

This is a repository copy of *No evidence for tactile communication of direction in foraging Lasius ants*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/156497/>

Version: Accepted Version

Article:

Popp, Stefan, Buckham-Bonnett, Phillip, Evison, Sophie E. F. et al. (2 more authors)
(2018) No evidence for tactile communication of direction in foraging *Lasius* ants. *Insectes Sociaux*. pp. 37-46. ISSN 1420-9098

<https://doi.org/10.1007/s00040-017-0583-6>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

Stefan Popp^{1,2}, Phillip Buckham-Bonnett³, Sophie E. F. Evison^{4,*}, Elva J. H. Robinson^{3,*}, and Tomer J. Czaczkes^{1,*}

¹ Institute of Zoology, Universität Regensburg, Universitätsstraße 31, D-93053 Regensburg, Germany

² Department of Behavioral Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

³ Department of Biology and York Centre for Complex Systems Analysis, Heslington, York, YO10 5DD, UK

⁴ Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield, S10 2TN, UK

* Corresponding authors

ORCID IDs

Stephan Popp	0000-0003-4384-2294
Phillip Buckham-Bonnett	0000-0002-2102-7486
Sophie E. F. Evison	0000-0002-6210-533X
Elva J. H. Robinson	0000-0003-4914-9327
Tomer J. Czaczkes	0000-0002-1350-4975

Abstract

The idea that ants communicate when meeting on a trail is beguiling, but evidence for this is scarce. Physical communication in ants has been demonstrated to play a role as a modulator of behaviours such as alarm and recruitment. Honeybees can communicate the location of a resource using an advanced motor display – the waggle dance. However, no equivalent of the waggle dance has been described for any ant species, and it is widely believed that ants cannot communicate the location of resources using motor displays. One group of researchers report several demonstrations of such communication in *Formica* ants; however, these results have been largely ignored. More recently some evidence arose that *Lasius niger* foragers returning from a food source can communicate to outgoing foragers the direction that should be taken at the next bifurcation by means of physical contact on the trail. Here, we make a concerted effort to replicate these results. Although initial results seemed to indicate physical communication, once stringent controls to eliminate pheromone cues were put in place, no evidence for physical communication of food location could be found. This null result was replicated independently by a different research group on a closely related species, *L. neglectus*. We conclude that neither *L. niger* nor *L. neglectus* foragers communicate resource location using physical contact. Our results increase the burden of proof required for other claims of physical communication of direction in ants, but do not completely rule out this possibility.

Key words

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

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

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,

contamination by trail pheromone must be entirely eliminated. Our first attempt to do this failed (see supplement S1). Thus, in this experiment separate T-mazes were used for informed and naïve ants.

Detailed description of methods – experiment 1

The experiment was carried out in a laboratory space with many high contrast objects which could act as landmarks. The experimenter always sat at the head end of the apparatus. A colony was connected to the testing apparatus via a paper covered drawbridge. The apparatus was constructed out of Perspex, and consisted of two 80mm long, 5mm wide paths (the ‘communication section’), an additional 80mm long path (the ‘buffer section’) and a T-maze (see figure 1). The stem of the T-maze was 150mm long and 5mm wide, and the head was 220mm long and 20mm wide. The entire apparatus was raised on stilts over water moats, to prevent ants from escaping. Two identical T-mazes were constructed arranged next to each other on a board. This allowed the T-mazes to be rapidly exchanged by sliding the board back and forth. One of the T-mazes was used exclusively for the informed ants, and the other exclusively for the naïve ants. The entire apparatus was covered with disposable paper overlays. The stem overlays had been kept in the nest for at least 24 hours prior to use, to ensure that they were marked with colony-specific home range markings and encourage direct walking and reduce U-turning (Devigne and Detrain 2006; Lenoir et al. 2009). A drop of 1M sucrose solution on a 20x20mm acetate sheet was placed at the end of one arm of the T-maze and acted as a sugar feeder.

Several ants were allowed onto the apparatus, and the first two to find the feeder were marked individually on the abdomen with acrylic paints. These ants would become the informed ants. All other ants were removed from the apparatus. The marked ants were allowed to feed, return to the nest, unload the sucrose, and make three more return visits to the feeder. During this initial training phase, no other ants were allowed onto the apparatus. The paper overlays on the T-maze head, but not the stem, were replaced with unmarked paper every time the ants walked over them. This was done so as to ensure that the informed ants had to rely on their memories for navigation, rather than their previously deposited pheromone trail. The maze was cleaned with ethanol after every 5 return visits of the informed ants to remove any traces of pheromone which may have reached the plastic.

After the informed ant had fed for the fourth time and was about to return to the nest, several naïve ants were allowed onto the bridge and one of them was further allowed onto the first platform of the meeting section. As soon as the informed ant stepped onto the second platform, the segments were connected to allow physical contact between the two ants. Ants could thus make contact at any point on the communication sections, or occasionally on the buffer section. Data were collected from contacted naïve ants only if they were contacted by the informed ant with both antennae on the

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.

243

244 Confirmatory experiment on *Lasius neglectus* - Experiment 2

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

254

255 *Study species and animal maintenance*

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

260

261 *Experimental procedure*

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

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

275

276 In this experiment, rather than using two highly informed ants, which make many return visits to the
277 feeder, each informed ant only made one visit to the feeder. Thus, an ant was allowed onto the
278 experimental setup, allowed to find the sucrose and drink, and as it returned a naïve ant was brought
279 onto the experimental setup and allowed to contact the informed ant on the stem of the T maze. The
280 informed ant was then removed just before it left the T-maze, and prevented from returning to the
281 nest. This method has the benefit of having a much larger range of informed ants, making each data-
282 point more independent. However, this method has the drawback of low information certainty in the
283 informed ant: *Lasius niger* can reliably learn the location of a feeder at the end of a T-maze in
284 between 1 and 3 visits: After one visit foragers show between 75% and 80% accuracy (Grüter et al.
285 2011; Czaczkes et al. 2015a). Thus, we can assume that between 20% and 25% of ants considered
286 'informed' did not possess accurate information. Indeed, this might be even higher, even uninformed
287 ants choose the correct side half the time, by chance. However, even disregarding this, and assuming
288 100% accurate and effective ant-ant physical communication, the maximum accuracy we could
289 expect in this experiment is 75-80%.

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

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

293

294 *Statistical analysis*

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

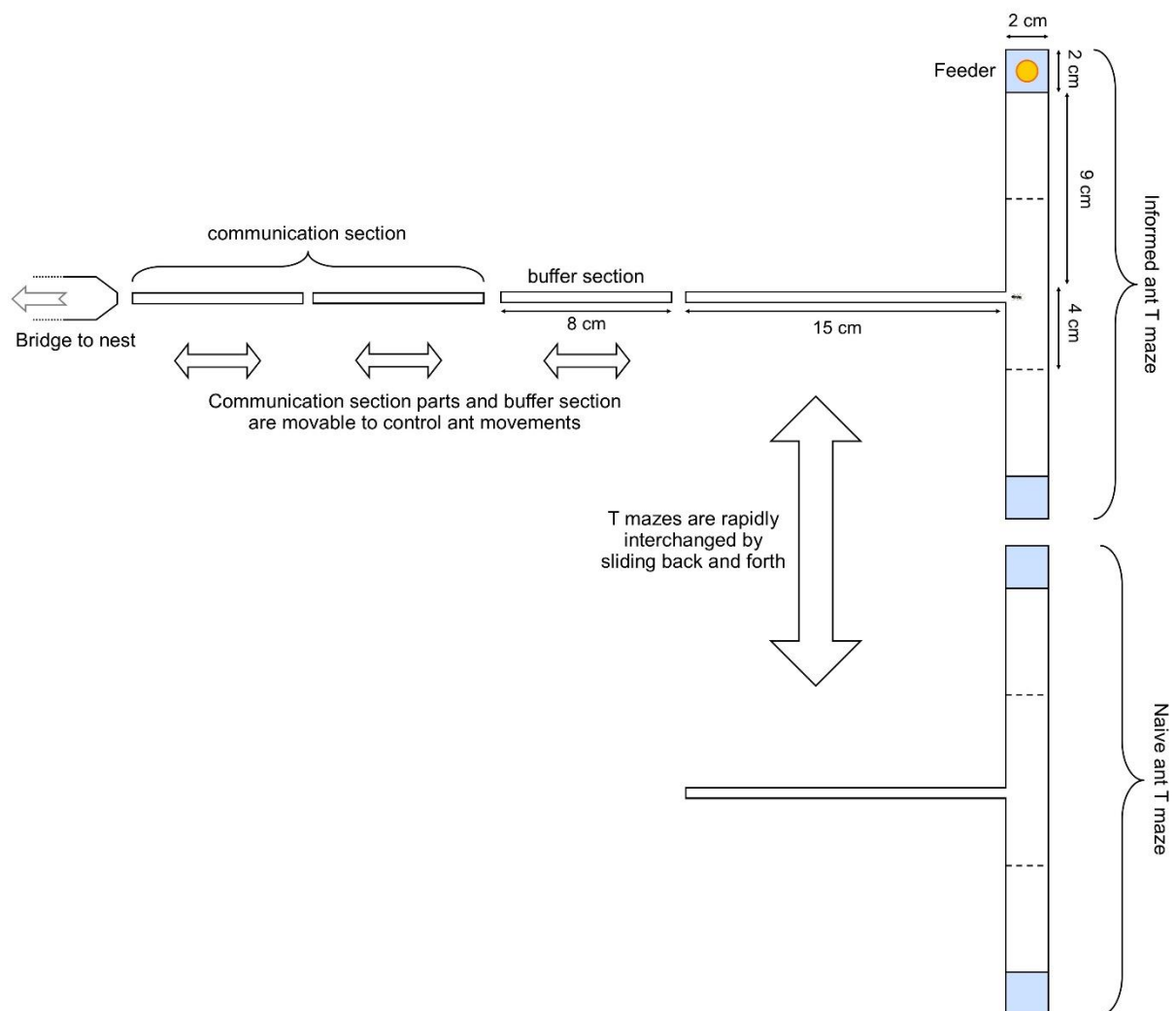
301 To test whether treatment affected the accuracy of the ants, we used the following model formula:

302
$$\text{Decision} = \text{treatment} * \text{ant order} + (\text{trialID as a random effect})$$

303 Ant order is the order in which the naïve ants were tested. We added this factor to test for possible
304 pheromone contamination (see S1), as if pheromone contamination was occurring, it would result in
305 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.

310



311

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.

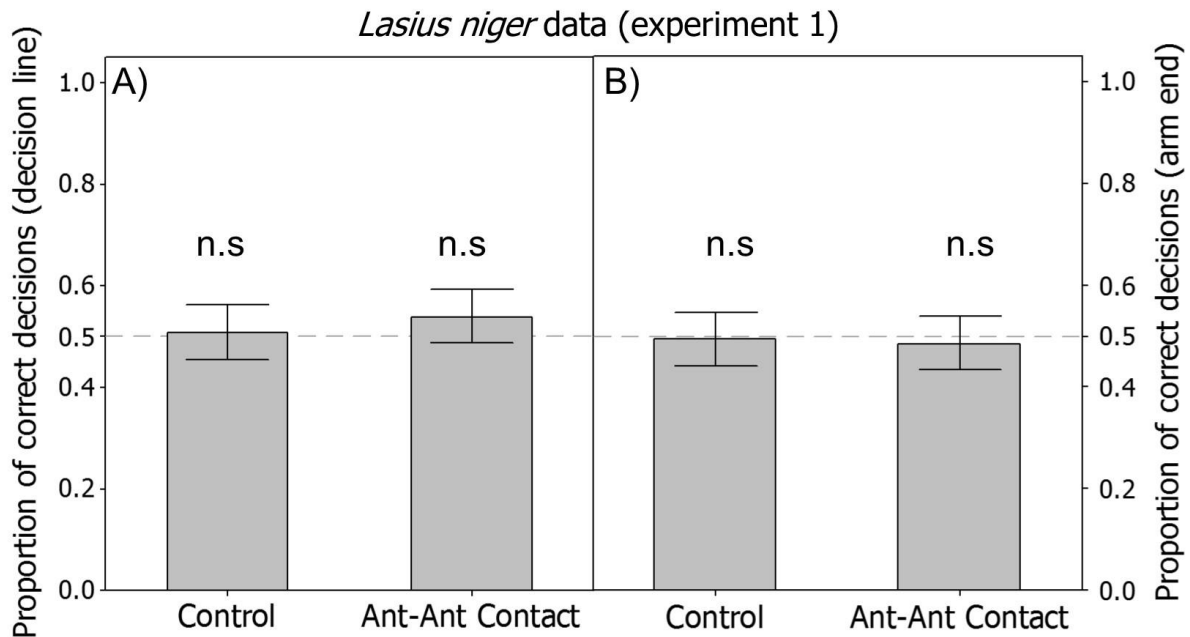


Figure 2 – No evidence of tactile communication of direction in *Lasius niger* ants. Naïve ants heading towards a food source which had made antennal contact with well-informed ants returning from a food source were no more likely to choose the correct route than naïve ants that had not made antennal 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, $P = 0.437$).

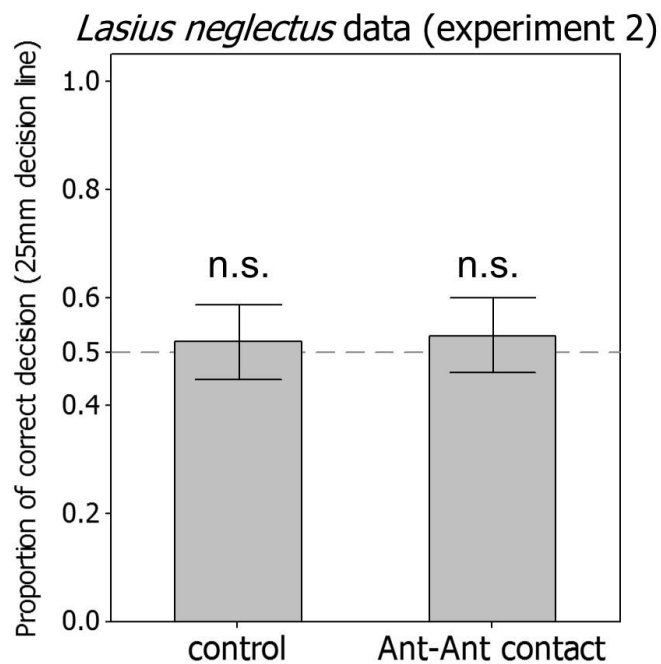


Figure 3 – Decision accuracy of *Lasius neglectus* ants in the confirmatory experiment.

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).

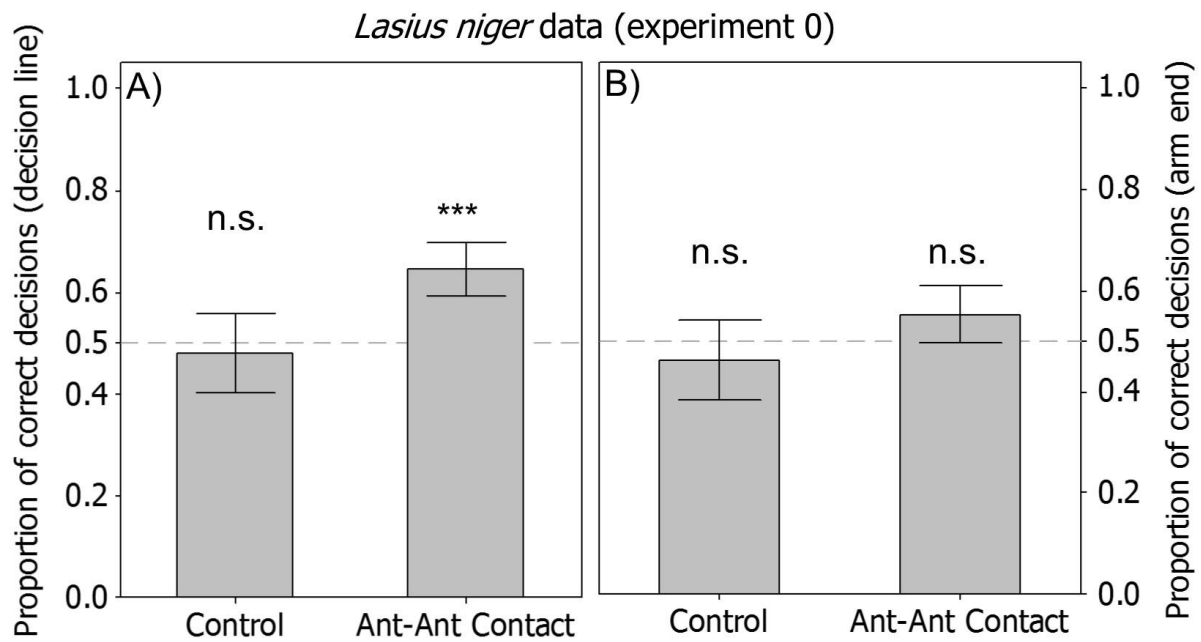


Figure 4 – Decision accuracy of *Lasius niger* ants in the initial, flawed trial. The initial choice of contacted naïve ants (A, measured by crossing a decision line 4 cm from the centre of the T-maze stem) were correct significantly more often than expected by chance. The initial choices of uncontacted 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

pheromone accumulation. Secondly, the increase in accuracy is only evident when the initial decision of the ants, as defined by crossing a decision line close to the junction, is considered. When the final decision of the naïve ants is considered, as defined by the end of the T-maze reached first, the pattern disappears. This indicates local pheromone contamination around the T-maze junction. Lastly, when completely separate T-mazes are used for informed and naïve ants (as in the main experiment and in the confirmatory experiment), contacted ants do no better than uncontacted ants.

We included a detailed analysis of the flawed experiment 0 (see supplement S1), as we feel that important lessons can be learned from it. It is worth noting that pilot experiments by PBB and EJHR (unpublished data) found similar results to the flawed experiment reported in S1, but that again once the stringent control for pheromone contamination was implemented these effects also disappeared (Experiment 2). That both groups initially failed to control the experiments properly demonstrates how difficult it can be to exclude all biases in the data. It is likely that the results reported by Evison (2008) are similarly flawed. In these experiments, the choice zone was replaced between each trial, but the zone leading up to this was never replaced and would have been contaminated with pheromones that may have biased decisions leading up to the branch point. The use of disposable paper overlays to remove pheromones deposited during an experiment is a widespread technique, as it is rapid, simple, and does not involve using cleaning solvents that might disturb the ants. However, the results of experiment 0 suggest that this method is not sufficient to ensure the complete removal of pheromone trails, especially in experiments involving many ant passages.

Do our results also cast doubt on those of Reznikova and Raybako (1994; 2008), and Novgorodova (2006)? Parallels must be drawn with caution. Firstly, Reznikova and Raybako (1994) mention in passing that two species of ants tested, *Myrmica rubra* and *Formica cunicularia*, showed no evidence for tactile communication of food location. Reznikova (2008) argues that tactile communication of food location will only arise in ants which form very large and complex colonies, and forage over very large areas, and will only be used in complex environmental situations (i.e. multiple bifurcations). *L. niger* form moderately sized colonies of several thousand workers or more – a comparable size to that of *F. sanguinea* (Seifert 2007), in which such communication was reported. Nonetheless, their territory size is smaller than that of the three *Formica* species in which physical communication was found. Furthermore, while the *Formica* species and *Lasius* species all rely heavily on honeydew, and must solve broadly similar problems to forage successfully, their foraging organisation is no doubt different. Indeed, foraging teams consisting of specialised workers performing specific roles (such as trophobiont guarding, honeydew harvesting, and honeydew transporting) have been described for *F. polyctena*. *Lasius niger*, on the other hand, are reported to

show much less specialisation during foraging, with no stable task partitioning via ‘foraging teams’ (Novgorodova 2015). The physical communication described by Reznikova and Ryabko (1994) relied on the presence of these stable foraging teams consisting of one scout and 5-8 recruits, and scouts would communicate food location only with their team mates. It is not clear why such specialisation is beneficial, although it may allow long-term specialisation of different teams in different foraging locations (Salo and Rosengren 2001; Czaczkes et al. 2015a). While there seems no *a priori* reason to expect physical communication of food location only when robust foraging teams are present, this is a possibility. Lastly, the character of the ant-ant contacts in the two studies was very different. In the work of Reznikova and Ryabko (1994, 2001) and Novgorodova (2006), information transfer contacts occurred mainly in the nest, and required many tens of seconds. The exact definition of ‘contact duration’ in these studies is somewhat unclear. The contacts used in Evison (2008) and in the present study occurred on the foraging platform, and lasted only a few seconds. Thus, the two different groups of studies may have been studying different types of contacts.

In spite of the large differences between the current study and the work of Reznikova et al., our results do increase the burden of proof required to fully accept physical communication of food location by ants. Our study demonstrates how easy it is to miss critical experimental flaws, resulting in overlooked chemical directional information being available to the ants. While we could detect no major flaws in the methodology of Reznikova and Ryabko (1994) or that of Novgorodova (2006), it is notoriously difficult to fully describe an experimental design in prose. With such extraordinary claims, extraordinarily robust evidence must be brought forward. This may take the form of repeated video documentation of these effects, or better yet, a replication of these results by an unaffiliated research group. While direct replication of experiments may be unappealing to most researchers, similar research in a different group of ants might be more attractive. *Oecophylla longinoda* forms large, dominant colonies with complex organisation, and has been demonstrated to make extensive use of motor displays (Hölldobler 1976). If physical communication of food location is to be searched for in an ant group unrelated to the previous demonstrations, we feel *O. longinoda* would be a good place to start.

In this study we set out to test whether brief contacts on a foraging trail between an informed and uninformed *Lasius niger* worker transfer directional information. Our results demonstrate that they do not. The difficulties we had in performing a fair experiment, despite three experienced groups leading their own trajectory, highlight the importance of very stringent controls for such experiments. Multi-group efforts have brought many challenging fields of research in diverse topics forward. Such successful multi-group efforts may be competitive, such as in the question of metacognition in animals (Smith et al. 2008) or cognitive maps in insects (Wehner and Menzel 1990;

Collett et al. 2013), or collaborative, for example in understanding the evolution of (eu)sociality (Kennedy et al. 2017). Our results also raise the burden of proof for claims of physical communication of food location in ants. However, our results do not rule out that such communication may happen in other situations and in other species. Reliable, independent, well-documented replication of any such findings will be necessary for claims of physical communication of location by ants to be broadly accepted by the scientific community.

Acknowledgements

Many thanks to two anonymous reviewers for comments on an earlier version of this manuscript. TJC was supported by a DFG Emmy Noether group leader grant (grant number CZ 237/1-1). PBB was supported by a NERC Industrial CASE studentship with Hymettus awarded to EJHR (NE/L008904/1).

References

- Akino T, Yamamura K, Wakamura S, Yamaoka R (2004) Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). *Appl Entomol Zool* 39:381–387.
- Aron S, Beckers R, Deneubourg J, Pasteels JM (1993) Memory and chemical communication the orientation of two mass-recruiting ant species. *Insectes Sociaux* 40:369–380. doi: 10.1007/BF01253900
- Balbuena MS, Molinas J, Farina WM (2012) Honeybee recruitment to scented food sources: correlations between in-hive social interactions and foraging decisions. *Behav Ecol Sociobiol* 66:445–452. doi: 10.1007/s00265-011-1290-3
- Bates D, Maechler M, Bolker B, et al (2014) lme4: Linear mixed-effects models using Eigen and S4.
- Beckers R, Deneubourg JL, Goss S (1993) Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *J Insect Behav* 6:751–759. doi: 10.1007/BF01201674
- Beckers R, Deneubourg JL, Goss S, Pasteels JM (1990) Collective decision making through food recruitment. *Insectes Sociaux* 37:258–267.
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 57:289–300.
- Bhatkar A, Whitcomb WH (1970) Artificial diet for rearing various species of ants. *Fla Entomol* 53:229–232.
- Bonavita-Cougourdan A, Morel L (1984) Les activités antennaires au cours des contacts trophallactiques chez la fourmi *Camponotus vagus* Scop. Ont-elles valeur de signal? *Insectes Sociaux* 31:113–131. doi: 10.1007/BF02232709

496 Collett M, Chittka L, Collett TS (2013) Spatial memory in insect navigation. *Curr Biol* 23:R789–R800.
 497 doi: 10.1016/j.cub.2013.07.020

498 Czaczkes TJ, Castorena M, Schürch R, Heinze J (2016a) Pheromone trail following in the ant *Lasius*
 499 *niger*: high accuracy and variability but no effect of task state.

500 Czaczkes TJ, Czaczkes B, Iglhaut C, Heinze J (2015a) Composite collective decision-making.

501 Czaczkes TJ, Grüter C, Ratnieks FLW (2015b) Trail pheromones: an integrative view of their role in
 502 colony organisation. *Annu Rev Entomol* 60:581–599. doi: 10.1146/annurev-ento-010814-
 503 020627

504 Czaczkes TJ, Heinze J (2015) Ants respond to a changing environment and making errors by adjusting
 505 pheromone deposition. *Proc R Soc B-Biol Sci*. doi: 10.1098/rspb.2015.0679

506 Czaczkes TJ, Salmane AK, Heinze J, Klampfleuthner FAM (2016b) Private information alone can cause
 507 trapping of ant colonies in local feeding optima. *J Exp Biol* 219:744–751. doi:
 508 10.1242/jeb.131847

509 Czaczkes TJ, Schlosser L, Heinze J, Witte V (2014) Ants use directionless odour cues to recall odour-
 510 associated locations. *Behav Ecol Sociobiol* 68:981–988. doi: 10.1007/s00265-014-1710-2

511 Dacke M, Srinivasan MV (2008) Evidence for counting in insects. *Anim Cogn* 11:683–689. doi:
 512 10.1007/s10071-008-0159-y

513 Devigne C, Detrain C (2006) How does food distance influence foraging in the ant *Lasius niger*: the
 514 importance of home-range marking. *Insectes Sociaux* 53:46–55. doi: 10.1007/s00040-005-
 515 0834-9

516 Evison SEF (2008) Foraging Organisation in Ants. PhD Thesis, University of Sheffield

517 Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and
 518 visual landmarks by the common garden ant *Lasius niger*. *Behav Ecol Sociobiol* 63:261–267.
 519 doi: 10.1007/s00265-008-0657-6

520 Farina WM, Grüter C, Díaz PC (2005) Social learning of floral odours inside the honeybee hive. *Proc R*
 521 *Soc B Biol Sci* 272:1923–1928. doi: 10.1098/rspb.2005.3172

522 Forstmeier W, Schielzeth H (2011) Cryptic multiple hypotheses testing in linear models:
 523 overestimated effect sizes and the winner’s curse. *Behav Ecol Sociobiol* 65:47–55. doi:
 524 10.1007/s00265-010-1038-5

525 Goss S, Aron S, Deneubourg JL, Pasteels JM (1989) Self-organized shortcuts in the Argentine ant.
 526 *Naturwissenschaften* 76:579–581. doi: 10.1007/BF00462870

527 Grüter C, Balbuena MS, Farina WM (2008) Informational conflicts created by the waggle dance. *Proc*
 528 *R Soc B Biol Sci* 275:1321–1327. doi: 10.1098/rspb.2008.0186

529 Grüter C, Czaczkes TJ, Ratnieks FLW (2011) Decision making in ant foragers (*Lasius niger*) facing
 530 conflicting private and social information. *Behav Ecol Sociobiol* 64:141–148. doi:
 531 10.1007/s00265-010-1020-2

532 Hölldobler B (1971) Recruitment behavior in *Camponotus socius* (Hym. Formicidae). *J Comp Physiol A*
 533 *Neuroethol Sens Neural Behav Physiol* 75:123–142. doi: 10.1007/BF00335259

- 534 Hölldobler B (1976) Recruitment behavior, home range orientation and territoriality in harvester
535 ants, *Pogonomyrmex*. Behav Ecol Sociobiol 1:3–44. doi: 10.1007/BF00299951
- 536 Hölldobler B, Wilson EO (1978) The multiple recruitment systems of the african weaver ant
537 *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae). Behav Ecol Sociobiol 3:19–60.
538 doi: 10.1007/BF00300045
- 539 Hölldobler B, Wilson EO (1990) The Ants. Springer Verlag, Berlin, Heidelberg
- 540 Huber P (1810) Recherchez sur les Mœurs des Fourmis Indigenes. JJ Paschoud, Paris
- 541 Kennedy P, Baron G, Qiu B, et al (2017) Deconstructing Superorganisms and Societies to Address Big
542 Questions in Biology. Trends Ecol Evol. doi: 10.1016/j.tree.2017.08.004
- 543 Le Breton J, Fourcassie V (2004) Information transfer during recruitment in the ant *Lasius niger* L.
544 (Hymenoptera: Formicidae). Behav Ecol Sociobiol 242–250.
- 545 Lenoir A (1982) An informational analysis of antennal communication during trophallaxis in the ants
546 *Myrmica Rubra* L. Behav Processes 7:27–35.
- 547 Lenoir A, Depickère S, Devers S, et al (2009) Hydrocarbons in the ant *Lasius niger*: from the cuticle to
548 the nest and home range marking. J Chem Ecol 35:913–921. doi: 10.1007/s10886-009-9669-6
- 549 McCabe S, Farina W, Josens R (2006) Antennation of nectar-receivers encodes colony needs and
550 food-source profitability in the ant *Camponotus mus*. Insectes Sociaux 53:356–361. doi:
551 10.1007/s00040-006-0881-x
- 552 Novgorodova TA (2006) Experimental investigation of information transmission in *Formica pratensis*
553 (Hymenoptera, Formicidae) using “binary tree” maze. Entomol Rev 86:287–293. doi:
554 10.1134/S0013873806030043
- 555 Novgorodova TA (2015) Organization of honeydew collection by foragers of different species of ants
556 (Hymenoptera: Formicidae): Effect of colony size and species specificity. Eur J Entomol
557 112:688–697. doi: 10.14411/eje.2015.077
- 558 Ozaki M, Wada-Katsumata A, Fujikawa K, et al (2005) Ant nestmate and non-Nestmate discrimination
559 by a chemosensory sensillum. Science 309:311–314. doi: 10.1126/science.1105244
- 560 R Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for
561 Statistical Computing, Vienna, Austria
- 562 Reznikova Z (2008) Experimental paradigms for studying cognition and communication in ants
563 (Hymenoptera: Formicidae). Myrmecol News 11:201–214.
- 564 Reznikova Z (2017) Studying Animal Language Without Translation: An Insight from Ants. Springer,
565 Cham, Switzerland
- 566 Reznikova Z (2007) Dialog with black box: using Information Theory to study animal language
567 behaviour. Acta Ethologica 10:1–12.
- 568 Reznikova Z, Ryabko B (1994) Experimental study of the ants’ communication system with the
569 application of the Information Theory approach. Memorab Zool 48:219–236.
- 570 Reznikova Z, Ryabko B (2001) A study of ants’ numerical competence.

571 Roces F (1990) Olfactory conditioning during the recruitment process in a leaf-cutting ant. *Oecologia*
572 83:261–262. doi: 10.1007/BF00317762

573 Roces F (1994) Odour learning and decision-making during food collection in the leaf-cutting
574 ant *Acromyrmex lundi*. *Insectes Sociaux* 41:235–239. doi: 10.1007/BF01242294

575 Salo O, Rosengren R (2001) Memory of location and site recognition in the ant *Formica uralensis*
576 (Hymenoptera : Formicidae). *Ethology* 107:737–752.

577 Seifert B (2007) *Die Ameisen Mittel-und Nordeuropas*, Augsburg. Lutra

578 Smith JD, Beran MJ, Couchman JJ, Coutinho MVC (2008) The comparative study of metacognition:
579 Sharper paradigms, safer inferences. *Psychon Bull Rev* 15:679–691. doi:
580 10.3758/PBR.15.4.679

581 Sudd JH (1967) *An introduction to the behaviour of ants*. Edward Arnold, London

582 von Frisch K (1923) Über die "Sprache" der Bienen. *Zool Jb Physiol* 40:1–186.

583 von Frisch K (1967) *The dance language and orientation of bees*. Harvard University Press

584 von Thienen W, Metzler D, Choe D-H, Witte V (2014) Pheromone communication in ants: a detailed
585 analysis of concentration-dependent decisions in three species. *Behav Ecol Sociobiol*
586 68:1611–1627. doi: 10.1007/s00265-014-1770-3

587 Wasmann E (1905) *Comparative studies in the psychology of ants and of higher animals*, 2nd edn. B.
588 Herder, London

589 Wehner R, Menzel R (1990) Do insects have cognitive maps? *Annu Rev Neurosci* 13:403–414.

590

591