. 1991 Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction. *Am. Nat.* **137**, 244–259.

15. Cole BJ. 1991 Is Animal Behaviour Chaotic? Evidence from the Activity of Ants. *Proc. Biol. Sci.* **244**, 253–259.

16. Cole BJ. 1991 Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. *J. Insect Behav.* **4**, 129–137. (doi:10.1007/BF01054607)

17. Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF. 1999 Coupled Oscillators and Activity Waves in Ant Colonies. *Proc. Biol. Sci.* **266**, 371–378.

18. Wheeler WM. 1903 A Revision of the North American Ants of the Genus *Leptothorax* Mayr. *Proc. Acad. Nat. Sci. Phila.* **55**, 215–260.

19. Goss S, Deneubourg JL. 1988 Autocatalysis as a source of synchronised rhythmical activity in social insects. *Insectes Sociaux* **35**, 310–315. (doi:10.1007/BF02224063)

20. Solé RV, Miramontes O, Goodwin BC. 1993 Oscillations and Chaos in Ant Societies. *J. Theor. Biol.* **161**, 343–357. (doi:10.1006/jtbi.1993.1060)

21. Couzin ID. 2018 Synchronization: The Key to Effective Communication in Animal Collectives. *Trends Cogn. Sci.* **22**, 844–846. (doi:10.1016/j.tics.2018.08.001)

22. Tsimring LS. 2014 Noise in Biology. *Rep. Prog. Phys. Phys. Soc. G. B.* **77**, 026601. (doi:10.1088/0034-4885/77/2/026601)

23. Muratov CB, Vanden-Eijnden E, E W. 2007 Noise can play an organizing role for the recurrent dynamics in excitable media. *Proc. Natl. Acad. Sci.* **104**, 702–707. (doi:10.1073/pnas.0607433104)

24. Hatcher MJ. 1992 Activity patterns and organization within ant nests. PhD Thesis, University of Bath.

25. Mallon E, Pratt S, Franks N. 2001 Individual and collective decision-making during nest site selection by the ant *Leptothorax albipennis*. *Behav. Ecol. Sociobiol.* **50**, 352–359. (doi:10.1007/s002650100377)

26. Deneubourg JL, Pasteels JM, Verhaeghe JC. 1983 Probabilistic behaviour in ants: A strategy of errors? *J. Theor. Biol.* **105**, 259–271. (doi:10.1016/S0022-5193(83)80007-1)

27. Yan J, Goldbeter A. 2019 Multi-rhythmicity generated by coupling two cellular rhythms. *J. R. Soc. Interface* **16**, 20180835. (doi:10.1098/rsif.2018.0835)

28. Neiman A, Schimansky-Geier L, Cornell-Bell A, Moss F. 1999 Noise-Enhanced Phase Synchronization in Excitable Media. *Phys. Rev. Lett.* **83**, 4896–4899. (doi:10.1103/PhysRevLett.83.4896)

29. Tofts C, Hatcher M, Franks NR. 1992 The autosynchronization of the ant *Leptothorax acervorum* (Fabricius): Theory, testability and experiment. *J. Theor. Biol.* **157**, 71–82. (doi:10.1016/S0022-5193(05)80757-X)

30. Bradley D, Roth G. 2007 Adaptive Thresholding using the Integral Image. *J. Graph. Tools* **12**, 13–21. (doi:10.1080/2151237X.2007.10129236)

31. Cole BJ, Hoeg L. 1996 The influence of brood type on activity cycles in *Leptothorax allardycei* (Hymenoptera: Faruicidae). *J. Insect Behav.* **9**, 539–547. (doi:10.1007/BF02213878)

32. Richardson TO, Liechti JI, Stroeymeyt N, Bonhoeffer S, Keller L. 2017 Short-term activity cycles impede information transmission in ant colonies. *PLOS Comput. Biol.* **13**, e1005527. (doi:10.1371/journal.pcbi.1005527)

33. Heinze J, Stahl M, Hölldobler B. 1996 Ecophysiology of hibernation in boreal *Leptothorax* ants (Hymenoptera: Formicidae). *Écoscience* **3**, 429–435.

34. Beshers SN, Fewell JH. 2001 Models of Division of Labor in Social Insects. *Annu. Rev. Entomol.* **46**, 413–440. (doi:10.1146/annurev.ento.46.1.413)

35. Beshers SN, Robinson GE, Mittenthal JE. 1999 Response thresholds and division of labor in insect colonies. In *Information Processing in Social Insects* (eds C Detrain, JL Deneubourg, JM Pasteels), pp. 115–139. Basel: Birkhäuser. (doi:10.1007/978-3-0348-8739-7_7)

36. Robinson EJH, Franks NR, Ellis S, Okuda S, Marshall JAR. 2011 A Simple Threshold Rule Is Sufficient to Explain Sophisticated Collective Decision-Making. *PLOS ONE* **6**, e19981. (doi:10.1371/journal.pone.0019981)

37. Baddeley R, Abbott LF, Booth MCA, Sengpiel F, Freeman T, Wakeman EA, Rolls ET. 1997 Responses of neurons in primary and inferior temporal visual cortices to natural scenes. *Proc. R. Soc. Lond. B Biol. Sci.* **264**, 1775–1783. (doi:10.1098/rspb.1997.0246)

38. Smeal RM, Ermentrout GB, White JA. 2010 Phase-response curves and synchronized neural networks. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 2407–2422. (doi:10.1098/rstb.2009.0292)

39. White JA, Chow CC, Rit J, Soto-Treviño C, Kopell N. 1998 Synchronization and Oscillatory Dynamics in Heterogeneous, Mutually Inhibited Neurons. *J. Comput. Neurosci.* **5**, 5–16. (doi:10.1023/A:1008841325921)

40. Wheeler WM. 1911 The ant-colony as an organism. *J. Morphol.* **22**, 307–325. (doi:10.1002/jmor.1050220206)

41. Robson SK, Traniello JFA. 1999 Key individuals and the organisation of labor in ants. In *Information Processing in Social Insects* (eds C Detrain, JL Deneubourg, JM Pasteels), pp. 239–259. Basel: Birkhäuser Basel. (doi:10.1007/978-3-0348-8739-7_13)

42. O'Shea-Wheller TA, Masuda N, Sendova-Franks AB, Franks NR. 2017 Variability in individual assessment behaviour and its implications for collective decision-making. *Proc. R. Soc. B Biol. Sci.* **284**, 20162237. (doi:10.1098/rspb.2016.2237)

43. Valentini G, Masuda N, Shaffer Z, Hanson JR, Sasaki T, Walker SI, Pavlic TP, Pratt SC. 2020 Division of labour promotes the spread of information in colony emigrations by the ant *Temnothorax rugatulus*. *Proc. R. Soc. B Biol. Sci.* **287**, 20192950. (doi:10.1098/rspb.2019.2950)

44. Dussutour A, Beekman M, Nicolis SC, Meyer B. 2009 Noise improves collective decision-making by ants in dynamic environments. *Proc. R. Soc. B Biol. Sci.* **276**, 4353–4361. (doi:10.1098/rspb.2009.1235)

45. Gelblum A, Pinkoviezky I, Fonio E, Ghosh A, Gov N, Feinerman O. 2015 Ant groups optimally amplify the effect of transiently informed individuals. *Nat. Commun.* **6**, ncomms8729. (doi:10.1038/ncomms8729)

46. Dornhaus A, Lynch N, Mallmann-Trenn F, Pajak D, Radeva T. 2020 Self-Stabilizing Task Allocation In Spite of Noise. In *Proceedings of the 32nd ACM Symposium on Parallelism in Algorithms and Architectures*, pp. 201–211. Virtual Event, USA: Association for Computing Machinery. (doi:10.1145/3350755.3400226)

47. Rozenblit F, Copelli M. 2011 Collective oscillations of excitable elements: order parameters, bistability and the role of stochasticity. *J. Stat. Mech. Theory Exp.* **2011**, P01012. (doi:10.1088/1742-5468/2011/01/P01012)

48. McDonnell MD, Abbott D. 2009 What Is Stochastic Resonance? Definitions, Misconceptions, Debates, and Its Relevance to Biology. *PLOS Comput. Biol.* **5**, e1000348. (doi:10.1371/journal.pcbi.1000348)

49. Nicolaou ZG, Sebek M, Kiss IZ, Motter AE. 2020 Coherent Dynamics Enhanced by Uncorrelated Noise. *Phys. Rev. Lett.* **125**, 094101. (doi:10.1103/PhysRevLett.125.094101)

50. Gregor T, Fujimoto K, Masaki N, Sawai S. 2010 The Onset of Collective Behavior in Social Amoebae. *Science* **328**, 1021–1025. (doi:10.1126/science.1183415)

51. Sarfati R, Hayes JC, Sarfati É, Peleg O. 2020 Spatio-temporal reconstruction of emergent flash synchronization in firefly swarms via stereoscopic 360-degree cameras. *J. R. Soc. Interface* **17**, 20200179. (doi:10.1098/rsif.2020.0179)

52. Biswas D, Banerjee T, Kurths J. 2017 Control of birhythmicity: A self-feedback approach. *Chaos Interdiscip. J. Nonlinear Sci.* **27**, 063110. (doi:10.1063/1.4985561)

53. Miramontes O, Solé RV, Goodwin BC. 1993 Collective behaviour of random-activated mobile cellular automata. *Phys. Nonlinear Phenom.* **63**, 145–160. (doi:10.1016/0167-2789(93)90152-Q)

54. Charbonneau D, Dornhaus A. 2015 Workers ‘specialized’ on inactivity: Behavioral consistency of inactive workers and their role in task allocation. *Behav. Ecol. Sociobiol.* **69**, 1459–1472. (doi:10.1007/s00265-015-1958-1)

55. Sendova-franks AB, Franks NR. 1995 Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. *Anim. Behav.* **50**, 121–136. (doi:10.1006/anbe.1995.0226)

56. Sendova-Franks AB, Franks NR. 1995 Demonstrating new social interactions in ant colonies through randomization tests: separating seeing from believing. *Anim. Behav.* **50**, 1683–1696. (doi:10.1016/0003-3472(95)80021-2)

57. Cole BJ. 1981 Dominance Hierarchies in *Leptothorax* Ants. *Science* **212**, 83–84. (doi:10.1126/science.212.4490.83)

58. Heinze J, Puchinger W, Hölldobler B. 1997 Worker reproduction and social hierarchies in *Leptothorax* ants. *Anim. Behav.* **54**, 849–864. (doi:10.1006/anbe.1996.0511)

59. Wilson EO. 1974 Aversive Behavior and Competition within Colonies of the Ant *Leptothorax curvispinosus*. *Ann. Entomol. Soc. Am.* **67**, 777–780. (doi:10.1093/aesa/67.5.777)

Figure 3.1

Empirical data from colonies. (A) *Leptothorax crassipilis* workers with brood. (B) Histograms of STAC periods expressed by colonies of both *Leptothorax* species. (C) *Leptothorax* sp. W workers with brood. (D) Time series of collective locomotor activity from two representative *L. crassipilis* colonies and (E) two *L.* sp. W colonies. Black dots in time series represent unprocessed data points and lines show the smoothed weighted moving average. *Leptothorax* sp. W colonies show little variation in collective frequencies, but *L. crassipilis* can express a range of cycle frequencies.

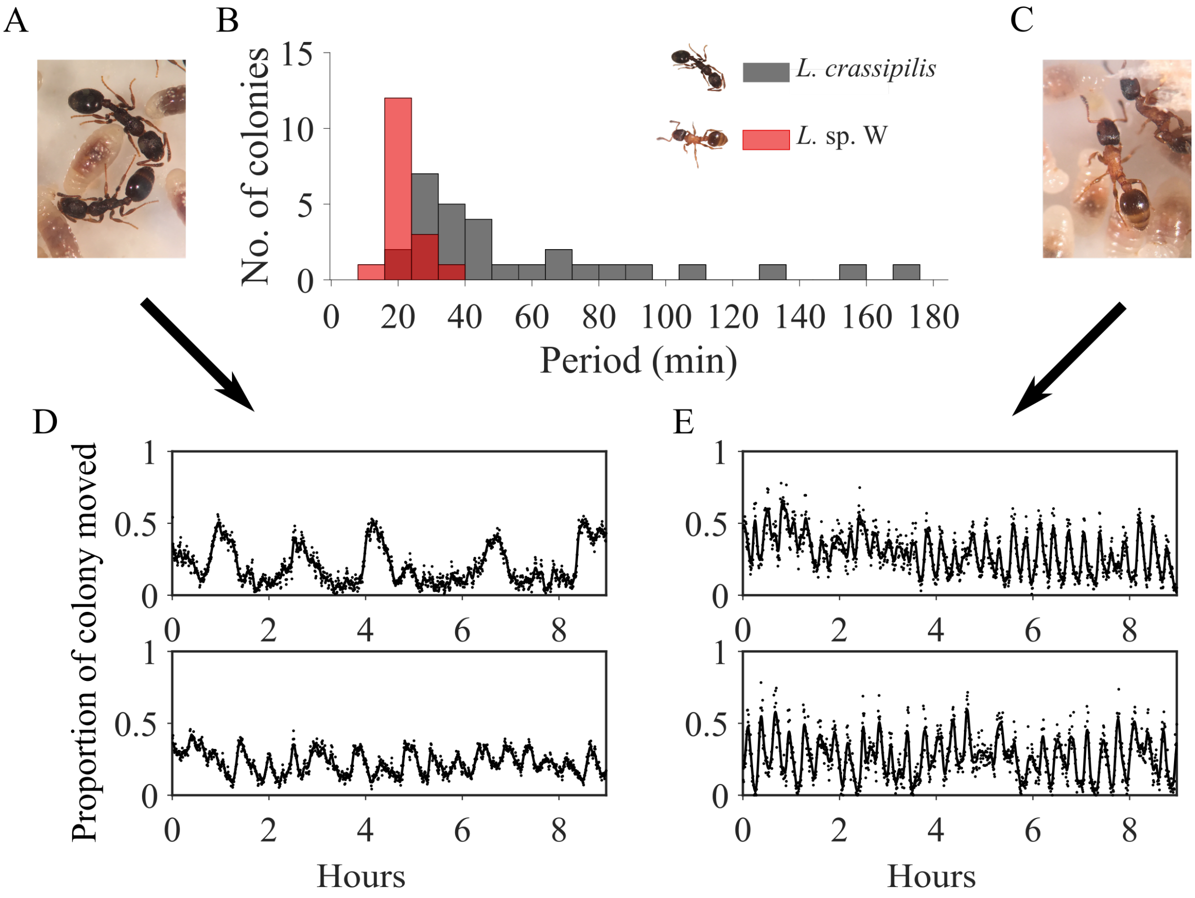


Figure 3.2

Longer time series from colonies. Example time series from 35-hour recordings of a *L*. sp W colony (A) and three *L. crassipilis* colonies (B-C). The black curves in each plot depict the collective activity of colonies after being smoothed using a Gaussian-weighted moving average with a window of 15 points. The translucent green curves depict the same time series as the black curves expect with a smoothing window of 200 data points. The time series have been rescaled to fall between 0 and 1 in the panels. Lomb-Scargle periodograms are plotted to the right of their corresponding time series. The periodograms were made using the detrended time series, and no smoothing was applied to the time series prior to this particular analysis. Multiple collective oscillation frequencies can occur in the same activity record.

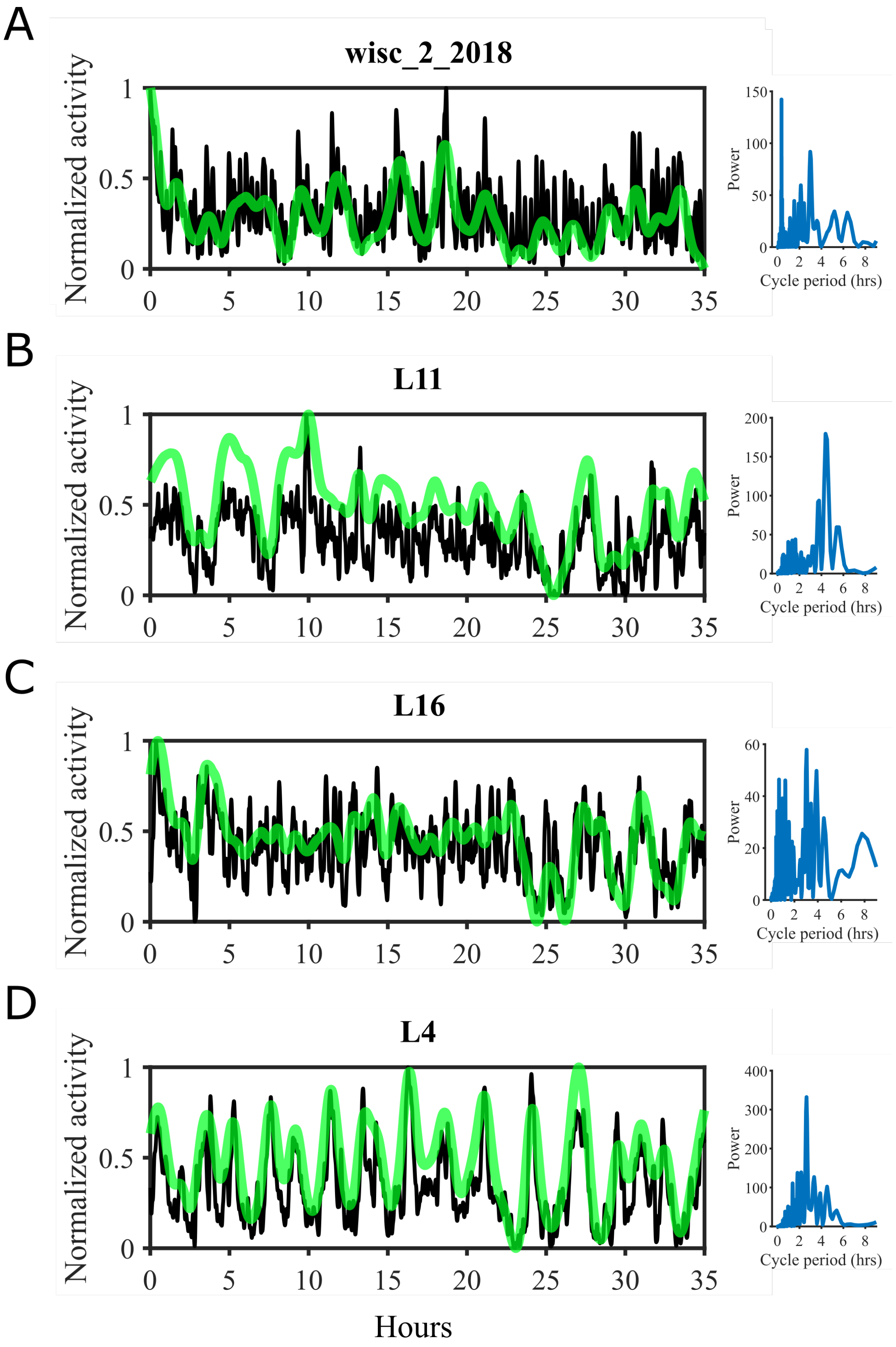

A
wisc_2_2018
Normalized activity
1
0.5
0
0 5 10 15 20 25 30 35
Power
150
100
50
0
0 2 4 6 8
Cycle period (hrs)
B
L11
Normalized activity
1
0.5
0
0 5 10 15 20 25 30 35
Power
200
150
100
50
0
0 2 4 6 8
Cycle period (hrs)
C
L16
Normalized activity
1
0.5
0
0 5 10 15 20 25 30 35
Power
60
40
20
0
0 2 4 6 8
Cycle period (hrs)
D
L4
Normalized activity
1
0.5
0
0 5 10 15 20 25 30 35
Power
400
300
200
100
0
0 2 4 6 8
Cycle period (hrs)
Hours

Figure 3.3

Summed power spectra from all 35-hour colony time series. The periodogram depicted here was created by rescaling the Lomb-Scargle power spectra of all 8 of the 35-hour colony recordings and summing them together. There are two distinct peaks, which indicates that multiple colonies exhibit these particular periodicities.

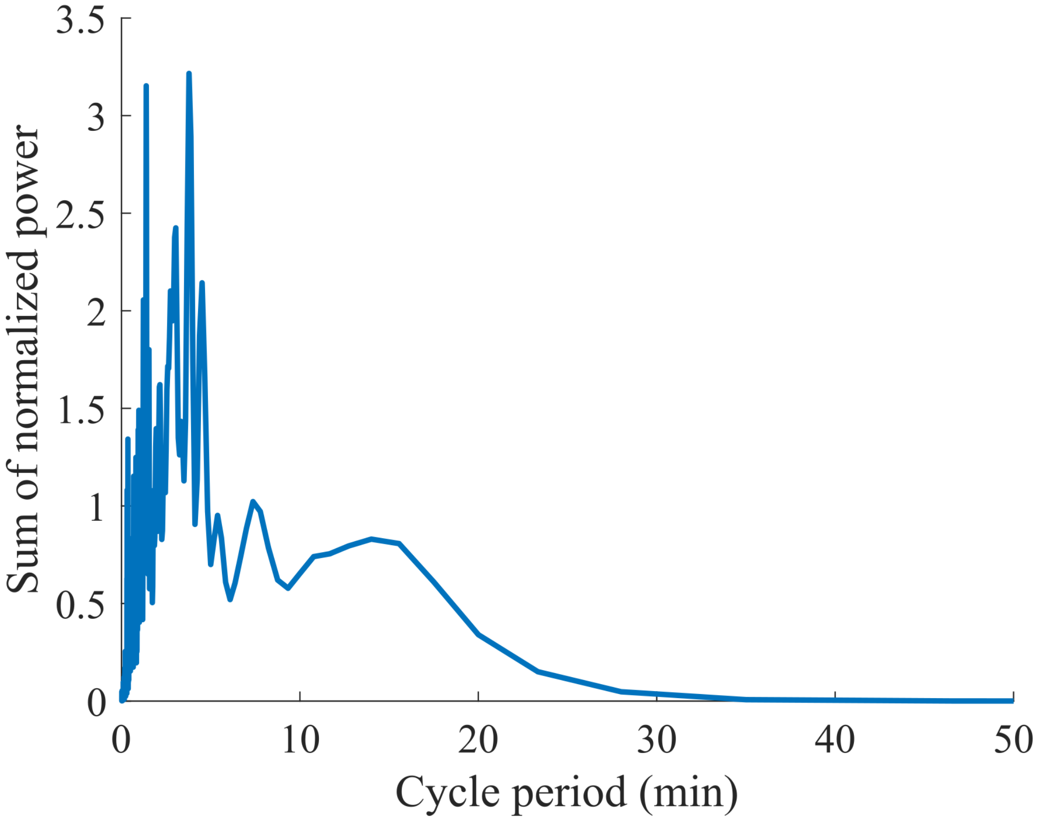


Figure 3.4.

Empirical data from individuals. (A) Time series of locomotor activity from three representative isolated *L. crassipilis* singletons and (B) three *L.* sp. W singletons. Activity time series of individuals were normalized so that each ant's movement distances (originally in pixels) were rescaled to fall between 0 and 1. Box plots comparing (C) the CV and (D) mean IBI of activity time series from isolated singletons between species. Box plot points are horizontally offset for visibility. Isolated *L.* sp. W singletons have greater variance in CV and often have longer spike intervals than *L. crassipilis*. (E) Kaplan-Meier "survival" curve estimates of the probability that an ant will ignore a stimulation as a function of the time an ant has spent inactive. Shaded areas represent log-log 95% confidence intervals. (F) Differing distributions of individual ant inactivity durations between *Leptothorax* species. Data for each species is combined from observations from 4 colonies. The dotted lines depict the best fit exponential distribution for *L. crassipilis* and best fit log-logistic distribution for *L*. sp W.

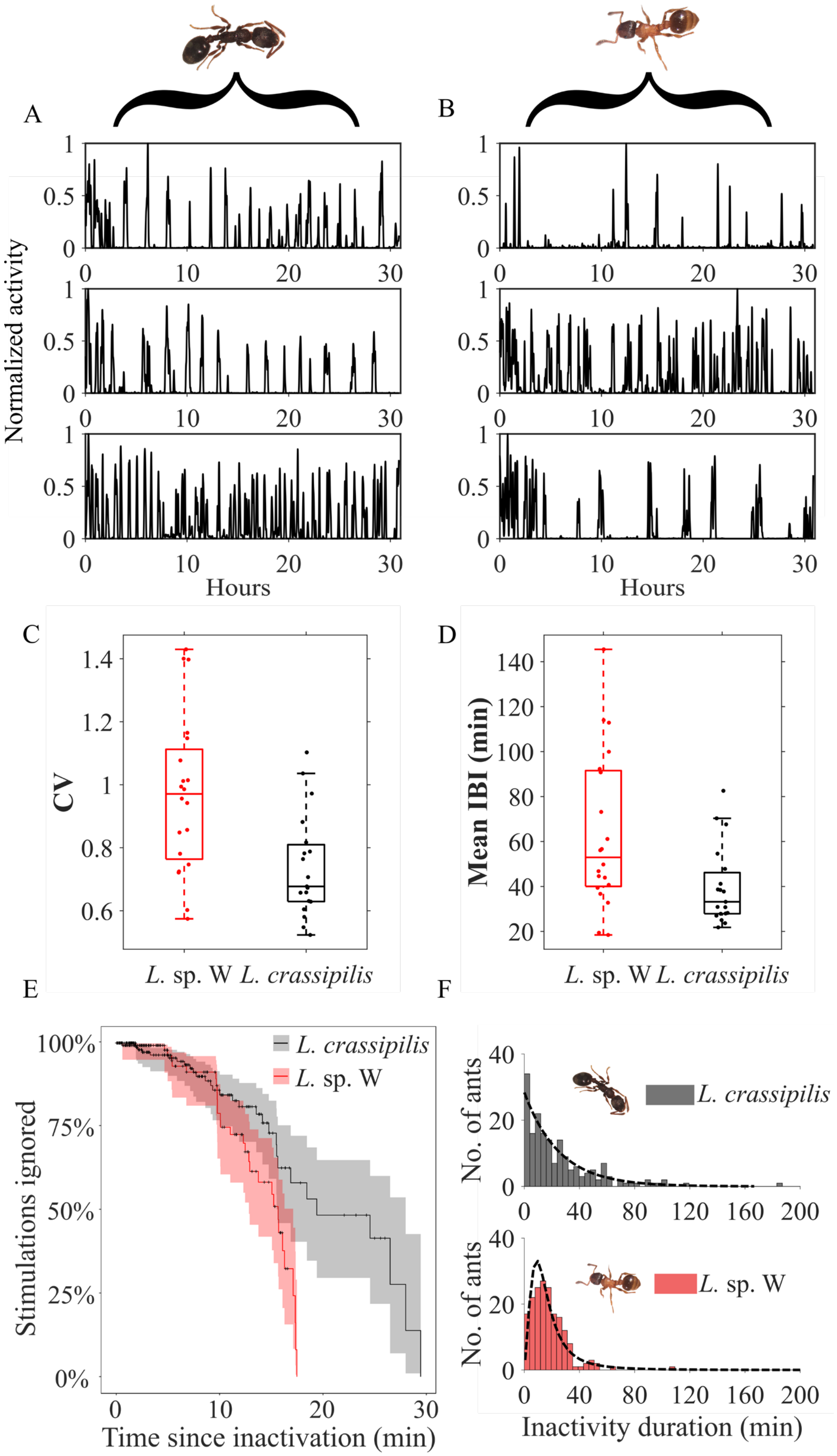

A
B
Normalized activity
1
0.5
0
0
10
20
30
Hours
C
CV
1.4
1.2
1
0.8
0.6
L. sp. W
L. crassipilis
D
Mean IBI (min)
140
120
100
80
60
40
20
L. sp. W
L. crassipilis
E
Stimulations ignored
100%
75%
50%
25%
0%
L. crassipilis
L. sp. W
0
10
20
30
Time since inactivation (min)
F
No. of ants
40
20
0
L. crassipilis
L. sp. W
0
40
80
120
160
200
Inactivity duration (min)

Figure 3.5 Algorithm used by modelled ants, and simulated colonies vs. real colonies. (A) Schematic diagram of individual ant behaviour used in the model. Boxes depict the two possible states ants may occupy. Text in each box describes the sequence of behaviours executed by an ant during each time step (i.e., second) spent in the corresponding state. Arrows indicate the ways that ants may transition between the two states. Whenever an ant switches states it sets its personal clock *T* to 0. (B) Plots of activity traces for an example *L.* sp. W colony (black line) and a segment of a simulated *L.* sp. W colony (blue line) over approx. 9 hours (32,400 time steps of the simulation). Data was normalized to fall between 0 and 1. (C) Box plots showing the cycle periods of real (black) vs. simulated (blue) colonies of both *Leptothorax* species. The simulated data for *L. crassipilis and L.* sp. w used one-hundred separate simulations (100,001 time steps long) for each species.

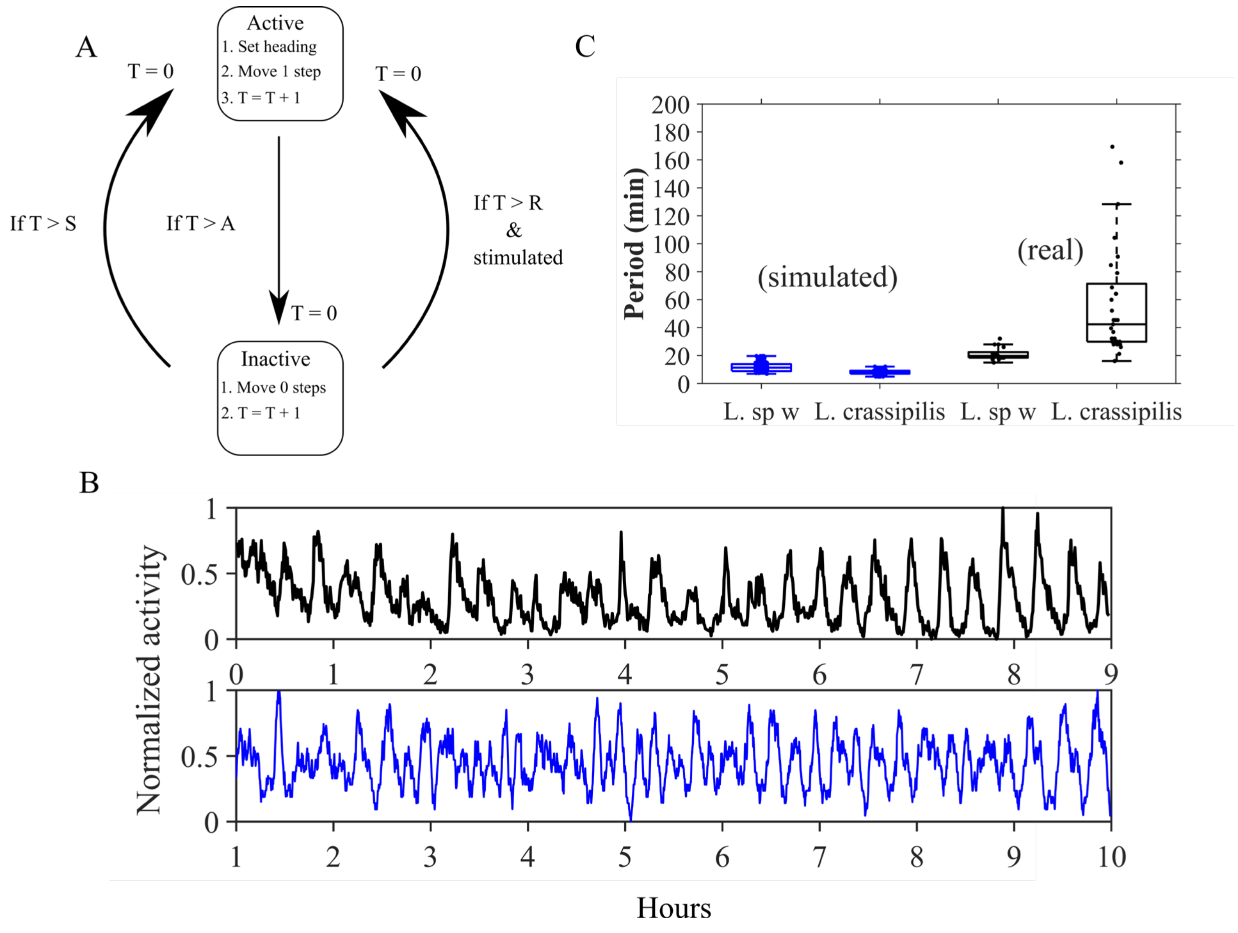
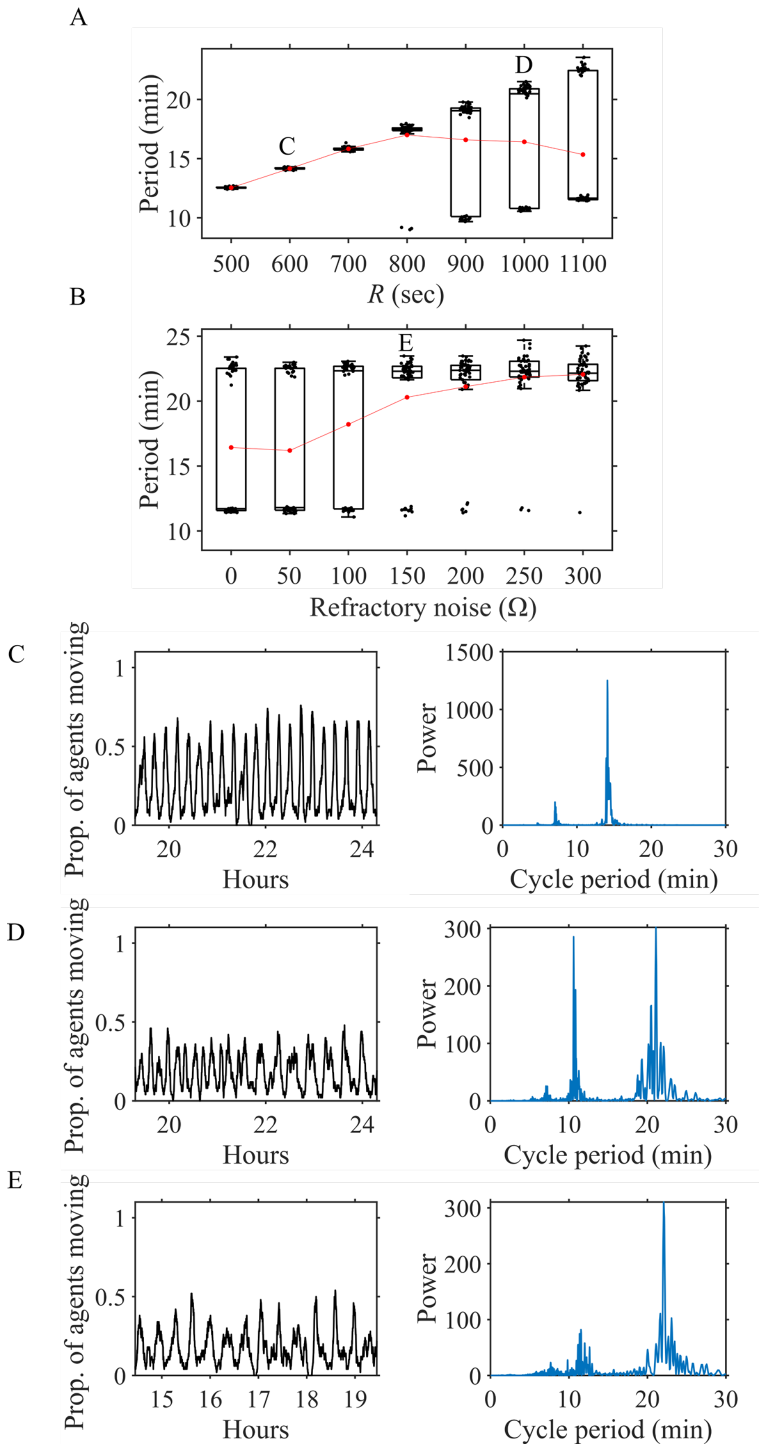


Figure 3.6. Effect of refractory period length and refractory noise on collective oscillations. (A) Boxplots of the dominant period (determined through Lomb-Scargle periodograms) for simulations using different values of *R* (refractive period). The red dots indicate the mean of each box. There are 50 simulations of 100,001 time steps for each *R* value, and the other model parameters were held constant at: <S> = 4050 seconds, A = 216 seconds, and Ω = 0. (B) Boxplots of the dominant period for simulations using different levels of refractory noise Ω. There are 50 simulations of 100,001 time steps for each Ω value. The other model parameters were held constant at: <S> = 4050 seconds, A = 216 seconds, and <R> = 1100 seconds. (C-E) Plots of unprocessed time series segments showing the proportion of agents active over time for selected simulation runs along with the associated Lomb-Scargle periodograms of the entire time series. Letters C through E in panels A & B correspond to the respective locations of the example time series and periodograms. Increasing the refractive period of individual ants results in longer collective cycles but also encourages multirhythmicity. The addition of refractory noise reduces the amount of switching between collective frequencies.


A
Period (min)
10
15
20
C
D
500
600
700
800
900
1000
1100
R (sec)
B
Period (min)
10
15
20
25
E
0
50
100
150
200
250
300
Refractory noise (Ω)
C
Prop. of agents moving
0
0.5
1
20
22
24
Hours
Power
0
500
1000
1500
0
10
20
30
Cycle period (min)
D
Prop. of agents moving
0
0.5
1
20
22
24
Hours
Power
0
100
200
300
0
10
20
30
Cycle period (min)
E
Prop. of agents moving
0
0.5
1
15
16
17
18
19
Hours
Power
0
100
200
300
0
10
20
30
Cycle period (min)

CHAPTER 4 – DISSIMILAR EFFECTS OF LIGHT ON SHORT VERSUS LONG PERIOD COLLECTIVE RHYTHMS IN ANT COLONIES.

4.1 Abstract

Rhythmic cycles of rest and activity are widespread in animal behavior. Light pays a critical role in structuring the activity rhythms of solitary animals, but the effect of light input on the emergence of collective-level circadian and ultradian activity inside the nests of social insects has never been systematically investigated. We observed the collective activity levels of entire colonies from 10 different temperate ant species under three different light regimes (constant darkness, constant light, and alternating light and dark). We found that 24-hour collective rhythms were significantly weaker in colonies that experienced constant light and constant darkness when compared to colonies living in a 12-hour light/dark cycle. However, the external light regime had no effect on the rhythmicity nor the oscillation frequency of colonies' ultradian rhythms. A combination of social and environmental factors thus regulates collective rhythms inside ant nests, and the loss of 24-hour rhythms does not entail losing ultradian rhythms. These results add to our understanding of the factors that influence synchronized rhythms in social animals.

4.2 Introduction

Rhythmic behaviors are nearly ubiquitous in animals. Numerous processes such as egg laying (Manjunatha et al. 2008), mating (Baldridge et al. 1980; Staab and Kleineidam 2014), and locomotion (Delgado-Garcia et al. 1976; Lumineau et al. 2001; Guzmán et al. 2017) often occur in predictable cycles. These cycles may take on a 24-hour period (i.e., have a circadian rhythm), but rhythms that are faster (ultradian) or longer (infradian) than 24-hours can also be observed in various behaviors from a wide range of species (Baldridge et al. 1980; Lloyd and Stupfel 1991; Lumineau et al. 2001; Tran et al. 2011; Seki and Tanimura 2014; Berberich et al. 2019). Rhythms can become entrained to external stimuli (e.g., temperature, light, etc.) and can be maintained through endogenous timekeeping mechanisms that allow an organism to retain its periodicity even when environmental cues are absent. Animals that live in groups are also capable of expressing cyclic behavior, but, unlike solitary organisms, rhythms that occur in a social context can also be affected by interactions with conspecifics that live in the same group (Regal and Connolly 1980; Bloch et al. 2013). Due to their sophisticated division of labor, eusocial insects are especially sensitive to input from nestmates, and their patterns of activity can consequently be modified based on their social environment (Gordon and Mehdiabadi 1999; Nicolis et al. 2013; Fuchikawa et al. 2016; Fujioka et al. 2017). Rhythms in social insects can also be defined at the collective level. For example, the rate of ants leaving their nest to forage can adhere to ultradian, circadian, and infradian schedules (Lewis et al. 1974; Nicolis et al. 2013; Berberich et al. 2019). Ants are the most abundant group of eusocial insects (Wilson 1987), and many species can be easily maintained in a laboratory setting, which makes them an excellent model system for exploring the characteristics of different types of collective-level behavioral rhythms.

Besides the daily rhythms of foraging outside the nest, a handful of species are also known to exhibit a type of collective-level *intranidal* (i.e., occurring inside the nest) ultradian rhythm that has been referred to as the "short-term activity cycle" (Barnes 1941; Cole 1991a; Hatcher 1992; Cole and Trampus 1999; Richardson et al. 2017; Tennenbaum and Fernandez-Nieves 2017; Doering et al. 2019). During short-term activity cycles, workers within a nest cavity synchronize their locomotor activity so that they move together in bursts. Most workers become quiescent and remain completely motionless between cycles. Cycles of activity can also be highly rhythmic (supplementary figure S1) with periods of oscillation that range from 20 min to more than 2 hours depending on the species and particular colony (Cole 1991a; Hatcher 1992; Doering et. al. 2021). Activity bursts do not appear to be associated with any external signal but are instead created by the interactions between ants (Cole and Trampus 1999). The activity bursts spread through the nest like a wave; active ants physically bump into and cause inactive nestmates to start moving, who in turn stimulate more activity (Goss and Deneubourg 1988; Cole 1991a, b; Doering et. al. 2021).

A major theme of chronobiology research is the characterization of the various mechanisms that regulate biological rhythms in organisms. Light has long been known as an important zeitgeber for solitary organisms (Dunlap et al. 2004), but few studies consider its effects on collective activity patterns (Sinhuber et al. 2019). It is not currently known if ambient light intensity has any influence on the oscillation frequency of colonies' ultradian activity cycles, nor is not known if collective intranidal activity patterns have an endogenous *circadian* rhythm that persists in the absence of alternating light levels. To our knowledge, observations of colony-level intranidal locomotor activity patterns that last for at least 24hrs have been reported for seven species. Research using *Temnothorax longispinosus, T. rugatulus,* and *Leptothorax acervorum* found evidence of diurnal activity (a higher proportion of workers were moving in the nest during the day) and were conducted under an alternating light/dark cycle (Charbonneau and Dornhaus 2015; Richardson et al. 2017; Libbrecht et al. 2020). In contrast, work with *Diacamma indicum*, *T. corticalis*, *L. crassipilis* and *L.* sp. W conducted under constant lighting conditions found no clear evidence of collective intranidal circadian activity (Chauvin 1944; Fujioka et al. 2021; Doering et. al. 2022). However, the primary motivation of these prior studies was not to look at whether collective 24-hr intranidal rhythms are generated endogenously or are a direct result of external influences like light. The daily cycles observed in *T. longispinosus*, *T. rugatulus*, and *L. acervorum* may therefore be responses to external changes in light intensity. Work with *L. acervorum* also reported that, in addition to diurnal rhythms, the oscillation frequency of the ultradian short-term activity cycles increases during the day (Richardson et al. 2017). These changes in ultradian frequency may similarly stem from the imposed light cycle.

To address these interrelated topics, we used automated tracking to measure the intranidal colony-level activity of ants from several species. We then tested whether 24-hour rhythmicity in colonies' collective activity (i.e., within-nest circadian rhythms) becomes stronger when colonies are subjected to an alternating light/dark cycle compared to conditions of either constant light or darkness. Finally, we also sought to analyze whether the oscillation frequencies of colonies' ultradian, short-term activity cycles depend on the imposed light/dark cycle (i.e., speeding up during the day for diurnal colonies).

4.3 Methods

Colony collection & maintenance

48 colonies in total were used in the experiment (see supplementary file S1 for data on each colony used) and were evenly divided between the three treatments (16 colonies per treatment). The assignment of colonies to each of three treatments (constant dark, constant light, and light/dark) was randomized using the function *sample* in R. Ten species were used in the experiment: *Formica subaenescens* (1 colony), *Lasius americanus* (5 colonies), *Leptothorax* AF-can (3 colonies), *Leptothorax crassipilis* (1 colony), *Myrmecina americana* (5 colonies), *Myrmica punctiventris* (6 colonies), *Tapinoma sessile* (4 colonies), *Temnothorax caguatan* (1 colony), *Temnothorax longispinosus* (19 colonies), and *Temnothorax stenotyle* (3 colonies). In natural habitats, all of these species typically form small colonies of less than 1000 workers and also live in pre-formed cavities. The colonies used in this study were collected from rotting acorns, sticks and between rock crevices. The preference of these species for dwelling in existing cavities facilitated the collection of entire nest populations. Colony sizes ranged from approximately 6 to 146 adult individuals (mean: 38.1, standard deviation: 25.3). *Temnothorax longispinosus* was the most represented species in our experiment because other researchers have already documented diurnal intranidal rhythms in this species under a 12hr light/dark cycle (Libbrecht et al. 2020), allowing us the opportunity to build off of this work, potentially replicate these findings in our light/dark treatment, and better understand collective rhythms in this species. The additional species were included in the experiment because we wanted to increase the phylogenetic diversity of the study and see if any detected effects of the three treatments were true of other ants, not just *T. longispinosus*.

Colonies were maintained in artificial nests that were constructed by drilling a 38mm diameter hole in a 75x50mm balsa wood slat (approx. 3.6 mm thick). Slats were sandwiched between two glass microscope slides, which provided a ceiling and floor to each nest. An approximately 4 mm slit was cut into the side of each slat to allow ants to enter and leave the nest cavity. All colonies were fed weekly with a diet of Spam (Hormel Foods, Minnesota) and honey. Colonies had constant access to water through cotton plugged vials that were checked weekly to prevent depletion. Colonies were kept in a room with a 12:12 hr LD cycle and at a constant temperature of 23° C prior to being used in the experiment.

Circadian experiment

Like prior experiments that have characterized the circadian rhythms of ants in captivity, we used low intensity red light (approx. 6.5 lux) to illuminate colonies in the constant dark (DD) treatment and to produce the "night" portions of the light/dark treatment (Fujioka et al. 2017, 2021; Libbrecht et al. 2020). Specifically, the red light was produced with a single 8-Watt LED light bulb (Philips, model number 463216). The "day" conditions in the LD treatment as well as the constant light (LL) treatment were created by turning on the overhead florescent lamps that were built into the ceiling of the rooms used in the experiment. The photoperiod for the LD treatment was programmed so that "day" conditions fell between 10:00 hours and 22:00 hours, and "night" conditions fell between 22:00 and 10:00 hours. These times were chosen to coincide with the timing of LD cycle that the colonies experienced before being used in the experiment. Colonies were left in each treatment for 96 hours and recorded using camcorders (Canon VIXIA). Due to capacity limits, the SD cards in the cameras needed to be exchanged with empty cards once during the 96-hour recordings. All colonies in the three treatments were filmed concurrently over the same 96-hour interval.

Activity time series & image & image processing

We applied a simple image analysis technique to automatically track the total level of movement colonies exhibited within their nests over time. We first sampled frames every 30 seconds from each video recording. Using the resulting images, we cropped out the area in each frame containing the nest cavity and applied an adaptive binary threshold (Bradley and Roth 2007) in order to segment the darker worker ants in the image from the lighter background. This process converted each frame into a binary matrix where every element that had a value of 1 was a region where a part of an ant's body was detected. For every pair of consecutive frames, we calculated the number of pixels that had changed from 0 to 1 between the two images. We then divided this quantity by the total number of pixels in the first frame in the pair to determine the approximate proportion of the colony that had moved between successive images (Boi et al. 1999; Doering et al. 2019). For the constant dark treatment and during the portions of the recordings from the LD treatment that were filmed under red light, sensor noise in the cameras caused by the weak illumination made tracking colony-level activity more challenging. For both the LD and constant dark (DD) treatments, we therefore applied a two-dimensional Wiener filter to each processed video frame in our activity tracking analyses to denoise the video. We used the MATLAB function *wiener2* with a pixel neighborhood of 5x5 (Lim 1990) to calculate the local mean and variance of the pixels in each frame. This allowed us to successfully detect ants in the low-light conditions (supplementary figure S2).

Previous work has found that different colonies from the same species can vary in the amplitude of their ultradian cycles (the proportion of ants moving during activity peaks) depending on colony size and whether a queen is present, but variation in cycle length (i.e., oscillation frequency) has not been linked with these factors (Cole 1991a; Hatcher 1992; Doering et al.

2019). We thus chose to focus exclusively on the oscillation frequencies of the collective activity patterns, and we normalized all time series to fall between 0 and 1 in order to discard any amplitude differences between colonies. All time series were also smoothed with a Gaussian moving average filter with a window length of 15 data points (equal to 7.5 minutes) prior to being used in any analyses.

One colony in the "dark" treatment needed to be excluded from our analysis. This colony (*Lasius americanus*) had fully absconded its nest after 33 hours of filming and emigrated to the area surrounding the moist cotton of its water tube. The colony was thus out of view and its behavior could not be observed.

Data analysis

We used wavelet analysis to determine 1) the strength of 24-hr rhythmicity, 2) the strength of short-term (ultradian) activity cycle rhythmicity, and 3) the dominant oscillation period for every time series. Wavelet analysis is a signal processing technique that has previously been used to identify frequencies in short-term activity cycles in ants (Richardson et al. 2017; Doering et al. 2019), and it is also commonly used to assess the strength of circadian rhythms (Leise and Harrington 2011). We used the MATLAB function *cwt* to compute the 1D continuous wavelet transform of activity time series using Morse wavelets (Lilly and Olhede 2012). We then found the frequency band in the resulting transform associated with the largest wavelength magnitude and noted this as the being dominant oscillation frequency of the given time series. All portions of each wavelet transform that fell within the "cone-of influence" were excluded in this analysis to avoid the inaccuracies presented by edge effects (Torrence and Compo 1998). To measure the strength of colonies' 24-hour rhythms, we found the sum of the wavelet magnitudes of each colony's activity time series associated with the frequency band closest to a frequency of 1/24 hours. The continuous wavelet transform in MATLAB returns the wavelet magnitudes of time series in discrete frequency bands, so we could not directly find the sum of wavelet magnitudes at a frequency of exactly 1/24 hours. The closest frequency, and the one we used, was 1/24.2 hours. To complement this analysis, we also tested for the strength of colonies' circadian rhythms using cosinor analysis (Cornelissen 2014; Libbrecht et al. 2020) using the cosinor2 R package. Cosinor analysis is an alternative method of testing for a pre-specified periodicity in time-series data by fitting a cosine function (Cornelissen 2014). The magnitude of the transformed amplitude parameter (Cornelissen 2014) determined by the cosinor analysis was used as our secondary metric for circadian rhythmicity strength in colonies. The wavelet magnitudes measuring the strength of colonies' 24-hour rhythms should, in principle, be highly correlated with the results of the cosinor analysis. Using two approaches can help validate that we are indeed measuring 24-hour rhythm strength. We did not apply cosinor analysis to colonies' short-term activity rhythms since these ultradian rhythms can vary both interspecifically and intraspecifically (Cole 1991a; Hatcher 1992; Doering et al. 2019), so there is no specific *a priori* oscillation frequency to test for, which would be required for cosinor analysis.

We used a linear mixed effects model (LME) to assess whether there were differences between light regime treatments in the strength of colonies' 24-hour activity. We set colonies' location of origin and species ID as nested random effects in our model. Not all of the colonies we used contained brood items. Although we can confirm that the majority of the colonies contained brood, we could not tell if brood items were present in the *Lasius*, *Myrmecina*, and three of the

Tapinoma colonies using our videos. We therefore also reran our LME model while only including colonies that were known to have brood items.

In order to test whether colonies in the light/dark treatment synchronized with the light cycle, we used an LME model to determine if the "day" and "night" conditions were correlated with colonies' average level of intranidal locomotor activity; we again set location and species ID as nested random effects. We additionally used LME models to test each of the species in L:D treatment separately to see if different species were diurnal, nocturnal, or did not entrain to the photoperiod. Finally, we also used wavelet analysis to determine the dominant short-term activity cycle period of each colony in the light/dark treatment during every "day" segment and every "night" segment. We then used this information to test whether the oscillation frequency of ultradian short term activity cycles change based on the photoperiod using an LME model where colonies' location of origin, species ID and colony ID were included as nested random effects. All statistical tests were performed using R version 4.0.3 (https://www.r-project.org).

4.4 Results

Collective circadian rhythms

Light regime had a strong influence on the appearance of intranidal circadian rhythms (Figure 1a; supplementary video S3). The L:D regime resulted in colonies with significantly stronger (as determined through wavelet analysis) 24-hour cycles of intranidal collective activity than colonies housed in constant darkness (LME: $t_{32} = 3.404$, $p = 0.0018$) or in constant light (LME: $t_{32} = 4.640$, $p < 0.001$). There was no difference in the strength of circadian rhythms between colonies in the constant light and constant darkness treatments (LME: $t_{32} = -1.071$, $p = 0.292$).

The results of these tests do not change significantly when only colonies that were confirmed to have brood are included (L:D vs. constant dark LME: $t_{20} = 3.163$, $p = 0.0049$; L:D vs. constant light LME: $t_{20} = 4.182$, $p = 0.0005$; constant light vs. constant dark LME: $t_{20} = -0.758$, $p = 0.458$). The results of our wavelet metrics were also strongly correlated with our secondary cosinor analysis (Figure S4; Pearson correlation: $r = 0.873$, $p < .0001$).

Short-term activity cycles

There were no differences between treatments in the strength of colonies' short-term activity rhythms (Figure 1b; L:D vs. DD LME: $t_{32} = -0.081$, $p = 0.936$; L:D vs. LL LME: $t_{32} = 0.513$, $p = 0.611$; LL vs. DD LME $t_{32} = 0.405$, $p = 0.688$), nor were there any differences between treatments in the dominant period of oscillation for short-term activity cycles (L:D vs. DD LME: $t_{32} = -0.497$, $p = 0.622$; L:D vs. LL LME: $t_{32} = 1.225$, $p = 0.230$; LL vs. DD LME: $t_{32} = 0.652$, $p = 0.520$). All tests for differences in the strength of colonies' short-term activity rhythms remain non-significant when only colonies that were known to contain brood are considered (L:D vs. constant dark LME: $t_{20} = 0.336$, $p = 0.740$; L:D vs. constant light LME: $t_{20} = 0.302$, $p = 0.766$; constant light vs. constant dark LME: $t_{20} = 0.626$, $p = 0.539$). All tests for differences between treatments in the dominant period of oscillation for short-term activity cycles likewise remain non-significant (L:D vs. constant dark LME: $t_{20} = 0.916$, $p = 0.371$; L:D vs. constant light LME: $t_{20} = 1.167$, $p = 0.257$; constant light vs. constant dark LME: $t_{20} = 2.014$, $p = 0.058$).

Activity and rhythm variation during light/dark cycles

Overall, there was a significant association between colonies' activity levels and the photoperiod in the L:D treatment (LME: $t_{127} = -2.512$, $p = 0.013$). Two species exhibited diurnal activity, becoming more active in the nest during the "day" (Figure 2c,d; *Temnothorax longispinosus*

LME: $t_{55} = -4.312$, $p < 0.001$; *Myrmica punctiventris* LME: $t_{15} = -3.326$, $p = 0.005$; supplementary video S3). One species exhibited nocturnal activity, becoming more active in the nest at “night” (Figure 2e; *Tapinoma sessile* LME: $t_{15} = 6.024$, $p < 0.001$). The activity patterns of three species did not significantly entrain to the photoperiod (*Temnothorax stenotyle* LME: $t_7 = -1.016$, $p = 0.343$; *Myrmecina americana* LME: $t_{23} = 1.413$, $p = 0.171$; *Leptothorax* AF-can LME: $t_{127} = -1.428$, $p = 0.196$). There were colonies in all three treatments that showed little evidence for 24-hour rhythms at all and only exhibited ultradian cycles (Figure 2a,b,f). We found no evidence overall for an effect of photoperiod on the oscillation rhythm of colonies’ short-term activity cycles (LME: $t_{127} = 0.0134$, $p = 0.989$).

4.5 Discussion

Our experiment investigated if light plays a role in controlling the collective rhythms of ants within their nests. We found that different types of collective activity patterns within ant nests are not affected by exposure to light in the same way. The short-term (ultradian) activity cycles of the species investigated in this study did not become weaker when confronted with unchanging light levels, and colonies in the LD treatment did not alter the period of their ultradian oscillations according to the photoperiod. In contrast, 24-hour rhythms of collective activity were the most prominent in colonies that experienced an alternating light/dark cycle, and the 24-hour rhythms of colonies in this treatment were synchronized with the photoperiod, with colonies expressing diurnal and nocturnal rhythms. Our results suggest that social circadian rhythms in ants are more fragile than ultradian collective rhythms, being disrupted simply by living in constant environmental conditions.

Why should short-term activity cycles be less sensitive to the effects of light regime than 24-hour rhythms? The ultimate reasons colonies evolved synchronized intranidal ultradian rhythms is still unknown, but empirical data and modelling work has uncovered several plausible benefits of ultradian collective cycles for colonies, such as improved brood care (Hatcher et al. 1992), controlling information transfer (Richardson et al. 2017), easier worker and queen locomotion within the nest (Wilson 1974, Doering et. al Unpublished data), and faster completion of tasks (Delgado and Solé 2000). Because short-term activity cycles are known to be generated through interactions between workers (Cole 1991a) and occur inside the nest, it makes sense that varying the light cycle alone would not induce any changes to this particular type of synchronization. Contrary to our findings, work with *Leptothorax acervorum* documented slower short-term activity cycles at night (Richardson et al. 2017). In addition to varying light intensity, this study also lowered the temperature colonies experienced at night compared to the day. Because colder temperatures can make ants move more slowly (Barnes and Kohn 1932; Angilletta et al. 2008), this might be the source of the discrepancy between our results.

The result that colonies' 24-hour rhythms were weakened under constant lighting conditions suggests that colonies of several species do not have strong endogenous clocks that regulate the collective activity inside nests. Research on the Asian ant *Diacamma indicum* found that isolated workers do have robust circadian rhythms of locomotion under constant conditions (Fujioka et al. 2017, 2019). However, individuals of this same species lose their circadian rhythms when paired with larvae (Fujioka et al. 2017), and entire colonies do not exhibit 24-hour rhythms of intranidal activity in constant darkness (Fujioka et al. 2021). A study that took measurements of colony-level respiration (instead of locomotor activity) inside nests using *Temnothorax unifasciatus* in constant temperature conditions likewise found no evidence of circadian rhythms

(Martin 1991). Field and laboratory studies have revealed that foraging workers in several social insect species have strong circadian activity rhythms *outside* the nest and that foragers can entrain to signals like temperature, humidity, daylight, and the rate of encounters with other foragers (Talbot 1946; McCluskey 1973, 1974; Bernstein 1974, 1979; Fellers 1989; Crailsheim et al. 1996; Lone and Sharma 2011; Berberich et al. 2019). Despite the clear evidence of endogenous timekeeping processes for foraging outside the nest, there is no strict reason that any oscillations of intranidal activity need to be coupled with foraging activity, though this is sometimes assumed in theoretical models (Bonabeau et al. 1998; Nicolis et al. 2013). In fact, preliminary data from at least one species (*Leptothorax acervorum*) points to a very weak association between short-term activity cycles and foraging (Hatcher 1992).

This raises the question of whether it is beneficial for ants to have circadian rhythms inside their nests at all. Unlike foraging, where the availability of food (Mildner and Roces 2017) and competition with other ant colonies (Fellers 1989) may select for robust circadian rhythms in the proportion of ants leaving the nest to forage at specific times of the day, the conditions inside a colony's nest chambers might be relatively static for long periods of time. The temperature and humidity inside nests are regulated by workers in several species (Korb and Linsenmair 2000; Jones and Oldroyd 2006), and ants in particular often prefer to live in nests with dark interiors where changes in the level of daylight could be difficult to sense (Franks et al. 2003). Social insect nurse workers also often exhibit activity around the clock when inside the nest to care for larvae and eggs (Crailsheim et al. 1996; Mildner and Roces 2017; Fujioka et al. 2017) and organizing collective activity within the nest so that fewer individuals are active at certain portions of the day (e.g., at night) might be detrimental to brood care. Peculiar ecological or physiological characteristics are believed to make circadian rhythms less important or impractical in some organisms. For example, reindeer that live in the high arctic and experience long periods of constant daylight in the summer do not appear to have circadian rhythms (Lu et al. 2010). Ram ventilating sharks that require constant swimming for respiration also lack circadian activity (Kelly et al. 2020). Ant colonies may similarly not gain any functional benefits for having circadian cycles of intranidal activity. The circadian rhythms of ants inside their nests under natural conditions has never been investigated. It therefore remains to be seen if colonies have active-rest rhythms inside their nests when in the wild. Investigating this question along with the potential relationship between intranidal activity and foraging activity will likely be a promising subject for future research into how the collective behavior of ants in structured in time.

4.6 Acknowledgements

We thank Robin Stuart for advice on finding *Leptothorax* colonies, and we thank Tovah Kashestsky, and Janice Yan for help collecting colonies from Ontario. We are grateful to Lisa Staton of the Heartland Forest in Niagara Falls, Ontario for allowing us to collect ants on their property. Reuven Dukas kindly provided us access to his dissecting microscope.

4.7 Data availability

All time series used in this study along with all code used for the data analysis are available as a single supplementary zip file.

4.8 Funding

This research was supported by the Canada 150 Research Chair of JNP.

4.9 Author contributions

GND conceived and designed the study, performed all analyses, conducted the experiment, processed and handled all the data, and wrote the first draft of the manuscript. All authors helped acquire ant colonies and contributed to the editing and writing of the manuscript.

4.10 References

Angilletta MJ, Ii TCR, Wilson RS, et al (2008) The fast and the fractalous: speed and tortuosity trade off in running ants. Funct Ecol 22:78–83. https://doi.org/10.1111/j.1365-2435.2007.01348.x

Baldridge RS, Rettenmeyer CW, Watkins JF (1980) Seasonal, Nocturnal and Diurnal Flight Periodicities of Nearctic Army Ant Males (Hymenoptera: Formicidae). J Kans Entomol Soc 53:189–204

Barnes TC (1941) Rhythms of activity in ant colonies. J Gen Psychol 25:249–255. https://doi.org/10.1080/00221309.1941.10544396

Barnes TC, Kohn HI (1932) The Effect of Temperature on the Leg Posture and Speed of Creeping in the Ant *Lasius*. Biol Bull 62:306–312. https://doi.org/10.2307/1537511

Berberich GM, Berberich MB, Ellison AM, et al (2019) First In Situ Identification of Ultradian and Infradian Rhythms, and Nocturnal Locomotion Activities of Four Colonies of Red Wood Ants (*Formica rufa*-Group). J Biol Rhythms 34:19–38. https://doi.org/10.1177/0748730418821446

Bernstein RA (1974) Seasonal Food Abundance and Foraging Activity in Some Desert Ants. Am Nat 108:490–498. https://doi.org/10.1086/282928

Bernstein RA (1979) Schedules of Foraging Activity in Species of Ants. J Anim Ecol 48:921–930. https://doi.org/10.2307/4204

Bloch G, Herzog ED, Levine JD, Schwartz WJ (2013) Socially synchronized circadian oscillators. Proc R Soc B Biol Sci 280:20130035. https://doi.org/10.1098/rspb.2013.0035

Boi S, Couzin ID, Del Buono N, et al (1999) Coupled Oscillators and Activity Waves in Ant Colonies. Proc Biol Sci 266:371–378

Bonabeau E, Theraulaz G, Deneubourg J-L (1998) The synchronization of recruitment-based activities in ants. Biosystems 45:195–211. https://doi.org/10.1016/S0303-2647(98)00004-5

Bradley D, Roth G (2007) Adaptive Thresholding using the Integral Image. J Graph Tools 12:13–21. https://doi.org/10.1080/2151237X.2007.10129236

Charbonneau D, Dornhaus A (2015) Workers "specialized" on inactivity: Behavioral consistency of inactive workers and their role in task allocation. Behav Ecol Sociobiol 69:1459–1472. https://doi.org/10.1007/s00265-015-1958-1

Chauvin R (1944) L'effet de groupe et la régulation sociale chez les fourmis du genre Leptothorax étudiés au moyen du microactographe optique-I. Bull Biol Fr Belg 78:1–9

Cole BJ (1991a) Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction. Am Nat 137:244–259

Cole BJ (1991b) Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav 4:129–137. https://doi.org/10.1007/BF01054607

Cole BJ, Trampus FI (1999) Activity cycles in ant colonies: worker interactions and decentralized control. In: Detrain C, Deneubourg JL, Pasteels JM (eds) Information Processing in Social Insects. Birkhäuser Basel, Basel, pp 289–307

Cornelissen G (2014) Cosinor-based rhythmometry. Theor Biol Med Model 11:16. https://doi.org/10.1186/1742-4682-11-16

Crailsheim K, Hrassnigg N, Stabentheiner A (1996) Diurnal behavioural differences in forager and nurse honey bees (Apis mellifera carnica Pollm). Apidologie 27:235–244. https://doi.org/10.1051/apido:19960406

Delgado J, Solé RV (2000) Self-synchronization and Task Fulfilment in Ant Colonies. J Theor Biol 205:433–441. https://doi.org/10.1006/jtbi.2000.2077

Delgado-Garcia JM, Grau C, DeFeudis P, et al (1976) Ultradian rhythms in the mobility and behavior of rhesus monkeys. Exp Brain Res 25:79–91

Doering GN, Sheehy KA, Lichtenstein JLL, et al (2019) Sources of intraspecific variation in the collective tempo and synchrony of ant societies. Behav Ecol 30:1682–1690. https://doi.org/10.1093/beheco/arz135

Dunlap JC, Loros JJ, DeCoursey PJ (2004) Chronobiology: biological timekeeping. Sinauer Associates

Fellers JH (1989) Daily and seasonal activity in woodland ants. Oecologia 78:69–76. https://doi.org/10.1007/BF00377199

Franks NR, Mallon EB, Bray HE, et al (2003) Strategies for choosing between alternatives with different attributes: exemplified by house-hunting ants. Anim Behav 65:215–223. https://doi.org/10.1006/anbe.2002.2032

Fuchikawa T, Eban-Rothschild A, Nagari M, et al (2016) Potent social synchronization can override photic entrainment of circadian rhythms. Nat Commun 7:11662. https://doi.org/10.1038/ncomms11662

Fujioka H, Abe MS, Fuchikawa T, et al (2017) Ant circadian activity associated with brood care type. Biol Lett 13:20160743. https://doi.org/10.1098/rsbl.2016.0743

Fujioka H, Abe MS, Okada Y (2021) Individual Ants Do Not Show Activity-Rest Rhythms in Nest Conditions. J Biol Rhythms 07487304211002934. https://doi.org/10.1177/07487304211002934

Fujioka H, Abe MS, Okada Y (2019) Ant activity-rest rhythms vary with age and interaction frequencies of workers. Behav Ecol Sociobiol 73:30. https://doi.org/10.1007/s00265-019-2641-8

Gordon DM, Mehdiabadi NJ (1999) Encounter rate and task allocation in harvester ants. Behav Ecol Sociobiol 45:370–377. https://doi.org/10.1007/s002650050573

Goss S, Deneubourg JL (1988) Autocatalysis as a source of synchronised rhythmical activity in social insects. Insectes Sociaux 35:310–315. https://doi.org/10.1007/BF02224063

Guzmán DA, Flesia AG, Aon MA, et al (2017) The fractal organization of ultradian rhythms in avian behavior. Sci Rep 7:684. https://doi.org/10.1038/s41598-017-00743-2

Hatcher MJ (1992) Activity patterns and organization within ant nests. PhD Thesis, University of Bath

Hatcher MJ, Tofts C, Franks NR (1992) Mutual exclusion as a mechanism for information exchange within ant nests. Naturwissenschaften 79:32–34. https://doi.org/10.1007/BF01132279

Jones JC, Oldroyd BP (2006) Nest Thermoregulation in Social Insects. In: Simpson SJ (ed) Advances in Insect Physiology. Academic Press, pp 153–191

Kelly ML, Murray ERP, Kerr CC, et al (2020) Diverse Activity Rhythms in Sharks (Elasmobranchii). J Biol Rhythms 35:476–488. https://doi.org/10.1177/0748730420932066

Korb J, Linsenmair KE (2000) Ventilation of termite mounds: new results require a new model. Behav Ecol 11:486–494. https://doi.org/10.1093/beheco/11.5.486

Leise TL, Harrington ME (2011) Wavelet-Based Time Series Analysis of Circadian Rhythms. J Biol Rhythms 26:454–463. https://doi.org/10.1177/0748730411416330

Lewis T, Pollard GV, Dibley GC (1974) Rhythmic Foraging in the Leaf-Cutting Ant *Atta cephalotes* (L.) (Formicidae: Attini). J Anim Ecol 43:129–141. https://doi.org/10.2307/3162

Libbrecht R, Nadrau D, Foitzik S (2020) A Role of Histone Acetylation in the Regulation of Circadian Rhythm in Ants. iScience 23:100846. https://doi.org/10.1016/j.isci.2020.100846

Lilly JM, Olhede SC (2012) Generalized Morse Wavelets as a Superfamily of Analytic Wavelets. IEEE Trans Signal Process 60:6036–6041. https://doi.org/10.1109/TSP.2012.2210890

Lim JS (1990) Two-dimensional signal and image processing. Englewood Cliffs NJ Prentice Hall 1990 710 P

Lloyd D, Stupfel M (1991) The Occurrence and Functions of Ultradian Rhythms. Biol Rev 66:275–299. https://doi.org/10.1111/j.1469-185X.1991.tb01143.x

Lone SR, Sharma VK (2011) Timekeeping Through Social Contacts: Social Synchronization of Circadian Locomotor Activity Rhythm in the Carpenter Ant *Camponotus paria*. Chronobiol Int 28:862–872. https://doi.org/10.3109/07420528.2011.622676

Lu W, Meng Q-J, Tyler NJC, et al (2010) A Circadian Clock Is Not Required in an Arctic Mammal. Curr Biol 20:533–537. https://doi.org/10.1016/j.cub.2010.01.042

Lumineau S, Guyomarc'h C, Richard J-P (2001) Ultradian Rhythm of Activity in Japanese Quail Groups under Semi-Natural Conditions during Ontogeny: Functional Aspects and Relation to Circadian Rhythm. Biol Rhythm Res 32:373–400. https://doi.org/10.1076/brhm.32.3.373.1339

Manjunatha T, Dass SH, Sharma VK (2008) Egg-laying rhythm in *Drosophila melanogaster*. J Genet 87:495–504. https://doi.org/10.1007/s12041-008-0072-9

Martin PJ (1991) Respiration of the ant *Leptothorax unifasciatus* (Hymenoptera, Formicidae) at individual and society levels. J Insect Physiol 37:311–318. https://doi.org/10.1016/0022-1910(91)90065-8

McCluskey ES (1973) Generic Diversity in Phase of Rhythm in Formicine Ants. Psyche (Stuttg) 80:295–304. https://doi.org/10.1155/1973/91652

McCluskey ES (1974) Generic Diversity in Phase of Rhythm in Myrmicine Ants. J N Y Entomol Soc 82:93–102

Mildner S, Roces F (2017) Plasticity of Daily Behavioral Rhythms in Foragers and Nurses of the Ant *Camponotus rufipes*: Influence of Social Context and Feeding Times. PLOS ONE 12:e0169244. https://doi.org/10.1371/journal.pone.0169244

Nicolis SC, Fernández J, Pérez-Penichet C, et al (2013) Foraging at the Edge of Chaos: Internal Clock versus External Forcing. Phys Rev Lett 110:268104. https://doi.org/10.1103/PhysRevLett.110.268104

Regal PJ, Connolly MS (1980) Social Influences on Biological Rhythms. Behaviour 72:171–199

Richardson TO, Liechti JI, Stroeymeyt N, et al (2017) Short-term activity cycles impede information transmission in ant colonies. PLOS Comput Biol 13:e1005527. https://doi.org/10.1371/journal.pcbi.1005527

Seki Y, Tanimura T (2014) Ultradian rhythm unmasked in the Pdf clock mutant of *Drosophila*. J Biosci 39:585–594. https://doi.org/10.1007/s12038-014-9450-z

Sinhuber M, van der Vaart K, Ouellette NT (2019) Response of insect swarms to dynamic illumination perturbations. J R Soc Interface 16:20180739. https://doi.org/10.1098/rsif.2018.0739

Staab M, Kleineidam CJ (2014) Initiation of swarming behavior and synchronization of mating flights in the leaf-cutting ant *Atta vollenweideri* FOREL, 1893 (Hymenoptera: Formicidae). Myrmecol News 19:93–102

Talbot M (1946) Daily Fluctuations in Aboveground Activity of Three Species of Ants. Ecology 27:65–70. https://doi.org/10.2307/1931019

Tennenbaum M, Fernandez-Nieves A (2017) Activity-driven changes in the mechanical properties of fire ant aggregations. Phys Rev E 96:052601. https://doi.org/10.1103/PhysRevE.96.052601

Torrence C, Compo GP (1998) A Practical Guide to Wavelet Analysis. Bull Am Meteorol Soc 79:61–78. https://doi.org/10.1175/1520-0477(1998)079<0061:APGTWA>2.0.CO;2

Tran D, Nadau A, Durrieu G, et al (2011) Field Chronobiology of a Molluscan Bivalve: How the Moon and Sun Cycles Interact to Drive Oyster Activity Rhythms. Chronobiol Int 28:307–317. https://doi.org/10.3109/07420528.2011.565897

Wilson EO (1987) Causes of Ecological Success: The Case of the Ants. J Anim Ecol 56:1–9. https://doi.org/10.2307/4795

Wilson EO (1974) Aversive Behavior and Competition within Colonies of the Ant *Leptothorax curvispinosus*. Ann Entomol Soc Am 67:777–780. https://doi.org/10.1093/aesa/67.5.777

Figure 4.1

Box plots depicting the strength of the 24-hr (circadian) rhythmicity (a) and the strength of short-term activity cycle (STAC) rhythmicity (b) for each colony in the three light regimes as measured by wavelet analysis. Each point represents a separate colony, which have been jittered for clarity. Colonies that were filmed in conditions with a 12-hour light/dark cycle possessed stronger 24-hr activity cycles within their nests, but the treatment groups did not differ in the strength of short-term activity rhythms.

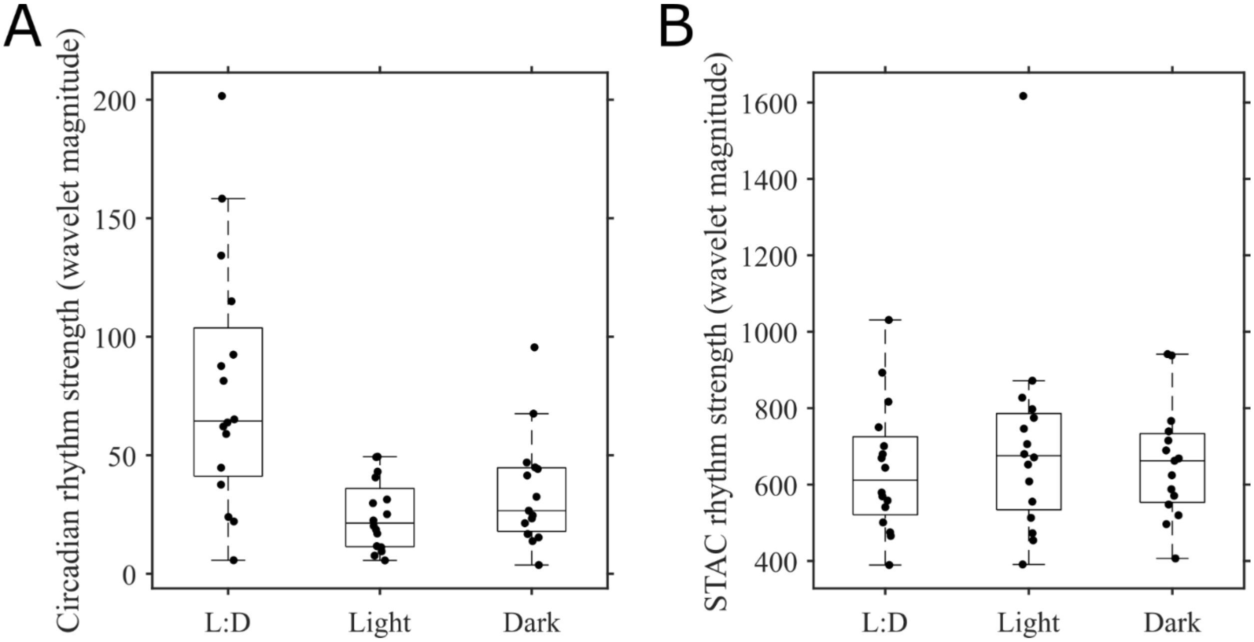
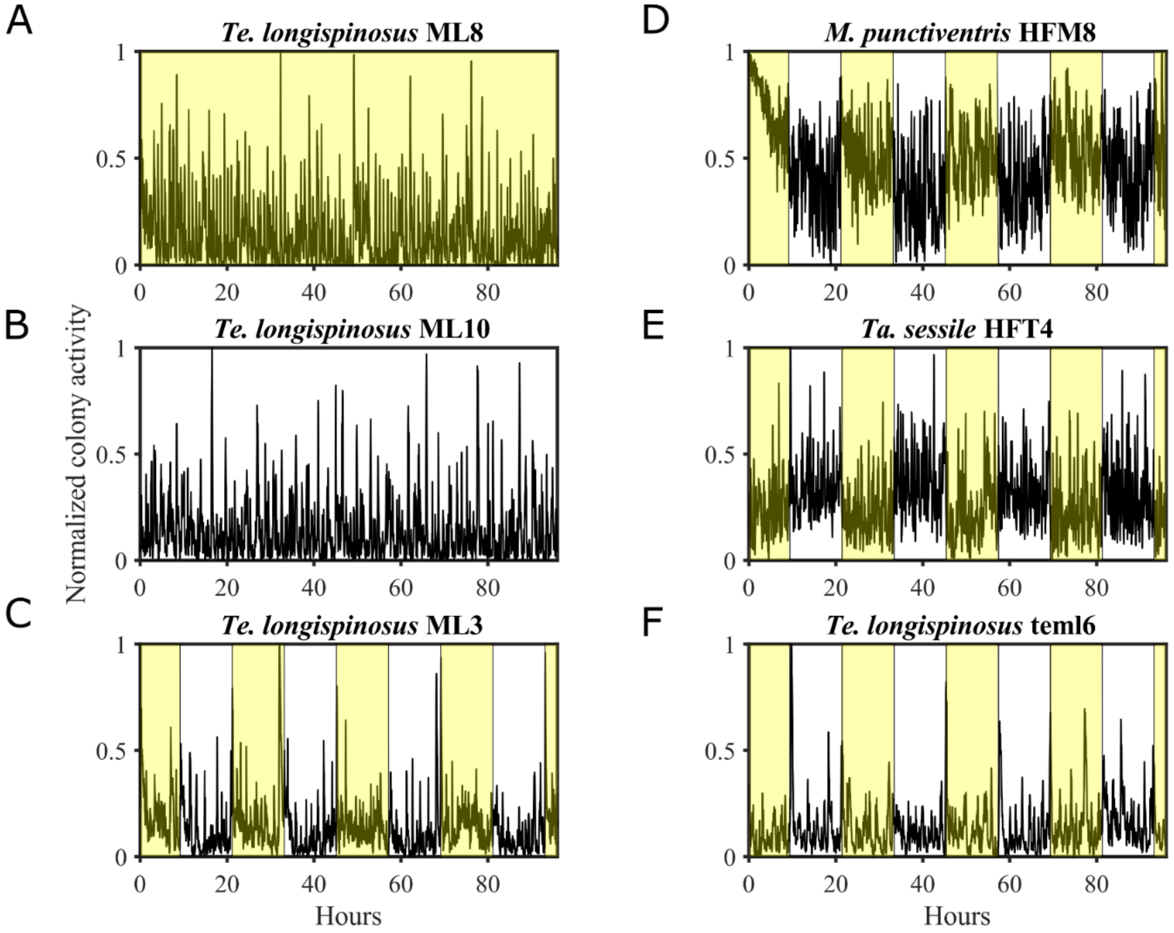


Figure 4.2

Representative within-nest collective activity time series (a-f) from all three light regime treatments in the circadian experiment. The time series have been rescaled to fall between 0 - 1 and have been smoothed as described in the methods. The corresponding species and colony ID appears above each time series. Regions that are shaded yellow indicate times when a colony was experiencing "day" conditions. Areas that are not highlighted in yellow indicate "night" conditions (i.e., illuminated with dim red light). Colonies exhibited nocturnal (e) and diurnal (c,d) rhythms, but other colonies showed no evidence of any circadian patterns (a,b,f).



CHAPTER 5 – SYNCHRONIZED LOCOMOTION IMPROVES SPATIAL ACCESSIBILITY IN ANT COLONIES AND IN OSCILLATING ACTIVE PARTICLES.

5.1 Abstract

Synchronization is a common and important collective phenomenon in many biological and physical systems. Ant colonies from genus *Leptothorax* exhibit a form of synchronized behavior where workers inside colonies' nests become active together in rhythmic cycles that have a period of approximately 20 minutes. However, it is not currently known if these synchronized rhythms of locomotion confer any functional benefit to colonies. By using a combination of multiple image analysis techniques, we show that inactive ants act as immobile obstacles to moving ants and that, compared with asynchronous locomotion, synchronized activity reduces the amount of time that active ants encounter clusters of inactive ants that impede access to regions of the nest. We demonstrate qualitatively similar findings using a computational model of confined, oscillating active particles where the level of particle phase synchrony, average activity level, and particle density can be directly manipulated. Our model simulations reveal that synchronous activity provides the greatest improvements to spatial accessibility when particle density is high and when the duration particles spend inactive is long.

5.2 Introduction

Synchronization is a conspicuous form of collective behavior found in numerous physical, biological, and artificial systems **[1]**. Synchronization also appears in the socially coordinated behaviors of many different organisms. Collective oscillations in swarms of bacteria **[2]**, fireflies flashing in unison **[3]**, duetting songbirds **[4]**, and human musicians playing in a string quartet **[5]** are all examples of synchronization in a social context.

Ants are among the most abundant social animals on Earth, and workers ants in colonies exhibit multiple forms of complex synchronized behavior, such as the collective transport of prey **[6]**, and robust consensus-decision making when choosing a nest **[7,8]**. One relatively poorly studied variety of behavioral synchronization in ant colonies is a type of ultradian collective activity rhythm that occurs within the nests of ants from several genera, including *Leptothorax* **[9–13]**. *Leptothorax* species form small colonies typically made up of only a few hundred workers **[14]**. Colonies live in pre-formed cavities (e.g., within rotting sticks, acorns, between rock crevices, etc.). During collective activity rhythms in *Leptothorax,* most workers become active simultaneously every 20-30 min and begin walking around inside the nest **[9,15]**. Between these moments of heightened activity, most ants will rest and remain motionless (supplementary video 1). Previous research has shown that these synchronized cycles of activity are generated through physical contact between ants; active ants stimulate inactive ants into moving, and activity spreads like a wave through the colony not unlike an excitable medium **[9,11,16]**.

The benefits that this pattern of locomotion might provide to colonies are unknown **[12,17]**. One plausible *physical* advantage of synchronizing times of rest and activity is improved spatial accessibility within the nest. Because ants cannot simply walk through each other, inactive ants will act as immobile obstacles that active individuals must either walk around or climb over. The presence of immobile obstacles within the nest can therefore be problematic for at least two reasons: 1) if the nest cavity is long and narrow (e.g., *Leptothorax* colonies that live in narrow rotting sticks), then just one or two inactive individuals could clog the motion of ants through the nest, and 2) aggregations of inactive ants may congregate near regions of the nest where larvae are present, limiting the ability of active ants to walk into such regions assess whether any tasks like tending brood need attention in the area.

The capacity of individuals to coordinate their movements as a group is a key part of animal collective behavior. Spatial constraints can have significant effects on collective motion, and such effects have therefore been studied both in the context of ant traffic and in active matter more generally **[18–25]**. Ants are adept at adjusting their walking speed and headings to optimizing the flow of workers in nest tunnels and on foraging trails while minimizing instances of jamming **[19–21]**. Theoretical models have also shown that adding noise **[22]** and delivering periodic perturbations **[25]** are both capable of improving the flow of moving particles when in the presence of static obstacles that have a heterogeneous spatial arrangement. However, the benefits of synchronized active-rest rhythms to the collective mobility of active matter are less well understood. In particular, it is not known if synchronized rhythms of locomotion inside an ant nest will reduce the likelihood that individual ants will encounter regions of immobile obstacles that impede access to portions of the nest. In this letter, we tested this hypothesis using observations of live *Leptothorax* colonies that exhibited highly rhythmic within-nest activity oscillations. We then built a simple agent-based model of non-interpenetrating mobile oscillators to support our empirical observations and investigate what conditions make synchronous activity most beneficial for special accessibility.

5.3 Results

We obtained colony-level activity time series of the approximate proportion of ants in a colony that were moving over time using the same methods employed in several previous studies **[9,11,16]**. Our tracking algorithm extracts frames from a video recording every 30 seconds and then creates binary images by using adaptive thresholding to segment ants inside the nest from the comparatively lighter background. We then compute the number of pixels classified as ants that change from 0 to 1 in each pair of frames and divide this quantity by the total number of pixels detected in the first frame in each pair to estimate the proportion of the colony that has moved between successive frames. Video recordings (each approx. 9-hours long) of four colonies (Three colonies of *Leptothorax canadensis* **[26]** and one colony of *Leptothorax retractus* **[27]**) were used in our study. *Leptothorax canadensis* is a species complex in need of taxonomic revision, and our assessment of the colonies' morphology suggests that all three belong to the undescribed *L*. AF-can **[28]**.

Colonies were collected from rotting sticks in Royun-Noranda, Quebec in September 2019, and kept in artificial nests **[29]** that consisted of a balsa wood slat with a 38mm diameter hole drilled through the slat's center. Two glass microscope slides (50 x 75 mm) acted as a roof and ceiling for each nest, and an approximately 4mm nest entrance was cut through the side of each slat, allowing ants to freely leave and return to their nest. Colonies were feed weekly with Spam (Hormel foods, Minnesota) and honey and had *ad libitum* access to water.

In addition to within-nest worker activity, we also recorded larval interactions for one colony (RN 19) by haphazardly selecting 20 larvae and noting every time any ant fed or groomed the chosen larvae over the first 3 hours of the colony's video recording. This was done to validate the anecdotal observation reported for the related ant *Temnothorax allardycei*, where synchronized activity oscillations appear to correlate with times of larval care **[11]**, a vital colony maintenance task.

The four recorded colonies exhibited activity cycles with a period of approximately 20 min (Figure 1). Larval interactions in colony RN19 were also cyclic and synchronized with locomotor activity level (Figure 1; Pearson correlation: $r = 0.776$, $p < 0.00001$). A visual inspection of our videos gives the impression that inactive ants do indeed act as immobile obstacles; ants that are moving tend to walk around inactive nestmates (supplementary video 2). To verify this observation more rigorously, we used optical flow to measure the amount of motion passing through inactive ants and compared this to the amount of motion at random locations within the nest. Optical flow is an image analysis method similar to particle image velocimetry; the technique compares consecutive frames in a video to determine the relative speeds and directions of moving objects in a video. We used a MATLAB implementation of the Farneback optical flow algorithm **[30]** to find a displacement magnitude for each pixel in every frame pair from a 2.8-minute long segment (at 1 fps) of the 9-hour recording of colony RN23 (Figure 2a). We found the cumulative sum ($\boldsymbol{\sigma_{Px}}$) of each pixel's displacement magnitudes over the 2.8-minute segment.

$$\boldsymbol{\sigma_{Px}} = \sum_{t=1}^{T} \boldsymbol{M_t} \tag{1}$$

Here, $\boldsymbol{T}$ denotes the total number of frame pairs from the video segment, and $\boldsymbol{M_t}$ is the displacement magnitude of pixel $\boldsymbol{Px}$ at frame pair *t*. This enabled us to estimate the total amount of motion that occurred in each pixel of the video segment over its entire duration. We also automatically segmented all ants that remained inactive over this 2.8-minute clip by finding pixels that had a value of 1 in both the first and last binarized frames from the video segment. Finally, we compared the $\boldsymbol{\sigma_{Px}}$ values of pixels classified as comprising inactive ants with an equal number of randomly chosen pixels. If active ants do not avoid walking over inactive ants, the average $\boldsymbol{\sigma_{Px}}$ values for pixels that correspond with inactive ant locations should be no different than randomly chosen pixels. However, mean $\boldsymbol{\sigma_{Px}}$ was lower for pixels associated with inactive ants (Figure 2b; two sample t-test: $t_{46897} = -43.56$, $p < 0.0001$), indicating that active ants do in fact maneuver around inactive ants.

We defined two metrics to quantify the "spatial accessibility" experienced by workers inside a nest over time. The first of these measured the size of the largest aggregation of inactive ants inside the nest at each moment of the activity time-series. To compute this, we partitioned each video frame into a grid with 16 sectors and counted the number of pixels that made up the inactive ants in each sector. For each data point of an activity time series, we could thereby find the region with the largest number of inactive ants. The number of pixels in this sector was then divided by the approximate number of pixels that made up a single ant (62 pixels in the case of *L. retractus* (RN6), and 80 pixels for the three *L. canadensis* colonies) to estimate the number of individuals in the sector. We used this as the value of our first metric, which we called maximum local density (*MLD*).

The second metric, which we refer to as *local openness* (*O*), measured approximately how many inactive ants surrounded each active ant. At each point in an activity time-series, we located the active ants in the corresponding video frames and overlaid a square of 31x31 pixels on the centroid of each detected active ant. We then counted the number of pixels inside each of these squares that was classified as belonging to an inactive ant and divided this quantity by the approximate number of pixels that made up a single ant. At each point of an activity time-series we thus obtained a vector containing an *O* value for each active ant. Taking the average of each local openness vector at each time point *t* could then be used to create a time-series of the average local openness $\langle \boldsymbol{O_t} \rangle$ experienced by active ants at each time point. A low value of $\langle \boldsymbol{O_t} \rangle$ indicates that the active ants at a particular time point are (on average) not closely surrounded by inactive ants that could impede their choice of where to take their next step. Neither of these metrics is trivially guaranteed to vary with colony activity level. For example, even if most ants are moving during an activity burst, the ants that do remain inactive could conceivably still be concentrated in a dense aggregate around the brood pile.

Using the time-series of these two metrics, we calculated three indices for each metric that summarized different aspects of a colony's overall level of spatial accessibility during its 9-hour recording. We refer to our first index as the *weighted index* ($\boldsymbol{\bar{\phi}}$), which was the weighted arithmetic mean of a colony's *MLD* time-series. The weights were the number of ants active at each time point ($\boldsymbol{N_{a_t}}$):

$$\bar{\varphi} = \frac{\sum_{t=1}^{T} N_{a_t} * MLD_t}{\sum_{t=1}^{T} N_{a_t}} \tag{2}$$

The rationale for this index is that captures the typical *MLD* experienced by ants when they are active by accounting for the fact the number of active ants is not constant but cyclical. We created a second index called the *ordinary index* or $\boldsymbol{\phi}$, which was just the time average of a colony's *MLD* metric. If there was no synchronization among ants, then a colony's activity level would fluctuate around a roughly constant proportion of ants active. For example, if individual workers typically spend about 30% of their time active over a given timeframe, then in a nonsynchronous colony you would expect to always find around 30% of the colony active but with different individuals making up the population of active individuals at different time points. In such a scenario, the weighted index would be nearly equal to the ordinary index $\bar{\boldsymbol{\phi}} \approx \boldsymbol{\phi}.$ The average index therefore approximates what the weighted index ($\bar{\boldsymbol{\phi}}$) would be for a colony if the activity of individuals were asynchronous but still had the same *average* level of activity. Because most brood tending occurs during peaks of activity, we also calculated a third index (the *peak index* $\boldsymbol{\phi}^*$) that was defined as the average of the *MLD* values corresponding to the activity peaks of a colony's activity time-series. This index is thus a measure of the mean spatial accessibility experienced by workers during the times when larval tending is at its busiest. The peaks in the activity time series were automatically detected using the MATLAB function *findpeaks*.

The corresponding indices associated with the $\langle \boldsymbol{O_t} \rangle$ metric were calculated in the same way as they were for *MLD* except for the weighted index $\bar{\boldsymbol{\phi}}$. This was because unlike *MLD*, which is defined for the colony as a whole at each time point, we had separate values of $\boldsymbol{O}$ for each active ant at every time point. The index $\bar{\boldsymbol{\phi}}$ was therefore found by simply concatenating all $\boldsymbol{O}$ vectors and calculating the average of this set. The fact that the size of the $\boldsymbol{O}$ vectors is based on the number of active ants means that this computation will effectively be a weighted average.

We found that *MLD* was significantly negatively correlated with colony-level activity in all four colonies (RN22 - Pearson correlation: r = -0.713, p < 0.0001, Figure 3a; RN23 - Pearson correlation: r = -0.575, p < 0.0001, Figure S1a; RN19 - Pearson correlation: r = -0.452, p < 0.0001, Figure S1b; RN6 - Pearson correlation: r = -0.706, p < 0.0001, Figure S1c). This was also true of the second metric, local openness $\langle \boldsymbol{O_t} \rangle$ (RN22 - Pearson correlation: r = -0.489, p <

0.0001, Figure 3b; RN23 - Pearson correlation: $r = -0.451$, $p < 0.0001$, Figure S2a; RN19 - Pearson correlation: $r = -0.500$, $p < 0.0001$, Figure S2b; RN6 - Pearson correlation: $r = -0.217$, $p < 0.0001$, Figure S2c). These analyses demonstrate that during activity peaks, clusters of inactive ants tend to dissipate, and that active ants are, on average, less likely to be near an inactive nestmate. The negative relationships between activity and the two metrics also do not depend on specific choices regarding the number of regions we used for calculating *MLD* nor the dimensions of the overlaid squares that we used to calculate local openness (supplementary figure S3). The weighted and peak *MLD* indices ($\bar{\boldsymbol{\phi}}$, and $\boldsymbol{\phi}^*$) were lower than ordinary index ($\boldsymbol{\phi}$) in all four colonies (Figure 3a, figure S1a-c). This was also true for the weighted and peak indices of the $\langle \boldsymbol{O_t} \rangle$ metric, which were lower than the ordinary index in all four colonies (Figure 3b, figure S2a-c). For these four colonies, the synchronized activation of workers therefore results in better spatial accessibility than random individual-level activity.

We designed a simple agent-based model to reinforce our empirical observations. This discrete-time model consisted of a square, continuous 2D space of size L x L (arbitrary length units) where agents (circular particles with a radius of 1 unit) could move around. Each agent could be in either one of two states at any one time: active or inactive. When inactive, an agent would remain motionless at its current coordinates. When active, agents would perform a correlated random walk through the simulation space; at every time step each agent would independently decide on the direction of its next movement by randomly selecting a heading ±45° of its current heading and then proceed to walk 1 unit of distance in the chosen direction. Agents were prohibited from spatially overlapping with other agents (i.e., they could not interpenetrate). If an active agent attempted to make an invalid movement during its random walk (this would either be a movement that would result in overlap with another agent or cause it to step outside the bounds of the simulation arena), it would randomly select a new heading and attempt to walk in that direction.

Each agent transitioned between being active and inactive by following an internal oscillator whose phase spanned 0-360°. The phase of each agent's oscillator would advance by one degree at each time-step of a simulation. All agents were always made to start in the inactive state at the beginning of a simulation. Whenever an agent's phase passed 350° it would enter the active state and remain active until its phase progressed by *A* degrees, at which point it would enter the inactive state. The value of *A* thus controlled the proportion of time that ants spent active versus inactive. We quantified the level of phase synchrony between agents using the Kuramoto order parameter *R* **[31]**.

$$R = \left| \frac{1}{N} \sum_{j=1}^{N} e^{i\vartheta_j} \right| \tag{3}$$

In eq. 3, *N* represents the number of oscillators (i.e., ants/agents), and $\boldsymbol{\vartheta_j}$ represents the phase of oscillator *j*, where $\boldsymbol{j} \in \{\mathbf{1} \ldots \boldsymbol{N}\}$. The order parameter ranges from 0 (no phase synchrony) to 1 (complete phase synchrony). To set a specific level of synchrony between the oscillators in the model, we randomly assigned phases to each oscillator at the start of a simulation until the resulting value of *R* was within 0.01 of the desired value. This particular modelling approach allowed us to study the effects of synchrony on spatial accessibility in general while being agnostic to the underlying mechanism the agents use to synchronize. Every simulation run of our model lasted 4000 time-steps.

We measured the spatial accessibility of simulated nests using the same metrics that we defined for the empirical data from real colonies. Our model parameters were chosen to approximate the behavior of real colonies. We simulated an arena with 120 agents, set the activity level of individuals to be $A = 100$, and set the synchrony of the agents to be $R = 0.5$. Density, which was defined as the number of agents divided by the area of the simulation arena $\boldsymbol{D_a = N/L^2}$, was set to $0.192 = 120/25^2$. This level of density is similar to that of real colonies from this study. Since the approximate body length of a *L. canadensis* worker is 3mm and the diameter of an agent in our model is 2 units, we can compare the densities in both cases by standardizing the units of measurement by the body length of individual ants/agents. The standardized density of the model is $120/(25/2)^2 = 0.768$ agents per body length squared, and the standardized density of a colony of 120 ants in one of our circular artificial nests (19mm radius) is $\boldsymbol{120/(\pi * \left(\frac{19}{3}\right)^2})$ = 0.952 ants per body length squared. To calculate the spatial accessibility metrics, we divided the simulation arena into 16 grid sectors to calculate *MLD* and overlaid circles with a radius of 3 units on each agent when calculating local openness. We ran 10 simulations when $R = 0.5$ and we also ran 10 simulations when $R = 0$ (see figure S4 for examples of activity time series from the model) to test if the weighted indices of spatial accessibility are indeed predictably lower for asynchronous agents versus synchronous agents when agent density and colony activity level fall within the same ranges that occur in real colonies.

The behavior of the model qualitatively matches that seen in real colonies. Both *MLD* and mean local openness are reduced at higher activity levels (Figure 3c,d). In our model simulations using synchronous agents ($R = 0.5$), the weighted index of the *MLD* metric was significantly lower than the ordinary index of *MLD* (Figure 3c inset; two sample t-test: $t_{16.479} = -12.28$, $p < 0.0001$). When agents are not synchronized ($R = 0$), the weighted index of *MLD* is significantly higher compared to the weighted index of *MLD* for synchronized agents (Figure 3c inset; two sample t-test: $t_{17.98} = 16.50$, $p < 0.0001$), but the weighted index of non-synchronized agents is not significantly different from the ordinary index of synchronized agents (Figure 3c inset; two sample t-test: $t_{16.75} = 1.70$, $p = 0.108$). For conditions that approximate those found in nests of actual colonies, activity synchronization thus results in consistently better spatial accessibility than asynchrony in our model. The ordinary index of *MLD* for synchronous agents is also thus a good predictor of what the weighted index of *MLD* would be for a synchronous colony if it was instead asynchronous but otherwise retained the same population density and average activity level.

Finally, we ran simulations where the three parameters of the model (individual activity level (A), level of synchronization (R), and the agent density (D_a)) were systematically varied to determine the effect of each parameter on the two metrics of spatial accessibility. We began by running simulations to determine the relationship between synchrony and the weighted index $\boldsymbol{\overline{\phi}}$ for both *MLD* and local openness. We used synchrony values that ranged from $R = 0$ to $R = 1$ in increments of 0.1 For each synchrony level, we also ran simulations with different agent densities. Increasing the synchronization of the agents' activity causes a decline in the weighted index of both *MLD* and local openness (Figure 4a,b). As one would expect, the relation between R and the weighted indices depends on the density of the agents in the simulation because both *MLD* and local openness are capable of being larger in denser simulation arenas. The difference between the weighted index values at $R = 0$ versus $R = 1$ is also larger when agent density is higher (Figure 4a,b).

To better understand how agent density and activity level mutually influence spatial accessibility, we ran simulations at six different $\boldsymbol{D_a}$ values and five different A values when synchrony was either maximized ($R = 1$) or minimized ($R = 0$). We then calculated the resulting difference ($\Delta\overline{\boldsymbol{\phi}}$) between weighted index values at $R = 0$ versus $R = 1$ to determine how much synchrony could improve the weighted indices of spatial accessibility at different combinations of A and $\boldsymbol{D_a}$. Higher agent densities increased the disparity between synchrony and asynchrony in the weighted index of *MLD* (Figure 4c) and in the weighted index of local openness (Figure 4d). Lower activity levels also increased this disparity (Figure 4c,d). We fit a linear function to each of the five curves associated with the different A values in our simulation output. The slope of the linear fit deceases as A increases for both the $\Delta\overline{\boldsymbol{\phi}}$ associated with *MLD* (Figure 4c inset) and the $\Delta\overline{\boldsymbol{\phi}}$ associated with local openness (Figure 4d inset). Synchronization thus provides the greatest improvement to spatial accessibility in situations where many agents are packed in a small space and agents do not spend much time active.

5.4 Discussion

Our results show that synchronizing movements of activity and rest can lead to better spatial accessibility inside ant nests when compared to asynchronous individuals with the same average level of activity. Inactive ants form piles that active ants have difficulty walking through. The synchronization of worker activity thereby makes it possible for ants to inspect a larger portion of the nest during the times that they are active. Our model simulations also point to the intuitive conclusion that synchrony provides the greatest benefit to spatial accessibility in dense nests and when ants do not spend much time active.

Synchronous active-rest oscillations may be present in other social situations that share some of the same characteristics as ant nest cavities, such as bacteria colonies, other social insects, or gregarious vertebrates restricted to living in small habitat patches. In such cases, synchrony might similarly improve spatial accessibility. Indeed, our results resemble the coordinated motions observed in huddles of emperor penguins; these birds stand in tight formations and intermittently become active together to allow individuals at the periphery of the huddle to access the crowd's warmer interior **[32]**. The simultaneous flashing of fireflies in mating swarms also appears to have a somewhat analogous benefit. Asynchronous flashing would cause the flash patterns of males to interfere with each other, reducing visibility for females seeking mates **[33]**. Instead of clearing visual "clutter" in the case of fireflies, synchrony inside *Leptothorax* nests reduces the spatial clutter caused by immobile ants.

The findings of this study may also have implications for robotics. Achieving synchronization in swarms of mobile robots is often desirable **[34,35]**. If superior spatial accessibility is also a design objective for a team of robots that operates in and repeatedly patrols a dedicated two-dimensional territory, then the groups' collective behavior could benefit from activity synchronization if the agents must conserve energy and need to work at high densities.

Several other hypotheses have previously been proposed for short-term activity cycles including faster task completion **[36]**, rapid information transfer **[37]**, and control over foraging rates **[38]**. These hypotheses are not mutually exclusive and all of them might end up being correct. Although our work is the first demonstration of a functional benefit from synchronized short-term activity rhythms, one limitation is that it is not yet evident whether improved spatial accessibility inside the nest has any fitness implications for colonies. Our results do add support to the notion that short-term activity cycles may be an adaptation for efficient brood tending, but this needs more research to be conclusively demonstrated. The logic is that because inactive ants congregate near larvae, poor accessibility of these regions could interfere with the ability of workers to inspect which larvae need tending. This, in turn, could lead to less frequent feeding and grooming, harming eclosion rates. Other lines of evidence point in this direction. For example, collective activity cycles are stronger near the brood pile **[16]**. The coherence of colony oscillations are also weaker when just pupae (which do not require tending) are present or when a colony has no brood at all **[39]**. Another form of rhythmic activity in ants (circadian rhythms) also disappears in the presence of pupae **[40]**. Queens can also trigger large activity waves **[9]** and this appears to clear a path for the queen and gives her improved access to larval secretions **[41]**.

Entomologists have documented substantial variation between ant species in colonies' preferred nest types **[42]** and in tempo (i.e., how quickly and often individual ants move) **[43,44]**. Our findings raise the hypothesis that more coherent colony rhythms are more likely to evolve in species that live in densely packed colonies and/or in species that are of a slower tempo. If every ant inside a nest always remained in motion, the issue of immobile ants blocking nest regions would not exist. However, nearly constant levels of activity may not be sustainable for many slower tempo species since motionless ants consume less energy than walking ants **[45,46]**. Because agent density and activity level exhibit nearly linear effects on the spatial accessibility indices, it is reasonable to expect that activity synchronization is not a binary social phenotype in ants. Instead, we might observe a range of synchrony levels that are correlated with the typical nestmate population density and tempo of species. This speculative hypothesis can be tested by observing the within-nest activity patterns of more species across different ant genera.

The synchronization of activity rhythms in group-living animals is a crucial element of the sociality of many species from birds to insects **[47,48]**. The results presented here enrich this rapidly growing literature. Fortunately, due to the ease with which individuals and entire colonies can be manipulated and observed, *Leptothorax* ants are a useful taxon for studying synchronization in social systems and collective motion in cramped spaces.

5.5 Acknowledgements

We thank Raul Costa-Pereira and Brendan McEwen for help with colony collection. We also thank Robin Stuart for advice on finding *Leptothorax* colonies in Quebec. Finally, we thank Jonathan N. Pruitt for providing funding and lab space.

5.6 Data availability

All time series used in this study, the code for our agent-based model simulations, and the code used for our statistical analyses are available in a single supplementary zip file.

5.7 Funding

This research was supported by the Canada 150 Research Chair of Jonathan N. Pruitt at McMaster University.

5.8 References

[1] S. Boccaletti, A. N. Pisarchik, C. I. del Genio, and A. Amann, *Synchronization: From Coupled Systems to Complex Networks* (Cambridge University Press, 2018).
[2] C. Chen, S. Liu, X. Shi, H. Chaté, and Y. Wu, *Weak Synchronization and Large-Scale Collective Oscillation in Dense Bacterial Suspensions*, Nature **542**, 210 (2017).
[3] J. Buck and E. Buck, *Mechanism of Rhythmic Synchronous Flashing of Fireflies: Fireflies of Southeast Asia May Use Anticipatory Time-Measuring in Synchronizing Their Flashing*, Science **159**, 1319 (1968).
[4] M. L. Hall, *Chapter 3 A Review of Vocal Duetting in Birds*, in *Advances in the Study of Behavior*, Vol. 40 (Academic Press, 2009), pp. 67–121.
[5] A. Chang, S. R. Livingstone, D. J. Bosnyak, and L. J. Trainor, *Body Sway Reflects Leadership in Joint Music Performance*, PNAS **114**, E4134 (2017).
[6] O. Feinerman, I. Pinkoviezky, A. Gelblum, E. Fonio, and N. S. Gov, *The Physics of Cooperative Transport in Groups of Ants*, Nature Phys **14**, 683 (2018).
[7] S. C. Pratt, *Quorum Sensing by Encounter Rates in the Ant* Temnothorax Albipennis, Behav. Ecol. **16**, 488 (2005).

[8] G. N. Doering and S. C. Pratt, *Symmetry Breaking and Pivotal Individuals during the Reunification of Ant Colonies*, Journal of Experimental Biology jeb.194019 (2019).

[9] G. N. Doering, K. A. Sheehy, J. L. L. Lichtenstein, B. Drawert, L. R. Petzold, and J. N. Pruitt, *Sources of Intraspecific Variation in the Collective Tempo and Synchrony of Ant Societies*, Behav Ecol **30**, 1682 (2019).

[10] M. Tennenbaum and A. Fernandez-Nieves, *Activity-Driven Changes in the Mechanical Properties of Fire Ant Aggregations*, Phys. Rev. E **96**, 052601 (2017).

[11] B. J. Cole, *Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction*, The American Naturalist **137**, 244 (1991).

[12] B. J. Cole and F. I. Trampus, *Activity Cycles in Ant Colonies: Worker Interactions and Decentralized Control*, in *Information Processing in Social Insects*, edited by C. Detrain, J. L. Deneubourg, and J. M. Pasteels (Birkhäuser Basel, Basel, 1999), pp. 289–307.

[13] C. Tofts, M. Hatcher, and N. R. Franks, *The Autosynchronization of the Ant* Leptothorax Acervorum *(Fabricius): Theory, Testability and Experiment*, Journal of Theoretical Biology **157**, 71 (1992).

[14] W. M. Wheeler, *A Revision of the North American Ants of the Genus* Leptothorax *Mayr*, Proceedings of the Academy of Natural Sciences of Philadelphia **55**, 215 (1903).

[15] T. O. Richardson, J. I. Liechti, N. Stroeymeyt, S. Bonhoeffer, and L. Keller, *Short-Term Activity Cycles Impede Information Transmission in Ant Colonies*, PLOS Computational Biology **13**, e1005527 (2017).

[16] S. Boi, I. D. Couzin, N. Del Buono, N. R. Franks, and N. F. Britton, *Coupled Oscillators and Activity Waves in Ant Colonies*, Proceedings: Biological Sciences **266**, 371 (1999).

[17] I. D. Couzin, *Synchronization: The Key to Effective Communication in Animal Collectives*, Trends in Cognitive Sciences **22**, 844 (2018).

[18] G. Gompper, R. G. Winkler, T. Speck, A. Solon, C. Nardini, F. Peruani, H. Löwen, R. Golestanian, U. B. Kaupp, L. Alvarez, T. Kiørboe, E. Lauga, W. C. K. Poon, A. DeSimone, S. Muiños-Landin, A. Fischer, N. A. Söker, F. Cichos, R. Kapral, P. Gaspard, M. Ripoll, F. Sagues, A. Doostmohammadi, J. M. Yeomans, I. S. Aranson, C. Bechinger, H. Stark, C. K. Hemelrijk, F. J. Nedelec, T. Sarkar, T. Aryaksama, M. Lacroix, G. Duclos, V. Yashunsky, P. Silberzan, M. Arroyo, and S. Kale, *The 2020 Motile Active Matter Roadmap*, J. Phys.: Condens. Matter **32**, 193001 (2020).

[19] A. Dussutour, V. Fourcassié, D. Helbing, and J.-L. Deneubourg, *Optimal Traffic Organization in Ants under Crowded Conditions*, Nature **428**, 70 (2004).

[20] N. Gravish, G. Gold, A. Zangwill, M. A. D. Goodisman, and D. I. Goldman, *Glass-like Dynamics in Confined and Congested Ant Traffic*, Soft Matter **11**, 6552 (2015).

[21] J. Aguilar, D. Monaenkova, V. Linevich, W. Savoie, B. Dutta, H.-S. Kuan, M. D. Betterton, M. a. D. Goodisman, and D. I. Goldman, *Collective Clog Control: Optimizing Traffic Flow in Confined Biological and Robophysical Excavation*, Science **361**, 672 (2018).

[22] O. Chepizhko, E. G. Altmann, and F. Peruani, *Optimal Noise Maximizes Collective Motion in Heterogeneous Media*, Phys. Rev. Lett. **110**, 238101 (2013).

[23] O. Chepizhko and T. Franosch, *Ideal Circle Microswimmers in Crowded Media*, Soft Matter **15**, 452 (2019).

[24] X. Yang, M. L. Manning, and M. C. Marchetti, *Aggregation and Segregation of Confined Active Particles*, Soft Matter **10**, 6477 (2014).

[25] C. Reichhardt and C. J. O. Reichhardt, *Controlled Fluidization, Mobility, and Clogging in Obstacle Arrays Using Periodic Perturbations*, Phys. Rev. Lett. **121**, 068001 (2018).

[26] S. Schär, G. Talavera, X. Espadaler, J. D. Rana, A. A. Andersen, S. P. Cover, and R. Vila, *Do Holarctic Ant Species Exist? Trans-Beringian Dispersal and Homoplasy in the Formicidae*, Journal of Biogeography **45**, 1917 (2018).

[27] A. Francoeur, *Deux Nouvelles Fourmis Néarctiques:* Leptothorax Retractus *et* L. Sphagnicolus *(Formicidae, Hymenoptera)*, Canadian Entomologist **118**, 1151 (1986).

[28] A. M. Ellison, N. J. Gotelli, E. J. Farnsworth, and G. D. Alpert, *A Field Guide to the Ants of New England* (Yale University Press, 2012).

[29] T. Sasaki, B. Colling, A. Sonnenschein, M. M. Boggess, and S. C. Pratt, *Flexibility of Collective Decision Making during House Hunting in* Temnothorax *Ants*, Behavioral Ecology and Sociobiology **69**, 707 (2015).

[30] G. Farnebäck, *Two-Frame Motion Estimation Based on Polynomial Expansion*, in *Image Analysis*, edited by J. Bigun and T. Gustavsson (Springer, Berlin, Heidelberg, 2003), pp. 363–370.

[31] Y. Kuramoto, *Self-Entrainment of a Population of Coupled Non-Linear Oscillators*, in *International Symposium on Mathematical Problems in Theoretical Physics* (Springer, 1975), pp. 420–422.

[32] D. P. Zitterbart, B. Wienecke, J. P. Butler, and B. Fabry, *Coordinated Movements Prevent Jamming in an Emperor Penguin Huddle*, PLOS ONE **6**, e20260 (2011).

[33] A. Moiseff and J. Copeland, *Firefly Synchrony: A Behavioral Strategy to Minimize Visual Clutter*, Science **329**, 181 (2010).

[34] F. Perez-Diaz, R. Zillmer, and R. Groß, *Control of Synchronization Regimes in Networks of Mobile Interacting Agents*, Phys. Rev. Applied **7**, 054002 (2017).

[35] M. Schranz, M. Umlauft, M. Sende, and W. Elmenreich, *Swarm Robotic Behaviors and Current Applications*, Frontiers in Robotics and AI **7**, 36 (2020).

[36] J. Delgado and R. V. Solé, *Self-Synchronization and Task Fulfilment in Ant Colonies*, Journal of Theoretical Biology **205**, 433 (2000).

[37] B. Hölldobler and E. O. Wilson, *The Ants* (Harvard University Press, 1990).

[38] E. Bonabeau, G. Theraulaz, and J.-L. Deneubourg, *The Synchronization of Recruitment-Based Activities in Ants*, Biosystems **45**, 195 (1998).

[39] B. J. Cole and L. Hoeg, *The Influence of Brood Type on Activity Cycles in* Leptothorax Allardycei *(Hymenoptera: Faruicidae)*, J Insect Behav **9**, 539 (1996).

[40] H. Fujioka, M. S. Abe, T. Fuchikawa, K. Tsuji, M. Shimada, and Y. Okada, *Ant Circadian Activity Associated with Brood Care Type*, Biology Letters **13**, 20160743 (2017).

[41] E. O. Wilson, *Aversive Behavior and Competition within Colonies of the Ant* Leptothorax Curvispinosus, Ann Entomol Soc Am **67**, 777 (1974).

[42] W. M. Wheeler, *Ants; Their Structure, Development and Behavior* (New York, Columbia university press, 1910).

[43] K. S. Mason, C. L. Kwapich, and W. R. Tschinkel, *Respiration, Worker Body Size, Tempo and Activity in Whole Colonies of Ants*, Physiological Entomology **40**, 149 (2015).

[44] G. F. Oster and E. O. Wilson, *Caste and Ecology in the Social Insects* (Princeton University Press, 1978).

[45] A. Lipp, H. Wolf, and F.-O. Lehmann, *Walking on Inclines: Energetics of Locomotion in the Ant* Camponotus, Journal of Experimental Biology **208**, 707 (2005).

[46] N. Ferral, K. Holloway, M. Li, Z. Yin, and C. Hou, *Heterogeneous Activity Causes a Nonlinear Increase in the Group Energy Use of Ant Workers Isolated from Queen and Brood*, Insect Science **25**, 487 (2018).

[47] S. C. Nicolis, J. Fernández, C. Pérez-Penichet, C. Noda, F. Tejera, O. Ramos, D. J. T. Sumpter, and E. Altshuler, *Foraging at the Edge of Chaos: Internal Clock versus External Forcing*, Phys. Rev. Lett. **110**, 268104 (2013).

[48] M. Bulla, M. Valcu, A. M. Dokter, A. G. Dondua, A. Kosztolányi, A. L. Rutten, B. Helm, B. K. Sandercock, B. Casler, B. J. Ens, C. S. Spiegel, C. J. Hassell, C. Küpper, C. Minton, D. Burgas, D. B. Lank, D. C. Payer, E. Y. Loktionov, E. Nol, E. Kwon, F. Smith, H. R. Gates, H. Vitnerová, H. Prüter, J. A. Johnson, J. J. H. St Clair, J.-F. Lamarre, J. Rausch, J. Reneerkens, J. R. Conklin, J. Burger, J. Liebezeit, J. Bêty, J. T. Coleman, J. Figuerola, J. C. E. W. Hooijmeijer, J. A. Alves, J. A. M. Smith, K. Weidinger, K. Koivula, K. Gosbell, K.-M. Exo, L. Niles, L. Koloski, L. McKinnon, L. Praus, M. Klaassen, M.-A. Giroux, M. Sládeček, M. L. Boldenow, M. I. Goldstein, M. Šálek, N. Senner, N. Rönkä, N. Lecomte, O. Gilg, O. Vincze, O. W. Johnson, P. A. Smith, P. F. Woodard, P. S. Tomkovich, P. F. Battley, R. Bentzen, R. B. Lanctot, R. Porter, S. T. Saalfeld, S. Freeman, S. C. Brown, S. Yezerinac, T. Székely, T. Montalvo, T. Piersma, V. Loverti, V.-M. Pakanen, W. Tijsen, and B. Kempenaers, *Unexpected Diversity in Socially Synchronized Rhythms of Shorebirds*, Nature **540**, 7631 (2016).

Figure 5.1

Time series of collective activity in the four *Leptothorax* colonies observed in this study. Colony RN6 was an *L. retractus* colony and the others were *L. canadensis* colonies. Colony activity refers to the approximate proportion of individuals moving inside each nest cavity. The panel of colony RN19 includes the 3-hour time series of the number of larval interactions that occurred with the 20 focal larvae every 30 seconds (transparent orange line). The plotted data on larval interactions was smoothed using a Gaussian-weighted moving average. The collective activity of colonies occurs in synchronized, rhythmic cycles. Peaks of collective activity are also correlated with peaks in larval interaction rate.

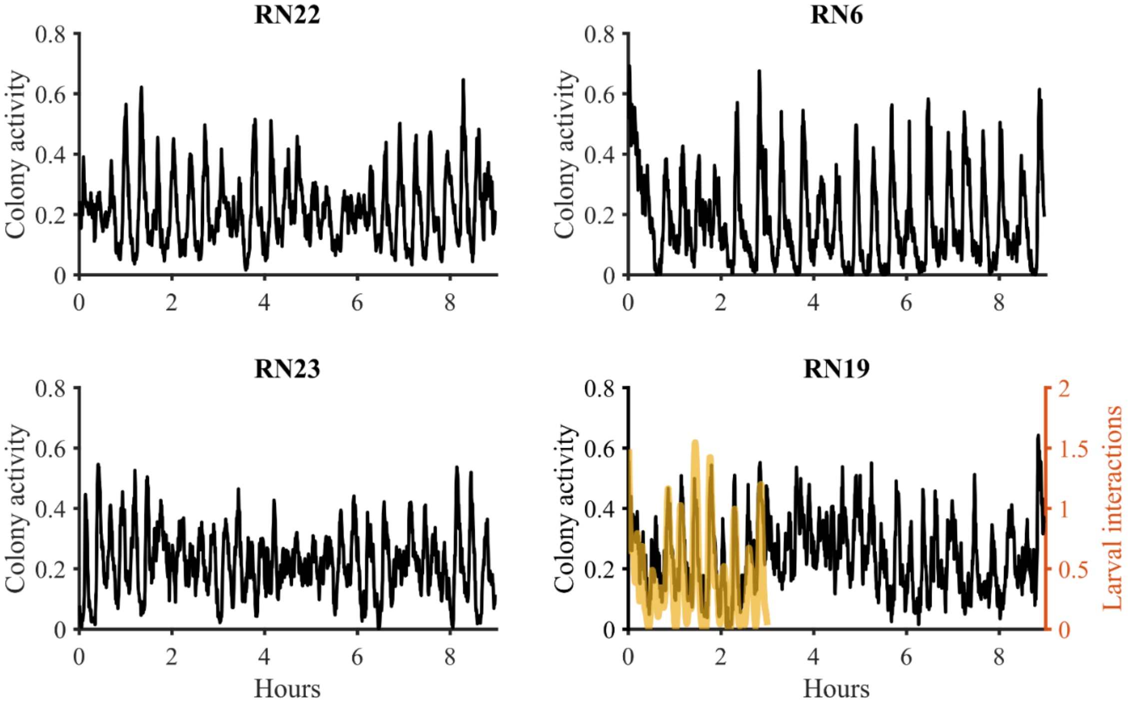


Figure 5.2.

The artificial nest of a *L. canadensis* colony (RN 23) observed in our study (a). The yellow oval-shaped objects with dark centers are larvae. The optical flow vectors associated with each pixel in this frame have been overlaid on the image. The length of each vector indicates the amount of motion occurring at specific pixels in the current fame compared to the prior frame, and the direction of each the vector indicates the direction of the motion. Relatively long blue vector lines can be seen over several ants in the image, indicating that these ants are active (i.e., moving). The boxplot (b) compares the summed optical flow magnitudes ($\boldsymbol{\sigma_{Px}}$) for pixels associated with the locations of inactive ants in the 2.8-minute video clip versus an equal number of randomly sampled pixels. Outlier points have been omitted from the boxplot (but were not omitted from the associated t-test) for visual clarity.

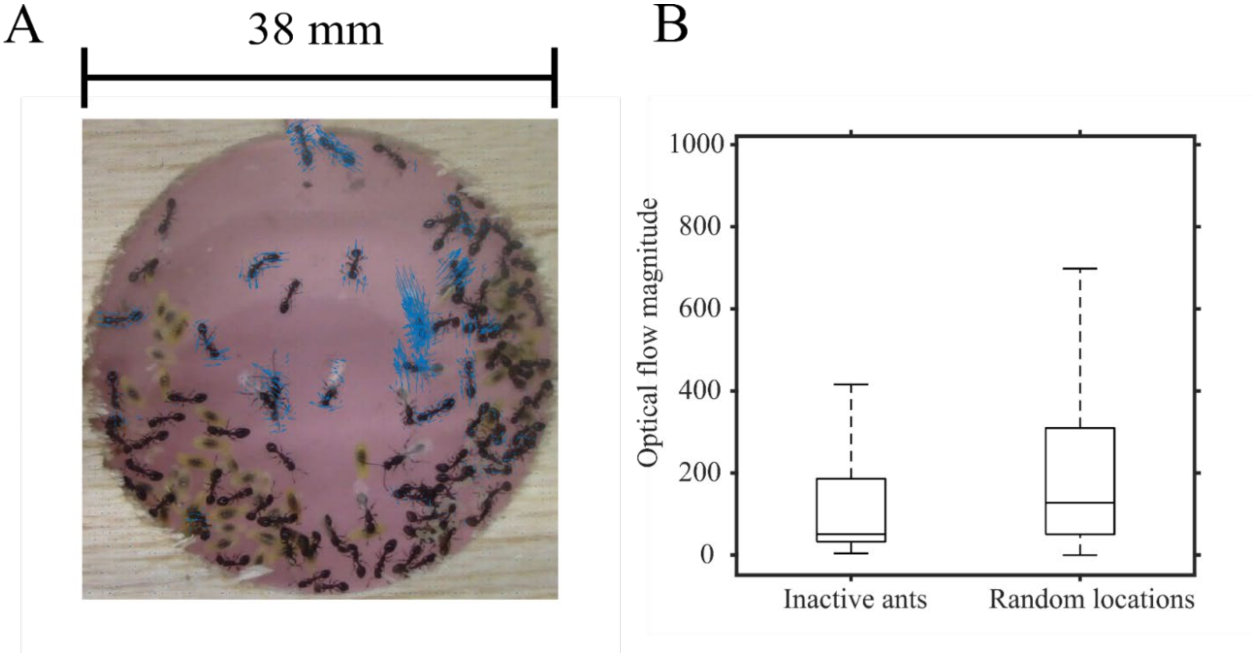


Figure 5.3.

The relationship between colony activity and the spatial accessibility metrics of max local density (a) and average local openness (b) for colony RN22. The relationship between collective activity and the spatial accessibility metrics of max local density (c) and average local openness (d) for a 4000 time-step simulation of our agent-based model where parameters were set to mimic real colonies ($A = 100$, $R = 0.5$, $D_a = 0.192$). The inset in panel (c) shows a boxplot of the weighted index of *MLD* (blue dots and blue boxes) and the ordinary index of *MLD* (red dots and red box) for the 10 simulations were $R = 0$ and the 10 simulations when $R = 0.5$. In all four panels, the red cross represents ordinary index $\boldsymbol{\phi}$, the blue dot represents the weighted index $\bar{\boldsymbol{\phi}}$, and the greed dot represents the peak index $\boldsymbol{\phi}^*$. The translucent gray dots depict the raw data points, and the solid black line in each panel is the least squares fit of the data.

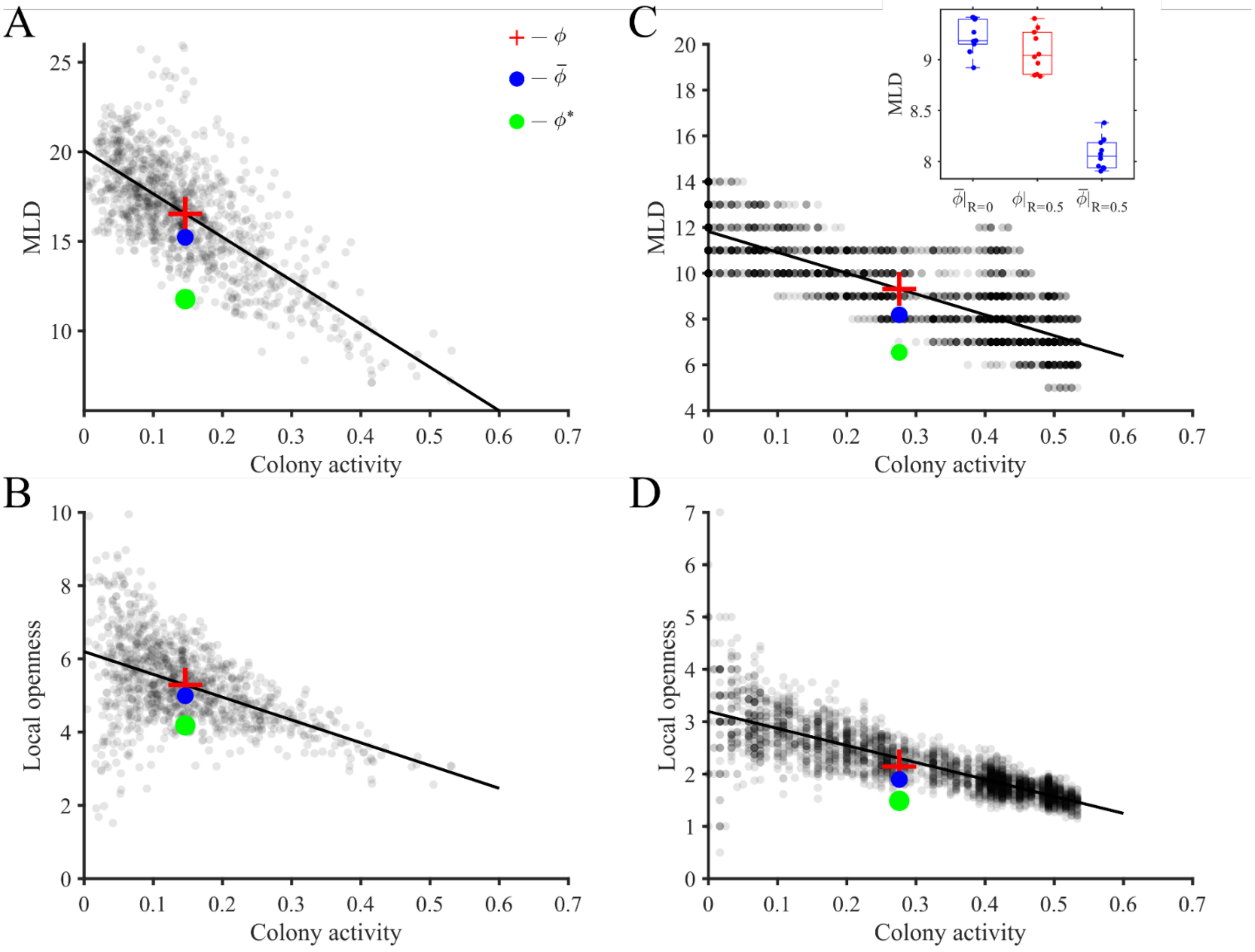
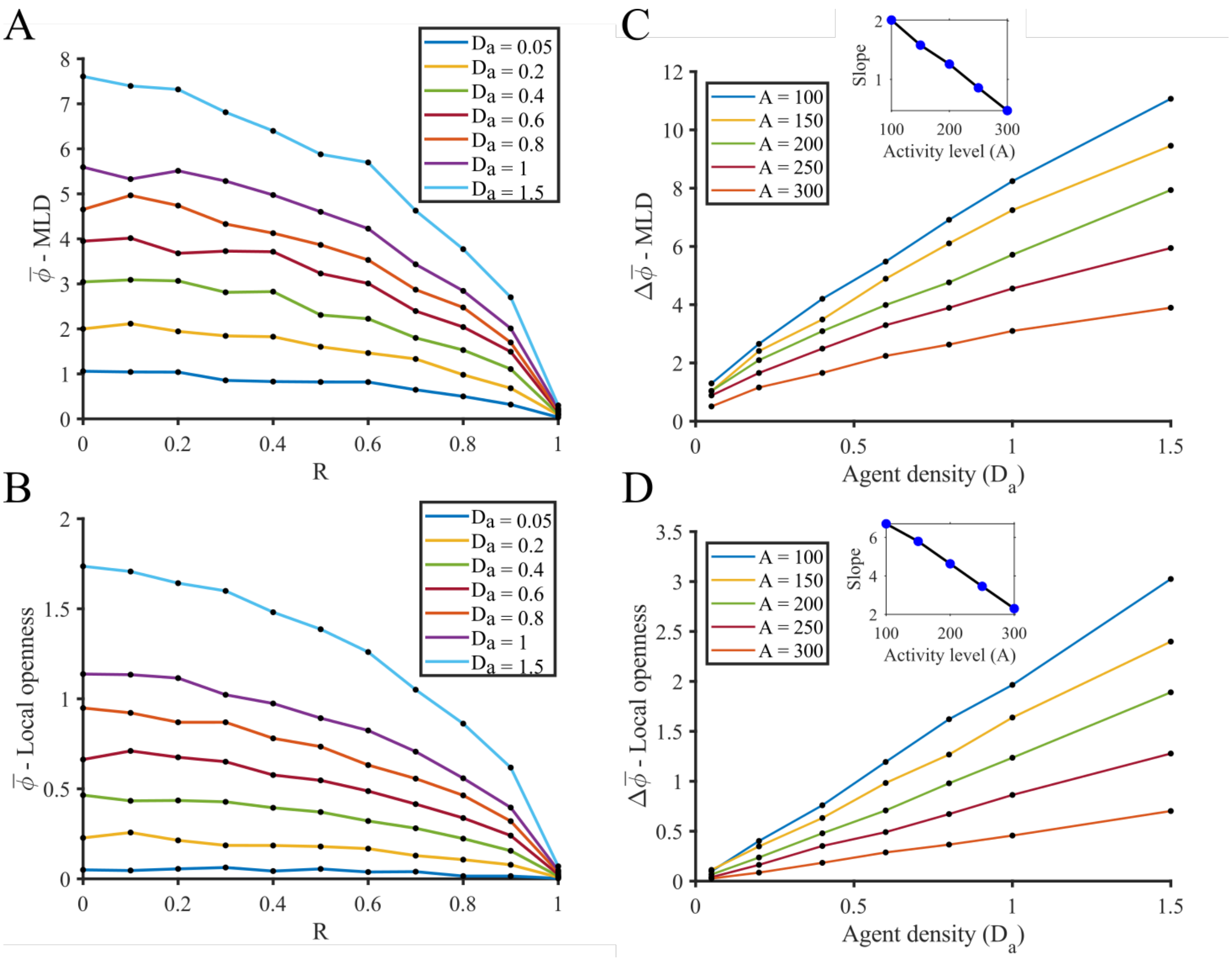


Figure 5.4.

The relationship between synchrony and the weighted index of *MLD* (a) and the weighted index of local openness (b). Each line corresponds to a different agent density, and each black dot represents the weighted index from a single simulation of 4000 time-steps. The activity parameter *A* was set to 216 for all simulations in panels (a) and (b). Panels (c) and (d) depict, respectively, the relationship between agent density and the difference of the weighted indices of either *MLD* (c) or local openness (d) when synchrony is maximized ($R = 1$) vs. when it is minimized ($R = 0$). Each line in these two panels corresponds to a different setting of the activity parameter *A*. The inset in these two panels depicts how, for both *MLD* and local openness, the slope of a least-squares fit of $\Delta\overline{\boldsymbol{\phi}}$ vs. agent density decreases when the activity parameter *A* is increased. To calculate the spatial accessibility metrics in these simulations, we divided the simulation arena into 36 grid sectors when computing *MLD* and overlaid circles with a radius of 10 units on each agent when computing local openness.



CHAPTER 6 – DISCUSSION

6.1 Overview

The research presented here documents novel aspects of the biology and mechanistic basis of synchronized short-term activity cycles in ant colonies. My work from chapter 2 found that different colonies in a single species can have consistent variation in the properties of their collective activity oscillations, and that this variation is correlated with the presence of a queen, the number of workers in a colony, and the amount of brood. In chapter 3, I demonstrated how there are robust colony-level differences between two species in the range of frequencies at which they tend to oscillate. I also show that colonies can modulate between distinct collective frequencies, that individual ants have imprecise refractory periods, and that a basic model of excitable agents can reproduce some aspects of colony behaviour. In chapter 4, I found that short-term activity cycles are not dependent on circadian rhythms of collective activity. The periodicity of short-term activity cycles does not change based on an external light cycle, but colonies show weaker 24-hour rhythms when kept in conditions with constant light or constant darkness. Finally, chapter 5 offers theoretical and empirical evidence for a functional advantage of synchronized active-rest rhythms: improved spatial accessibility within the nest for active workers. With these new results at hand, it is now possible to review what we currently know about short-term activity cycles in ants and sketch an outline of what future research on the subject might look like.

6.2 A brief history of short-term activity cycle research

Studies on rhythms in social insects have an extensive past. In the 19th and 20th centuries, research on ant rhythms were overwhelmingly focused on the daily and seasonal foraging patterns of colonies (Talbot 1946; Urbani 1965; Bernstein 1974; Bernstein 1979; Claborn and Phillips 1986). Many species will also have predictable seasonal rhythms of nest relocation or modification, moving farther underground to better tolerate colder months of the year (Kondoh 1968; Smallwood 1982; Herbers 1985; Herbers 1989; McGlynn 2012). Colonies of some species can undergo a cycle referred to as *seasonal polydomy* where colonies break apart and live concurrently in multiple nests in the summer but reunite at a single nest in the autumn (Partridge et al. 1997; Roberts et al. 1999; Buczkowski and Bennett 2008; McGlynn 2012). Older research from past centuries that did look at activity within nests was primarily oriented towards the topics of division of labour (Buckingham 1911; Weir 1958; Herbers 1983; Herbers and Cunningham 1983; Fresneau and Dupuy 1988; Pratt et al. 1994), assembling ethograms of behaviour (Wilson and Fagen 1974; Wilson 1975), and reproductive conflict (Franks and Scovell 1983; Hölldobler and Carlin 1985; Heinze 1990; Bourke 1991). These studies therefore did not seek to measure the level of synchronization among workers nor the periodicity of activity, partly due to the technical difficulties brought on by trying to collect high-resolution activity data spanning hours or days. However, observers of *Temnothorax* colonies in captivity have long noted that workers often spend much of their time inactive and motionless, with such moments of repose being interspersed with occasional sessions of locomotor activity (Herbers and Cunningham 1983; Cole 1986). As early as the 1870's, the Swiss myrmecologist Auguste Forel remarked how workers of *T. interruptus* (formerly *L. tuberoaffinis*) spent much less time moving within their nest compared to *Tetramorium caespitum* workers (Forel 1874; Wheeler 1903).

The earliest study that I can find on intranidal colony-level and individual-level activity patterns in ants that explicitly considered either *rhythmic* or *synchronous* behaviour was published by Thomas Cunliffe Barnes in 1941 (Barnes 1941). This study was an exploratory investigation into intra-colony variation in individual worker ants and how such variation affects colony-level activity. The paper reported data from four species: *Aphaenogaster fulva, Lasius flavus, Formica exsectoides,* and *Formica fusca.* The origin of the colonies is not provided in the article, but the *Lasius flavus* used is likely to actually have been what is now referred to as *Lasius brevicornis* (Schär et al. 2018). The number of active individuals (those exhibiting any locomotor activity) was manually recorded for approximately 1-1.5 hours for groups of workers from the same nest. Similar data was also obtained for isolated individuals. Barnes attempted to partially automate his data collection by casting shadows of the ants in a nest onto a sheet of photographic paper that was periodically changed by a machine. This method failed due to contemporary technical limitations; his apparatus would vibrate and disturb colonies. Although Barnes did not succeed in implementing his automated system, the idea to compare images of the inside of a colony's nest at different points in time formed the basis of the computer image analysis technique used by investigators decades later to study the same topic.

Another early study on collective activity rhythms inside ant nests was conducted by R. Chauvin in 1944 (Chauvin 1944). Chauvin was able to automatically collect time-series of locomotor activity in isolated individuals and small colonies (20 ants) of *Temnothorax corticalis* (formerly *Leptothorax tuberum* var. *corticalis*) by using a device he called a "microactographe". The microactographe was conceptually similar to Barnes' device, but Chuavin was able to operate it without causing disruptive vibrations. This study found that neither workers nor colonies showed any evidence of circadian (24-hour) rhythms when kept under constant conditions. The methodology described in the paper is unfortunately vague. Chauvin also did not conduct any rigorous analyses to test for the presence of ultradian rhythms (e.g., cycles shorter than 24-hours), so the paper thus could not evaluate the possibility of short-term activity cycles in *T.*

corticalis. Both Chauvin's and Barnes' papers were largely forgotten in the following decades. This may have been because Barnes published his work in a general psychology journal, and Chauvin's article appeared in an obscure French-only publication.

In the 1980's, two ant biologists (Blaine Cole and Nigel Franks) began independently revisiting the topic of cyclic within-nest activity in ants (Franks et al. 1990; Cole 1991a). Advances in computer image processing and time-series analysis from that era made it possible to rigorously demonstrate that the collective activity of both *Temnothorax allardycei* and *Leptothorax acervorum* followed a coherent, predictable rhythm of about 20-30 minutes. Cole and Franks took still images of colony nests in the laboratory every 30 seconds (in Cole's setup) or every minute (in Franks' setup), used thresholding to automatically segment ants from the image background, and calculated the amount of pixel changes between successive images. This is the same basic technique that I used in this thesis. Later in the 1990's, Melanie Hatcher and Chris Tofts (both colleagues of Nigel Franks) performed additional empirical and modelling work on short-term activity cycles using *L. acervorum* and *T. albipennis* (Hatcher 1992; Hatcher et al. 1992; Tofts et al. 1992). Franks, Cole, and their collaborators continued to publish on the subject throughout the 1990's (Cole and Cheshire 1996; Cole and Hoeg 1996; Boi et al. 1999; Cole and Trampus 1999). Several alternative theoretical models of short-term activity cycles accompanied this decade's surge of empirical research (Goss and Deneubourg 1988; Miramontes et al. 1993; Solé et al. 1993; Miramontes 1995; Delgado and Solé 2000; Sumpter et al. 2001). After 1999, there was a nadir in interest regarding the subject, and another paper on activity synchronization in ants would not appear until 2012 (Hayashi et al. 2012). This article examined the activity rhythms of isolated individuals along with the coupled activity of worker pairs of *Diacamma* ants but did not address colony-level activity cycles. Eventually, a paper was released a few years later that specifically dealt with the colony-level phenomenon of short-term activity cycles, this time returning to *Leptothorax* (Richardson et al. 2017).

6.3 Short-term activity cycle diversity

The basic sequence of events that occurs during a short-term activity cycle appears consistent among the several species who's within-nest activity patterns have been recorded. During moments of low activity, most ants inside the nest cavity will be almost entirely motionless, only making occasional, subtle movements with their antennae or legs. Once a handful of workers spontaneously begin moving, these active ants will walk into inactive nestmates, causing them to start moving as well. This process spreads the activity of moving ants to neighboring inactive individuals. The number of active ants will eventually reach a peak and will then begin to decline until most workers return to being immobile, at which point the cycle restarts. Although such superficial aspects of short-term activity cycles are somewhat stereotyped, the average properties of the activity oscillations (e.g., frequency, amplitude, level of synchrony, etc.) can vary substantially between and within species.

The studies of *L. acervorum* and *T. allardycei* in the 1990's noted that both species shared very similar collective oscillation periods of approximately 20-30 minutes. The observation of additional species in this thesis has now widened our view of what kinds of short-term activity cycles are possible in ants. Colonies of *Leptothorax* sp. W for example, typically oscillate with a period of around 20 min, thus closely resembling *L. acervorum* and *T. allardycei*. In contrast, I found that colonies of *T. rugatulus*, on average, have longer collective cycle periods; peaks of activity are separated by approximately 50 minutes (Chapter 2). Much longer dominant collective oscillations can be found in *Leptothorax crassipilis*, where the interval between activity peaks can span more than two hours (Chapter 3). *Leptothorax crassipilis* is also notable for the fact that colonies can exhibit fast oscillations (e.g., 30 min period) and slow oscillations (e.g., > 1 hour period). Colonies of both *L.* sp W and *L. crassipilis* can also be considered multirhythmic (Chapter 3), meaning they can express multiple periodicities (Yan and Goldbeter 2019). I have also observed sustained long period oscillations of 50 minutes or longer in *T. longispinosus*, *T. ambiguus*, *T. stenotyle*, *T. rudis*, *T. nitens, T. albipennis*, and *T. caguatan* (Chapter 1). The recently described arboreal species *T. quercicola* (Prebus 2021) is capable of noticeably faster oscillations (an approximately 11-minute period) than other species examined in this thesis (Chapter 1). For most of the species that I recorded (*T. stenotyle*, *T. rudis*, *T. nitens, T. albipennis*, *T. congruus*, *T. crassipinus*, *T. caguatan, T. obturator*, *T. emmae*, *T. tricarinatus*, *L. retractus*, *H. canadensis*, and *T. carinatus*), fewer than 10 colonies were recorded, so it is not yet possible to know the extent of intraspecific variation in collective frequencies in these species.

In addition to interspecific variation, my thesis contributes new information about what factors influence intraspecific variation in short-term activity cycles. Early work with *L. acervorum* and *T. allardycei* enumerated several important predictors of short-term activity cycle properties. An experiment with *L. acervorum* suggested that depriving colonies of food caused synchrony to deteriorate by increasing the average level of collective activity inside nests (Franks et al. 1990). However, additional experiments with the same species could not replicate these results and instead found that the only effect of starvation on colonies' cycles was a non-significant trend towards faster oscillations (Hatcher 1992). Observations of *T. rugatulus* also yielded contrary results; starved colonies seem to *decrease* their average level of activity (Rueppell and Kirkman 2005). However, this conflicting finding may have been due to methodological differences between the studies in question. The data from *T. rugatulus* was collected by manually identifying the occurrence and duration of collective activity bursts, but the data on *L. acervorum* was collected using automated image analysis. The number of brood items and their developmental stages have also been linked with short-term activity cycles. The strength of the rhythmicity of short-term activity cycles (as measured by Fourier analysis) becomes stronger when workers are with eggs and larvae compared to when they are with pupae alone or without brood altogether (Cole and Hoeg 1996). This result resembles a recent finding with workers of *Diacamma indicum*; isolated nurse workers and isolated nurse workers that were paired with pupae have strong circadian rhythms, but isolated nurses paired with either larvae or eggs exhibit round the clock activity. In both species, larvae modify worker locomotor behaviour differently than pupae, which do not require care from workers. My work with *T. rugatulus* found that colonies with more brood items (counting all developmental stages together) had less rhythmic and less synchronous short-term activity cycles, but slower oscillations (Chapter 2). The spatial arrangement of brood within nests is also relevant. The activity of workers near the brood plie are more synchronized and rhythmic than that of workers near the nest entrance (Boi et al. 1999).

Colony size is also important to short-term activity cycles. Rhythmic activity appears gradually in *T. allardycei*. Small groups of workers are arrhythmic and poorly synchronized, but full-sized colonies are synchronized and rhythmic (Cole 1991a; Cole and Hoeg 1996). In *T. rugatulus*, I found that colonies with large worker populations have more erratic collective oscillations (Chapter 2). The combination of these two results suggests the possibility that there may be an intermediate colony size that maximizes the rhythmicity and synchrony of short-term activity cycles. Although synchrony and rhythmicity are related to colony size, there does not appear to be any link between colony size and the average oscillation period of colonies. In *T. allardycei*, *L. crassipilis*, *T. rugatulus*, and *L.* sp. W, there is no association between the number of workers in a colony and the dominant frequency of a colony's short-term activity cycles (Chapter 2; Chapter 3; Cole 1991a).

My work with *T. rugatulus* highlights the influence that queens can exert on short-term activity cycles. Removing a colony's queen (compared to removing a random worker) results in less synchronized activity, but the underlying rhythm of the short-term activity cycle is not altered (Chapter 2). Queens also appear to begin moving before other ants in a colony, thereby being the initiator of many bursts of activity (Chapter 2). This observation aligns with an old paper, which reported that queens can instigate worker activity in *T. curvispinosus* (Wilson 1974).

Short-term activity cycles persist in conditions of constant temperature, constant darkness, or constant light (Chapter 4). Daily fluctuations in ambient lighting also do not appear to change the rhythmicity or average oscillation period of short-term activity cycles (Chapter 4). There are several other factors that are potentially important predictors of short-term activity cycle properties, but their effects are poorly understood. It is known for example that there is heterogeneity between workers in intrinsic activity levels (Chapter 3; Cole 1992). Worker heterogeneity is likely to be relevant to collective activity just as it is to other collective behaviours (Nishikawa and Motter 2016; O'Shea-Wheller et al. 2017; Jolles et al. 2020), but much more research is required to identify the extent of inter-individual behavioural differences and work out precisely how such variation affects collective activity cycles. Different chemical substances and changes in nest temperature are also factors that could plausibly influence short-term activity cycles but have yet to be studied in great detail (Barnes 1941; Hatcher 1992). A recent study identified circadian rhythms of within-nest collective activity in *L. acervorum*; colonies maintained their short-term activity cycles throughout the day, but their mean activity level declined at night and rose during the day (Richardson et al. 2017). The typical oscillation period of colonies also slowed down at night versus during the day. This study had light levels and temperatures that fluctuated based on the time of day. One possible interpretation of the data from my thesis (Chapter 3) and the aforementioned study may be that cooler temperatures outside of the nest can cause colonies to slow down their short-term activity cycle tempo.

Lastly, my work with *T. rugatulus* indicates that colonies are repeatable in the properties of their short-term activity cycles but may also drift over time (Chapter 2). Although much of this intraspecific variation is explained by the factors that I have mentioned here, they do not yet fully account for the differences between colonies of the same species. More research on this topic is thus necessary. To conclude, I have summarized the factors that are known to be associated with the properties of short-term activity cycles in Table 6.1.

6.4 Mechanisms of synchronization

To my knowledge, there are currently six species were some data on short-term activity patterns at both the colony-level and individual-level exist: *Temnothorax allardycei* (Cole 1991a; Cole 1991b; Cole and Cheshire 1996), *T. rugatulus* (Chapter 2), *Leptothorax crassipilis* (Chapter 3), *L. acervorum* (Hatcher 1992; Richardson et al. 2017), *Diacamma indicum* (Hayashi et al. 2012; Fujioka et al. 2019; Fujioka et al. 2021), and *L.* sp W (Chapter 3). The data from these species provides an updated picture of how individual ants synchronize and bring about short-term activity cycles.

Physical contact between workers is a fundamental part of how synchronized short-term activity cycles emerge in ants. The persistence of cycles in unchanging temperature and unchanging lighting environments and the lack of frequency modifications based on an external light/dark regime negates the idea that specific photoperiods or circadian rhythms are responsible for generating short-term activity cycles. Additionally, experiments with *T. allardycei* found that partitioning nests into two sectors separated by a screen permeable to air but impermeable by workers causes the two halves of the colony to oscillate asynchronously (Cole and Trampus 1999). This evidence discounts the idea that any sort of diffuse pheromone is key to regulating cycles. Individual ants have refractive-like states where they are less likely to be stimulated by a nestmate and subsequently begin moving. This means that the longer an individual ant has been inactive, the more likely it is that physical contact from an active individual will cause the inactive ant to begin moving (Chapter 3). The length of time that an ant will ignore stimulations from nestmates is not fixed, and there is some randomness in whether or not an ant will be triggered into activity by another ant. (Chapter 3). Individual ants have bursts of activity even when they are isolated and kept apart from the rest of their colony. The bursts of activity in isolated individuals are often erratic and non-rhythmic (Chapters 2 and 3, Cole 1991c). However, this is not universally true. As I show in the third chapter of this thesis, isolated *Leptothorax crassipilis* individuals have more predictable bursts of activity than isolated *L.* sp W individuals. In the case of *Diacamma indicum*, isolated workers have a strong periodic component to their activity rhythms (Hayashi et al. 2012), yet full colonies do not appear to have short-term activity cycles (Fujioka et al. 2021). Moreover, I have found highly rhythmic oscillations in *both* isolated individuals and full-size colonies of the ant *Myrmica punctiventris* (unpublished data; Figure 6.1). The synchronization of individual activity rhythms in a colony therefore appears to be at least partially explained by the interactions between individuals who have erratic, quasi-periodic, or periodic spontaneous intrinsic activity and noisy refractive states. Essentially, once a worker becomes inactive, she will either spontaneously become active or be triggered into becoming active through contact with an active nestmate. Noisy refractory states prevent ants from repeatedly restimulating each other, which would lead to constant levels of high activity. The combination of these features is reminiscent of an excitable medium like cardiac tissue, where periodic and synchronized activity can also appear (Bub et al. 2005).

Several different approaches have been used to model the phenomenon of short-term activity cycles mathematically. One such class of models is inspired by compartmental models from epidemiology, where ants shift between being active, inactive, and refractive based on a system of differential equations (Goss and Deneubourg 1988; Hemerik et al. 1990; Cole 1992; Tennenbaum and Fernandez-Nieves 2017). Other models of the phenomenon use either agent-based models (Solé et al. 1993) or process algebra (Tofts 1990; Sumpter et al. 2001). These modelling efforts have confirmed that synchronized and rhythmic collective activity can emerge in a population of individuals that are themselves aperiodic in their intrinsic activity patterns. My own model simulations show that synchrony and rhythmicity are not contingent on an absolute refractive period, and that it is possible for multirhythmicity to occur in simulated colonies (Chapter 3). However, none of the current models can currently capture some important features of short-term activity cycles. An outstanding issue is the origin of oscillations with long periods greater than 50 minutes like those seen in *L. crassipilis* and *T. rudis* for example. Models that assume erratic individual-level activity struggle with producing coherent longer rhythms. Some older agent-based models (referred to in their respective publications as mobile cellular automata models) can produce simulations with arbitrarily long periods, but these oscillations are substantially different from those seen in real colonies. More data on how individual ants interact could potentially help answer this riddle.

Another element to consider in a mechanistic account of how synchronization is accomplished in colonies is the role of specific individuals like the queen or unusually active workers. Even though colonies become less synchronized after the loss of their queen, queenless colonies still exhibit detectable short-term activity cycles (Chapter 2). A speculative possibility is that some ordinary workers in a colony may likewise play outsize roles in contributing to synchrony. Thomas Barnes spoke about the existence of what he referred to as "catalyst" ants that he believed were crucial to sustaining colony-level activity (Barnes 1941). In many ants, including *Temnothorax* and *Leptothorax,* a minority of workers (sometimes referred to as "keystone" individuals (Modlmeier et al. 2014)) often contribute a majority of the labour in various tasks like nest emigration for instance (Dornhaus et al. 2008; Richardson et al. 2018; Doering and Pratt 2019). As I mentioned in the previous section, worker heterogeneity in intrinsic activity patterns or refractoriness should indeed be more closely evaluated in the future. Data on *T. allardycei* suggests that older workers may spend less time active than younger workers, which could have consequences for the synchronizability of activity at the colony level (Cole 1992). The mechanism that causes queens to improve colony synchrony is unknown. However, it is conceivable that identifying this mechanism along with cataloging the effects of heterogeneity among workers may help resolve the unexplained aspects of short-term activity cycle generation. Additional research into individual-level ant behaviour during activity cycles would thus likely be worthwhile.

6.5 Biological significance of short-term activity cycles

Early work on activity cycles debated whether the phenomenon has any adaptive significance to colonies or whether it is merely an epiphenomenon that has a neutral or even detrimental effect on colony fitness. This question can be further broken down to treat the importance of the rhythmicity and synchrony of within-nest collective activity separately. Namely, it may be asked whether the *rhythms* of short-term activity cycles are beneficial to colonies and/or if the *synchrony* associated with short-term activity cycles is beneficial to colonies. The fact that short-term activity cycles are rhythmic and the fact that colonies are synchronized could both have their own set of benefits and drawbacks. Alternatively, one or both of these properties may be an epiphenomenon, that arose as a consequence of selection on some other phenotype (Cole 1991a; Hatcher 1992; Cole and Trampus 1999). Besides the idea that both the rhythms and synchrony of ant collective activity are epiphenomena, several different (but not necessarily mutually exclusive) hypotheses have been proposed over the years to explain the evolution of short-term activity cycles (Cole and Trampus 1999). I have compiled the major contenders in Table 6.2. I am only aware of empirical tests for two of these hypotheses. Future experiments may well uncover support for some of the other proposed hypotheses.

Novel results from this thesis along with more recent work on *L. acervorum* have now made it clear that there are indeed some functional implications of both *rhythmic* and *synchronized* activity, and at least one of the consequences of *synchrony* ostensibly provides a benefit to colonies (Chapter 5). In the case of synchrony, the average spatial accessibility inside the nest is improved. More specifically, inactive ants aggregate into piles that active ants have difficulty penetrating. Synchronizing when workers are active thus allows ants to inspect different portions of the nest interior more fully when they are active. This finding is concordant with observations made in *T. curvispinosus* that reported that active workers move out of the way of the queen to make it easier for her to engage in larval trophallaxis (Wilson 1974). In the case of collective rhythm, it was shown that predictable bursts of locomotor activity slows the spread of information inside *L. acervorum* nests (Richardson et al. 2017). It is not clear if arresting the flow of information would actually be beneficial in any way for colonies. The authors of that study also acknowledge that it is uncertain if any meaningful information is typically carried during short-term activity cycles. Nevertheless, the study highlights another possible functional by-product of short-term activity cycles in colonies.

It remains to be seen if either of the demonstrated functional implications lead to any *fitness* advantages for colonies. The logical chain connecting most of the discussed hypotheses to fitness advantages are intuitive. For example, if synchronized foraging yields more food for the colony than asynchronous worker foraging, larvae might receive better nutrition and thus enjoy higher rates of successful eclosion. However, no experiment has definitively shown any link between short-term activity cycles and increased survival or reproductive output in colonies. It should be noted that this situation is not unique to short-term activity cycles. The fitness implications of several collective behaviours in ants living in natural conditions have yet to be thoroughly studied. For example, because studies of nest relocation in the wild are rare, we do not yet have a full understanding of how the ability to reach a consensus on a single nest site during an emigration might improve a colony's fitness (Kaur et al. 2012). Other collective rhythms in ants like circadian cycles and ultradian foraging also lack definitive demonstrations of being adaptive traits. Ultradian rhythms (less than a 24-hour period) such as short-term activity cycles are also present in non-eusocial organisms like fruit flies, monkeys, and birds (Delgado-Garcia et al. 1976; Lumineau et al. 2001; Seki and Tanimura 2014). The purpose and evolution of these rhythms are somewhat enigmatic in these taxa as well (Lloyd and Stupfel 1991). Even if there are no direct fitness benefits to colony survival or reproduction, organizing activity into periodic bursts has implications for other dimensions of a colony's collective behaviour because physical interactions between workers (e.g., trophallaxis) and brood tending all require movement and are correlated with the bursts of colony locomotor activity (Chapter 5; Cole 1991a; Richardson et al. 2017). Like the visually impressive simultaneous flashing of firefly swarms (Hanson et al. 1971), the short-term activity cycles of ants will remain a striking example of synchronization in organismal biology regardless of their adaptive value.

The realization that different species can have large differences in the typical frequency of their short-term activity cycles raises more questions about the functional implications of having *rhythmic* collective activity. Why should some species oscillate more slowly than others? Perhaps species that oscillate more slowly have slower metabolic rates than higher tempo species. Existing data on colony energetics have shown that the proportion of workers active at any one time is correlated with energy expenditure (Mason et al. 2015; Ferral et al. 2018). Species with consistently longer period short-term activity cycles are likely to have lower average colony-level activity, which may implicate selective pressures to conserve energy. This speculative hypothesis should be tested in future work. Whether different oscillation frequencies are indeed adaptations remains to be seen.

6.6 Conclusion

Collective activity and collective motion are important aspects of social behaviour in animals. The sophistication of collective behaviour is magnified in social insects like ants, where colonies must skillfully coordinate their actions in order to gather food (Feinerman et al. 2018), build and repair nests (Franks et al. 1992), and reproduce (Staab and Kleineidam 2014). This thesis has uncovered new details on the natural history, mechanisms, and function of synchronization in ants. Studying ants and their collective activity, rhythms, and various forms of synchronization, will continue to deliver new breakthroughs about the biology of these ecologically important animals.

6.7 References

Barnes TC. 1941. Rhythms of activity in ant colonies. The Journal of General Psychology. 25:249–255.

Bernstein RA. 1974. Seasonal Food Abundance and Foraging Activity in Some Desert Ants. The American Naturalist. 108:490–498.

Bernstein RA. 1979. Schedules of Foraging Activity in Species of Ants. Journal of Animal Ecology. 48:921–930.

Boi S, Couzin ID, Del Buono N, Franks NR, Britton NF. 1999. Coupled Oscillators and Activity Waves in Ant Colonies. Proceedings: Biological Sciences. 266:371–378.

Bonabeau E, Theraulaz G, Deneubourg J-L. 1998. The synchronization of recruitment-based activities in ants. Biosystems. 45:195–211.

Bourke AFG. 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. Animal Behaviour. 42:295–310.

Bub G, Shrier A, Glass L. 2005. Global Organization of Dynamics in Oscillatory Heterogeneous Excitable Media. Phys Rev Lett. 94:028105.

Buckingham EN. 1911. Division of Labor among Ants. Proceedings of the American Academy of Arts and Sciences. 46:425–508.

Buczkowski G, Bennett G. 2008. Seasonal polydomy in a polygynous supercolony of the odorous house ant, Tapinoma sessile. Ecological Entomology. 33:780–788.

Chauvin R. 1944. L’effet de groupe et la régulation sociale chez les fourmis du genre *Leptothorax* étudiés au moyen du microactographe optique-I. Bull Biol Fr Belg. 78:1–9.

Claborn DM, Phillips SA. 1986. Temporal Foraging Activities of *Solenopsis invicta* (Hymenoptera: Formicidae) and Other Predominant Ants of Central Texas. The Southwestern Naturalist. 31:555–557.

Cole BJ. 1986. The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. Behav Ecol Sociobiol. 18:165–173.

Cole BJ. 1991a. Short-Term Activity Cycles in Ants: Generation of Periodicity by Worker Interaction. The American Naturalist. 137:244–259.

Cole BJ. 1991b. Short-term activity cycles in ants: A phase-response curve and phase resetting in worker activity. J Insect Behav. 4:129–137.

Cole BJ. 1991c. Is Animal Behaviour Chaotic? Evidence from the Activity of Ants. Proceedings: Biological Sciences. 244:253–259.

Cole BJ. 1992. Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. Behav Ecol Sociobiol. 31:181–187.

Cole BJ, Cheshire D. 1996. Mobile Cellular Automata Models of Ant Behavior: Movement Activity of *Leptothorax allardycei*. The American Naturalist. 148:1–15.

Cole BJ, Hoeg L. 1996. The influence of brood type on activity cycles in *Leptothorax allardycei* (Hymenoptera: Faruicidae). J Insect Behav. 9:539–547.

Cole BJ, Trampus FI. 1999. Activity cycles in ant colonies: worker interactions and decentralized control. In: Detrain C, Deneubourg JL, Pasteels JM, editors. Information Processing in Social Insects. Basel: Birkhäuser Basel. p. 289–307. [accessed 2018 Dec 24]. https://doi.org/10.1007/978-3-0348-8739-7_16.

Delgado J, Solé RV. 2000. Self-synchronization and Task Fulfilment in Ant Colonies. Journal of Theoretical Biology. 205:433–441.

Delgado-Garcia JM, Grau C, DeFeudis P, Del Pozo F, Jimenez JM, Delgado JMR. 1976. Ultradian rhythms in the mobility and behavior of rhesus monkeys. Experimental brain research. 25:79–91.

Doering GN, Pratt SC. 2019. Symmetry breaking and pivotal individuals during the reunification of ant colonies. Journal of Experimental Biology. 222:jeb194019.

Dornhaus A, Holley J-A, Pook VG, Worswick G, Franks NR. 2008. Why do not all workers work? Colony size and workload during emigrations in the ant *Temnothorax albipennis*. Behav Ecol Sociobiol. 63:43–51.

Feinerman O, Pinkoviezky I, Gelblum A, Fonio E, Gov NS. 2018. The physics of cooperative transport in groups of ants. Nature Phys. 14:683–693.

Ferral N, Holloway K, Li M, Yin Z, Hou C. 2018. Heterogeneous activity causes a nonlinear increase in the group energy use of ant workers isolated from queen and brood. Insect Science. 25:487–498.

Forel A. 1874. Les fourmis de la Suisse: systématique, notices anatomiques et physiologiques, architecture, distribution géographique, nouvelles expériences et observations de moeurs. Société Helvétique des Sciences Naturelles.

Franks NR, Bryant S, Griffiths R, Hemerik L. 1990. Synchronization of the behaviour within nests of the ant *Leptothorax acervorum* (Fabricius)—I. Discovering the phenomenon and its relation to the level of starvation. Bltn Mathcal Biology. 52:597–612.

Franks NR, Scovell E. 1983. Dominance and reproductive success among slave-making worker ants. Nature. 304:724–725.

Franks NR, Wilby A, Silverman BW, Tofts C. 1992. Self-organizing nest construction in ants: sophisticated building by blind bulldozing. Animal Behaviour. 44:357–375.

Fresneau D, Dupuy P. 1988. A study of polyethism in a ponerine ant: *Neoponera apicalis* (Hymenoptera, formicidae). Animal Behaviour. 36:1389–1399.

Fujioka H, Abe MS, Okada Y. 2019. Ant activity-rest rhythms vary with age and interaction frequencies of workers. Behav Ecol Sociobiol. 73:30.

Fujioka H, Abe MS, Okada Y. 2021. Individual Ants Do Not Show Activity-Rest Rhythms in Nest Conditions. Journal of Biological Rhythms. 36:297–310.

Goss S, Deneubourg JL. 1988. Autocatalysis as a source of synchronised rhythmical activity in social insects. Ins Soc. 35:310–315.

Hanson FE, Case JF, Buck E, Buck J. 1971. Synchrony and Flash Entrainment in a New Guinea Firefly. Science. 174:161–164.

Hatcher MJ. 1992. Activity patterns and organization within ant nests [PhD Thesis]. University of Bath.

Hatcher MJ, Tofts C, Franks NR. 1992. Mutual exclusion as a mechanism for information exchange within ant nests. Naturwissenschaften. 79:32–34.

Hayashi Y, Yuki M, Sugawara K, Kikuchi T, Tsuji K. 2012. Rhythmic behavior of social insects from single to multibody. Robotics and Autonomous Systems. 60:714–721.

Heinze J. 1990. Dominance behavior among ant females. Naturwissenschaften. 77:41–43.

Hemerik L, Britton NF, Franks NR. 1990. Synchronization of the behaviour within nests of the ant *Leptothorax acervorum* (Fabricius)—II. Modelling the phenomenon and predictions from the model. Bltn Mathcal Biology. 52:613–628.

Herbers JM. 1983. Social Organization in *Leptothorax* Ants: Within-And Between-Species Patterns. Psyche. 90:361–386.

Herbers JM. 1985. Seasonal structuring of a north temperature ant community. Ins Soc. 32:224–240.

Herbers JM. 1989. Community Structure in North Temperate Ants: Temporal and Spatial Variation. Oecologia. 81:201–211.

Herbers JM, Cunningham M. 1983. Social organization in *Leptothorax longispinosus* Mayr. Animal Behaviour. 31:759–771.

Hölldobler B, Carlin NF. 1985. Colony founding, queen dominance and oligogyny in the Australian meat ant *Iridomyrmex purpureus*. Behav Ecol Sociobiol. 18:45–58.

Hölldobler B, Wilson EO. 1990. The Ants. Harvard University Press.

Jolles JW, King AJ, Killen SS. 2020. The Role of Individual Heterogeneity in Collective Animal Behaviour. Trends in Ecology & Evolution. 35:278–291.

Kaur R, Anoop K, Sumana A. 2012. Leaders follow leaders to reunite the colony: relocation dynamics of an Indian queenless ant in its natural habitat. Anim Behav. 83:1345–1353.

Kondoh M. 1968. Bioeconomic studies on the colony of an ant species, *Formica japonica*. I. Nest structure and seasonal changes of the colony members. Japanese Journal of Ecology. 18:124–133.

Lloyd D, Stupfel M. 1991. The Occurrence and Functions of Ultradian Rhythms. Biological Reviews. 66:275–299.

Lumineau S, Guyomarc'h C, Richard J-P. 2001. Ultradian Rhythm of Activity in Japanese Quail Groups under Semi-Natural Conditions during Ontogeny: Functional Aspects and Relation to Circadian Rhythm. Biological Rhythm Research. 32:373–400.

Mason KS, Kwapich CL, Tschinkel WR. 2015. Respiration, worker body size, tempo and activity in whole colonies of ants. Physiological Entomology. 40:149–165.

McGlynn TP. 2012. The ecology of nest movement in social insects. Annual review of entomology. 57:291–308.

Miramontes O. 1995. Order-disorder transitions in the behavior of ant societies. Complexity. 1:56–60.

Miramontes O, Solé RV, Goodwin BC. 1993. Collective behaviour of random-activated mobile cellular automata. Physica D: Nonlinear Phenomena. 63:145–160.

Modlmeier AP, Keiser CN, Watters JV, Sih A, Pruitt JN. 2014. The keystone individual concept: an ecological and evolutionary overview. Animal Behaviour. 89:53–62.

Nishikawa T, Motter AE. 2016. Symmetric States Requiring System Asymmetry. Phys Rev Lett. 117:114101.

O'Shea-Wheller TA, Masuda N, Sendova-Franks AB, Franks NR. 2017. Variability in individual assessment behaviour and its implications for collective decision-making. Proc Biol Sci. 284.

Partridge LW, Partridge KA, Franks NR. 1997. Field survey of a monogynous leptothoracine ant (Hymenoptera, Formicidae): Evidence of seasonal polydomy? Insect Soc. 44:75–83.

Pratt SC, Carlin NF, Calabi P. 1994. Division of labor in *Ponera pennsylvannica* (Formicidae: Ponerinae). Insectes sociaux. 41:43–61.

Prebus MM. 2021. Taxonomic revision of the *Temnothorax salvini* clade (Hymenoptera: Formicidae), with a key to the clades of New World Temnothorax. PeerJ. 9:e11514.

Richardson TO, Liechti JI, Stroeymeyt N, Bonhoeffer S, Keller L. 2017. Short-term activity cycles impede information transmission in ant colonies. PLOS Computational Biology. 13:e1005527.

Richardson TO, Mullon C, Marshall JAR, Franks NR, Schlegel T. 2018. The influence of the few: a stable 'oligarchy' controls information flow in house-hunting ants. Proc R Soc B. 285:20172726.

Roberts ER, Daniels S, Wardlaw JC, Raybould AF, Pearson JE, Pearson B. 1999. Seasonal polydomy: Its possible causes and its consequences for kinship and intra-specific parasitism in *Leptothorax tuberum* (Hymenoptera : Formicidae). Sociobiology. 33:199–214.

Rueppell O, Kirkman RW. 2005. Extraordinary starvation resistance in *Temnothorax rugatulus* (Hymenoptera, Formicidae) colonies: Demography and adaptive behavior. Insectes Soc. 52:282–290.

Schär S, Talavera G, Espadaler X, Rana JD, Andersen AA, Cover SP, Vila R. 2018. Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. Journal of Biogeography. 45:1917–1928.

Seki Y, Tanimura T. 2014. Ultradian rhythm unmasked in the Pdf clock mutant of *Drosophila*. J Biosci. 39:585–594.

Smallwood J. 1982. Nest relocations in ants. Ins Soc. 29:138–147.

Solé RV, Miramontes O, Goodwin BC. 1993. Oscillations and Chaos in Ant Societies. Journal of Theoretical Biology. 161:343–357.

Staab M, Kleineidam CJ. 2014. Initiation of swarming behavior and synchronization of mating flights in the leaf-cutting ant *Atta vollenweideri* FOREL, 1893 (Hymenoptera: Formicidae). Myrmecol News. 19:93–102.

Sumpter DJ, Blanchard GB, Broomhead DS. 2001. Ants and agents: a process algebra approach to modelling ant colony behaviour. Bulletin of Mathematical Biology. 63:951–980.

Talbot M. 1946. Daily Fluctuations in Aboveground Activity of Three Species of Ants. Ecology. 27:65–70.

Tennenbaum M, Fernandez-Nieves A. 2017. Activity-driven changes in the mechanical properties of fire ant aggregations. Phys Rev E. 96:052601.

Tofts C. 1990. The Autosynchronisation of *Leptothorax acervorum* (*Fabricius*) Described in WSCCS. University of Edinburgh, Department of Computer Science

Tofts C, Hatcher M, Franks NR. 1992. The autosynchronization of the ant *Leptothorax acervorum* (Fabricius): Theory, testability and experiment. Journal of Theoretical Biology. 157:71–82.

Urbani CB. 1965. Sull'attivita' di foraggiamento notturna del *Camponotus nylanderi* Em. Insect Soc. 12:253–263.

Weir JS. 1958. Polyethism in workers of the ant *Myrmica*. Insectes sociaux. 5:97–128.

Wheeler WM. 1903. A Revision of the North American Ants of the Genus *Leptothorax* Mayr. Proceedings of the Academy of Natural Sciences of Philadelphia. 55:215–260.

Wilson EO. 1974. Aversive Behavior and Competition within Colonies of the Ant *Leptothorax curvispinosus*. Ann Entomol Soc Am. 67:777–780.

Wilson EO. 1975. *Leptothorax duloticus* and the Beginnings of Slavery in Ants. Evolution. 29:108–119.

Wilson EO, Fagen RM. 1974. On the Estimation of Total Behavioral Repertories in Ants. Journal of the New York Entomological Society. 82:106–112.

Yan J, Goldbeter A. 2019. Multi-rhythmicity generated by coupling two cellular rhythms. Journal of The Royal Society Interface. 16:20180835.

Figure 6.1

A time series of collective activity from a colony of *Myrmica punctiventris* (a), and a sample activity time-series from an isolated individual worker that was removed from the same colony (b). The black dots depict the original data (rescaled between 0 and 1), and the solid black lines were obtained by smoothing the data. Lomb-Scargle periodograms of the collective (c) and individual-level (d) activity time-series respectively. Periodic activity is possible in both colonies and isolated individuals, and the dominant oscillation period of each happen to be very similar.

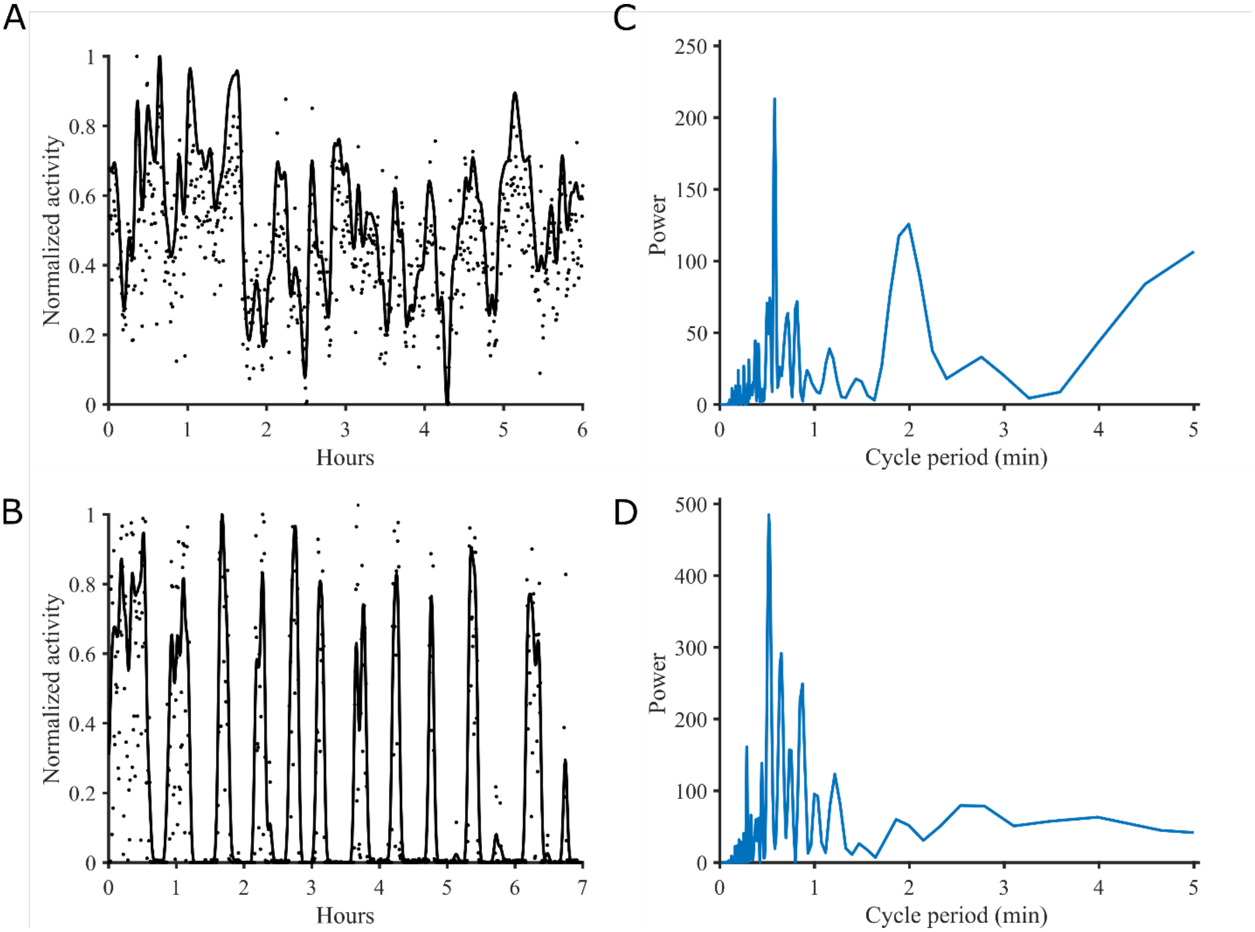


Table 6.1.

Factors that are associated with variation in short-term activity cycles.

Factor	**Effect**	**References**
No. and type of Brood	Activity rhythm synchrony is stronger near larvae. Large brood populations can reduce synchrony and rhythmicity in *T. rugatulus.*	(Chapter 2) (Cole and Hoeg 1996; Boi et al. 1999)
No. of workers and worker density	Unlike colonies, isolated individuals and small groups of workers do not possess rhythmic activity in some species. Higher worker densities can make oscillations less rhythmic in *T. rugatulus*.	(Chapter 2)(Chauvin 1944; Cole 1991a; Cole and Hoeg 1996)
Queen presence	Having a queen can make colony activity more synchronized. Queens can trigger bursts of activity.	(Chapter 2; Wilson 1974)
Satiation	Starved colonies may have higher average levels of collective activity and faster cycles.	(Franks et al. 1990; Hatcher 1992; Rueppell and Kirkman 2005)
Light/Dark cycle	A 12-hour light/dark cycle does not affect short-term activity cycles.	(Chapter 4)
Temperature cycle	Colder ambient temperature may cause colonies to oscillate more slowly.	(Richardson et al. 2017)
Intra-colony variation in workers (aka "Individual personality")	Colonies with older workers may be more synchronous than colonies comprised of mainly younger workers.	(Cole 1992)
Interspecific colony variation	Different species have significant differences in oscillation frequency.	(Chapters 1, 2 and 3)

Table 6.2.

Hypotheses of the function of short-term activity cycles

Hypothesis	**Description**	**Reference**	**Empirical data**
Spatial accessibility	Synchronized activity makes it less likely for ants to be blocked from accessing regions of the nest or larvae.	(Chapter 5; Wilson 1974)	Yes (Chapter 5)
Information flow	Rhythmic activity results in the faster transmission of information throughout the colony.	(Hölldobler and Wilson 1990)	Yes (Richardson et al. 2017)
Foraging control	Synchronized and rhythmic activity regulates the optimal number of ants foraging at specific times.	(Bonabeau et al. 1998)	No
Mutual exclusion	Synchronized activity facilitates more equitable brood tending by preventing multiple ants from repeatedly feeding the same larvae over a short time frame.	(Hatcher 1992; Hatcher et al. 1992)	No
Task efficiency	Synchronized activity allows workers to complete tasks faster.	(Goss and Deneubourg 1988; Delgado and Solé 2000)	No
Epiphenomenon	Neither synchronized nor rhythmic activity is adaptive; activity cycles are a by-product of some unidentified social trait.	(Cole 1991a; Cole 1992)	No
Responsiveness to stimuli	Synchronized activity allows workers to quickly mobilize if a threat appears.	(Hatcher 1992)	No