Original Article

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

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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 have 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 the queen's 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.

Key words: **activity, ants, synchronization, queens, *Temnothorax*, tempo.**

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, 1991c; Cole and Cheshire 1996; Cole and Trampus 1999; Richardson et al. 2017). Several species of ants 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

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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 toward 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 preformed 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 with 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.

MATERIALS AND METHODS
Colony collection and maintenance

The 29 colonies of *T. rugatulus* used in this study were collected in February 2018 on Madera Peak in the Pinal Mountains of Arizona (33.317N and 110.876W). All colonies were monogynous and had approximately 100–260 workers and approximately 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, which 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 and Fancy Feast salmon pâté) and sugar (maple syrup and 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

Eighteen colonies had their activity patterns assessed four times. Trials for each colony were separated by 48 h. Twelve hours before starting a 9-h recording session, colony nest boxes were arrayed on a laboratory bench and placed beneath camcorders (Canon VIXIA, Tokyo, Japan) mounted on 18-cm 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 three-stop light-filter paper (Rosco Cinegel, Stamford, Connecticut) to mitigate any disturbances caused by ambient light. Colonies were recorded for 9 h (approximately from 12 noon to 9 PM). No colonies were recorded on days that they received food.

Three weeks after filming the fourth trial, colonies were filmed for two additional 9-h recording sessions to see how their activity patterns changed over a longer interval than the 9-day span needed to complete the first four 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, $P \sim 0$; workers: PCC = 0.996, $P \sim 0$).

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-s intervals. Each frame was sequentially and automatically evaluated using a custom MATLAB (Version 9.4 R2018a, MathWorks, Natick, Massachusetts) 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 10 recordings from the first run of trials in experiment 1. These 10 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 s for the first 2 h of each 9-h 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-h 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 four trials of experiment 1, resulting in 9-h queen activity records from 40 separate trials.

Experiment 2: queen removal

Colony-level activity measurements

Twelve 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 six randomly chosen colonies, were filmed for 6 h in the morning (approximately 7 AM to 1 PM) and for 6 h in the evening (approximately 1 to 7 PM), 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 postremoval used the same schedule as the initial round of filming (i.e., with colonies in the morning cohort being filmed from approximately 7 AM to 1 PM and those in the evening cohort being filmed from approximately 1 to 7 PM).

Because 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 24 h 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 nest-mate interactions. Because each nest contained only a single ant, location and movement data could be obtained automatically. Video frames, again spaced in intervals of 30 s, were converted to binary images and the ant's centroid displacement between frames was stored. Workers and queens were filmed approximately 42 h after they had been removed. Thus, depending on the block assignment of their source colony, six individuals were filmed in the morning and six 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 preset 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 s 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 and 2.

RESULTS

Experiment 1: variation in activity oscillations

Colony-level activity

Looking at all 108 activity records, the period of group oscillations ranged from 16 to 104 min (mean: 48.4 min ± standard deviation [SD]: 16.0 min). There was a substantial degree of intercolony variability in the shape of group activity oscillations (Figure 1; Supplementary Figures S1 and S2). These differences were highly repeatable across the first four trials: synchrony ($r = 0.84$, confidence interval [CI] = 0.67–0.92, $P < 0.0001$), period ($r = 0.59$, CI = 0.33–0.77, $P < 0.0001$), and rhythmicity ($r = 0.65$, CI = 0.39–0.81, $P < 0.0001$). When all six trials are included, colonies are still repeatable, but their repeatability is reduced: synchrony ($r = 0.68$, CI = 0.46–0.81, $P < 0.0001$), period ($r = 0.40$, CI = 0.17–0.58, $P < 0.0001$), and rhythmicity ($r = 0.51$, CI = 0.24–0.68, $P < 0.0001$). 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 (PCC: −0.643, $P < 0.0001$) along with an increase in synchrony (PCC: 0.319, $P = 0.0008$) but not rhythmicity (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
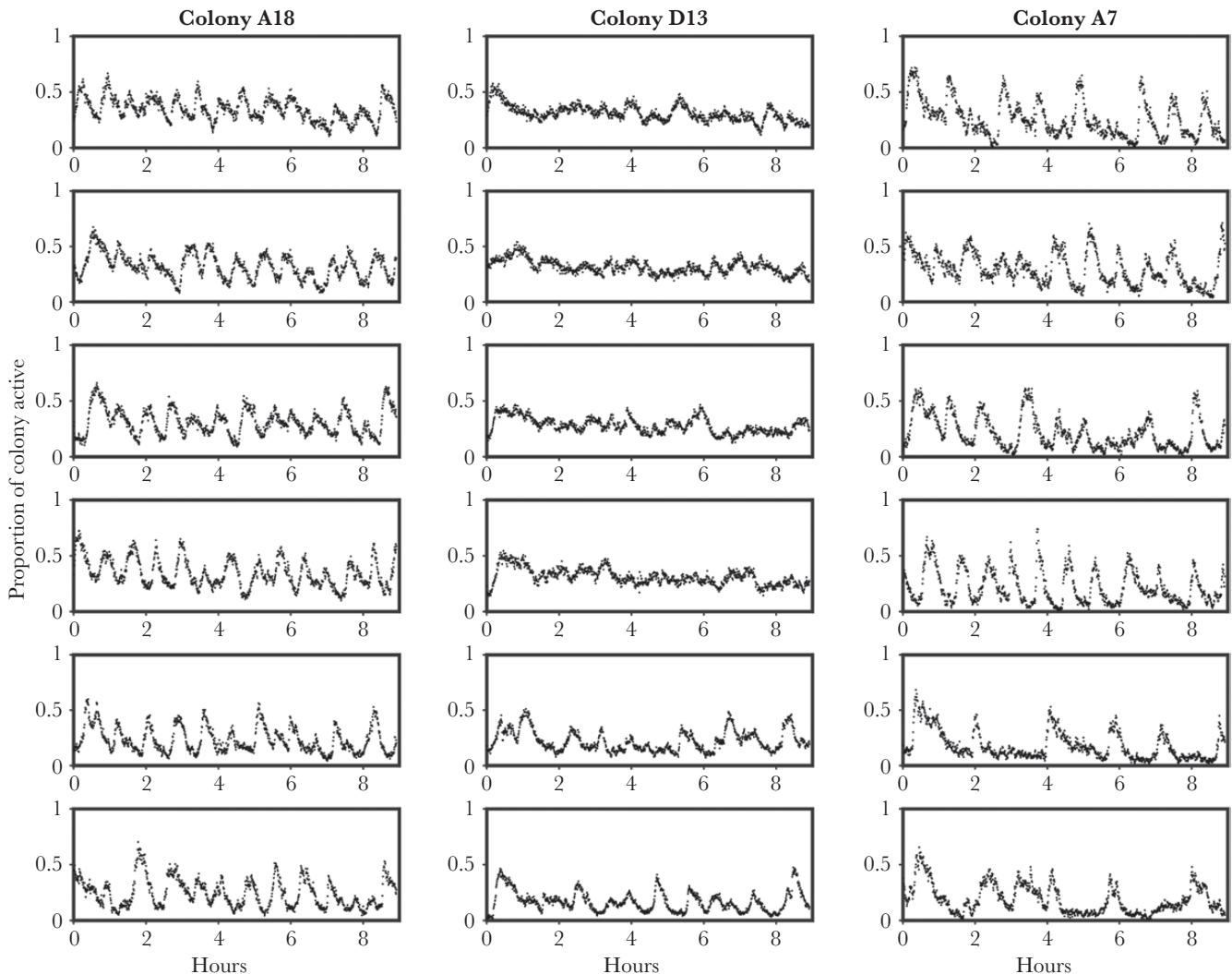
Figure 1
Data points of collective activity plotted for three selected colonies across six trials. Colonies A18 (178 workers, 92 brood) and A7 (101 workers, 52 brood) exhibit rhythmic and synchronous oscillations. D13 (262 workers, 131 brood) is erratic. The bottom two plots in each column represent trials 5 and 6, which were separated from trial 4 by 3 weeks. Colonies can show consistent differences in rhythmicity, period, and synchrony.

across the six trials to give each colony a single overall score for each colony a single overall score for each metric. We then evaluated the correlation between these metrics and colony size by computing 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. Because 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.

Table 1

A summary of the *P* values for the relationships between the six 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

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 Figures S1a and S2a), workers in erratic colonies are not more active than
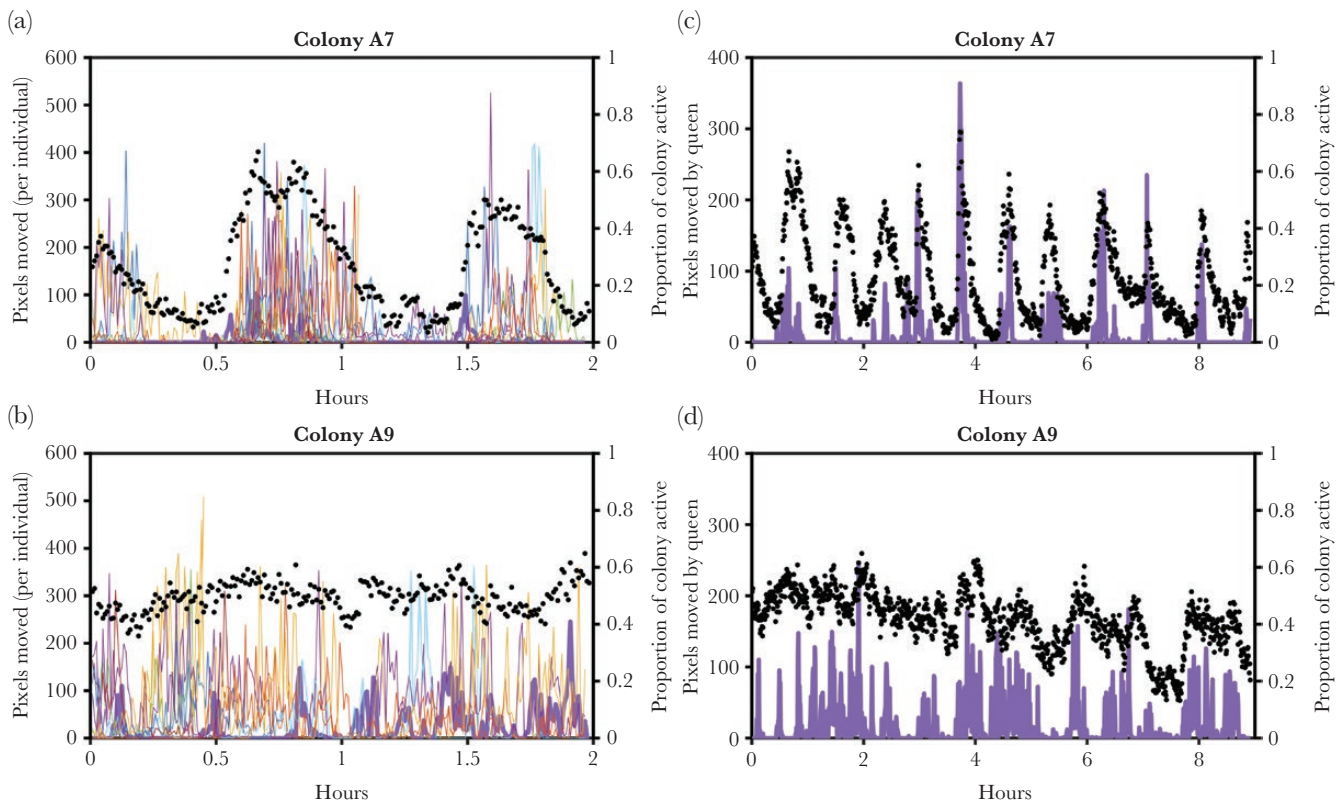
Figure 2
Data points of collective activity and movement patterns of individuals for two colonies (A7's fourth trial and A9's third trial). Panels a and b are activity records over the first 2 h of a trial, panels c and d are the activity records over the entire 9 h of the same trials. Colony A7 (101 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 h), 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.

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 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 ($t = -3.453$, $df = 32.107$, $P = 0.002$). The statistically significant differences in queen behavior between erratic and rhythmic colonies is detectable when queens are analyzed over the full 9 h of their activity (above) *and* when only the first 2 h 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 ($r = 0.80$, $CI = 0.51–0.92$, $P < 0.0001$), P_a ($r = 0.78$, $CI = 0.48–0.91$, $P < 0.0001$) and P_i ($r = 0.48$, $CI = 0.08–0.74$, $P = 0.001$). Colony synchrony is also highly negatively correlated with a queen's *inactive to active* transition probability ($PCC = -0.535$, $P = 0.0004$; Supplementary 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 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 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 Figure S4; paired t-test: $t = -10.253$, $df = 5$, $P = 0.0002$) but not after worker removal (Figure 3; Supplementary 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
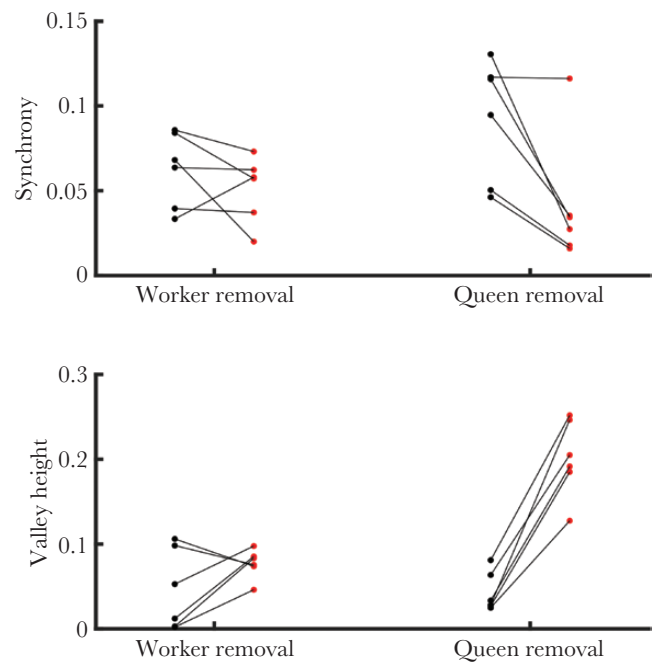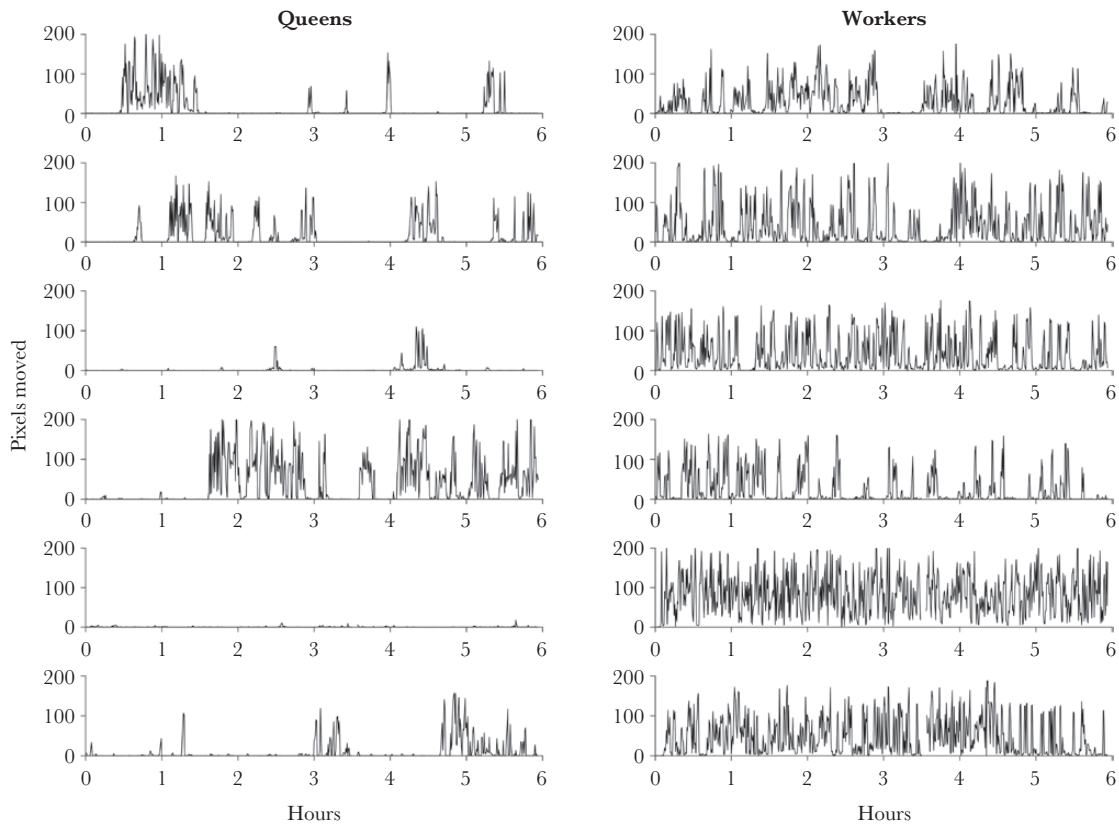

Figure 3
The synchrony and average valley heights (point of lowest activity) for colonies before and after the removal of queens/workers. Black dots indicate initial readings from colonies. Red dots indicate readings from colonies after individuals were removed. Lines between dots connect readings from the same colony. Removing queens (but not workers) reduces colony synchrony and increases the average proportion of ants moving during valleys of activity.

populated nests ($t = -1.372$, $df = 5.503$, $P = 0.223$), but isolated queens do spend less time active than isolated workers ($t = 7.2$, $df = 8.261$, $P < 0.0001$).

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 have 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

Figure 4

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

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 (Richardson et al. 2017). 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 toward 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 *Temnothorax 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 Figure S3). However, the present study cannot 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 *Temnothorax 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, 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 lead 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. Although 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.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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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 Biol Sci. 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. Gainesville (FL): 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 Soc. 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 Soc. 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 (Switzerland): 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 Soc. 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]. Bath (United Kingdom): 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, Holldobler 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 DJ, 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. Physica D. 63:145–160.

Mitrus S. 2015. The cavity-nest ant *Temnothorax crassispinus* prefers larger nests. Insectes Soc. 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 Soc. 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 DJ. 2006. A tunable algorithm for collective decision-making. Proc Natl Acad Sci USA. 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 Soc. 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. New York (NY): Springer Science and 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.