



The digging dynamics of ant tunnels: movement, encounters, and nest space

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Abstract

Underground ant nests are constructed by decentralised self-organisation wherein the ants respond to local stimuli and produce coordinated structures through globally regulated behaviours. One such regulation is the reduction in digging effort that occurs when available nest space has reached an adequate size. Tunnels have a distinct configuration relative to other nest elements and the processes regulating their excavation are poorly understood. We examined the relationship between digging effort and tunnel space by presenting groups of 10 *Acromyrmex lundii* workers with either short or long tunnel spaces and demonstrated that they will dig significantly less over time in a tunnel that is already long compared to one that is short. Additionally, we provided the same treatment to groups of 100 workers and found no significant effect of length, suggesting that group size has an important impact on tunnel excavation dynamics. Automated tracking was then used to examine tunnel digging in greater detail. Groups of 10 *Atta colombica* ants were tracked while excavating sand in a tunnel apparatus. There was a significant correlation between mean walking speed and excavation rate. Additionally, the ants would maintain a consistent level of proximity with each other over time. This suggests that as tunnel space expands, several factors combine to lower the chance of ants encountering the tunnel digging face and taking up excavation.

Keywords Self-organisation · Tunnel excavation · Behavioural regulation

Introduction

Many ant species construct underground nests, which can be both large and sophisticated, to protect their colony from inimical environment variation (Jones and Oldroyd 2007) and predators (Powell and Clark 2004). Ants can therefore be important soil engineers (Jouquet et al. 2006) that affect their local environment by changing soil properties such as runoff (Cammeraat et al. 2002), by turning over soil (de Bruyn and Conacher 1990), altering plant dynamics (Bieber et al. 2011), or enhancing soil nutrients (Shukla et al. 2013). While nests have also been shown to play a role in behavioural organisation (Gordon 1989; Franks and Tofts

1994; Tschinkel 2004; Pinter-Wollman et al. 2013), the behavioural rules that facilitate their construction are not well-understood. These rules are thought to produce self-organised collective action (Buhl et al. 2005), and therefore to operate without central control or direct communication. Instead, organisation is thought to be regulated by mechanisms such as response thresholds (Theraulaz et al. 1998; Halboth and Roces 2017), local communication (Pielström and Roces 2012), and stigmergic communication (Khuong et al. 2016). How such mechanisms lead to the construction of nest space remains yet to be fully understood.

Nest construction is costly in terms of work time, energy, and wear and tear (Mikheyev and Tschinkel 2003), all of which could be invested elsewhere. Ants should therefore carefully tailor their nest size to their space requirements to minimize costs whilst still meeting the needs of the colony (Halley et al. 2005). It has been observed that ants gradually reduce their digging rate as they excavate subterranean chambers and nest space (Rasse and Deneubourg 2001; Buhl et al. 2004, 2005; Pielström 2013). Indeed, Rasse and Deneubourg (2001) have found that this mechanism allows nest volume to be adapted to

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group size in the ant *Lasius niger*. Pielström (2013) found that equal numbers of the leaf-cutting ant *Atta vollenweideri* will dig less in a large chamber than in a small one. These results suggest that a behavioural mechanism exists to regulate the expansion of nest and chamber size during nest excavation, so that it is not enlarged more than necessary. However, the space in an ant nest is made up of both tunnels and chambers (Tschinkel 2004), which differ in shape, size, and orientation. Tunnels contribute volume to the nest and also serve as the connecting ducts among the different parts of the nest. The distinguishing features and functions of tunnels may require separate self-organised mechanisms to regulate their construction. Furthermore, in some ant species, tunnels make a large proportion of nest volume (Cassill et al. 2002; Tschinkel 2003). Tunnel design varies widely among the nests of different species. They can come in many shapes and sizes, such as long straight corridors of uniform width, helices, tunnels that widen and narrow and change direction, and funnel shapes that descend from a nest entrance to an initial chamber (Tschinkel 2003; Forti et al. 2007). Research, however, has been generally focused on chamber and whole nest excavation (chambers plus tunnels), while investigations into tunnels (Sudd 1975; Gravish et al. 2012; Gravish et al. 2013) have not yet revealed the underlying behavioural regulation systems of digging dynamics, particularly in regards to inter-ant dynamics.

Patterns of encounters and behavioural responses are a possible mechanism by which the construction of tunnels may be regulated. Much of the activity of any ant colony is known to be organised by patterns of encounters between workers (Gordon et al. 1993; Gordon and Mehdiabadi 1999; Pratt 2005; Greene and Gordon 2007; Czaczkes et al. 2013; Bouchebti et al. 2015). The rate at which ants encounter nest mates performing various tasks provides information about current activities and about the needs of the colony. Depending on context or recent experience, ants may switch tasks, from digging to foraging, for example, or increase their fidelity to a current task, i.e., continuing digging when they might have stopped, in response to the pattern of encounters they have with others (Gordon and Mehdiabadi 1999; Greene and Gordon 2007; Bouchebti et al. 2015; Pless et al. 2015).

This paper aims to examine the influence of tunnel length on digging rates in tunnels. We investigated whether workers can use the length of a tunnel as a cue to regulate their excavation, and whether the interaction patterns between ants may serve as regulatory cues for tunnel excavation. Two leaf-cutter ant species were used in this research, *Acromyrmex lundii* and *Atta colombica*. Both *Acromyrmex* and *Atta* cultivate fungus gardens and excavate nests and, while the maximum colony sizes of *Atta* are far larger than *Acromyrmex*, they both have systems of tunnels linked to fungus

garden chambers and can look especially similar in the early stages of nest formation (Weber 1972).

We first exposed workers of the species *Acromyrmex lundii* to tunnels of different initial lengths and measured the corresponding digging rates. Digging was constrained within a tube to a perpendicular digging face, essentially reducing digging opportunities to one dimension. This was done to remove variables that might occur with free excavation, such as changing geometry, division of digging effort into two places, and altered surface area/volume ratio. We anticipated that the motivation to lengthen a tunnel would vary with the apparent density of workers in the existing tunnel space. Thus, the shorter initial lengths would provide a greater impetus to excavation behaviours than longer tunnels. In a second experiment, we aimed to examine the detail of that digging behaviour so as to understand its dynamics, such as how movement and interactions influenced digging rate. Therefore, we tracked the movement of the leaf-cutting ants *Atta colombica* in an artificially restricted tunnel system and recorded their digging behaviour.

Experiment 1: manipulation of initial tunnel length

This experiment examined whether ants vary their digging rate in response to the available tunnel length they initially encounter.

Experiment 1: methods

Study species

The ants investigated were six colonies of *Acromyrmex lundii* that were collected in 2007 from Argentina, fed with rose and blackberry leaves as well as water and honey water, and kept in a 12 h light/dark cycle at a constant temperature of 25 °C. All experiments were carried out at 25 °C.

Experimental design

To begin an experiment, workers were removed from the foraging box of their nest, counted out, and placed into the experimental arena. The experimental arena consisted of a 9.5 cm by 9.5 cm by 5.5 cm transparent plastic box with a transparent plastic lid, in which sugar water and tap water were provided ad libitum in two small feeders placed on two slightly elevated platforms that prevented them from being immediately contaminated by excavated soil. Damp tissue paper was used to humidify the air. Despite the food, water, and humidity, ants would die on occasion. Any replicates that suffered a mortality greater than 30% were not included in the analysis. A

plastic tube (1 cm inner diameter) was attached to the box through a hole in the side and a small plastic ‘bridge’ (half a plastic tube) was installed to provide the ants easy access to the tube. The tube was filled with a mixture of 70% sand, 15% clay, and 15% water by weight. This mixture was the substrate that workers excavated. The high sand content allowed rapid extraction from the tunnel (Espinoza and Santamarina 2010), while the water and clay were sufficient to allow pellets to be formed for rapid transport. The rapid excavation allowed the behavioural signal of digging rate to be amplified. Digging progress was measured as the maximum distance that the ants could be observed to have dug. Initial tunnel length was varied and excavated space was manipulated in two separate treatments, as follows:

Two kinds of tubes were connected to the box: one presented the ants with a short length of empty tunnel in front of the substrate, and the other presented a long empty length. Groups of either 10 ants or 100 ants were tested so as to examine the relationship between space, digging rate, and group size. The short length treatment had 5 cm of empty tunnel space followed by 55 cm of soil-filled tube for both 10 ant ($n=15$) and 100 ant ($n=14$) groups. The long length treatment had 75 cm ($n=10$) or 80 cm ($n=7$) of empty tunnel space followed by 55 cm of soil-filled tube when presented to groups of 10 ants ($n=17$) or by 80 cm of soil-filled tube for the 100 ants ($n=10$). The slight variation of 75 cm or 80 cm of empty tunnel in the 10 ants treatment was caused by these two treatments originally serving as controls for separate, non-reported experiments. An ANCOVA showed no significant difference in the slopes of the two groups [$F(1,132)=1.7244$, $p=0.19$] and so the results were pooled for analysis. In all cases, different lengths of tubes were joined together using silicone tube joiners that provide an airtight seal. Ants were allowed to dig for 4 days. In cases where ant excavation approached the end of their tube, an extra soil-filled tube length was added using these joiners.

Statistical analysis

The ‘initial length’ experiments were analysed by hierarchical linear modelling (HLM), where digging tubes at level 1 were nested within the treatment (long or short space) at level 2. We looked for significant differences in the excavation rates in centimetres (CM) between treatment levels by examining the time in hours since the beginning of the experiment (TIME) \times treatment (TREATMENT) interaction using the mixed model:

$$CM_{ti} = \beta_{00} + \beta_{01} * TREATMENT_i + \beta_{10} * TIME_{ti} + \beta_{11} * TREATMENT_i * TIME_{ti} + e_{ti}$$

Hierarchical linear modelling analysis was performed using HLM 7.03 Student (Raudenbush et al. 2017).

Experiment 1: results

Manipulation of initial tunnel length

We found that groups of 10 ants dig significantly more in short tunnels than in long ones over time (Fig. 1a, $t=-4.516$, $p<0.001$ for the significant cross-level interaction between digging tubes—level 1—and treatment—level 2). In contrast, groups of 100 ants dig equally in both kinds of tunnels (Fig. 1c, $t=1.001$, $p=0.318$ for the cross-level interaction between digging tubes—level 1—and treatment—level 2). Additionally, the rate at which ants dig declines over time (Fig. 1b, d).

Experiment 2: encounters and excavation

Experiment 1 demonstrated that workers in small groups will excavate tunnels at a lower rate when presented with a larger tunnel space. If workers assess the adequacy of tunnel length through encounters with nestmates, we expect to find that contact rates decline and inter-ant distances increase as tunnel length increases. To understand how the ants reduce their activity in response to increasing space, more detail on the dynamics of the excavation process is required. In particular, the process of declining digging rate over time (Fig. 1b, d) appears to be key in matching space to ant number and so it requires an explanation. This experiment was designed to track in detail the movements and excavation behaviours of a small groups of ants in a tunnel environment.

Experiment 2: methods

Study species

Experiments were performed during March and April, 2014, at Barro Colorado Island, Panama. For each replicate, ten laden ants were taken from the active foraging trail of a single large *Atta colombica* colony. *Acromyrmex lundii* was not available at the field station and additional *A. colombica* colonies were not available due to the recent local absence of this species.

Experimental design

Ten workers were removed from their foraging trail and introduced into an experimental tunnel digging arena (Fig. 2) for six video series. The use of laden foragers required task switching from foraging to digging but ensured that the experimental ants were relatively

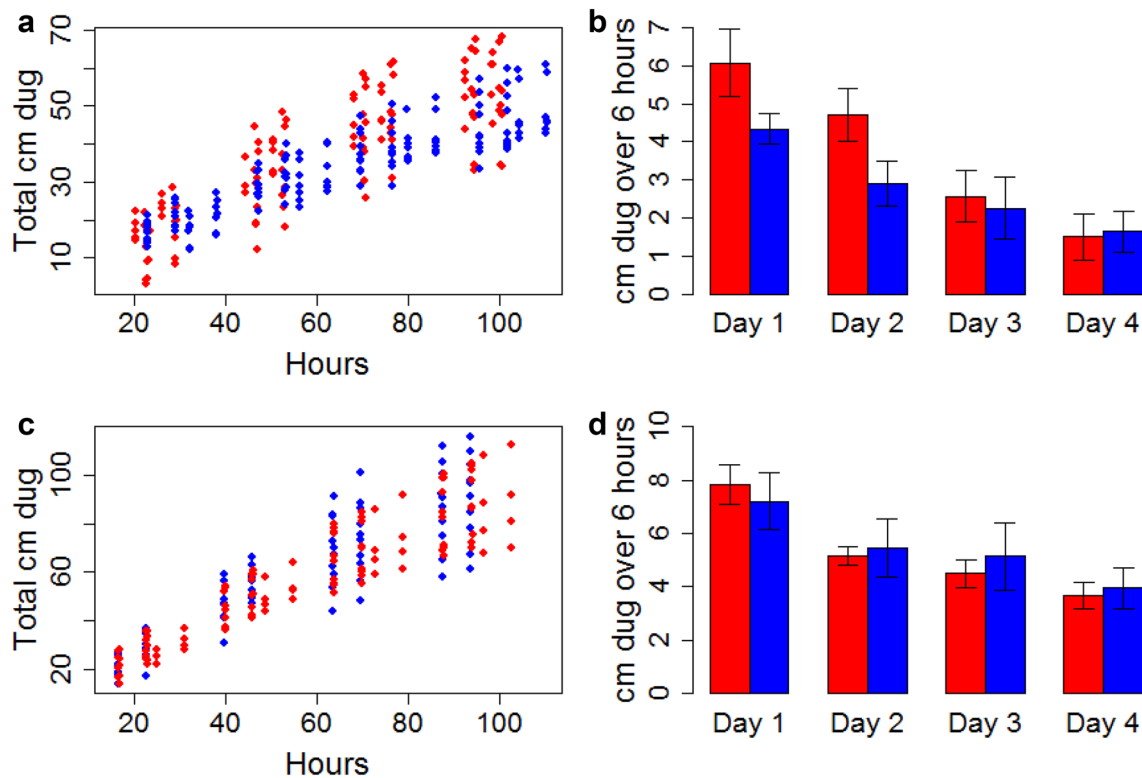


Fig. 1 **a** Total centimetres dug over time for 10 ants for short tunnel length in red (5 cm initial tunnel space, $n=15$) and long tunnel length treatment in blue (ten 75 cm and seven 80 cm initial tunnel space, $n=17$). **b** Distance dug over 6 h using the same data as (a) showing means and confidence intervals. **c** Total centimetres dug over time

for 100 ants for short tunnel length in red (5 cm initial tunnel space, $n=14$) and long tunnel length in blue (75 cm initial tunnel space, $n=10$). **d** Distance dug over 6 h using the same data as **c** showing means and confidence intervals. (Color figure online)

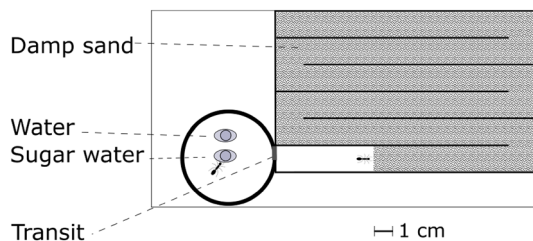


Fig. 2 Diagram of the tunnel digging arena from above. The tunnel section is sealed with transparent plexiglass but the round entrance is open to the air; ants can transit from one to the other. The round entrance is a high cylinder that is coated with talcum powder to prevent climbing and escape. The damp sand was excavated by the ants and transported to the entrance and periodically removed by the experimenter

uniform in behavioural caste. All experimental groups successfully took up tunnel excavation in the apparatus. The digging arena contained a small, open atrium with a circular entrance tube leading to a gallery system with fixed plastic walls defining a potential tunnel width. The gallery system was filled with fine white, moist sand (10%

water by weight). The tunnel was arranged with a series of 180° U-turns so that a long total length was compactly presented within the viewing frame of a camera. The apparatus used a clear Perspex roof to allow ants to be seen and the progress of tunnel excavation to be recorded from above. The experiment was conducted under constant fluorescent lighting, although some indirect sunlight was present. While the intrusion of light was not a natural condition for excavating *Atta* ants, this setup was necessary to allow the video recording of their behaviour. The experiments were carried on at ambient temperature that varied slightly over day–night cycles and at natural humidity that was high enough (67%, $SD=3.9$) that the ants could live comfortably. Video recordings of six replicates of the experiment (using different groups of ants each time) were made using a 60D Canon camera with a 50 mm lens in video mode. Magic Lantern software was used to enable the camera to record 30 min of high-definition video (1080p) at 25 frames s^{-1} every 6 h for 72 h, (two replicates continued for 80 h).

Video analysis and data extraction

Post-processing with Adobe Premiere Pro CC was used to remove shadows and dark patches from the recording. The high contrast nature of the video recording allowed us to use the tracking program idTracker (Pérez-Escudero et al. 2014) to characterise ant movements during the course of excavation. Condensation on the perspex cover prevented idTracker from reliably identifying and following individual ants. However, the tracking did allow collection of important variables on worker movement that did not require unique identification of individuals.

Six variables were measured. Three were measured by idTracker: mean inter-ant distance (cm), close-proximity frequency, and mean speed (cm/s). Three were extracted manually: tunnel length (cm), mean excavation rate at the distal end of the tunnel ('digging face'), and mean excavation rate elsewhere in the tunnel. Close-proximity frequency was defined as the frequency in which the centres of two ants were within a set minimum distance defined by the mean length from the centre of each ant to the tips of its antennae. The reasoning behind this decision was that if the antennae of one ant could reach the centre of the other ant, then they were likely to be in some form of contact or aware of the other. Speed could be extracted only from identified ants and sometimes, due to the aforementioned tracking problems, identities of ants would randomly switch. To control for the effect of such erroneous switching on speed, all movements of more than 25 pixels in one frame were excluded such that implausibly high speed spikes were removed from the data set and speed could be reliably extracted. Mean inter-ant distance (cm), close-proximity frequency, speed (cm/s), were averaged over the half hour of each observation. Tunnel length was measured digitally from the video and the mean excavation rate was determined by sampling a random 1 min section per half-hour observation and counting every digging event, consisting of bite, rake, and move as a 'grab' (thus, the rate was measured in grabs per minute). The use of two measurements, excavation rate at the digging face and elsewhere, allowed us to determine if activity patterns of workers differed according to spatial context. The sum of the two measurements yields the total excavation rate occurring at the time of observation.

Model of random movement of ants in tunnels

A model of random ant movement was required to determine if ant movement was significantly different from random. As tunnels are excavated and their floor area increases, one would expect that a fixed number of ants moving independently would spend less and less time in proximity with each other. The null expectation under random, independent motion of a fixed number of ants is that contact time

would vary inversely with tunnel length. Consider a tunnel of length l and width w —thus area lw —and a focal ant at a given position in the tunnel. Suppose an ant can detect the presence of another ant within a radius r from its centre, so that an interaction can be defined as the occurrence of a second ant within a circle of area πr^2 centred on the focal ant. If the second ant is placed randomly on the floor area of tunnel, it therefore has a probability $p = \pi r^2/lw$ of being in contact with the focal ant. As the contact radius r and the tunnel width w are fixed, this probability is an inverse function of tunnel length l . Some adjustment must be made if the focal ant is closer than r to the wall of a tunnel, as the circle of potential contact around the ant is then censored by the wall. Given our tunnel widths of 13 mm and an estimated value of $r = 6.11$ mm, the average area of the censored circles for randomly placed focal ants will be only about 80.1% of the maximum πr^2 . However, this merely means that the adjusted probability that a second ant occurs within contact of a focal ant is $p' \approx 3.204\pi r^2/lw$, and p' remains an inverse function of l .

If N ants move independently in random walks within the tunnel, they will occur at random positions at a randomly selected moment. The number of contacts, k ($0 \leq k \leq [N^2 - N]/2$), among N randomly positioned ants will follow a binomial distribution with a probability p' of "success," and the mean of the distribution will be Np' . As p' is an inverse function of length l , the total number of contacts under random motion should be an inverse function of l . If every contact lasts a mean of s seconds, then the total contact time among N randomly moving ants remains an inverse function of tunnel length l .

Statistics

Where visual inspection of bivariate relationships showed a strongly linear pattern, we used linear regressions to establish the relationship between variables. Model II regressions were used to compensate for the error in both axes of the data. The relationship between tunnel length and elapsed time was nonlinear and asymptotic. We fit a Michaelis–Menten curve, $\text{length} = a \cdot \text{time} / (1 + b \cdot \text{time})$, to these data. Parameter estimates were obtained using regression to fit a line to the reciprocals of tunnel length and elapsed time and the values of a and b were back-calculated from the slope and intercept of the linear model. This double-reciprocal method is the standard technique for parameter estimation for the Michaelis–Menten curve (Crawley 2013). We also used a Michaelis–Menten model to analyse the excavation rate over time. The first derivative of a curve relating tunnel length to elapsed time represents the excavation rate of the tunnel (length per unit time) at a given moment. We therefore fit our data on excavation activity rate, measured as grabs per minute, to the first derivative of a Michaelis–Menten

curve, using nonlinear least-squares regression to estimate the parameters. Statistical analyses were performed with R (R Development Core Team 2015).

Experiment 2: results

Tunnel excavation occurred initially at a high rate but declined over time, so that tunnel length increased rapidly at the start of the experiments but eventually reached an approximate plateau (Fig. 3). Linear regression through the double-reciprocal data ($1/\text{length}$ versus $1/\text{time}$) provided a highly significant fit ($F_{1,68} = 126.1$, $p \ll 0.0001$, $R^2 = 0.65$). Back calculation of the parameters of a Michaelis–Menton curve yielded values of $a = 3.076$ and $b = 0.097$, which provided a good fit to the asymptotic pattern (Fig. 3). Fitting Michaelis–Menton curves to the individual replicates also yielded highly significant results, with average parameter values among the six replicates of $a = 3.187$ and $b = 0.105$. There is a strong and significant positive linear relationship between digging activity and ant walking speed (Fig. 4, $p < 0.001$, $R^2 = 0.57$, $df = 74$) and a significant negative linear relationship between walking speed and elapsed time (Fig. 5, $p < 0.001$, $R^2 = 0.18$, $df = 74$). The contact frequency among the workers, as a percentage of the total possible contact time, was statistically constant (slopes not significantly different from zero) over time ($p = 0.81$, $R^2 = 0.0008$, $df = 74$) and with respect to tunnel length ($p = 0.47$, $R^2 = 0.007$, $df = 74$). Evaluated against the null model of random movement, this constancy of the summed contact frequency was significantly different from the expected inverse relationship between contact and tunnel length. An inverse relationship

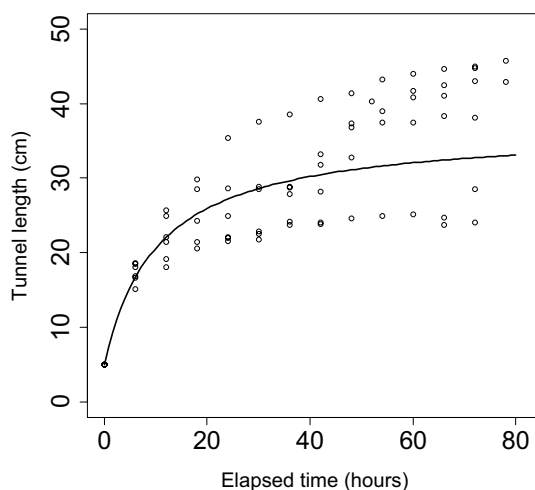


Fig. 3 Tunnel length in relation to elapsed time since excavation began. Symbols represent observations from six replicates. The curve shows a Michaelis–Menton curve, $\text{length} = a \cdot \text{time} / (1 + b \cdot \text{time})$, with best-fit parameters $a = 3.076$ and $b = 0.097$

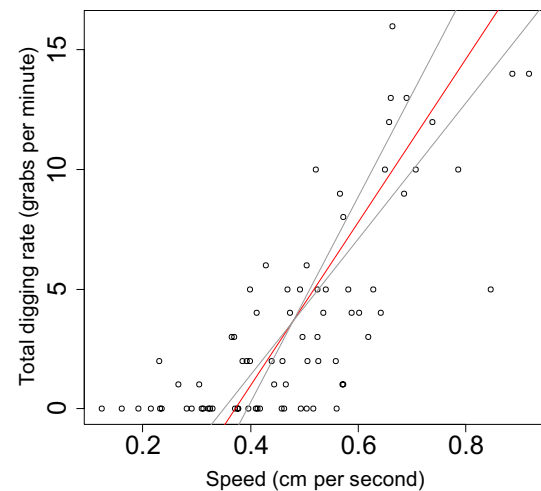


Fig. 4 Relationship between ant speed (cm/s) and digging rate (digging events in 1 min) ($p < 0.001$, $R^2 = 0.57$, $df = 74$, CI = 95% of slope)

would produce a slope of -1 between the logarithms of these variables, but the observed slope was significantly greater (Fig. 6, $p < 0.001$).

The excavation rate at the digging face was high at the beginning of the experiments and dropped rapidly over time (Fig. 7a). Given that a Michaelis–Menton curve fit the data on tunnel length as a function of elapsed time in Fig. 3, the data in Fig. 7 on the behavioural rate of digging as a function of elapsed time would be expected to fit the first derivative of a Michaelis–Menton curve, $\text{rate} = a / (1 + b \cdot \text{time}) - a \cdot b \cdot \text{time} / (1 + b \cdot \text{time})^2$. The difference in units between the data of Fig. 3 and of Fig. 7 requires reparameterization. The best-fit parameter values determined by nonlinear least-squares regression, $a = 11.16$ and $b = 0.14$, provided a good fit to the behavioural rate data (Fig. 7a). By contrast, digging at

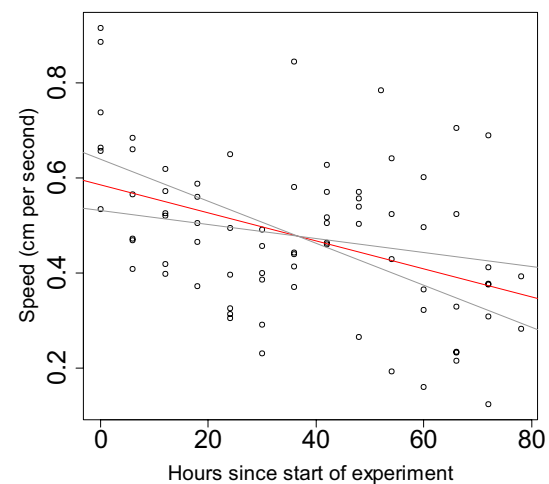


Fig. 5 Relationship between hours since the experiment commenced and speed (cm/s) ($p < 0.001$, $R^2 = 0.18$, $df = 74$, CI = 95% of slope)

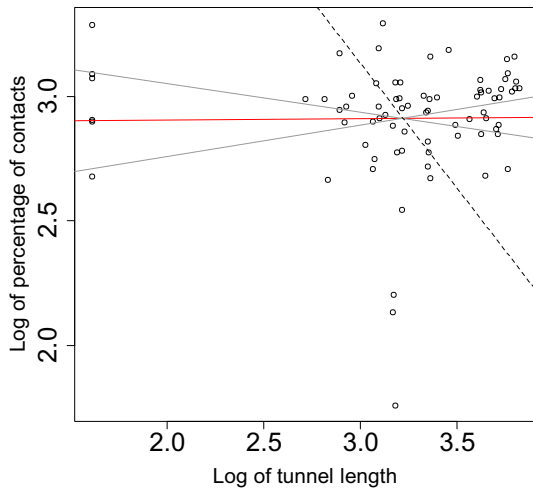


Fig. 6 The slope for the relationship between log proximity frequency (the percentage of contacts among all possible pairwise distance comparisons) and log tunnel length (middle red line, CI=95% of slope) differs significantly from the expected slope of -1 for random independent movement (broken line) ($p < 0.001$). (Color figure online)

locations other than the tunnel face was relatively constant over time (Fig. 7b). While the best-fit value of a was significantly different from zero ($a = 1.91, t_{74} = 3.61, p = 0.0006$), the best-fit value of b was not ($b = 1.91, t_{74} = 0.12, p = 0.90$), as reflected in the nearly flat, linear relationship despite the fundamentally nonlinear form of the equation. Thus, the reduction in excavation rate over time implied in Fig. 3 comes from a reduction of digging specifically at the tunnel face rather than from an overall reduction in excavation activity. Mean inter-ant distance increased over time as tunnel length expands (Fig. 8, $p < 0.001, R^2 = 0.183, df = 74$).

Discussion

We found that ants in small groups of ten individuals dug significantly more rapidly when given only a short length of tunnel space (5 cm) when compared with those given a long length of tunnel space (75 and 80 cm) (Fig. 1a). Furthermore, this difference disappears when worker numbers are increased to 100 (Fig. 1c). This suggests that the relationship between ant number and tunnel length is a key element in determining digging rate and that when the width of the tunnel is constrained, additional ants do not make a proportional difference. Previous results have shown that available space can affect digging rate in whole nests with both chambers and tunnels (Rasse and Deneubourg 2001) and in chambers by themselves (Pielström 2013) and this study shows that available space can also affect digging rate in the constrained conditions of a tunnel. The similar dynamics present in three different forms of space and three different

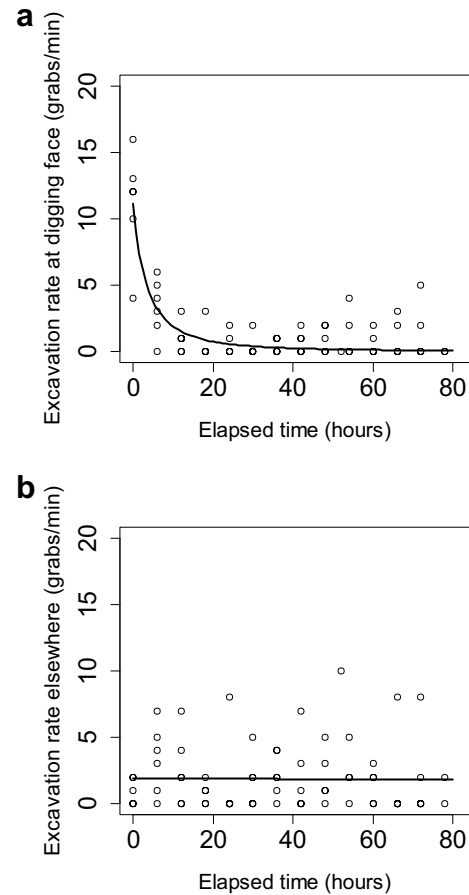


Fig. 7 Relationship between excavation rate (a) at the tunnel digging face and (b) elsewhere in the tunnel in relation to time since the experiment commenced. Curves represent least-squares fit of the equation: $\text{rate} = a/(1 + b \cdot \text{time}) - a \cdot b \cdot \text{time}/(1 + b \cdot \text{time})^2$ to the data (see text for parameter values and statistical results)

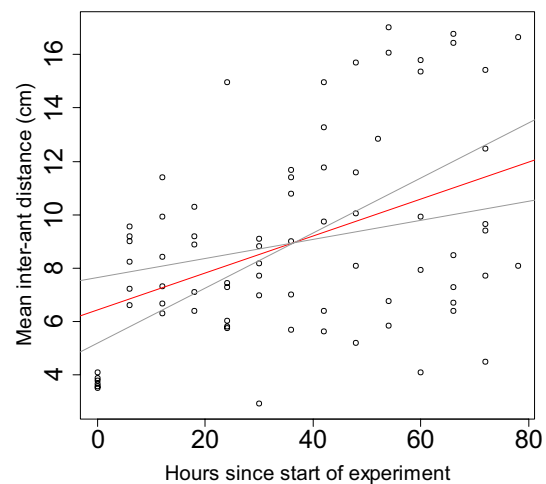


Fig. 8 Relationship between mean inter-ant distance (cm) and hours since the experiment commenced ($p < 0.001, R^2 = 0.183, df = 74, CI = 95\%$ of slope)

species (nests—*Lasius niger*, chambers—*Atta vollenweideri*, and tunnels—*Acromyrmex lundii*) suggest that the underlying mechanism could be the same for all three. Indeed, the similar pattern of reduction in digging rate over time for Figs. 1 and 3 despite the differences in experimental apparatus and species reinforce this possibility. The association of excavation rate with mean walking speed (Fig. 4) and the decline in mean walking speed over time (Fig. 5) lead to the implication that walking speed is an important factor in modulating digging rate, perhaps through increasing encounters with the digging face or other digging ants. However, we should remember that walking speed and digging rate are only correlated, and a third factor, such as arousal, may elevate the level of both. It has previously been shown that ants will dig at a high rate immediately following disturbance (Figs. 1b, c, 3, Buhl et al. 2005), even creating excess space that is later refilled (Römer and Roces 2015). It is likely that removal from the foraging box or trail and the imposition of new surroundings were a sufficient disturbance to cause a highly aroused state among the workers at the beginning of the experiment and thus their high digging rate. Exhaustion may have influenced the decline in walking speed and perhaps arousal over time. However, the ants had constant access to sugar, to which leaf-cutters will readily recruit (Roces and Núñez 1993), and so lack of energy is unlikely to be the primary determining factor.

If arousal were the only factor affecting digging rates then we would not expect to find that ants dig less in tunnels that were initially longer rather than initially shorter, as shown in Fig. 1a, as the ants could simply gather at the digging face and excavate at the accelerated rate. Therefore, a consistent explanation requires a mechanism that would result in the ants reducing their excavation activity in longer tunnels. Many authors have noted the tendency for ants to aggregate and regulate their rate of encounters with other ants (Gordon et al. 1993; Deneubourg et al. 2002; Depickère et al. 2004, 2008; Jeanson et al. 2004). If walking speed and other variables remain constant, ants would be less likely to encounter a distant tunnel face if they had a tendency to aggregate in locations other than the tunnel face. Consistent with the work of Gordon et al. (1993) and others, we found in experiment 2 that ants maintain a consistent level of proximity to one another over time (slope not significantly different from zero, $p = 0.81$, $R^2 = 0.0008$, $df = 74$) even as tunnels grow longer (slope not significantly different from zero, $p = 0.47$, $R^2 = 0.007$, $df = 74$) and the ants have more space available to them, resulting in a pattern of contact that is significantly different from random expectations (Fig. 6). The decline in excavation that occurs specifically at the tunnel face (Fig. 7a) relative to the consistent digging elsewhere in the tunnel (Fig. 7b) is likely due to a reduction in the number of ants encountering that point due to their aggregation elsewhere, such as at the tunnel entrance. Therefore, multiple factors,

primarily arousal and the tendency of ants to aggregate, likely interact to determine digging rate.

A complicating question is how it can be that ants can have increased inter-ant distances over time (Fig. 8) while proximity frequency remains relatively constant (Fig. 6). One might expect that as inter-ant distance tended to increase, proximity frequency would also tend to decrease. The answer is likely to be that ants seek out a minimum level of contact with others (Gordon et al. 1993; Pinter-Wollman et al. 2013; Pless et al. 2015) and this causes the formation of small groups, each of which provides the others in its group with sufficient contact but which may be out of immediate contact with the other subgroups. In reality, ants are likely to transit from group to group and area to area. Previous research suggests that these subgroups are likely to be important for the removal of pellets from the tunnel face to the entrance via task partitioning (Pielström and Roces 2013).

Here we have shed light on the mechanisms ants use to regulate their tunnel size, which includes ant number and walking speed. From this we hypothesise that arousal and the tendency of ants to aggregate with each other leads to the self-regulating reduction of digging at a digging face as the tunnel expands. It may be that differences in aggregation, movement patterns, and context-specific responses help to explain species typical differences in nest construction. Further investigations into these properties and the role of subgroups may contribute to improving our fundamental understanding of self-organised and emergent systems.

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