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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,*}

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

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

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

⁴Animal 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

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.

38

39 **Key words**

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

42

43 **Introduction**

44

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

48

49 An observation made by almost anybody who has ever watched ants forage is that ants encountering
50 nestmates on a trail will often pause and make antennal contact. As observers, we cannot help but
51 imagine that some form of communication is taking place. There is strong evidence that several ant
52 species use a series of motor displays to modulate their recruitment behaviour (Hölldobler 1971;
53 Hölldobler and Wilson 1978, 1990), such as priming nestmates to follow pheromone trails, or
54 signalling that a pheromone trail leads to a food source or a nest site (Hölldobler 1971). As ant trails
55 often form a branching network of paths, and much ant foraging occurs on plants (which again
56 constitute a ramifying system), it seems plausible that some sort of directional signalling of food
57 location would lead to more efficient foraging. This hypothesis was indeed suggested over two
58 centuries ago (Huber 1810) and found support from the eminent myrmecologist Erich Wasmann
59 (1905). In light of Karl von Frisch’s remarkable discovery of the honey bee waggle dance (von Frisch
60 1923, 1967), such a supposition seemed a lot more reasonable. Undoubtedly, ants meeting on a trail
61 ascertain each other’s colony identity (Akino et al. 2004; Ozaki et al. 2005). Odour cues from
62 successful ants returning to the nest are also likely to be gathered by the outgoing ant, which can
63 inform the foragers as to what type of food is available (Roces 1990, 1994; Le Breton and Fourcassie
64 2004). It is likely that odour cues on returning foragers can trigger previously learned associations
65 between food odours and foraging locations (Czaczkes et al. 2014), in a manner similar to odour cue
66 transfer via trophallaxis in honey bees (Farina et al. 2005; Grüter et al. 2008; Balbuena et al. 2012).
67 Despite the temptation to assume that more than simple cue-sensing is occurring during ant-ant
68 interactions, there remains very little support for anything more complex, such as signal exchange
69 (Sudd 1967). In their landmark book, Hölldobler and Wilson (1990) state that “ants antennate
70 nestmates in order to smell them, not to inform them”.

71

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

76 *sanguinea*) were able to communicate complex directional information to other foragers via physical
77 contact. Forager groups that could physically interact with an informed scout were able to find the
78 location of a food source at the end of a multiply-bifurcating maze much more accurately and rapidly
79 than groups that were not allowed to interact with an informed scout. These results implied that the
80 informed scout could communicate a series of turns to naïve foragers. In a second experiment
81 reported in the same paper, and replicated in Reznikova and Ryabko (2001), scout ants were allowed
82 to find a food source on one branch of a comb-like maze consisting of 25 or more branches, all
83 emerging from a single main stem in one direction. Groups of foragers subsequently contacted by
84 the informed scout then achieved remarkable accuracy in finding the food source: in one experiment
85 (Reznikova and Ryabko 2001) ant groups made zero mistakes in 117 of 152 trials. The authors
86 stressed that in every experiment steps were taken to ensure that no information apart from direct
87 physical contact from the informed scout was available to the otherwise naïve foragers. Using
88 variations of these experimental paradigms, and by measuring the time scout ants spent
89 communicating with their team of naïve foragers, Reznikova and Ryabko (1994, 2001) describe
90 further impressive information-processing feats by these ants. These include simple arithmetic
91 operations such as addition and subtraction, and information-compression abilities. Novgorodova
92 (2006) replicated some of the findings of Reznikova and Ryabko (1994) in a related species; *Formica*
93 *pratensis*. The results appeared to corroborate the previous findings, and showed that otherwise
94 naïve foragers which had contacted an informed scout spent significantly less time searching for a
95 feeder at the end of a maze than foragers that had no contact with informed scouts. However, as
96 decision accuracy was not provided, the results could equally well be explained by faster searching by
97 the contacted naïve ants.

98 The findings of Reznikova and colleagues are startling, but they have had little impact on the
99 scientific community, perhaps as the results seem unlikely. However, the uncovering of many
100 seemingly unlikely facts have been the cornerstone of scientific progress for centuries. Moreover, in
101 light of the honey bee waggle dance and the complex motor displays performed by other ants
102 (Hölldobler 1971, 1976; Hölldobler and Wilson 1978), such claims are perhaps not quite so far-
103 fetched. Indeed, one experiment suggests that honey bees can also count, albeit to a limit of five
104 items (Dacke and Srinivasan 2008). More concrete doubts on these findings are cast by analyses of
105 antennation during trophallaxis (Lenoir 1982; Bonavita-Cougourdan and Morel 1984), in which no
106 conclusive patterns could be found. Lenoir (1982) concludes that the Shannon information density of
107 antennal contact in *Myrmica rubra* is too low to support complex directional communication. Rather,
108 it is argued, such communication would be more suited to modulation, for example of trophallaxis
109 time or rate. Indeed, McCabe et al. (2006) support this claim by showing that antennation patterns
110 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

142

143

144 **Methods**

145 Three experiments were run in total: an initial experiment which was later found to be flawed
146 (experiment 0, see supplement S1 for details), an experiment in which all factors were adequately
147 controlled (experiment 1), and a confirmatory experiment run in a different laboratory to experiment
148 1 (experiment 2). Full details of experiment 1 will be presented below, followed by a more concise
149 description of experiment 2. Full details of experiment 0 are presented in supplement S1.

150

151 *Study species and animal maintenance for experiments 1*

152 We used 10 queenless colony fragments of the black garden ant, *Lasius niger* (Linnaeus), collected in
153 2014 from eight different colonies on the University of Regensburg campus. Each colony was housed
154 in a plastic box (40×30×20cm) with a layer of plaster on the bottom. Each box contained a circular
155 plaster nest (14cm diameter, 2cm high). Colonies contained c. 1000 workers and small amounts of
156 brood. The ants were fed three times per week with Bhatkar diet, a mixture of egg, agar, honey and
157 vitamins (Bhatkar and Whitcomb 1970). Colonies were deprived of food for four days prior to each
158 trial to give high and consistent motivation for foraging and pheromone deposition. Water was
159 provided *ad libitum*.

160

161 *Experimental procedure*

162 Overview

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

170 Food location learning in *L. niger* is rapid but not instantaneous. On average, foragers require 2-3
171 visits to a food source on one arm of a T-maze to make over 95% correct decisions (Grüter et al.
172 2011; Czaczkes and Heinze 2015). Thus, to ensure that informed ants were indeed informed, we
173 required them to make at least 3 visits to the food source before information transfer was tested.

174 Lastly, *L. niger* workers make extensive use of pheromone trails to guide nestmates to food sources
175 (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

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

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

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

237

238 Experiment 0

239 A similar experiment was carried out prior to experiment 1, which differed in some key
240 methodological details, and thus failed to adequately control for trail pheromone contamination. For
241 a detailed description of the methodological differences between these experiments, see online
242 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:

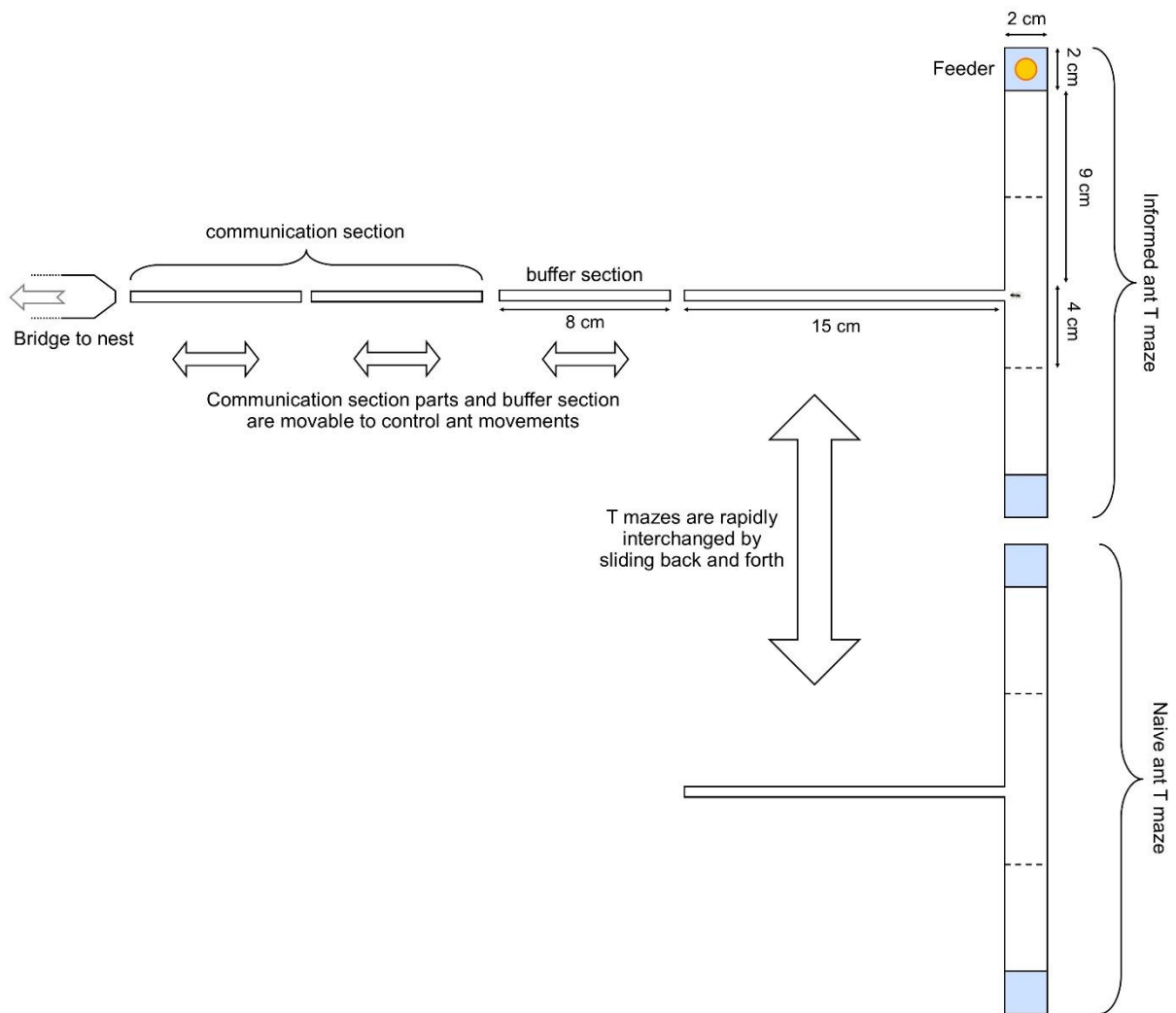
$$302 \text{ Decision} = \text{treatment} * \text{ant order} + (\text{trialID as a random effect})$$

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



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

320

321 **Results**

322

323 Experiment 1

324

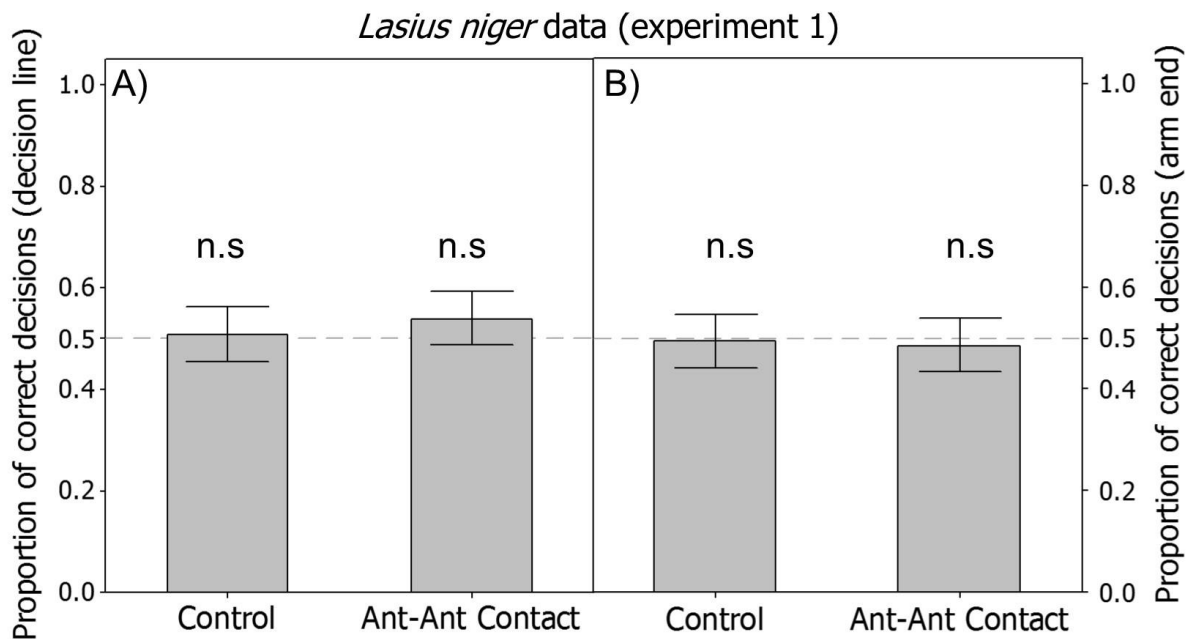
325 We found no evidence for tactile communication of direction between foraging ants. The initial
326 choice made by the contacted naïve ants which came into contact with informed ants did not differ
327 from random (exact binomial test, 248/460 correct decisions, probability of success 0.54, $P = 0.10$,
328 see figure 2A). Whether naïve ants contacted an informed ant or not did not significantly predict
329 decision accuracy (GLMM, $Z = 0.49$, $P = 0.95$). The order an ant was tested in, and the interaction
330 between order and treatment, were also not significant predictors of choice accuracy (order, $Z =$
331 0.304 , $P = 0.95$, interaction, $Z = -0.103$, $P = 0.95$).

332

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

340

341 The complete datasets for all experiments reported here are provided in supplement S2.



342

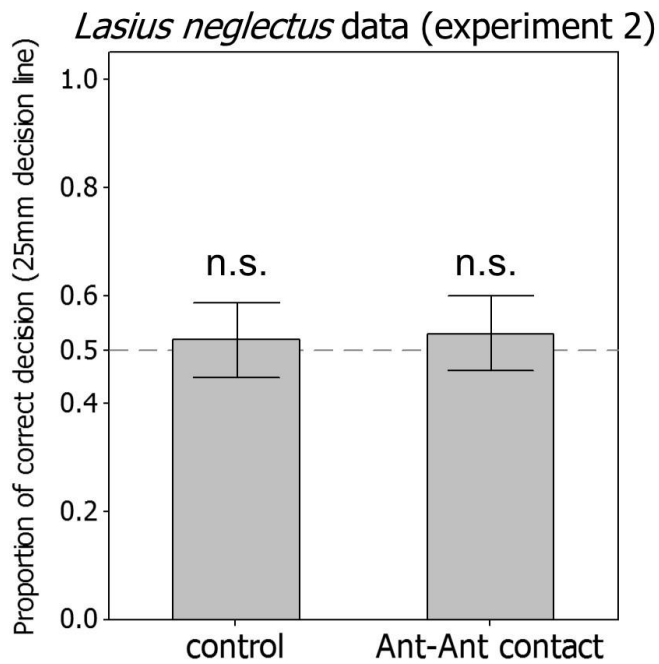
343 **Figure 2 – No evidence of tactile communication of direction in *Lasius niger* ants.** Naïve
 344 ants heading towards a food source which had made antennal contact with well-informed ants
 345 returning from a food source were no more likely to choose the correct route than naïve ants
 346 that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

350

351 Experiment 2

352 The proportion of correct decisions ants made on control and ant-ant contact trials was not different
 353 (GLMM, $Z = 0.26$, $P = 0.795$, see figure 3). The choices of both control and test ants did not differ
 354 from random (exact binomial test, control: 106 / 205 correct decisions, probability of success = 0.52,
 355 $P = 0.675$, test: 106 / 200 correct decisions, probability of success = 0.53, $P = 0.437$).

356



357

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

359 The arm choice of both the control and test (ant-ant contact) ants did not differ from chance.

360 Error bars show 95% confidence intervals for the mean.

361

362 Experiment 0 – initial experiment with flawed experimental design.

363 The initial choice of naïve ants which had made contact with informed ants was correct significantly
 364 more than half the time (exact binomial exact test, 206 / 299 correct decisions, probability of success

365 0.69, $P < 0.0001$, see figure 4A). This effect almost disappears, however, if the final decision is

366 considered (165 / 299 correct decisions, probability of success 0.55, $P = 0.08$, see figure 4B). Control

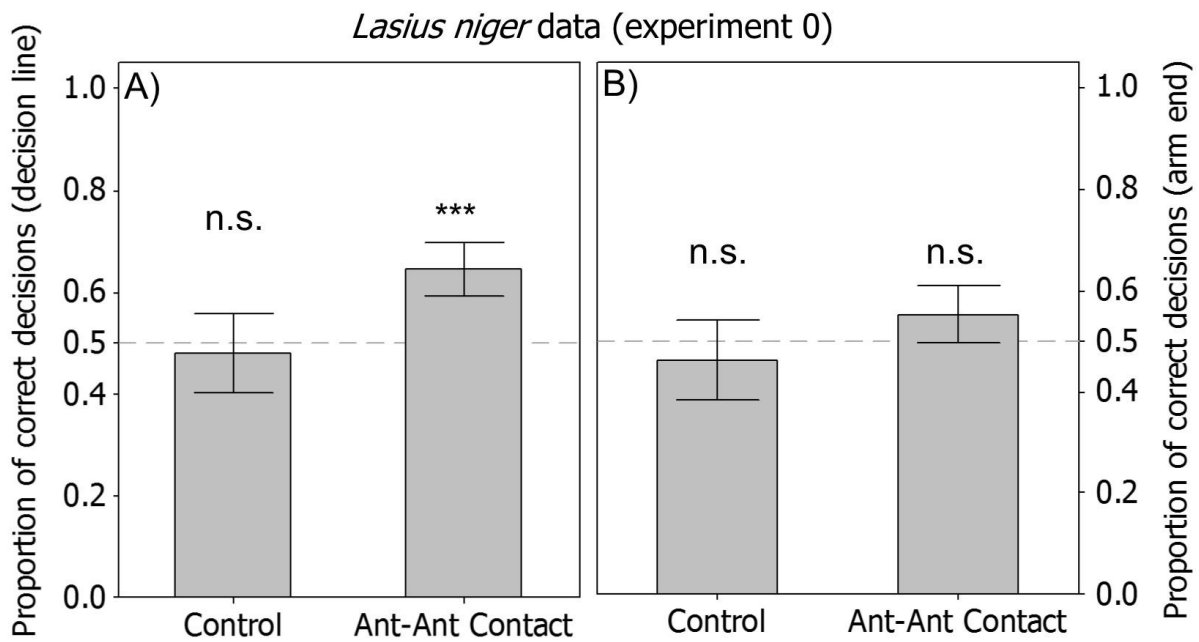
367 ants do not choose differently from chance either in terms of the initial decision (exact binomial

368 exact test, 77 / 160 correct decisions, probability of success 0.48, $P = 0.69$) or the final decision

369 (74/160 correct decision, probability of success 0.46, $P = 0.384$). Decision accuracy increases over the

370 course of the experiment ($Z = 2.59$, $P = 0.0095$, see figure S3), suggesting contamination by

371 pheromones over the course of each trial (see S1 for details).



372

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

378

379 Discussion

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

387

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

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 Ryabko (1994) or that of Novgorodova (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.

467

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