

1 **Trail-laying during tandem running recruitment in**

2 ***Temnothorax albipennis* ants**

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12
13 **Abstract**

14 **Tandem running is a recruitment strategy whereby one ant leads a single naïve nest mate**
15 **to a resource. While tandem running progresses towards the goal, the leader ant and the**
16 **follower ant maintain contact mainly by tactile signals. In this paper, we investigated**
17 **whether they also deposit chemical signals on the ground during tandem running. We**
18 **filmed tandem running ants and analysed the position of the gasters of leaders and**
19 **followers. Our results show that leader ants are more likely to press their gasters down**
20 **to the substrate compared to follower ants, single ants and transporter ants. Forward**
21 **tandem run leaders (those moving towards a new nest site) performed such trail marking**
22 **procedures three times more often than reverse tandem leaders (those moving towards**
23 **an old nest site). That leader ants marked the trails more often on forward tandem runs**
24 **may suggest that it is more important to maintain the bond with the follower ant on**
25 **forward tandem runs than on reverse tandem runs. Overall, our experiments provide**
26 **evidence that during tandem running in ants, particularly in *T. albipennis*, the leader ants**
27 **lay chemical trails probably to give the follower the freedom momentarily to stop**
28 **following and to look around to learn landmarks, and yet later successfully re-unite with**
29 **the tandem leader. The trails on the ground may serve as a safely line that improves both**
30 **the efficiency of tandem runs and their completion rates.**

31
32 **Keywords**

33 Navigation, communication, landmark, pheromone, tandem running, ant, *Temnothorax*
34 *albipennis*.

36

Introduction

37 Social insects use various types of communication to influence one another's behaviour
38 often so that another individual can also utilize useful resources (see review in Billen 2006).
39 Such communication leads to the spread of information among members of a colony (von
40 Frisch 1973; Jackson and Ratnieks 2006). In ants, communication via pheromone trails is
41 common and it is probably especially frequent in species with large colony sizes (Franks et al.
42 1991; Witte and Maschwitz 2002; Planqué et al. 2010; Loreto et al. 2013). The reason large
43 ant colonies use recruitment pheromone trails rather than one-to-one communication (see
44 Franks and Richardson 2006) is that in a large colony such broadcasting is likely to be efficient.
45 This is because with so many ants present the information is likely to be encountered and acted
46 on and conversely is unlikely to be lost. In fact, when there are many ants present, such
47 broadcast information can be rapidly reinforced if it is useful (Witte and Maschwitz 2002;
48 Jackson and Ratnieks 2006; Collignon and Detrain 2010; Czaczkes et al. 2013). By contrast,
49 in a small society information could easily be lost because with low numbers no ant may be
50 available to encounter a timely and pertinent pheromone trail. Therefore, one-to-one
51 communication, of which tandem running is an example, is a more effective solution for ants
52 living in small colonies (Franks and Richardson 2006). Moreover, tandem running is now of
53 general interest. It is widely known in the animal behaviour and behavioural ecology literature
54 as a prime example of teaching, orientation and social decision-making (e.g., Davies et al.
55 2012).

56 Recruitment by tandem running is important to many species of ant (Wilson 1959;
57 Hölldobler et al. 1974; Möglich et al. 1974; Lane 1977; Hölldobler and Traniello 1980;
58 Traniello and Hölldobler 1984; Pratt 2008). Various studies have been conducted in order to
59 understand the mechanisms of tandem running (Hölldobler et al. 1974; Maschwitz et al. 1974;
60 Möglich 1979; Traniello and Hölldobler 1984; Franks and Richardson 2006; Richardson et al.
61 2007). Previous study has shown that in certain species a leader ant may display “tandem
62 calling” behaviour by secreting pheromones into the air from its gaster to attract and recruit a
63 follower ant (Möglich et al. 1974; Möglich 1979). Researchers have also discovered that
64 feedback between leaders and followers occurs during tandem running such that each adjusts
65 their speed so that the gap between them does not become too great (Franks and Richardson
66 2006). This strategy reduces the chances that they will lose one another before reaching their
67 goal.

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69 During nest emigration in the ant *T. albipennis*, it is common to observe forward and
70 reverse tandem running (Franks et al. 2009). In forward tandem running, one ant leads one
71 other from the old nest to the new one and the follower maintains intermittent contact with its
72 leader by tapping the leader with her antennae. During nest emigrations such tandem running
73 is followed by social carrying. Typically the switch from tandem running to social carrying
74 occurs when a quorum threshold has been met in a new nest (Pratt 2005). Later in an
75 emigration, tandem runs may originate at the new nest and return to the old one (Franks et al.
76 2009). This is known as reverse tandem running (Richardson et al. 2007; Planqué et al. 2007;
77 Planqué et al. 2010). While forward tandem runs function mainly to teach naïve ants a route
78 between the old and the new nests (Franks and Richardson 2006), reverse tandem runs help to
79 speed up emigrations (Planqué et al. 2007). A need for this could arise if forward tandem runs
80 have been limited, as may occur when a colony has used a low quorum threshold in an
81 emergency (Planqué et al. 2007), or tandem run followers are in short supply at the old nest but
82 not at the new one (Franks et al. 2009).

83

84 Despite extensive studies of tandem running in *T. albipennis* (see review in Franklin
85 2014), very little is known about possible mechanistic differences between forward and reverse
86 tandem runs. An important question is whether or not tandem run leaders (or indeed followers)
87 lay trails on the ground during tandem running? Although it has been shown that these ants
88 produce an attractive tandem run pheromone (Möglich et al. 1974; Möglich 1979), it is not
89 clear if this is ever deposited on the substrate. *Temnothorax* ants lay individually specific
90 orientation trails rather than recruitment pheromone trails (Maschwitz and Buschinger 1986;
91 Aron et al. 1988; Mallon and Franks 2000). Thus, it is also possible that tandem run followers
92 may lay their own trail pheromones as they track their leader. Pratt et al. (2001) showed that in
93 an emigration experiment, pheromone trails are used by *T. albipennis* ants. Removal of an
94 acetate sheet that lay between the old nest and a new nest confused the ants. This indicates that
95 they lay a pheromone trail onto the substrate and may use it as one of their navigational cues
96 (Pratt et al. 2001). However, we still do not know whether these trails are laid mainly by single
97 ants or mostly by the members of tandem pairs.

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99 In another fairly closely related myrmicine ant, *Leptothorax acervorum*, tandem
100 leaders are known to extend their stings slightly whilst tandem running (Möglich 1979). This
101 may indicate that this ant secretes a tandem pheromone either from its poison gland or its
102 Dufour's gland. However, Möglich (1979) also noted that the sting of the leader was not

103 dragged over the surface as it is in other species that lay chemical trails. Thus it is far from
104 clear that tandem leaders continually secrete and deposit pheromones on the substrate during
105 tandem running. Hence, we believe that further investigations into the mechanics of the
106 behaviours that are utilised within tandem running are much needed.

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108 Fortunately, because the behaviour of pheromone depositing ants is highly stereotyped,
109 it is possible to use postural cues to infer if pheromone trails are being produced (Hölldobler et
110 al. 1974; Maschwitz and Buschinger 1986). Such postural cues can be highly informative
111 (Hölldobler and Wilson 1990). For example, if at some point during tandem running a leader
112 ant releases ‘calling pheromone’, typically one should see the leader’s gaster raised to a much
113 higher position than its normal horizontal one: i.e. the posture is very likely to be similar to that
114 exhibited by *L. acervorum* during tandem calling (Möglich et al. 1974). In addition, one should
115 expect to see that the leader’s gaster touches, or is dragged over, the substrate if such an ant is
116 laying a trail. Thus, one good way to study whether the ants secrete ‘calling pheromone’ or
117 whether they lay pheromone trails on the substrate during tandem running is by observing the
118 positions of the gasters of the ants during tandem running and comparing them with the gaster
119 positions of non-tandem running ants. We are aware, of course, that even if a tandem running
120 ant’s gaster is deployed downward so that it brushes the substrate, one cannot be completely
121 sure that this ant is laying a chemical trail. However, we can be fully confident that if the gaster
122 is held by the ant in a horizontal or higher position so that the gaster tip or sting cannot touch
123 the substrate the ant cannot be laying a trail from its poison gland or Dufour’s gland. Hence,
124 our approach is to compare the gaster positions of tandem leaders and tandem followers on
125 both forward and reverse tandem runs with ants running as isolated individuals or ants carrying
126 nestmates (either other adults or brood).

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128 In addition, the position of an ant’s antennae may indicate if it is following a pheromone
129 trail laid on the substrate. If such is the case, an ant will typically touch the substrate with its
130 antennae lowered rather than deporting them in a horizontal or raised position. We carried out
131 emigration experiments in which we filmed tandem running and non-tandem running ants, the
132 latter being individuals running alone or carrying. We then analysed the footage to determine
133 the positions of the gasters and the antennae of the ants: 1) during tandem runs, 2) when moving
134 alone and 3) when acting as transporters (ants that carry brood items or adult nestmates). We
135 also counted the number of trail-marking events by the leaders of tandem running ants. Our

136 goal is to understand in more detail the mechanisms of tandem running, especially in *T.*
137 *albipennis*.

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Materials and methods

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The sixteen colonies of *Temnothorax albipennis* (Curtis) used for these experiments were collected in September 2011, on the Dorset coast, England, UK. In the laboratory, each colony was housed in an artificial nest, consisting of a cardboard perimeter, sandwiched between two microscope slides (75 mm × 50 mm × 1 mm). The cardboard formed an inner nest cavity measuring 50 mm × 35 mm × 2 mm, with a 2 mm wide entrance. The colonies were provided with water, honey solution and *Drosophila* flies *ad libitum*.

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Each colony was given at least six ‘emigration-free’ days before it was used again for the next trial to make sure that any effect of previous emigration experience had faded (Langridge et al. 2004). Two Petri dishes were connected by a bridge of acetate sheet 1 mm wide and 102 mm long (Fig. 1). The bridge was narrow to make sure only one ant or tandem pair of ants could cross the bridge at one time. The narrowness of the bridge also ensured that the ants remained within the depth of focus of the camera’s lens to produce the clearest possible images. The ants started tandem running from either the new or the old nests inside the Petri dishes. Thus we could easily determine beforehand whether the ants that were running on the bridge were tandem running ants or single moving ants. In addition, because the body length of *T. albipennis* workers is just about 2 to 3 mm, these small ants can move along the 1-mm-wide bridge without difficulty.

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During the trials, the old nest was placed in the right Petri dish and the new nest in the other. To induce an emigration the roof of the old nest was removed. Once a pair of ants started tandem running across the bridge, all other ants not involved in the tandem run were removed and the entrance of the old nest Petri dish was closed to prevent other ants interrupting the tandem-run pair. These removed ants were then put back in the old nest Petri dish. After a tandem run had been recorded, the old nest Petri dish’s entrance was opened again to let another pair of tandem running ants cross the bridge. A Canon (EOS 60D) camera with a Canon Macro Photo Lens (MP-E) 65 mm 1-5x f/2.8 was set to focus at the centre of the bridge. This camera produces high definition videos and still images. Within the camera field of view, under effectively 3.2 times magnification, the maximum length of tandem run that could be measured

169 was 10 mm. Tandem run pairs were filmed as they travelled across the bridge (Fig. 1). Ants
170 that had been recorded were isolated from the rest of the nest before we started another
171 recording. They were reunited with their colony members only at the end of each trial. All
172 videos were recorded at 50 frames per second. After each trial, the bridge and the Petri dishes
173 were cleaned with water and 70% alcohol to remove any traces of pheromones. Single moving
174 ants and transporter ants were also filmed following the same protocol.

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176 *Analysis of tandem-running behaviour*

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178 Frame by frame video analyses were carried out using the VirtualDub video processing
179 utility v.1.10.4 (Lee 2001).

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181 To determine the position of the antennae and gaster, we used a reference picture as the
182 standard for determining posture: antennae or gaster ‘up’; ‘middle’; or ‘down’ (Fig. 2).
183 Normally, the gasters of these ants are in a horizontal position, i.e., at a ‘middle’ position
184 approximately level with the head and thorax. Thus in the gaster ‘up’ position, it can be seen
185 clearly that the gaster is raised higher than the head and thorax. While in the gaster ‘down’
186 position, the gaster is always deployed lower than the head and thorax. Analogous criteria were
187 applied to the analysis of the antennae position. The leader and follower of a tandem-run pair
188 were analysed independently when determining the positions of their antennae and gaster.

189

190 *Trail-marking*

191 We also counted the number of trail-marking events, i.e., the number of times the
192 leaders of forward and reverse tandem runs briefly dragged their stings on the substrate. For
193 this, we only re-analysed the videos for leader and follower ants exhibiting the gaster ‘down’
194 posture and only when the stings of the ants were clearly and visibly protruded from the tip of
195 the ants’ gasters. Our analyses were conservative and pragmatic and we restricted them to cases
196 in which the behaviours in question were very conspicuous.

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201 *Statistical analysis*

202 To determine the differences in the behaviour of both leaders and followers during
203 forward and reverse tandem runs, we pooled the results for the ants that deployed their gasters
204 ‘up’ (i.e., above horizontal) or ‘in the middle’ (i.e., at a horizontal position) into a gaster ‘not
205 down’ category. We then performed a Fisher’s (2 x 2) exact test to determine whether there
206 was a significant difference between the numbers of ants that deploy their gasters ‘down’
207 against the numbers with gasters ‘not down’. A Chi-square test for goodness-of-fit was
208 performed to test for any differences in gaster position between the leader and follower within
209 the same tandem pair during forward and reverse tandem runs.

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211 A binomial (two-tailed) test was performed to determine the difference between the
212 leaders and followers according to the frequency of keeping their gasters in the ‘up’ position.
213 In this test, we pooled the result of gaster ‘up’ from forward and reverse tandem runs for both
214 the leaders and followers. A Mann-Whitney test was conducted to determine whether there was
215 a significant difference in the number of trail-marking events between forward and reverse
216 tandem runs.

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218

Results

219 Overall, we analysed 50 forward tandem run pairs (50 tandem leaders and 50 tandem
220 followers) and 50 reverse tandem run pairs (50 tandem leaders and 50 tandem followers). In
221 addition we analyzed video sequences of 30 single ants and 63 transporters of brood or adult
222 nestmates to compare their behaviour with tandem-running ants.

223

224 We found that all non-tandem-running ants deployed their antennae down and their
225 gasters at the middle position (Fig. 2) irrespectively of whether they were moving alone (n =
226 30), transporting brood items (n = 30) or carrying adult nestmates (n = 33, Fig. 3). During
227 tandem running (combined data for forward and reverse tandem runs), 91% of the leaders (n =
228 100) and 80% of followers (n = 100) also deployed their antennae down. Thus for the statistical
229 analysis, we will focus only on the results of the gaster positions for the leaders and followers
230 during forward and reverse tandem runs.

231

232 During forward tandem runs, 66% of the leader ants deployed their gaster down while
233 the rest had their gaster either ‘up’ or in the ‘middle’ position (Table 1, Fig. 4a and b). In
234 contrast, during reverse tandem runs, only 42% of leaders deployed their gaster ‘down’ while

235 the rest of the reverse tandem leaders deployed their gaster in either the ‘up’ or ‘middle’
236 position (Table 1, Fig. 4c). This shows that the leader ants’ behave differently during forward
237 and reverse tandem runs (Fisher’s exact test, two-tailed, $p=0.03$, Table 1). Follower ants,
238 however, showed no significant differences in their behaviour during forward and reverse
239 tandem runs (Fisher’s exact test, two-tailed, $p=1.00$, Table 1).

240

241 We also found differences between forward and reverse tandem runs when we
242 compared the positions of the gasters of the leader and follower within the same tandem pair
243 (Table 2). Leaders and followers deploy their gasters differently during forward tandem runs
244 but not during reverse ones. In forward tandem runs, the vast majority of pairs were those where
245 the leader deployed her gaster ‘down’ and the follower deployed her gaster ‘not down’; pairs
246 with the reverse combination of gaster positions were rare (Chi-square test, $\chi^2=15.12$, d.f.=3,
247 $p=0.002$, Table 2). By contrast, in reverse tandem runs, the most common pairs were those
248 where both the leader and the follower had their gasters deployed ‘not down’ while pairs where
249 the leader had her gaster ‘not down’ and the follower had her gaster ‘down’ were rare as in
250 forward tandem runs ($\chi^2=16.08$, d.f.=3, $p=0.001$, Table 2). Finally, when we extracted the
251 results from the gaster ‘not down’ posture, we found that the total number of gaster ‘up’
252 observations (for both forward and reverse tandem runs) was also significantly different
253 between leaders and followers (11 leaders versus 2 followers; Binomial test, two-tailed,
254 $p=0.02$, Table 1).

255

256 The leaders of forward tandem runs briefly dragged their stings and gasters (Fig. 4d)
257 on the substrate on average three times per 10 mm distance (median \pm interquartile range = 3.0
258 ± 2.0) while reverse tandem leaders dragged their stings and gasters on average only once per
259 10 mm distance (median \pm interquartile range = 1.0 ± 0.0). There was a significant difference
260 between these two frequencies suggesting that leaders of forward tandem runs marked trails
261 more often compared to the leaders of reverse tandem runs (Mann-Whitney test, two-tailed,
262 $N_1=23$, $N_2=8$; $W=443.5$; $p<0.01$).

263

264

Discussion

265

266 Through analysing the positions of the gasters of the ants, we found that single ants and
267 transporting ants seem never to have their gasters in the downward position when running
268 between old and new nest sites. In contrast, when the ants engaged in tandem running, most of

269 the leader ants' gasters were deployed downward while most of the follower ants' gasters were
270 either horizontal (middle position) or were raised a bit higher than the horizontal position. Even
271 though the results of the current experiments showed that the gasters of single running ants
272 were mostly deployed in a horizontal position, previous experiments have demonstrated that
273 single *Temnothorax* ants do lay pheromone trails especially when they start exploring new
274 environments, for example to determine the interior floor area of a new potential nest site
275 (Mallon and Franks 2000) and for their orientation cues (Pratt et al. 2001). In contrast, we are
276 confident that transporter ants do not lay chemical trails because they did not drag their gasters
277 on the substrate. This is probably because they are not involved in recruitment. Furthermore,
278 single ants will only begin transporting when the quorum threshold has been met in the new
279 nest (Pratt et al. 2002; Pratt 2005; Langridge et al. 2008). Hence, there might be enough trails
280 on the floor laid by the other ants (or by themselves) previously, during tandem running,
281 making it unnecessary for them to lay any additional trails during carrying. Moreover, in
282 previous experiments transporter ants of *T. albipennis* had been observed to use different
283 pathways on their outbound and inbound journeys (Pratt et al. 2001). This suggests that
284 transporter ants do not retrace their own chemical trails on their inbound paths. They probably
285 depend on other cues such as landmarks and celestial cues or may also follow other colony
286 members' trails for homing (see later). In addition, carrying a nest mate to a new nest is three
287 time faster than tandem running (Franks and Richardson 2006) and so it seems likely that when
288 travelling at such a speed transporters may not be able to lay trails.

289

290 The behaviour of leaders is different during forward tandem runs compared to reverse
291 tandem runs. In forward tandem runs, the gasters of leaders were significantly more frequently
292 deployed downward compared to those of leaders of reverse tandem runs. Leaders changed the
293 way they deployed their gasters between forward and reverse tandem runs in a way that is
294 consistent with them doing some trail laying during both but doing so more often on forward
295 than reverse tandem runs. Comparing the gaster positions of leaders and followers during
296 forward tandem runs shows that they are significantly associated in a way consistent with much
297 more trail laying by leaders than by followers. In contrast, gaster positions for leaders and
298 followers during reverse tandem runs show no significant differences. It also seems unlikely
299 that the follower ants in forward tandem runs marked the trails when leaders did not. This is
300 because, as our results show, the most common posture combination within forward tandem
301 run pairs is for the leader's gaster to be 'down' and the follower's gaster to be 'not down' while
302 the opposite posture combination is rare. Taken altogether, this suggests that it may be

303 important for leaders to invest more in marking their route and maintaining the bond with their
304 follower during a forward rather than a reverse tandem run.

305

306 Our results also show that during forward tandem runs, leader ants mark the trails three
307 times more often than during reverse tandem runs. This is probably because nest emigrations
308 may not occur at all without a sufficient number of forward tandem runs. The ants need to reach
309 a quorum threshold in the new nest before they initiate an emigration of the whole colony (Pratt
310 et al. 2002). Thus the leader ant may provide a trail to ensure a connection between her and the
311 follower ant all the way from the old nest until they reach the goal (i.e., the new nest). Leaders
312 often lead more than one tandem run (Basari, personal observations) and hence a well-marked
313 path may help them to do this more efficiently. Perhaps the trail is also important to help other
314 ants to find the goal and thus for a colony to achieve a quorum threshold more rapidly. Although
315 *Temnothorax* may lay individual specific trails, this does not mean that such trails are
316 undetectable to their nestmates. Hence even though they may not be used for mass recruitment
317 in which specific narrow paths become stronger and stronger as they are reinforced by more
318 and more traffic (Beckers et al. 1993; Witte and Maschwitz 2002; Planqué et al. 2010;
319 Collignon and Detrain 2010), a loose set of trails heading more or less in the same direction
320 may still provide major orientation cues (Franks et al. 2010).

321

322 In tandem running ants, the leader may use a trail pheromone to help keep the bond
323 between the follower and herself and also to facilitate learning by the follower (Maschwitz et
324 al. 1974; Hölldobler and Traniello 1980; Traniello and Hölldobler 1984). Tandem runs are slow
325 in part because followers frequently produce tight loops in the wake of their leader (Franks and
326 Richardson 2006). During this time, leaders stand still waiting for further contact from their
327 follower. Such looping behaviour may help followers to learn landmarks (Pratt et al. 2001;
328 McLeman et al. 2002; Basari et al. 2014) but they also put completion of the tandem run at risk
329 if the pair does not come together again (Richardson et al. 2007; Pratt 2008; Franks et al. 2010).
330 Such risks may be minimized by leaders of forward tandem runs laying trails. While the leader
331 ant marks the trail with pheromone, the follower can concentrate on learning other cues along
332 the route such as landmarks. Hence such trail laying may facilitate landmark learning by
333 followers of forward tandem runs. Although the use of chemical signals during tandem running
334 had been reported previously in another species of ants (Maschwitz et al. 1974; Hölldobler and
335 Traniello 1980; Traniello and Hölldobler 1984; Maschwitz and Buschinger 1986; Jessen and

336 Maschwitz 1986), no reports to our knowledge have established, as we have done, a difference
337 in trail laying behaviour between forward and reverse tandem runs.

338

339 That tandem run leaders deploy their gasters down more frequently on forward rather
340 than reverse tandem runs may imply that it is more important to maintain the path and the bond
341 with follower ants on forward rather than reverse tandem runs. This is because, reverse tandem
342 runs only occur after many ants have made the journey between the old and new nest. Hence,
343 by this stage many ants may have learned the route through individual exploration. Even though
344 trail laying during forward tandem running may help prevent break-ups, perhaps they are less
345 needed on reverse tandem runs because if the tandem does break up, the follower ants should
346 still be able to get to the goal. In addition, because reverse tandem runs occur later in
347 emigrations by that time multiple pheromone trails are likely to indicate the general route
348 between the old and the new nest sites (Franks et al. 2009).

349

350 Overall, we have demonstrated that leaders of forward and reverse tandem runs in *T.*
351 *albipennis* may frequently be laying trails compared to the follower ants. In tandem running
352 ants, the presence of a pheromone trail can provide at least three benefits to the ants. Firstly, it
353 maintains the bond between the leader and the follower; secondly, it facilitates learning by
354 follower ants; and thirdly, it assists both leaders and followers in their navigational strategies.
355 The results of our experiments support earlier findings showing that both tactile and chemical
356 cues are important for successful tandem running recruitment (Maschwitz et al. 1974;
357 Hölldobler and Traniello 1980; Traniello and Hölldobler 1984; Maschwitz and Buschinger
358 1986; Jessen and Maschwitz 1986). However, our experiments provide additional evidence
359 that during tandem running in ants, particularly in *T. albipennis*, leaders lay chemical trails
360 probably to give followers the freedom momentarily to stop following and to look around to
361 learn landmarks and later re-unite with the tandem leader. In other words, trails on the ground
362 may serve as a safety line that improves both the efficiency of tandem runs and their completion
363 rates. Our experiments also show that among *T. albipennis* ants, both forward and reverse
364 tandem run leaders lay trails but do so more often during forward tandem runs, probably
365 because by the time reverse tandem runs start lots of pheromone trails have been laid and
366 followers are less likely to become lost.

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372

373 Table 1. The number of individual ants according to their role in a tandem (leader/follower),
 374 the tandem direction (forward/reverse) and the position of their gaster (down/not down).

Role	Tandem direction	Gaster		Fisher's exact test, 2-tailed
		Down	Not down: Either up or at middle position	
Tandem leader	Forward	33	17 (4u)	p=0.03
	Reverse	21	29 (7u)	
Tandem follower	Forward	14	36 (0u)	p=1.00
	Reverse	14	36 (2u)	

375 Numbers in brackets represent the number of ants with their gaster in the 'up' (u) position, this
 376 number is extracted from the total number of ants with gaster 'not down'.

377

378 Table 2. The number of pairs of ants according to the roles of the tandem members
 379 (leader/follower), the positions of the ants' gaster (down/not down) and the tandem direction
 380 (forward/reverse).

Gaster's posture (in pair)		Forward tandem	Reverse tandem
Leader	Follower		
Down	Down	10 (0.50)	9 (0.98)
Down	Not down	23 (8.82)	12 (0.02)
Not down	Down	4 (5.78)	5 (4.50)
Not down	Not down	13 (0.02)	24 (10.58)

381 Numbers in brackets represent the contribution of each cell to the χ^2 -value for the respective
 382 test; one for the forward tandems and one for the reverse tandems (see Results). For both,
 383 forward and reverse tandem runs, the goodness-of-fit χ^2 -test has the null hypothesis that all
 384 four categories of pairing between the gaster positions of leader and follower are equally
 385 likely, namely that the probability of each is 0.25.

386 **Figures legend**

387

388 **Fig. 1.** Diagram showing a top view of the experimental set-up (not drawn to scale).

389

390 **Fig. 2.** Reference picture for the position analysis of gaster and antennae.

391

392 **Fig. 3.** Transporter ants: a) carrying an adult nestmate; b) carrying a brood item.

393

394 **Fig. 4.** Tandem running ants. Examples of the leader ants' gasters at various positions. a)
395 'down' position; b) 'up' position (both (a) and (b) are in forward tandem runs), c) 'middle'
396 position in a reverse tandem run and d) the sting of the leader ant (in circle) was protruded from
397 the tip of her gaster for trail marking. The follower ants' gasters are all at the middle position.

398

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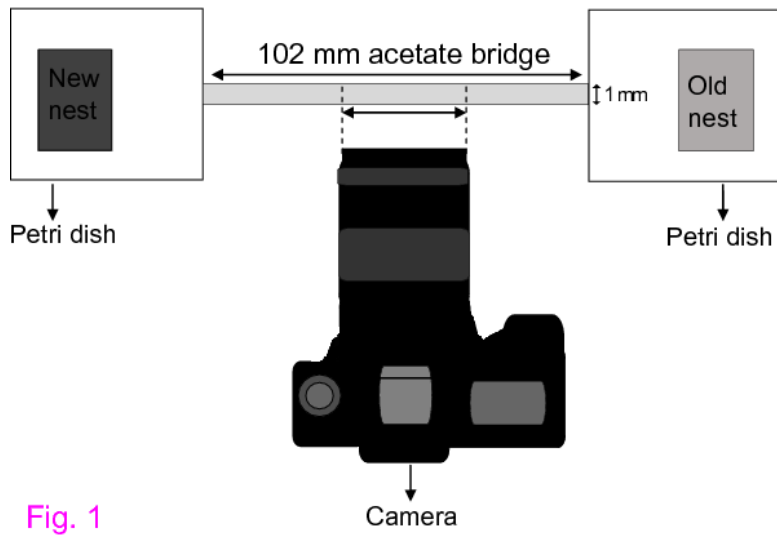


Fig. 1

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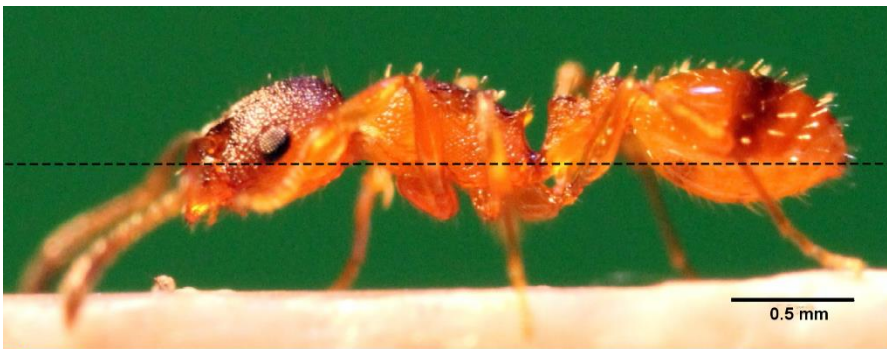


Fig. 2

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Figure 3

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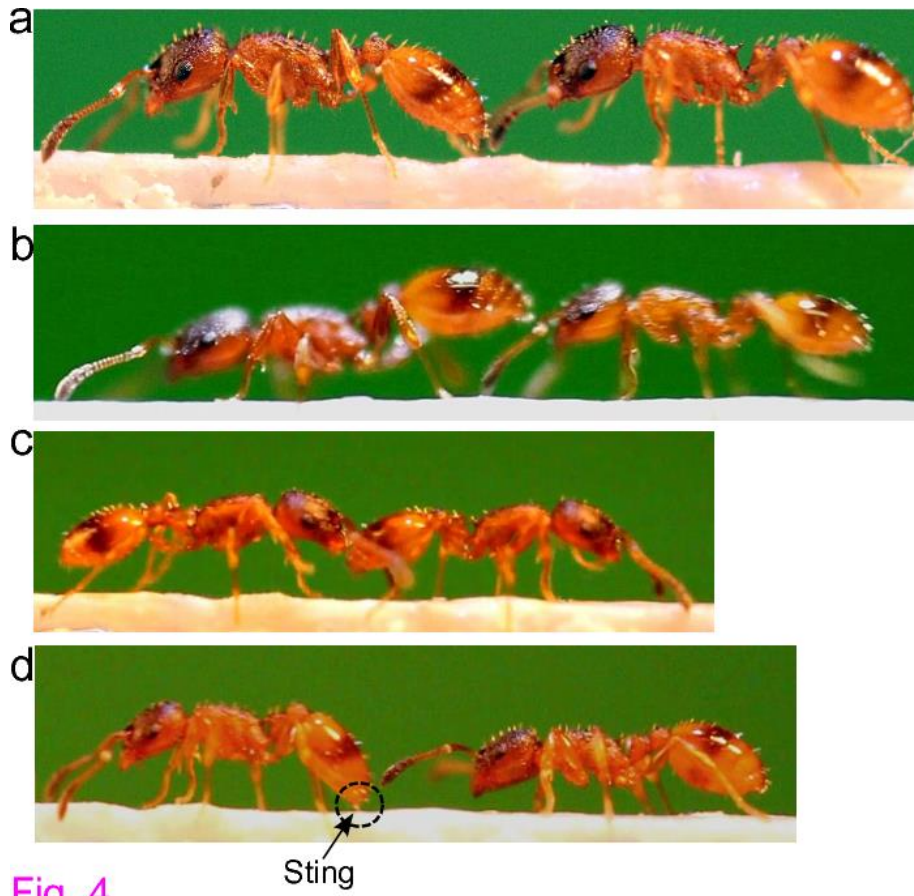


Fig. 4

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