SHORT COMMUNICATION

No specialist pheromone-ignoring ants in *Lasius niger*

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Abstract. 1. In insect societies, the balance between exploitation of known resources and exploration of new ones is important to ensure sufficient resources.

2. Mass recruiting ants, such as *Lasius niger*, use pheromone trails to recruit nestmates to a newly discovered food source. Pheromone following, however, shows characteristic non-following (lapse) rates among different species, with ~20% of *L. niger* foragers ignoring pheromone.

3. These characteristic lapse rates might simply be 'noise', or they might indicate a subset of specialised explorative foragers, a scouting caste, that consistently ignores pheromone in order to explore.

4. Here we show pheromone ignoring is not a repeatable behaviour in *L. niger* foragers – ants who did not follow a trail were no more likely to ignore it again an hour later than ants which did follow it.

5. Our findings suggest that there is no subset of specialised pheromone-ignoring *L. niger* foragers. This may be due to their moderate colony size and strong reliance on individual memories: species with larger colony sizes or a weaker reliance on private information (i.e. memory) may have specialist non-followers.

6. Our work raises the question: what is a scout ant? We encourage future research to investigate the presence of a scouting caste in other ant species using our straightforward methodology, as a social information-ignoring caste may be rarer than expected.

Key words. Behavioural castes, individual differences, pheromone trails, scouts, social information.

Introduction

Insect societies (such as honey bees) can produce specialised individuals, so called scouts, which search for new resources to balance the colony's exploration exploitation trade-off (Biesmeijer & de Vries, 2001). Newly discovered food sources can then be exploited by recruited nestmates. In ants, one recruitment system commonly used is chemical mass recruitment (Hölldobler & Wilson, 1990; Lanan, 2014). Here, a forager, often referred to as a scout, recruits nestmates to a newly discovered resource using a pheromone trail (Hölldobler & Wilson, 1990; de Biseau & Pasteels, 1994; Robson & Traniello, 2002).

Scouts form a distinct caste in honey bees, which search out new food sources even when food is plentiful (Beekman *et al.*, 2007). The term scout in ants, however, is used loosely to refer to individuals which go out in search of resources

(de Biseau & Pasteels, 1994; Robson & Traniello, 2002; Lanan, 2014; Kolay *et al.*, 2020). Specialist trail followers, used to re-establish old trails, have been reported (Jackson *et al.*, 2006; Evison *et al.*, 2008). While classical models of collective behaviour downplay individual differences between workers, both transient and long-lasting individual specialisation are common components of collective behaviours such as house hunting and prey retrieval (Robson & Traniello, 1999, 2002; Fonio *et al.*, 2016). While several studies have examined the consistency of individual ant behaviours such as exploration or aggression (Kolay *et al.*, 2020), none have examined consistency in pheromone following versus ignoring pheromone trails.

The pheromone following rate, or its counterpart, the non-following or 'lapse rate' (von Thienen *et al.*, 2014), has been shown to differ characteristically between species, with some variability between studies. A common method for quantifying trail following is by counting the proportion of ants freely choosing the pheromone-marked arm of a bifurcation (von Thienen *et al.*, 2014; Czaczkes *et al.*, 2017). A selection of pheromone following rates for various species is given in

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Table 1. A selection of pheromone following rates for different species.

Species	Pheromone following rate estimate	Method notes	Reference
Euprenolepis procera	95%	Y maze, gland extract	(von Thienen *et al.*, 2014)
Tapinoma simrothi	100%	Bifurcating ellipse, gland extract	(Simon & Hefetz, 1991)
Tapinoma nigerrimum	c. 98%	Y maze, gland extract	(van Oudenhove *et al.*, 2012)
Aphaenogaster senilis	c. 83%	Y maze, gland extract	(van Oudenhove *et al.*, 2012)
Monomorium pharaonis	70–80%	Y maze, naturally laid trail	(Jeanson *et al.*, 2003)
Linipithema humile	94%	Y maze, artificial trail pheromone	(von Thienen *et al.*, 2014)
↑	89%	Y maze, gland extract	(von Thienen *et al.*, 2014)
↑	99%	Y maze, naturally laid trail	(Aron *et al.*, 1993)
Lasius niger	82%	Y maze, gland extract	(von Thienen *et al.*, 2014)
↑	79–100%	Y maze, naturally laid trail	(Aron *et al.*, 1993)
↑	70%	T maze, naturally laid trail	(Grüter *et al.*, 2011)
↑	74–83%	T maze, naturally laid trail	(Czaczkes *et al.*, 2017)

We only show pheromone following rates where no other competing or conflicting information sources are available.

Table 1. For example, in *Lasius niger*, the lapse rate is relatively high at around 20%.

Some 'noise' during trail following can improve collective decision-making (Deneubourg *et al.*, 1983), but where does this noise come from? Here we ask: is there a discrete subgroup of ants which either ignore or actively avoid pheromone trails, or do all extranidal ants have the same baseline probability of ignoring trails? A subset of individuals that consistently ignore pheromone trails would indicate specialised explorative foragers, i.e. a scouting caste.

Materials and methods

Study species and maintenance

We used 15 queenless *Lasius niger* colony fragments (c. 1000 workers), collected from 15 different wild colonies. The ants were maintained on 1 M sucrose and water *ad libitum*, supplemented with *Drosophila melanogaster*. Colonies were deprived of food for 4 days prior to each trial.

Experimental procedure

Each trial began by allowing a colony access to a Y-maze. The arms of the maze were 10 cm long and 1 cm wide, narrowing to 2 mm wide at the junction, and were covered with paper overlays. A line 2 cm away from the end of each overlay marked a decision line. The set-up was oriented parallel to a wall, so that one arm faced the room, and the other wall. The overlay on one arm was marked with an artificial pheromone trail. This trail was produced by immersing eight worker hindgut glands in 2 ml of dichloromethane (DCM), following von Thienen *et al.* (2014). About 5.6 µl of this mixture was applied in an even line along the overlay using a capillary tube. This amount was calculated to produce a pheromone trail of a realistic strength (von Thienen *et al.*, 2014). The other arm was marked identically with 5.6 µl DCM. The Y-maze stem was unmarked. A fluon-coated container was placed underneath the end of each arm. Ants were allowed to freely run up the bridge onto the Y-maze. Ants crossing the decision line were counted and gently brushed off the Y-maze into the containers underneath. After 10 min, access to the Y-maze was stopped, and ants were allowed to rest for c. 1 h. Thereafter, each container was tested for pheromone following again, as above, although a small number of ants did not climb the bridge (n = 35, 1.8%), so were not retested. Note that this method allows us to know the previous decision of each ant, without requiring individual marking. The orientation of the apparatus, the arm marked with pheromone on the first test, and the arm marked with pheromone on the second test, was varied systematically, resulting in balanced sample sizes for all combinations (see ESM 1).

Statistical analysis

Data were analysed in R v.3.6.1 using generalised linear mixed models (GLMMs via LME4). We used the following model:

Decision (Phero/not)

= group (followed pheromone on first visit/not)

+ pheromone trail direction (left/right)

∗ maze orientation (wall on left/right)

+ random effect (colonyID, random intercept)

Effects of pheromone and maze orientation were added to account for the ants' tendency to walk to one side, either due to lateralization or spatial preference. Whether pheromone following was greater than chance was tested using a binomial test. Differences in following rates between the first and second visit were tested using a test for equal proportions.

The complete statistical analysis method, including all code and outputs, can be found at https://figshare.com/s/4447a791c552b08b408d, and as online supplements 1 & 2.

Results

About 75.1% of ants followed the pheromone trail on the first visit (Fig. 1a). Ants which had initially followed the pheromone
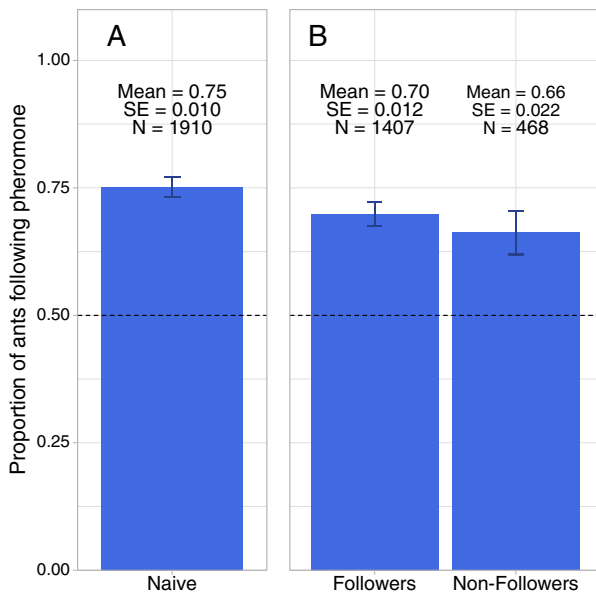
Fig 1. (a) The proportion and respective 95% confidence interval (CI) of ants following pheromone in the first assay. (b) The proportion and CI of ants following pheromone in the second assay, split by whether those ants had followed pheromone in the first assay. [Colour figure can be viewed at wileyonlinelibrary.com].

on the first visit (followers) following it at a rate of 69.9% on the second, slightly but significantly lower than on the first test (95% CI: 0.021–0.084, $X^2 = 11.106$, $P < 0.001$). Ants which had initially not followed the pheromone trail (non-followers) followed it at a rate of 66.2%, also significantly lower than on the first test (95CI: 0.041–0.137, $X^2 = 14.762$, $P < 0.001$). Pheromone following rates of followers and non-followers did not differ significantly (GLMM, $Z = -0.453$, $P = 0.65$, Fig. 1b). Both groups followed pheromone significantly more often than chance (binomial test: 983/1407 in follower-group and 310/468 in not-follower-group, $P < 0.001$).

Pheromone following was significantly stronger when the trail pointed away from the wall, towards the room (GLMM, $Z = 9.429$, $P < 0.001$).

The complete dataset can be found at: https://figshare.com/s/305f3043840a9ff676ff and as online supplement 3.

Discussion

Our results provide no evidence of a distinct group of pheromone-ignoring foragers in *L. niger*, i.e. no inter-individual differences in pheromone following. Interestingly, Beckers *et al.* (1992) found strong inter-individual differences in trail laying behaviour in *L. niger*. Recruited foragers lay significantly less pheromone than initial discoverers. Furthermore, independent of the group (recruited vs. recruiting) some individuals lay significantly more pheromone than others and deposition by any one forager decrease with the number of trips made, as confirmed elsewhere (Czaczkes & Heinze, 2015).

Why then do we not observe repeatable differences between individual *L. niger* foragers in pheromone following? *L. niger* often follow memory over pheromone trails when the two conflict (Grüter *et al.*, 2011). These knowledgeable individuals may be a sufficient source of pheromone-ignoring foragers: when following their memory (and ignoring pheromones) to a no-longer productive food source, they would begin scouting from that location, performing the role of a 'scout', without the need for specialization. However, specialist pheromone-ignorers may exist in other species. Very large colony sizes, for example, promote task specialization in ants (Gautrais *et al.*, 2002; Lanan, 2014).

Our study design might have caused stress, despite the hour-long resting period, possibly resulting in non-natural trail following and thus lower following rates in the second phase. Nonetheless, both groups in the second phase followed pheromone trails at higher-than-chance rates, so if a distinct non-follower caste was present, this would have been detected. The decrease in trail following may have been due to an increase in random choice stemming from escape behaviour, wherein ants attempt to escape rather than follow trails. The fact that ants preferentially chose the arm facing towards the room and away from the wall is likely explained by the paucity of visual cues on the wall side, and the innate attractiveness of visual cues to ants (Graham *et al.*, 2003). As we carefully balanced for arm side, this effect could be statistically controlled for.

We can only draw firm conclusions here about *L. niger*. However, they indicate that the term 'scout' has perhaps been used too loosely in myrmecology, and it may be useful to distinguish uninformed foragers (which would follow pheromone trails if encountered) from specialist scouts (which would not). Specialist scouts may be expected to show other traits, such as abandoning productive resources in favour of further scouting (as in honey bees (Beekman *et al.*, 2007)). Whether specialist scout ants actually exist is unclear. No other studies have explicitly tested for the presence of specialised pheromone-ignoring scouts. We encourage other researchers to apply our straightforward methodology to test for a scouting caste in other species. If these are found, the same ants should be tested for their willingness to ignore memory as well. Only then could a strong case be made for a scout caste in ants. More broadly, it may be valuable to periodically question the validity of the standard terms we use.

Data availability statement

The statistical analysis code and data that support the findings of this study are openly available in figshare [code: https://doi.org/10.6084/m9.figshare.12732281.v1, data:

https://doi.org/10.6084/m9.figshare.12732275.v1], as well as online supplements to the article via the publishers website.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

ESM 1 Code used for data management and cleaning

ESM 2 Full code and output for the statistical analyses

ESM 3 Raw data used for the analysis

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