No evidence for tactile communication of direction in foraging *Lasius* ants

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21 **Abstract**

22 The idea that ants communicate when meeting on a trail is beguiling, but evidence for this is scarce.

23 Physical communication in ants has been demonstrated to play a role as a modulator of behaviours

24 such as alarm and recruitment. Honeybees can communicate the location of a resource using an

25 advanced motor display – the waggle dance. However, no equivalent of the waggle dance has been

26 described for any ant species, and it is widely believed that ants cannot communicate the location of

27 resources using motor displays. One group of researchers report several demonstrations of such

28 communication in *Formica* ants; however, these results have been largely ignored. More recently

29 some evidence arose that *Lasius niger* foragers returning from a food source can communicate to

30 outgoing foragers the direction that should be taken at the next bifurcation by means of physical

31 contact on the trail. Here, we make a concerted effort to replicate these results. Although initial

32 results seemed to indicate physical communication, once stringent controls to eliminate pheromone

33 cues were put in place, no evidence for physical communication of food location could be found. This

34 null result was replicated independently by a different research group on a closely related species, *L.*

35 *neglectus*. We conclude that neither *L. niger* nor *L. neglectus* foragers communicate resource location

36 using physical contact. Our results increase the burden of proof required for other claims of physical

37 communication of direction in ants, but do not completely rule out this possibility.

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39 **Key words**

40 Motor displays – tactile communication – distance homing – *Lasius niger* – *Lasius neglectus* -

41 antennation

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Introduction

"The story that ants talk by touching antennae is probably the most deeply rooted idea most people have about ants. It is also a story of considerable age. Yet the evidence that ants do have an antennal language is extremely thin". Sudd (1967) – *An Introduction to The Behaviour of Ants*

An observation made by almost anybody who has ever watched ants forage is that ants encountering nestmates on a trail will often pause and make antennal contact. As observers, we cannot help but imagine that some form of communication is taking place. There is strong evidence that several ant species use a series of motor displays to modulate their recruitment behaviour (Hölldobler 1971; Hölldobler and Wilson 1978, 1990), such as priming nestmates to follow pheromone trails, or signalling that a pheromone trail leads to a food source or a nest site (Hölldobler 1971). As ant trails often form a branching network of paths, and much ant foraging occurs on plants (which again constitute a ramifying system), it seems plausible that some sort of directional signalling of food location would lead to more efficient foraging. This hypothesis was indeed suggested over two centuries ago (Huber 1810) and found support from the eminent myrmecologist Erich Wasmann (1905). In light of Karl von Frisch's remarkable discovery of the honey bee waggle dance (von Frisch 1923, 1967), such a supposition seemed a lot more reasonable. Undoubtedly, ants meeting on a trail ascertain each other's colony identity (Akino et al. 2004; Ozaki et al. 2005). Odour cues from successful ants returning to the nest are also likely to be gathered by the outgoing ant, which can inform the foragers as to what type of food is available (Roces 1990, 1994; Le Breton and Fourcassie 2004). It is likely that odour cues on returning foragers can trigger previously learned associations between food odours and foraging locations (Czaczkes et al. 2014), in a manner similar to odour cue transfer via trophallaxis in honey bees (Farina et al. 2005; Grüter et al. 2008; Balbuena et al. 2012). Despite the temptation to assume that more than simple cue-sensing is occurring during ant-ant interactions, there remains very little support for anything more complex, such as signal exchange (Sudd 1967). In their landmark book, Hölldobler and Wilson (1990) state that "ants antennate nestmates in order to smell them, not to inform them".

There is, however, one notable exception to the lack of support for tactile directional information transfer in ants: the findings of Reznikova and colleagues (reviewed in Reznikova 2008; Reznikova 2017), and the related work of Novgorodova (2006). Reznikova and Ryabako (1994) describe a series of experiments in which scouts from two *Formica* species (*F. polyctena* and *F.*

sanguinea) were able to communicate complex directional information to other foragers via physical contact. Forager groups that could physically interact with an informed scout were able to find the location of a food source at the end of a multiply-bifurcating maze much more accurately and rapidly than groups that were not allowed to interact with an informed scout. These results implied that the informed scout could communicate a series of turns to naïve foragers. In a second experiment reported in the same paper, and replicated in Reznikova and Ryabko (2001), scout ants were allowed to find a food source on one branch of a comb-like maze consisting of 25 or more branches, all emerging from a single main stem in one direction. Groups of foragers subsequently contacted by the informed scout then achieved remarkable accuracy in finding the food source: in one experiment (Reznikova and Ryabko 2001) ant groups made zero mistakes in 117 of 152 trials. The authors stressed that in every experiment steps were taken to ensure that no information apart from direct physical contact from the informed scout was available to the otherwise naïve foragers. Using variations of these experimental paradigms, and by measuring the time scout ants spent communicating with their team of naïve foragers, Reznikova and Ryabko (1994, 2001) describe further impressive information-processing feats by these ants. These include simple arithmetic operations such as addition and subtraction, and information-compression abilities. Novgorodova (2006) replicated some of the findings of Reznikova and Ryabko (1994) in a related species; *Formica pratensis*. The results appeared to corroborate the previous findings, and showed that otherwise naïve foragers which had contacted an informed scout spent significantly less time searching for a feeder at the end of a maze than foragers that had no contact with informed scouts. However, as decision accuracy was not provided, the results could equally well be explained by faster searching by the contacted naïve ants.

The findings of Reznikova and colleagues are startling, but they have had little impact on the scientific community, perhaps as the results seem unlikely. However, the uncovering of many seemingly unlikely facts have been the cornerstone of scientific progress for centuries. Moreover, in light of the honey bee waggle dance and the complex motor displays performed by other ants (Hölldobler 1971, 1976; Hölldobler and Wilson 1978), such claims are perhaps not quite so far-fetched. Indeed, one experiment suggests that honey bees can also count, albeit to a limit of five items (Dacke and Srinivasan 2008). More concrete doubts on these findings are cast by analyses of antennation during trophallaxis (Lenoir 1982; Bonavita-Cougourdan and Morel 1984), in which no conclusive patterns could be found. Lenoir (1982) concludes that the Shannon information density of antennal contact in *Myrmica rubra* is too low to support complex directional communication. Rather, it is argued, such communication would be more suited to modulation, for example of trophallaxis time or rate. Indeed, McCabe et al. (2006) support this claim by showing that antennation patterns during trophallaxis correlate with food quality and colony hunger levels in the ant *Camponotus mus*.

111   However, the communication periods observed by Reznikova et al. included more than just

112   trophallaxis, and Reznikova and Ryabko (1994, 2001) argue that numerical information is transmitted

113   by the duration of antennation, not the pattern of antennal strikes, as assumed by Lenoir. Indeed,

114   Reznikova et al. explicitly tested for, and found no evidence of, tactile communication of direction in

115   *M. rubra* (Reznikova and Ryabko 1994). Lastly, a major reason for the lack of acceptance of

116   antennation as a directional communication method is that, unlike the honey bee waggle dance, the

117   underlying mechanism has not been elucidated, and thus this putative communication system

118   remains a 'black box' (Reznikova 2007).

119   From a theoretical standpoint, the additional benefit of such a communication system is not

120   wholly clear. Chemical recruitment systems are already available to these ants, although their

121   reliance on pheromonal recruitment may vary (e.g. (Aron et al. 1993; von Thienen et al. 2014).

122   Antennation may add another source of information to the large array of information sources which

123   ants are known to use when making directional decisions (Czaczkes et al. 2015b). It may also be that

124   an additional physical system could help prevent ant colonies becoming 'trapped' by outdated

125   pheromone trails or memories, by acting to counter such information (Goss et al. 1989; Beckers et al.

126   1990; Czaczkes et al. 2016b).

127   The phenomenon of transfer of directional information via physical contact was investigated

128   in a different species of ant, *Lasius niger* in the doctoral thesis of Evison (2008). This study appeared

129   to suggest that ant-ant communication could convey directional information in this species, but in a

130   far more modest manner (e.g. 'go left', or 'go left then left', but not 'go left then right'), and with

131   more modest accuracy: 66-69% accuracy on a single bifurcation. This accuracy was somewhat lower

132   than the accuracy of foragers that had other information cues, such as visual memory and trail

133   pheromone (Evison 2008; Evison et al. 2008), even after having made only one previous visit to a

134   food location (Grüter et al. 2011; Czaczkes et al. 2015a), and lower than the trail following accuracy

135   of *L. niger* for moderately strong trails (Evison et al. 2008; von Thienen et al. 2014; Czaczkes et al.

136   2016a). Again, the results of Evison (2008) were critically received, and were published only in thesis

137   form. Here, we make a collaborative effort between three laboratory groups to add weight to the

138   findings of Reznikova et al., in an attempt to clarify this enigmatic phenomenon. Stringent control

139   experiments suggest that the effect initially found by three of the groups may have been

140   confounded. This study is therefore an important addition to the curious case of directional

141   information transfer via physical contact in ants

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Methods

Three experiments were run in total: an initial experiment which was later found to be flawed (experiment 0, see supplement S1 for details), an experiment in which all factors were adequately controlled (experiment 1), and a confirmatory experiment run in a different laboratory to experiment 1 (experiment 2). Full details of experiment 1 will be presented below, followed by a more concise description of experiment 2. Full details of experiment 0 are presented in supplement S1.

Study species and animal maintenance for experiments 1

We used 10 queenless colony fragments of the black garden ant, *Lasius niger* (Linnaeus), collected in 2014 from eight different colonies on the University of Regensburg campus. Each colony was housed in a plastic box (40×30×20cm) with a layer of plaster on the bottom. Each box contained a circular plaster nest (14cm diameter, 2cm high). Colonies contained c. 1000 workers and small amounts of brood. The ants were fed three times per week with Bhatkar diet, a mixture of egg, agar, honey and vitamins (Bhatkar and Whitcomb 1970). Colonies were deprived of food for four days prior to each trial to give high and consistent motivation for foraging and pheromone deposition. Water was provided *ad libitum.*

Experimental procedure

Overview

In all experiments ants that knew the location of a food source at the end of a T-maze (henceforth "informed ants") were allowed to make contact with ants that did not know the food location henceforth "contacted naïve ants". The contacted naïve ants were then tested for their arm choice on the T-maze. If information acquired by the informed ants is transferred to the contacted naïve ants, we expect these ants to choose the correct arm significantly more often than chance. In this experiment, as a control, the arm choice of uncontacted naïve ants (which were not allowed to make contact with an informed ant) was tested.

Food location learning in *L. niger* is rapid but not instantaneous. On average, foragers require 2-3 visits to a food source on one arm of a T-maze to make over 95% correct decisions (Grüter et al. 2011; Czaczkes and Heinze 2015). Thus, to ensure that informed ants were indeed informed, we required them to make at least 3 visits to the food source before information transfer was tested.

Lastly, *L. niger* workers make extensive use of pheromone trails to guide nestmates to food sources (Beckers et al. 1993; Evison et al. 2008). So as to test only for ant-ant physical communication,

176 contamination by trail pheromone must be entirely eliminated. Our first attempt to do this failed

177 (see supplement S1). Thus, in this experiment separate T-mazes were used for informed and naïve

178 ants.

179 Detailed description of methods – experiment 1

180 The experiment was carried out in a laboratory space with many high contrast objects which could

181 act as landmarks. The experimenter always sat at the head end of the apparatus. A colony was

182 connected to the testing apparatus via a paper covered drawbridge. The apparatus was constructed

183 out of Perspex, and consisted of two 80mm long, 5mm wide paths (the 'communication section'), an

184 additional 80mm long path (the 'buffer section') and a T-maze (see figure 1). The stem of the T-maze

185 was 150mm long and 5mm wide, and the head was 220mm long and 20mm wide. The entire

186 apparatus was raised on stilts over water moats, to prevent ants from escaping. Two identical T-

187 mazes were constructed arranged next to each other on a board. This allowed the T-mazes to be

188 rapidly exchanged by sliding the board back and forth. One of the T-mazes was used exclusively for

189 the informed ants, and the other exclusively for the naïve ants. The entire apparatus was covered

190 with disposable paper overlays. The stem overlays had been kept in the nest for at least 24 hours

191 prior to use, to ensure that they were marked with colony-specific home range markings and

192 encourage direct walking and reduce U-turning (Devigne and Detrain 2006; Lenoir et al. 2009). A

193 drop of 1M sucrose solution on a 20x20mm acetate sheet was placed at the end of one arm of the T-

194 maze and acted as a sugar feeder.

195 Several ants were allowed onto the apparatus, and the first two to find the feeder were marked

196 individually on the abdomen with acrylic paints. These ants would become the informed ants. All

197 other ants were removed from the apparatus. The marked ants were allowed to feed, return to the

198 nest, unload the sucrose, and make three more return visits to the feeder. During this initial training

199 phase, no other ants were allowed onto the apparatus. The paper overlays on the T-maze head, but

200 not the stem, were replaced with unmarked paper every time the ants walked over them. This was

201 done so as to ensure that the informed ants had to rely on their memories for navigation, rather than

202 their previously deposited pheromone trail. The maze was cleaned with ethanol after every 5 return

203 visits of the informed ants to remove any traces of pheromone which may have reached the plastic.

204 After the informed ant had fed for the fourth time and was about to return to the nest, several naïve

205 ants were allowed onto the bridge and one of them was further allowed onto the first platform of

206 the meeting section. As soon as the informed ant stepped onto the second platform, the segments

207 were connected to allow physical contact between the two ants. Ants could thus make contact at any

208 point on the communication sections, or occasionally on the buffer section. Data were collected from

209 contacted naïve ants only if they were contacted by the informed ant with both antennae on the

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head or antennae. The interactions between informed ant and contacted naïve ant lasted no longer than c. 1 second in the majority of the cases, and consisted of a stereotypical movement sequence- As soon as the ants touched each other with their antennae, they stopped running and occasionally even recoiled slightly. They then turned their heads toward each other and stroked the head of the opposite ant a few times with their antennae, after which both ants proceeded on their way. The contacted naïve forager sometimes turned its head after the returning ant, but quickly moved on in the direction of the food source. A few informed ants seemed to consistently avoid stopping for the interaction and ran past the outbound ants with very little interaction. No data were collected from these interactions; data was only collected from ants when they were contacted by the informed ant with both antenna on the head or the antenna.

After contact had been made, the informed ant was allowed to proceed back to the nest, and the outbound naïve ant was immediately allowed onto the buffer section. The T-maze the informed ant had walked on was then replaced by the naïve ant T-maze, and the naïve ant was allowed from the buffer section onto the T-maze. We recorded the initial decision of the naïve ant using decision lines located 4cm away from the middle line. We also recorded which end of the T-maze the informed ant reached first (henceforth the final decision). An ant was considered as having made a decision when both of its antennae crossed the decision line or the end of the T-maze head respectively. Additionally, we also recorded the delay from ant-ant contact to reaching the T-head and end of the maze. If an ant did not make a decision within 90 seconds after contacting the informed ant, it was considered not motivated and rejected for data collection. 15 out of 500 (=3%) ants were rejected for this reason. After the ant reached the end of the maze it was removed from the experiment and not reintroduced back into the colony, to prevent pseudoreplication.

The position of the feeder, and whether a control or an ant-ant contact trial was run, was varied between trials, and arranged in such a way that all colonies were tested with all side and control permutations equally, but with all permutations spaced equally over the course of the experiment. We aimed to test 20 ants per trial. In total 460 ants over 24 trials were tested with ant-ant contact, and 438 ants over 23 trials were tested in the control treatment (no contact).

Experiment 0

A similar experiment was carried out prior to experiment 1, which differed in some key methodological details, and thus failed to adequately control for trail pheromone contamination. For a detailed description of the methodological differences between these experiments, see online supplement 1.

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Confirmatory experiment on *Lasius neglectus* - Experiment 2

Concurrent to experiment 1 being run at the University of Regensburg by SP & TJC, PBB & EJHR were carrying out very similar experiments at the University of York. Initial pilot results seemed to suggest an effect of ant-ant communication on direction choice accuracy, but similar issues to those described for experiment 0 (see online supplement) likely played a role. To confirm the lack of effect we describe in experiment 1, a confirmatory experiment was carried out in the University of York by SP, PBB & EJHR. The methods used differed slightly due to differences in working style between the two labs. However, the key method of using different, sliding T-mazes for the informed and naïve ants was maintained. Rather than describe the methods in full, we will only describe the differences in experimental design between this experiment and experiment 1.

Study species and animal maintenance

Four queenless *Lasius neglectus* colonies, collected in 2015 at Hidcote, Gloucestershire were used in the experiment. Colonies contained between 500 and 2000 workers and small amounts of brood. Colonies were fed 3 times per week on a 50% honey solution and a chopped mealworm. Colonies were deprived of food for 3 - 5 days prior to testing.

Experimental procedure

All experiments were carried out at the University of York. C. 25% of the data was collected by SP, who collected the data for the other two experiments described. The remainder were collected by PBB.

Rather than having separate test and control trials, in this experiment naïve ants were simply brought onto the apparatus as the informed ants were returning. No attempt to force contact between the naïve and informed ant was made. Naïve ants which made contact with the informed ants were considered contacted naïve ants, and ants which by chance did not contact the informed ant were considered controls (uncontacted naïve ants). As such, no communication section was used in the experimental setup (see figure S5). Deliberate control trials, in which uncontacted naïve ants were tested after the informed ant had been removed, were also carried out. Decision lines were drawn 25mm from the centre of the T-maze. The T-maze stem did not have a constriction. Paper overlays were not used on the apparatus but the T-maze was cleaned with 80% ethanol between replicates.

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In this experiment, rather than using two highly informed ants, which make many return visits to the feeder, each informed ant only made one visit to the feeder. Thus, an ant was allowed onto the experimental setup, allowed to find the sucrose and drink, and as it returned a naïve ant was brought onto the experimental setup and allowed to contact the informed ant on the stem of the T maze. The informed ant was then removed just before it left the T-maze, and prevented from returning to the nest. This method has the benefit of having a much larger range of informed ants, making each data-point more independent. However, this method has the drawback of low information certainty in the informed ant: *Lasius niger* can reliably learn the location of a feeder at the end of a T-maze in between 1 and 3 visits: After one visit foragers show between 75% and 80% accuracy (Grüter et al. 2011; Czaczkes et al. 2015a). Thus, we can assume that between 20% and 25% of ants considered 'informed' did not possess accurate information. Indeed, this might be even higher, even uninformed ants choose the correct side half the time, by chance. However, even disregarding this, and assuming 100% accurate and effective ant-ant physical communication, the maximum accuracy we could expect in this experiment is 75-80%.

Lastly, rather than using a movable bridge to bring ants onto the apparatus, ants were allowed to climb onto a toothpick in their nest, and then allowed to climb off onto the apparatus.

The number of ants tested per trial was variable, ranging from one to 22.

Statistical analysis

Statistical analyses were carried out in R 3.1.0 (R Core Team 2012) using Generalised Linear Mixed Models (GLMMs) in the LME4 package (Bates et al. 2014). Following Forstmeier & Schielzeth (2011) we included in the tested models only factors and interactions for which we had a-priori reasons for including. As multiple ants were tested per trial, we added the trial identity as a random effect. The decisions of the ants (correct/incorrect) were modelled using a binomial distribution and logit link function.

To test whether treatment affected the accuracy of the ants, we used the following model formula:

$$\text{Decision} = \text{treatment} * \text{ant order} + (\text{trialID as a random effect})$$

Ant order is the order in which the naïve ants were tested. We added this factor to test for possible pheromone contamination (see S1), as if pheromone contamination was occurring, it would result in higher accuracy for ants tested later.

306   The same model formula was used to examine both the initial and final decisions of the ants. All

307   results reported were corrected for multiple testing using the Benjamini-Hochberg (1995) method.

308   Exact binomial tests were carried out in R using the binom.test function. All binomial tests were two-

309   tailed.

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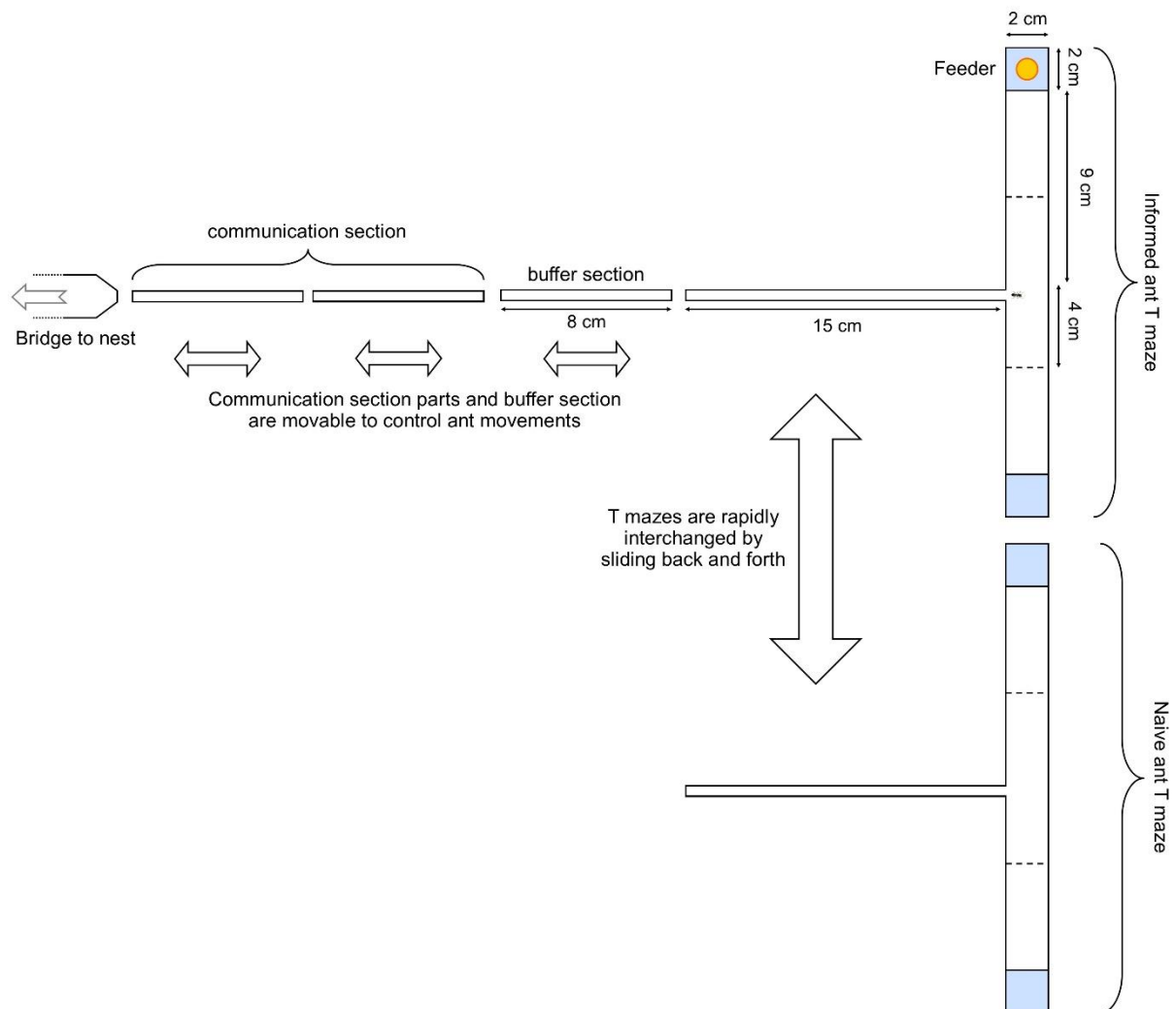
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Figure 1 – Experimental setup for experiment 1. Two marked (=informed) ants with knowledge of the feeder location are allowed to make repeated return visits to the feeder. On their return visits they may be allowed to encounter naïve ants on the communication section, by allowing a naïve ant onto the first section and the informed ant onto the second section, then joining the two sections. The T-mazes are slid along so as to replace the maze the informed ant walked on with a maze unmarked by pheromone. The contacted naïve ant is then allowed, via the buffer section, onto the maze, and its arm choice decision is noted. The figure, including ant entering the T-maze head, is to scale.

Results

Experiment 1

We found no evidence for tactile communication of direction between foraging ants. The initial choice made by the contacted naïve ants which came into contact with informed ants did not differ from random (exact binomial test, 248/460 correct decisions, probability of success 0.54, P = 0.10, see figure 2A). Whether naïve ants contacted an informed ant or not did not significantly predict decision accuracy (GLMM, Z = 0.49, P = 0.95). The order an ant was tested in, and the interaction between order and treatment, were also not significant predictors of choice accuracy (order, Z = 0.304, P = 0.95, interaction, Z = -0.103, P = 0.95).

If the final choices made by the ants is considered, the results remain qualitatively identical. Contacted naïve ants which came into contact with informed ants did not differ from random (exact binomial test, 223/460 correct decisions, probability of success 0.48, P = 0.54, see figure 2A). The treatment naïve ants underwent (contacting an informed ant or not) was not a predictor of decision accuracy (GLMM, Z = -0.84, P = 0.79). The order an ant was tested in, and the interaction between order and treatment, were also not significant predictors of choice accuracy (order, Z = 0.29, P = 0.79, interaction, Z = 0.90, P = 0.79).

The complete datasets for all experiments reported here are provided in supplement S2.
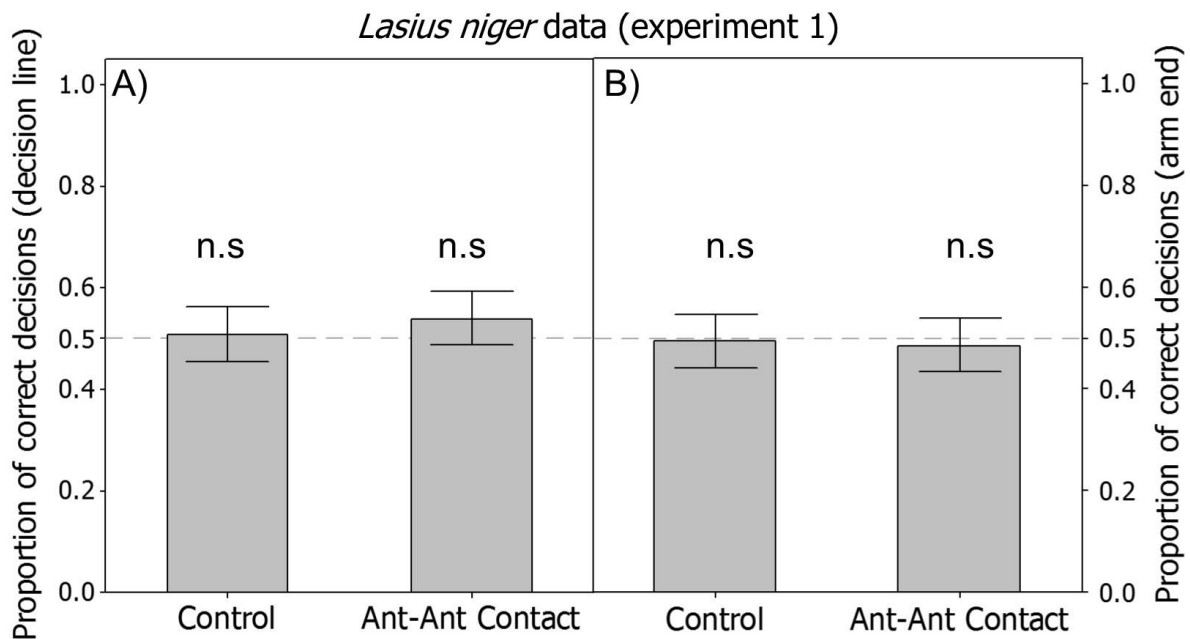
Figure 2 – No evidence of tactile communication of direction in *Lasius niger* ants. Naïve ants heading towards a food source which had made antennal contact with well-informed ants returning from a food source were no more likely to choose the correct route than naïve ants that had not made antennal contact with an informed ant. This is true both when considering the initial decision (A, 248 / 460 contacted ants and 223 / 438 control ants chose the correct arm) and the final decision (B, 223/460 contacted ants and 216 / 438 control ants chose the correct arm). Error bars show 95% confidence intervals for the mean.

Experiment 2

The proportion of correct decisions ants made on control and ant-ant contact trials was not different (GLMM, Z = 0.26, P = 0.795, see figure 3). The choices of both control and test ants did not differ from random (exact binomial test, control: 106 / 205 correct decisions, probability of success = 0.52, P = 0.675, test: 106 / 200 correct decisions, probability of success = 0.53, P = 0.437).
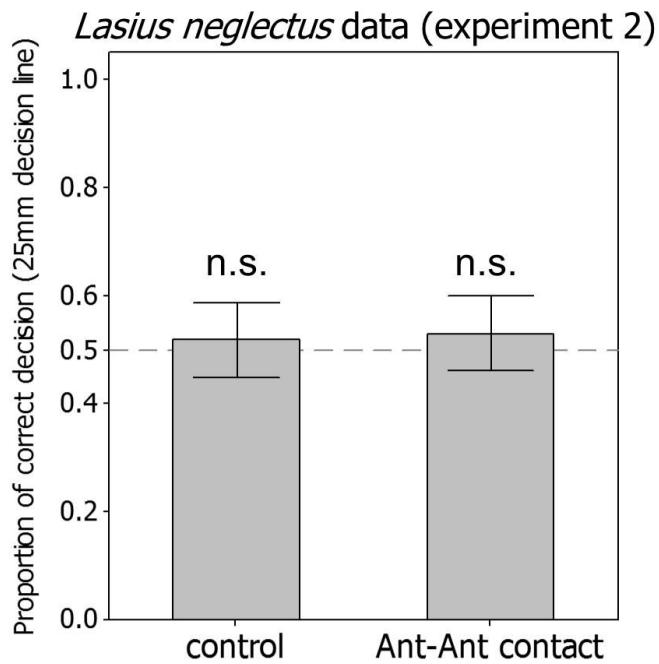
Figure 3 – Decision accuracy of *Lasius neglectus* ants in the confirmatory experiment.
The arm choice of both the control and test (ant-ant contact) ants did not differ from chance.
Error bars show 95% confidence intervals for the mean.

Experiment 0 – initial experiment with flawed experimental design.
The initial choice of naïve ants which had made contact with informed ants was correct significantly
more than half the time (exact binomial exact test, 206 / 299 correct decisions, probability of success
0.69, P < 0.0001, see figure 4A). This effect almost disappears, however, if the final decision is
considered (165 / 299 correct decisions, probability of success 0.55, P = 0.08, see figure 4B). Control
ants do not choose differently from chance either in terms of the initial decision (exact binomial
exact test, 77 / 160 correct decisions, probability of success 0.48, P = 0.69) or the final decision
(74/160 correct decision, probability of success 0.46, P = 0.384). Decision accuracy increases over the
course of the experiment (Z = 2.59, P = 0.0095, see figure S3), suggesting contamination by
pheromones over the course of each trial (see S1 for details).
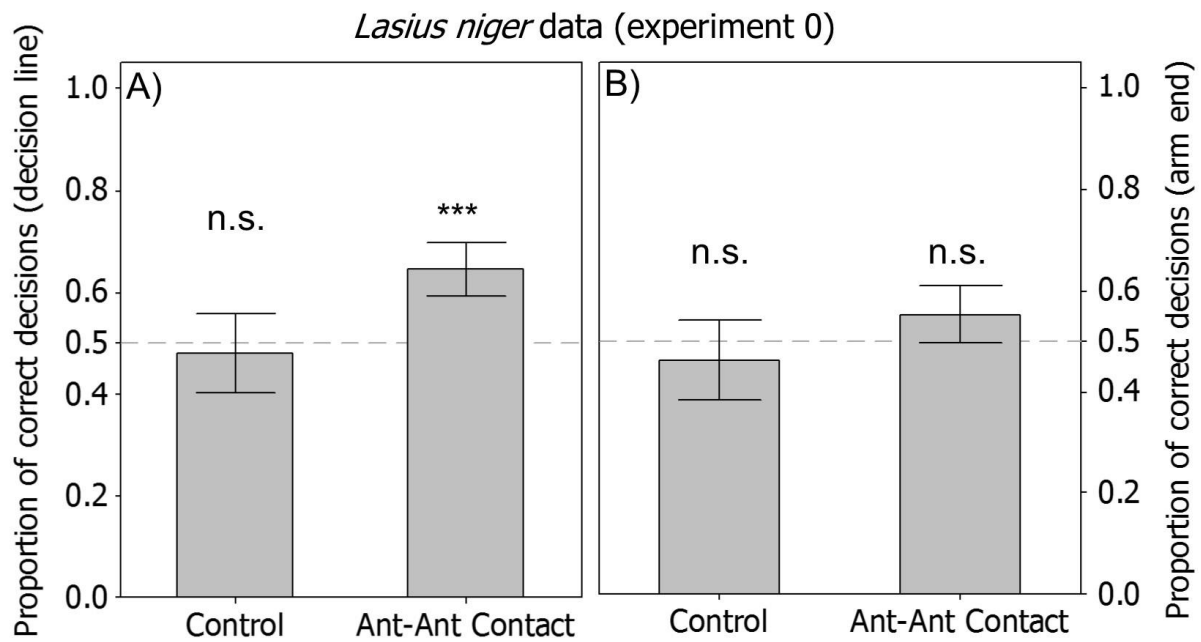
Lasius niger data (experiment 0)

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Figure 4 – Decision accuracy of *Lasius niger* ants in the initial, flawed trial. The initial
choice of contacted naïve ants (A, measured by crossing a decision line 4 cm from the centre
of the T-maze stem) were correct significantly more often than expected by chance. The
initial choices of uncontacted naïve ants (controls), and the final choice of both groups (B),
were not different from random. Error bars show 95% confidence intervals for the mean.

Discussion

Our experiments failed to find support for the hypothesis that ants can communicate food locations
by physical interaction. This null result was confirmed in both *L. niger* and in a second, independently
performed experiment using *L. neglectus*. We therefore add to the body of evidence that ants cannot
communicate direction via physical contact during foraging. We also believe that the combined effort
among our three groups is an important highlight to this almost decade long research. Each group
believed the initial positive results were sound; only the collaborative effort highlighted the
methodological flaw that led to these misleading findings.

While an initial experiment (experiment 0, see S1) seemed to find evidence for such
communication, a careful analysis of the data revealed that these results were due to a flawed
methodology. Specifically, it is likely that pheromonal contamination on the stem of the T-maze
resulted in the higher accuracy of the contacted naïve ants. We conclude this from three lines of
evidence: Firstly, the accuracy of naïve ants increases over the course of the experiment, suggesting

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393   pheromone accumulation. Secondly, the increase in accuracy is only evident when the initial decision

394   of the ants, as defined by crossing a decision line close to the junction, is considered. When the final

395   decision of the naïve ants is considered, as defined by the end of the T-maze reached first, the

396   pattern disappears. This indicates local pheromone contamination around the T-maze junction.

397   Lastly, when completely separate T-mazes are used for informed and naïve ants (as in the main

398   experiment and in the confirmatory experiment), contacted ants do no better than uncontacted ants.

399   We included a detailed analysis of the flawed experiment 0 (see supplement S1), as we feel

400   that important lessons can be learned from it. It is worth noting that pilot experiments by PBB and

401   EJHR (unpublished data) found similar results to the flawed experiment reported in S1, but that again

402   once the stringent control for pheromone contamination was implemented these effects also

403   disappeared (Experiment 2). That both groups initially failed to control the experiments properly

404   demonstrates how difficult it can be to exclude all biases in the data. It is likely that the results

405   reported by Evison (2008) are similarly flawed. In these experiments, the choice zone was replaced

406   between each trial, but the zone leading up to this was never replaced and would have been

407   contaminated with pheromones that may have biased decisions leading up to the branch point. The

408   use of disposable paper overlays to remove pheromones deposited during an experiment is a

409   widespread technique, as it is rapid, simple, and does not involve using cleaning solvents that might

410   disturb the ants. However, the results of experiment 0 suggest that this method is not sufficient to

411   ensure the complete removal of pheromone trails, especially in experiments involving many ant

412   passages.

413   Do our results also cast doubt on those of Reznikova and Raybako (1994; 2008), and

414   Novgorodova (2006)? Parallels must be drawn with caution. Firstly, Reznikova and Raybako (1994)

415   mention in passing that two species of ants tested, *Myrmica rubra* and *Formica cunicularia*, showed

416   no evidence for tactile communication of food location. Reznikova (2008) argues that tactile

417   communication of food location will only arise in ants which form very large and complex colonies,

418   and forage over very large areas, and will only be used in complex environmental situations (i.e.

419   multiple bifurcations). *L. niger* form moderately sized colonies of several thousand workers or more –

420   a comparable size to that of *F. sanguinea* (Seifert 2007)*,* in which such communication was reported.

421   Nonetheless, their territory size is smaller than that of the three *Formica* species in which physical

422   communication was found. Furthermore, while the *Formica* species and *Lasius* species all rely heavily

423   on honeydew, and must solve broadly similar problems to forage successfully, their foraging

424   organisation is no doubt different. Indeed, foraging teams consisting of specialised workers

425   performing specific roles (such as trophobiont guarding, honeydew harvesting, and honeydew

426   transporting) have been described for *F. polyctena*. *Lasius niger*, on the other hand, are reported to

427   show much less specialisation during foraging, with no stable task partitioning via 'foraging teams'

428   (Novgorodova 2015). The physical communication described by Reznikova and Ryabko (1994) relied

429   on the presence of these stable foraging teams consisting of one scout and 5-8 recruits, and scouts

430   would communicate food location only with their team mates. It is not clear why such specialisation

431   is beneficial, although it may allow long-term specialisation of different teams in different foraging

432   locations (Salo and Rosengren 2001; Czaczkes et al. 2015a). While there seems no *a priori* reason to

433   expect physical communication of food location only when robust foraging teams are present, this is

434   a possibility. Lastly, the character of the ant-ant contacts in the two studies was very different. In the

435   work of Reznikova and Ryabko (1994, 2001) and Novgorodova (2006), information transfer contacts

436   occurred mainly in the nest, and required many tens of seconds. The exact definition of 'contact

437   duration' in these studies is somewhat unclear. The contacts used in Evison (2008) and in the present

438   study occurred on the foraging platform, and lasted only a few seconds. Thus, the two different

439   groups of studies may have been studying different types of contacts.

440   In spite of the large differences between the current study and the work of Reznikova et al.,

441   our results do increase the burden of proof required to fully accept physical communication of food

442   location by ants. Our study demonstrates how easy it is to miss critical experimental flaws, resulting

443   in overlooked chemical directional information being available to the ants. While we could detect no

444   major flaws in the methodology of Reznikova and Rybako (1994) or that of Nogorodova (2006), it is

445   notoriously difficult to fully describe an experimental design in prose. With such extraordinary claims,

446   extraordinarily robust evidence must be brought forward. This may take the form of repeated video

447   documentation of these effects, or better yet, a replication of these results by an unaffiliated

448   research group. While direct replication of experiments may be unappealing to most researchers,

449   similar research in a different group of ants might be more attractive. *Oecophylla longinoda* forms

450   large, dominant colonies with complex organisation, and has been demonstrated to make extensive

451   use of motor displays (Hölldobler 1976). If physical communication of food location is to be searched

452   for in an ant group unrelated to the previous demonstrations, we feel *O. longinoda* would be a good

453   place to start.

454   In this study we set out to test whether brief contacts on a foraging trail between an

455   informed and uninformed *Lasius niger* worker transfer directional information. Our results

456   demonstrate that they do not. The difficulties we had in performing a fair experiment, despite three

457   experienced groups leading their own trajectory, highlight the importance of very stringent controls

458   for such experiments. Multi-group efforts have brought many challenging fields of research in diverse

459   topics forward. Such successful multi-group efforts may be competitive, such as in the question of

460   metacognition in animals (Smith et al. 2008) or cognitive maps in insects (Wehner and Menzel 1990;

461 Collett et al. 2013), or collaborative, for example in understanding the evolution of (eu)sociality

462 (Kennedy et al. 2017). Our results also raise the burden of proof for claims of physical communication

463 of food location in ants. However, our results do not rule out that such communication may happen

464 in other situations and in other species. Reliable, independent, well-documented replication of any

465 such findings will be necessary for claims of physical communication of location by ants to be broadly

466 accepted by the scientific community.

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