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Short communication

It is not all pheromones: No evidence that pheromones affect digging face choice during ant nest excavation

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ABSTRACT

Ants create nests of a size that is tailored to the number of individuals in a nest via a self-organized process. It is not yet clear how they accomplish this. Deposition and evaporation of pheromones at the digging face has been hypothesised by Deneubourg and Franks (1995) and Buhl et al. (2005) to be part of the nest construction process, with models being presented to support this contention.

This hypothesis was tested by allowing groups of 5 *Acromyrmex lundi* workers to choose between two excavation sites, one that was freshly exposed to digging and one where digging had ceased an hour previously. It was expected that if pheromones played a role in stimulating digging, then ants would show a preference for digging in the "fresh" sites rather than the "aged" sites where the putative digging pheromone had decayed.

No significant difference in digging activity between "fresh" and "aged" sites was detected. It is therefore likely that, while digging pheromones may play other roles in other parts of the digging system, they do not play an important role in regulation of soil excavation at the digging face.

1. Introduction

Nests are a key element in the success of eusocial insects (Wilson, 2008). They provide physical protection from predators and enemies (Herrera and Valenciaga, 2011; Theraulaz et al., 2003), help to regulate temperature (Bollazzi et al., 2008; Farji-Brener and Tadey, 2012; Jones and Oldroyd, 2007; Korb, 2003), moisture and humidity (Bollazzi and Roces, 2007, 2010; Hölldobler and Wilson, 1986), and carbon dioxide concentration (Kleineidam et al., 2001; Lüscher, 1961), as well as play a role in task allocation and colony activity (Gordon et al., 1993; Hölldobler and Wilson, 1990; Kwapich and Tschinkel, 2013; Stickland and Franks, 1994; Tschinkel, 1999a). However, the mechanisms by which social insects organize their nest construction to achieve the correct functional form and size for their species and state of colony growth are not fully understood. In this article, I investigate the hypothesis that ants regulate their nest construction through pheromone deposition on the surfaces of the growing nest structure.

The nests of most ant species are subterranean and are constructed by excavating soil from tunnels and chambers and transporting it to the surface (Sudd, 1967). Ants would be expected to avoid unnecessary excavation, and so a simple but fundamental design principal should be that nest size is adjusted to the needs of the colony. Specifically, nest volume should be tailored to the number of ants present in the colony. Such matching of form to function has been documented in several ant species by Tschinkel (1999b), Mikheyev and Tschinkel (2003), Tschinkel (2004), Tschinkel (2005), Tschinkel (2011), Hart and Tschinkel (2012), and Murdock and Tschinkel (2015).

Deneubourg and Franks (1995) and Buhl et al. (2005) have proposed a simple mechanism to explain how a self-organised process of digging might be regulated using pheromones. Suppose that ants deposit pheromones on soil particles at the digging face of a tunnel and that this pheromone prompts other ants to dig. As the excavation progresses, the increased size of the nest means that ants, moving at a constant speed, are less likely to be recruited to dig due to the decreased chance of encountering a pheromone laden site. As nest structure expands and the pheromone evaporates the impetus for continued excavation declines. Eventually work declines to near zero and the nest attains its final size relative to the population of ants. Models of this process predict logistic growth of nest size over time, and some experimental data conform approximately to this pattern (Buhl et al., 2005; Halley et al., 2005).

In addition, support for the proposed pheromonal mechanism was provided by Chen and Zhang (2013), who found that *Solenopsis invicta* would choose to dig significantly more in sand treated with mandibular gland extracts over sand without the treatment.

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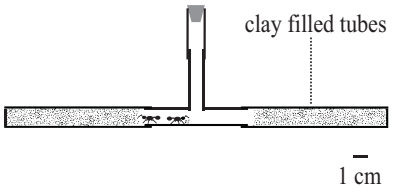

Fig. 1. Digging face choice setup. One side was recently exposed to digging ("fresh") while the other was also exposed to digging at least an hour before ("aged"). The "aged" tube was attached to an air pump and water bubbler to keep the surface of the clay from drying out. Tubes had a 10 mm inner dimension. The T-junction had an 8 mm internal diameter, 7 cm length, and 3.5 cm stem height.

Additionally, Pielström and Roces (2013) found that freshly excavated soil pellets guided the location of digging by *Atta vollenweideri* leaf-cutting ants, while pellets that were one hour old did not, suggesting that chemical cues that stimulate excavation may have evaporated from the older pellets.

Although the pheromone hypothesis has some empirical support, a behavioural effect of pheromones that the ants have themselves deposited has not been demonstrated. The present experiment investigates the possibility that fresh pheromones on the digging face of tunnels might significantly attract worker digging behaviour.

2. Methods

Five colonies of *Acromyrmex lundi,* collected in Argentina in 2007 and maintained at the Biocenter of the University of Würzburg, Germany, were used for this experiment. Colonies were maintained at 25 °C and 50% humidity under a light/dark cycle of 12 h/12 h. The experimental setup allowed groups of five workers from each colony to choose between two tubes from which to excavate a mixture of 75% clay and 25% water by weight. For each trial, the tubes were attached to opposite arms of a plastic T-junction and the ants were introduced through the stem (Fig. 1).

The experiment compared the quantity of soil excavated by groups of 5 *A. lundi* workers given a choice between two digging locations: a fresh tunnel face that recently had ants digging at it, and one that had not been exposed to excavation for one hour. The procedure did not measure pheromones directly, but used time since the end of excavation as a proxy for putative pheromone quantity based on the effect noted by Pielström and Roces (2013). Significantly greater excavation in the "fresh" than the "aged" soil face would therefore be consistent with stimulation of digging by pheromones, but would not directly confirm their presence. A negative result would be inconsistent with the digging pheromone hypothesis.

Because temperature can influence digging performance in this species (Bollazzi et al., 2008), digging tubes were sealed and left overnight to bring them to ambient temperature. Digging tubes were attached for two hours to boxes containing a large number of workers (more than 30) of *A. lundi* from the same colony that supplied workers for the experimental trial itself. Excavation usually occurred during this time, and if it did not, the tube was not used in any trials. Ants from all parts of the experiment were used only once.

One of the two tubes for each experimental trial was timed so that it could be left for at least one hour before commencement to allow evaporation of any pheromone present on the digging face of the clay substrate. This was the "aged" condition. The other tube was used immediately after excavating ants were removed. This was the "fresh" condition.

Because excavation had occurred at the surfaces in both experimental tubes, ants in a trial had to choose between two digging faces that were physically similar but might differ in the degree of pheromone evaporation. Water as well as pheromones could evaporate from the clay surface, and soil moisture differences can affect ant digging choices (Pielström and Roces, 2014). Therefore, in order to prevent physical changes to the digging face as a result of water evaporation, "aged" tubes were attached to a source of humidified air for an hour before a trial. In order to demonstrate that the humidified air did not bias digging behaviour, a control provided ants with the same choice between two tubes of clay substrate, but, unlike the experimental trials, without the pre-exposure to digging ants. All other conditions were the same.

In both the control and experimental trials the choice tubes were weighed (Kern ABT 120-5DM, accuracy 0.01 mg) before the experiment and attached to the T-junction. Five ants were then introduced into the T-junction (Fig. 1). The setup was directionally randomised and covered in black photography cloth to prevent bias. An average of 1.43 min ($n = 8$, SD = 6.5 s) elapsed between the end of preparatory exposure of tubes to digging ants in the "fresh" treatment and the insertion of the five experimental workers. It then took an ant an average of 15.9 s ($n = 16$, SD = 10.0 s) to encounter one of the digging faces.

Ants were left to dig in the setup for one hour. Preliminary tests had shown that excavation would begin well within the hour. Further measurements described below confirm this. Ants tend to dig where other ants are already digging (Sudd, 1970) and so it was expected that the initial choice of an ant to dig at a location would be amplified by other ants from the group also choosing to dig there. At the end of the hour, the ants were removed from the tubes and both tubes were weighed. The difference between the initial weight and the subsequent weight was taken to be the amount of soil excavated from that tube.

A separate test was performed to measure the lag times from introduction to the start of digging. The control protocol was used for these measurements, but without the black cloth so that the ants' activity could be observed. Each replicate was checked once each minute and the time of first digging was recorded.

In total, 103 trials of the control setup and 96 trials of the experimental setup were performed. Paired Wilcoxon tests were used to compare the amount of soil removed from the "fresh" and "aged" tubes. Results from each of the five colonies were tested separately to detect possible colony differences. To compensate for the inflation of Type 1 error by multiple testing, a Bonferroni-corrected probability value of 0.01 was used. When no significant effects within colonies were found, the data were pooled across colonies and again tested with a paired Wilcoxon test. Confidence intervals were formed for these tests via bootstrapping. Statistical calculations were performed with R (R Development Core Team, 2012).

3. Results

The first digging commenced after an average of 11 min ($n = 10$, SD = 4:03). Therefore, the total time from removal of the preparatory ants to the start of digging by the experimental ants was an average of approximately 12.4 min.

No colonies showed a significant difference between "aged" and "fresh" tubes for either the control or experimental condition (Table 1). Therefore, the results were pooled for further examination.

As expected, ants showed no preference between the two tubes of the control trials. On average, they removed 0.19 g (SD = 0.23) from control "fresh" tubes that had not been exposed to digging, and 0.23 g (SD = 0.19) from control "aged" tubes that received the sham treatment of attachment to the humidifying apparatus for an hour before the trial (Fig. 2a). This difference was not significant (Wilcoxon test, $p = 0.211$, $n = 103$). Workers also showed no discrimination between tubes in the experimental trials. They exca-