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Cite this article: Wendt S, Kleinhoelting N, Czaczkes TJ. 2020 Negative feedback: ants choose unoccupied over occupied food sources and lay more pheromone to them. *J. R. Soc. Interface* 20190661.
<http://dx.doi.org/10.1098/rsif.2019.0661>

Received: 23 September 2019

Accepted: 3 February 2020

Subject Category:

Life Sciences—Earth Science interface

Subject Areas:

computational biology, systems biology, environmental science

Keywords:

negative feedback, ants, crowding, foraging behaviour, food choice, pheromone deposition

Author for correspondence:

Stephanie Wendt

e-mail: wendtstephanie@outlook.deElectronic supplementary material is available online at rs.figshare.com.THE ROYAL SOCIETY
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Negative feedback: ants choose unoccupied over occupied food sources and lay more pheromone to them

Stephanie Wendt¹, Nico Kleinhoelting^{1,2} and Tomer J. Czaczkes¹¹Animal Comparative Economics laboratory, Institute of Zoology and Evolutionary Biology, University of Regensburg, 93053 Regensburg, Germany²Faculty of Medicine, University Hospital Regensburg, 93042 Regensburg, Germany

SW, 0000-0002-8950-2845; TJC, 0000-0002-1350-4975

In order to make effective collective decisions, ants lay pheromone trails to lead nest-mates to acceptable food sources. The strength of a trail informs other ants about the quality of a food source, allowing colonies to exploit the most profitable resources. However, recruiting too many ants to a single food source can lead to over-exploitation, queuing, and thus decreased food intake for the colony. The nonlinear nature of pheromonal recruitment can also lead colonies to become trapped in suboptimal decisions, if the environment changes. Negative feedback systems can ameliorate these problems. We investigated a potential source of negative feedback: whether the presence of nest-mates makes food sources more or less attractive. *Lasius niger* workers were trained to food sources of identical quality, scented with different odours. Ants fed alone at one odour. At the other odour ants fed either with other feeding nest-mates, or with dummy ants (black surface lipid-coated glass beads). Ants tended to avoid food sources at which other nest-mates were present. They also deposited less pheromone to occupied food sources, suggesting an active avoidance behaviour, and potentiating negative feedback. This effect may prevent crowding at a single food source when other profitable food sources are available elsewhere, leading to a higher collective food intake. It could also potentially protect colonies from becoming trapped in local feeding optima. However, ants did not avoid the food associated with dummy ants, suggesting that surface lipids and static visual cues alone may not be sufficient for nest-mate recognition in this context.

1. Introduction

Distributing labour and coordinating collective tasks is a challenge faced by both social insects and human societies. A critical challenge is to allocate effort to where it will be most productive, while avoiding crowding consequences and queuing costs. This must be achieved without centralised control in both social insect societies, and in many human endeavours (e.g. distributed computation, telecommunication networks). Social insects have developed numerous strategies to inform nest-mates about valuable food sources, allowing collective exploitation of resources in the surrounding environment. For example, honeybees can share information about the direction and distance of food sources with other nest-mates via the waggle dance [1]. Both the duration and the number of waggle runs increase when bees dance for higher food qualities, thus increasing the likelihood that foragers are recruited to better resources [2,3]. In ants, information about distance and direction is not directly shared with nest-mates. Instead, many ant species deposit pheromone trails when returning from a resource, such as a food source or new nest site. These pheromone trails lead other nest-mates to newly discovered food sources. The more pheromone ants lay when returning from a food source, the stronger the trail will be. Ants can modulate both their decision to deposit

pheromone, and the intensity of pheromone deposition [4,5]. Both trail lay rates and pheromone deposition intensity increase as the perceived value of the resources increases [6–8]. Stronger trails result in more ants being recruited from the nest and a higher proportion of ants following the trail at a bifurcation [5,9,10]. This simple system results in a positive feedback mechanism, leading to colonies often collectively focusing their foraging effort on the most valuable resources [4,11–14].

However, recruiting too many nest-mates to a food source can lead to crowding and an overexploitation of food sources, and thus decrease colony food intake [15,16]. For example, although crowding increases foraging efficiency in leaf-cutter ants [17], it decreases walking speed of ants affected by head-on collisions [18] and can have negative effects in other species as well. Many natural food sources are limited by quantity and replenishment time. For example, honeydew-producing aphids or extrafloral nectaries slowly produce a variable amount of food over the course of a day [19–21]. Depending on an ant's crop capacity, even a single individual may have to spend around 40 min at an extrafloral nectary in order to fill its crop [22], and ants were shown to invest much time in patrolling these food sources [20,23]. Due to long replenishment times, small groups of ants may be capable of fully exploiting even larger patches of aphid colonies or extrafloral nectaries. If a resource is fully exploited, recruiting more individuals will lead to increased waiting times and foragers returning to the nest without food. An optimal distribution of foragers would allocate foragers to a resource until the efforts of any additional forager would be better focused on a different resource.

Decentralised decision-making in non-limited situations also poses challenges for positive-feedback based coordination systems. If recruitment feedback is nonlinear, as is the case in mass-recruiting ants which deposit pheromone trails [7,11,24,25], recruitment can rapidly become extremely strong to one option. If the environment then changes, colonies may not be able to break out of their previous decision and become trapped in exploiting a temporal local optimum [11,12,26,27], but also see [28].

The problems of overexploitation and crowding, and of trapping in local optima, can be ameliorated or overcome by building negative feedback into the collective decision-making system. Social insects have developed a number of negative feedback systems which decrease the number of recruited nest-mates as recruitment progresses. These systems include both active and passive processes [29,30]. Honeybees, for example, use an acoustic signal as an active inhibitory stop signal, stopping returning foragers from recruiting [29,31–35]. Ants reduce pheromone deposition when walking on a pheromone laden path and when encountering other nest-mates on the trail [30,36].

In addition to active recruitment signals, social insects also rely on cues when deciding where to forage. A very important cue for a wide variety of animals is the presence of fellow foragers, both con- and heterospecific [37,38]. In ants, as in other social insects, cuticular hydrocarbons (CHCs) are used to identify and distinguish nest-mates from non-nest-mates [39–41]. The presence of nest-mates has successfully been mimicked by presenting glass beads coated with nest-mate CHCs in ants [42–45]. The presence of conspecifics provides information about the safety and productivity of a foraging patch. Naive bumblebees, for

example, prefer to visit food sources at which conspecifics are already present [46–49]. Ants show a similar behavioural pattern, preferentially choosing to follow paths on which other nest-mates are present [50]. This is somewhat at odds with the finding that ants reduce recruitment in the presence of others ants [30,36]. This highlights a trade-off foragers have to make: well-used patches imply productivity and safety, but also competition for resources and potential over-exploitation.

The aim of this study was twofold: Firstly, we ask whether the presence of nest-mates at a food source (as opposed to on a trail [30]) triggers a negative-feedback effect by reducing recruitment. Secondly, we ask whether unoccupied food sources are more attractive than otherwise equally profitable occupied food sources. We trained individual ants to two alternating food sources associated with different odours. At one food source, ants fed alone. At the other, either live nest-mates or black glass beads coated with surface lipids (containing CHCs and any other lipids soluble in dichloromethane) were present at the food source. After training, odour preference was tested. If nest-mate presence has an inhibitory effect in this context, the ants should follow the odour associated with feeding alone, and deposit less pheromone when returning from occupied food sources. By contrast, if nest-mate presence enhances the attractiveness of a food source, ants should prefer the odour associated with the presence of nest-mates or lipid-coated glass beads, and deposit more pheromone when returning from occupied feeders. Several studies have considered the effect of crowding on trails or at a food source on individual and collective path and food source choice [51,52], and shown that massive overcrowding can lead to individual ants being physically 'pushed' towards alternative options. Other studies have shown that the presence of nest-mates on paths can affect individual and collective path use by decreasing U-turning [50] and reduce pheromone deposition [30]. The presence of pheromone on paths has also been shown to reduce further pheromone deposition [36]. Our study is the first to investigate the effect of nest-mate presence (not massive crowding) at the food source on the evaluation of food sources by individual ants. It is also the first to investigate the effect of nest-mate presence on food source evaluation using associative learning and binary choice assays, as opposed to simply considering pheromone deposition.

2. Methods

2.1. Study animals

Eight stock colonies of the black garden ant *Lasius niger* were collected on the University of Regensburg campus. *Lasius niger* derive much of their carbohydrate intake from tending honeydew-producing insects [54], but do not show task specialisation within aphid-tenders (e.g. to guards, shepherds, and transporters, [55]). The colonies were housed in 30 × 30 × 10 cm foraging boxes with a layer of plaster covering the bottom. Each box contained a circular plaster nest-box (14 cm diameter, 2 cm height). The colonies were queenless with around 1000–2000 workers and small amounts of brood. Queenless colonies forage and lay pheromone trails, and are frequently used in foraging experiments [51,56]. As foragers rarely interact with the queen [57], a lack of queen (but not brood, see [58]) should have little effect on the details of forager behaviour. The colonies were fed with 0.5 M sucrose solution and received *Drosophila* fruit flies once a week. Water was

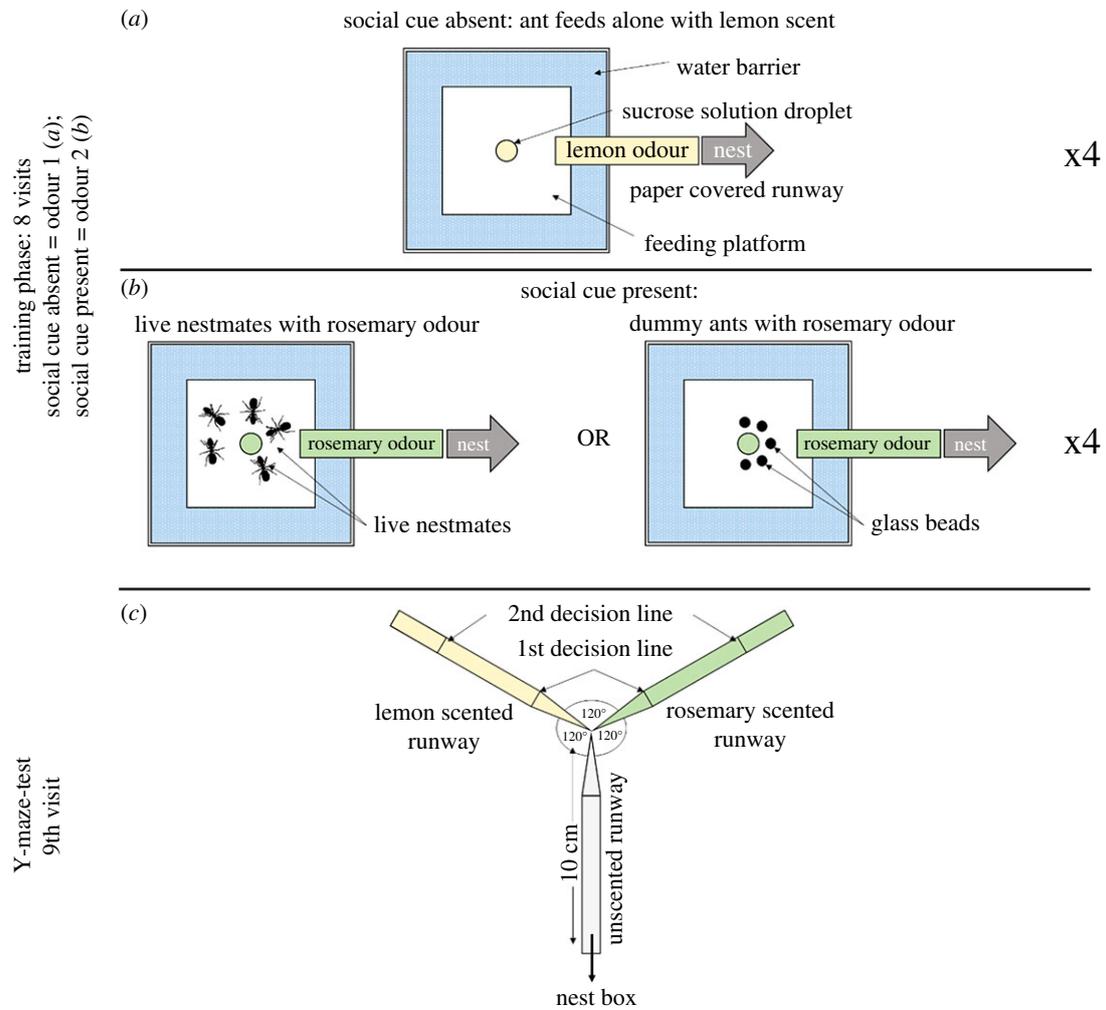


Figure 1. (a,b) Experimental set-up used during training visits 1–8. A 1 M sucrose solution droplet was placed in the centre of a platform surrounded by a water barrier. The platform was connected to the nest via a paper-covered 20 cm long runway and a 40 cm drawbridge. (a) The platform for visits on which the social cue was absent. In this case, sucrose solution was presented with one odour (lemon or rosemary; lemon odour in the example used in figure 1) on the runway and in the food. (b) The platform for visits on which the social cue was present. In this case, sucrose solution was presented with another odour on the runway and in the food (lemon or rosemary; rosemary odour in the example used in figure 1). Half of the tested ants were confronted with live nest-mates as a social cue, the other half was confronted with dummy ants (black surface lipid-coated glass beads). Social cue presence (and the associated odours) alternated each visit. (c) Y-maze used on the 9th (test) visit. All arms were 10 cm long. The arm connected to the nest-box was covered with unscented paper overlays while the other two arms were covered with lemon and rosemary scented paper overlays (one scent on each side). The first decision line was located 2.5 cm from the Y-maze centre and marked the initial decision of an ant while the second decision line was placed 7.5 cm from the centre and marked the final decision.

available *ad libitum*. Colonies were starved for 4 days prior to the experiments in order to achieve a uniform and high motivation for foraging [59,60]. During starvation, water was available *ad libitum*.

2.2. Set-up and experimental procedure

2.2.1. Overview

All ants underwent a similar training protocol: individual ants were allowed to make repeating visits to a food source, which was alternately surrounded by social cues (other nest-mates or dummy ants) and had alternating scents (figure 1). Pheromone deposition was measured on each return to the nest. After eight training visits ants were allowed to choose between social- or non-social feeding odour cues in a binary choice assay.

2.2.2. Training

Two to four foragers were given access to a 20 × 1 cm long plastic runway overlaid with scented paper via a 40 cm long drawbridge. Paper overlays were scented by storing them for at least 1 day in an airtight box containing a droplet of either lemon or

rosemary essential oil (rosemary: *Rosmarinus officinalis*; lemon: *Citrus limon*, Markl GbR, Grünwald) on filter paper in a Petri dish. Previous work has shown that *Lasius niger* foragers can form robust expectations of upcoming reward quality based on lemon or rosemary runway odour after just 1 visit to each odour/quality combination [8,61,62]. A 5 mm diameter drop of scented 1 M sucrose solution (Sigma-Aldrich) was placed in the centre of a feeding platform (4 × 4 cm; figure 1) surrounded by a water barrier (1.75 cm wide and 1.3 cm deep, platform size including the surrounding water barrier: 7.5 × 7.5 cm) at the end of the runway (60 cm from the nest). The solutions were scented using either rosemary or lemon essential oils (0.5 µl essential oil per ml sucrose solution). The first ant to reach the feeder was marked with a dot of acrylic paint (el Greco Acrylic Colors, C. Kreul, Germany) on its abdomen. The marked ant was allowed to drink to repletion at the food source, while all other ants were returned to the nest. When the ant had filled its crop, it was allowed to walk back into the nest. Inside the nest, the ant unloaded its crop to its nest-mates and was then allowed back onto the runway for another visit. The drawbridge was now used to selectively allow only the marked ant onto the runway.

The ant was allowed to make eight return visits to the feeder, with alternating odour cues on each subsequent visit: in half of the visits, ants were allowed to feed alone in the presence of one odour. In the other visits, ants fed together with either (i) five other nest-mates or (ii) five black lipid-coated glass beads (dummy ants which were placed in a semicircle around the sucrose droplet at a distance of 5 mm, allowing unlimited access to the food without disturbance) in the presence of a second odour (figure 1, see below for dummy ant creation details). Sucrose solutions and the runway overlays were scented, with the 'nest-mate' and 'alone' treatments each having a fixed odour. For half of the ants, lemon was associated with the 'nest-mate' treatment and rosemary with the 'alone' treatment, and vice versa for the other ants. Companion nest-mates were gently placed onto the feeding platform shortly before the test ant arrived by allowing them to walk onto a piece of paper, and walk off the paper onto the platform. They displayed no signs of alarm behaviour and fed calmly at the food source when they discovered it. It is thus unlikely that they emitted alarm pheromones which may have led to the test ant avoiding this food source [63]. Mimicking nest-mates with dummy ants allowed us to control for movement cues, potential local feeding cues [64], and pheromone which may be deposited on the runway or the feeding platform by foraging or returning nest-mates. In *Lasius niger*, black glass beads coated in nest-mate lipids were shown to have a greater effect on ant behaviour compared to clear beads [30]. In this study, we thus used black surface lipid-covered beads to mimic nest-mates. Blank beads which were not covered in nest-mate surface lipids did not affect ant behaviour in other studies simulating crowding [30,50], suggesting that ants note surface lipid-covered beads as nest-mates while blank beads are ignored.

As the ant returned to the nest from the food source, we counted the number of pheromone depositions performed. Individual pheromone deposition behaviour correlates with the (perceived) quality of a food source [4,5,8,61]. Individual ants can adapt the strength of a pheromone trail by either depositing pheromone or not, or varying the intensity of pheromone depositions [4,5]. Pheromone deposition behaviour in *Lasius niger* is highly stereotypic. To deposit pheromone, an ant briefly interrupts running to bend its gaster and press the tip onto the ground [6]. Pheromone depositions were measured each time the ant moved from the food source back to the nest (inward trip), and each time the ant moved from the nest towards the food source (outward trip). Because *Lasius niger* foragers almost never lay pheromone when they are not aware of a food source [6], we did not measure pheromone depositions for the very first outward trip (visit 1). The presence of trail pheromone on a path depresses further pheromone deposition [36]. Thus, each time an ant had passed the 20 cm runway, the paper overlay covering the runway was replaced by a fresh one.

2.2.3. Choice tests

On the ninth visit (the testing phase), the linear runway was replaced with a Y-maze (figure 1c), with two 10 cm long arms and a 10 cm long stem. The Y-maze stem was covered with an unscented paper overlay while one arm was covered with the odour overlay associated with the social cue present (e.g. rosemary), and the other with the odour overlay associated with the social cue absent (e.g. lemon). The trained ant was allowed to choose between the two arms, and its decision was recorded. We used two decision lines to define arm choice—an initial decision line (figure 1c, 2.5 cm after the bifurcation) and a final decision line (7.5 cm after the bifurcation). After an ant had made a choice, it was allowed to walk onto a piece of paper at the end of the Y-maze arm and moved to the beginning of the Y-maze in order to allow it to make another choice. This was repeated until an ant had made 3–8 choices in the Y-maze

(for a detailed overview of ant choices split by visit number in the Y-maze please refer to electronic supplementary material, figure S6). The number of choices made by an ant depended on its motivation to make another choice, but was limited to a maximum of eight choices.

After each experimental run, the ant was permanently removed from the colony. In addition to observations by a student sitting next to the experimental set-up, all experimental runs were recorded with a Panasonic DMC-FZ1000 camera.

While data could not be collected blind, as the presence of nest-mates or dummy ants could not be hidden, we used strict behavioural definitions. More importantly, data were collected by a naive experimenter blind to any *a priori* hypotheses about the data, thus protecting against unconscious bias.

2.3. Preparation of dummy ants

To simulate the presence of other nest-mates, we used black glass beads (dummy ants) coated in nest-mate surface lipids, which included CHCs. CHC profiles differ between colonies and allow ants to identify nest-mates and distinguish them from non-nest-mates [65]. CHC-coated glass beads are regularly used to mimic nest-mates [30,43,66] and non-nest-mates [42,45,67] in ants, including *Lasius niger*. Bead preparation followed Czaczkes *et al.* [30]: Clean black glass beads (diameter 2.5 mm, height 1 mm; KnorrPrandell GmbH, Lichtenfels, Germany) were first washed with pentane multiple times, then baked for 1–2 h at 300°C and again washed with pentane after baking to remove any substances or odours which may interfere with nest-mate identification. To coat the beads in ant CHC-profiles, 20 foragers out of the colony to be tested were freeze-killed at –20°C for about 10 min. The ants were then placed in a 2 ml extraction vial (Sigma-Aldrich) and covered in pentane. To dissolve the surface lipids from the ants' cuticle, the vial was agitated for 5 min at 30°C. To ensure that the dummy ants were as realistic as possible, no further steps to purify the CHCs were taken, and thus the beads were coated with nest-mates CHCs and other surface lipids which may have been present on the nest-mate cuticle. In the next step, the ants of which surface lipids had been dissolved were removed from the pentane solution containing ant surface lipids and eight black glass beads (diameter 2.3 mm, height 1.5 mm) were placed into the solution instead. The solution and beads were then again agitated at 30°C until all the pentane had evaporated. This procedure left the beads coated in surface lipids.

A pilot aggression test revealed that surface lipid-coated beads elicited aggressive behaviour such as mandible opening [68] when they were coated with non-nest-mate surface lipid, while no aggressive behaviour was shown when beads were coated with nest-mate surface lipid. This suggests that beads were sufficiently coated to allow ants to recognize them as other ants and differentiate between nest-mates and non-nest-mates.

2.4. Statistical analysis

Statistical analyses were carried out in R v. 3.5.0 [69] using generalized linear mixed models (GLMMs) in the LME4 package [70]. As multiple ants were tested per colony, and we took multiple measurements from each ant, colony identity and individual ant identity nested in colony identity were added as random effects to each model. GLMMs were tested for fit, dispersion and zero inflation using the DHARMA package [71]. The model predictors and interactions were defined *a priori*, following Forstmeier & Schielzeth [72]. All *p*-values presented were corrected for multiple testing using the Benjamini–Hochberg method [73]. A total of 49 ants (3–11 ants per colony) were confronted with real nest-mates as a social cue, making a total of 278

253 choices. In the bead treatment, a total of 43 ants (3–10 ants per
254 colony) was tested, of which 248 choices were made.

255 2.4.1. Choice tests

257 The initial and final choice of the ants matched in 92.4% of
258 choices, so for simplicity, we only considered final choices in
259 the statistical analysis. Choice preference was tested using a
260 GLMM with a binomial distribution. We included the fixed
261 factors social cue type (nest-mates or surface lipid-coated glass
262 beads; SocialCueType), the odour associated with the social
263 cue (lemon or rosemary, to test for innate odour preferences;
264 SocialCueOdour), the side of the social cue odour in the
265 Y-maze (right or left, to test for a side bias; SocialCueSide) and
266 a binomial factor indicating whether ants were confronted with
267 a social cue or fed alone on the first training visit (social cue
268 type at first training visit; SocialCueFirst) in order to test for pri-
269 macy and recency effects [74]. This resulted in the following
270 model formula:

271 $\text{finaldecision} \sim \text{socialcuetype} + \text{socialcueodour}$
272 $+ \text{socialcueside} + \text{socialcuefirst} + (\text{random factor: colony}/\text{AntID})$

273 2.4.2. Inbound pheromone depositions during training

276 Inbound pheromone deposition behaviour (number of phero-
277 mone depositions on the way back to the nest) was analysed
278 using GLMMs with a Poisson distribution. First, we attempted
279 to predict pheromone deposition using the fixed factor social
280 cue type in interaction with whether social cues were present
281 that visit and a scaled visit variable included to model changes
282 in pheromone deposition over subsequent visits. This resulted
283 in the following model formula:

284 complete model: $\text{inboundpheromonedepositions} \sim$
285 $\text{socialcuetype} * \text{socialcuepresence} + \text{visitnumber} +$
286 $(\text{random factor: colony}/\text{AntID})$

288 As the interaction was not significant (see results), but social cue
289 presence had a significant effect on ant choices and visual inspec-
290 tion of the data showed a clear difference between ants
291 confronted with live nest-mates and those confronted with
292 dummy ants (figure 3), we ran two further models in order to
293 explore the data in more detail. We thus subsetted the data
294 according to social cue type. This resulted in the following
295 model formula:

296 split by socialcuetype: $\text{inboundpheromonedepositions} \sim$
297 $\text{socialcuepresence} + \text{visitnumber} + (\text{random factor: colony}/\text{AntID})$

300 3. Results

301 3.1. Choice tests

303 Only 34.8% (97 of 278) of choices in the Y-maze were
304 made for the odour previously associated with the presence of
305 other nest-mates, which is significantly different from chance
306 (figure 2, GLMM: estimate = -0.68 , $z = -3.07$, $p = 0.002$).
307 By contrast, when the social cue was dummy ants, 52.8%
308 (131 of 248) of choices were made for the arm containing
309 the social-associated cue, which does not differ from chance
310 (GLMM: estimate = 0.16 , $z = 0.79$, $p = 0.43$). Social cue type
311 had a significant effect on ant choices, with ants being
312 more likely to choose the social cue side in the bead treatment
313 compared to the nest-mate treatment (GLMM: estimate =
314 0.84 , $z = 3.13$, $p = 0.003$). A significant effect of the first
315 presentation of the social cue during training was also

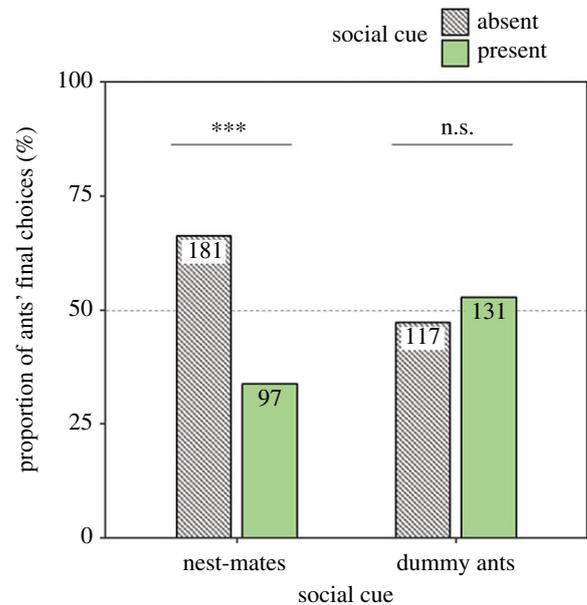


Figure 2. Proportions of all final choices made by ants in the Y-maze. Ants fed with either real nest-mates or with dummy ants (black surface lipid-coated glass beads) when the social cue was present and fed alone when the social cue was absent. Forty-nine ants were trained with real nest-mates and 43 with dummy ants. Numbers in the bars represent sample sizes (individual choices). *** $p < 0.001$, n.s.: $p > 0.05$.

found. Ants were significantly more likely to choose the social cue side in the Y-maze when the social cue was first presented on the first training visit compared to the second training visit (electronic supplementary material, figure S5; GLMM: estimate = 0.89 , $z = 4.11$, $p < 0.001$). Furthermore, ants showed side and odour biases, with significant preferences for lemon odour (electronic supplementary material, figures S1 and S2) and the left side (electronic supplementary material, figures S3 and S4) in the Y-maze (odour preference GLMM: estimate = -0.61 , $z = -2.71$, $p = 0.0083$; side preference GLMM: estimate = -0.69 , $z = 3.19$, $p = 0.0029$).

302 3.2. Inbound pheromone depositions during training

The GLMM analysing the complete dataset indicated a significant effect of the social cue presence on the number of pheromone depositions: ants deposited significantly less pheromone when returning from a food source at which a social cue (live nest-mates or dummy ants) was present (GLMM: estimate = -0.13 , $z = -2.38$, $p = 0.03$). Ants further deposited significantly more pheromone on later training visits (GLMM: estimate = 0.05 , $z = 2.76$, $p = 0.017$).

We did not find a significant effect of social cue type (GLMM: estimate = 0.1 , $z = 1.75$, $p = 0.10$) or the interaction between social cue type and social cue presence (GLMM: estimate = 0.11 , $z = 1.53$, $p = 0.13$) on choice (see electronic supplementary material, figure S6). However, visual inspection of the data (figure 3) clearly showed a difference between ants confronted with live nest-mates and those confronted with dummy ants. We thus examined the data more closely by splitting it by social cue type and ran another GLMM to check for the effect of social cue presence for each of the two social cue types respectively. Ants deposited significantly less pheromone when returning from a food source at which other nest-mates were present compared to when

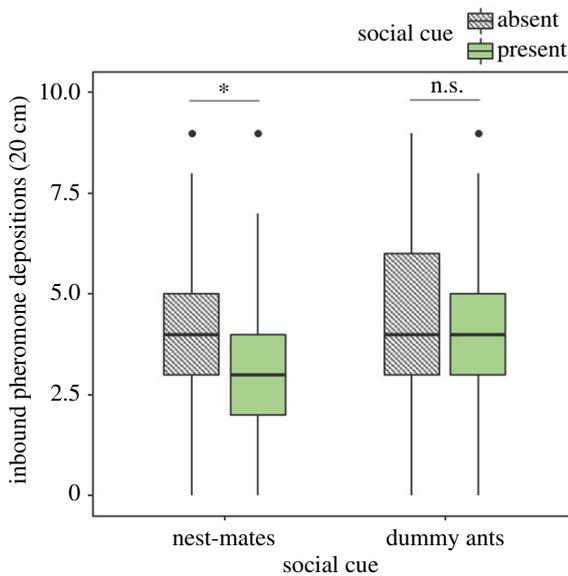


Figure 3. Number of pheromone depositions deposited during the way back to the nest on a 20 cm track right behind the feeding platform. Horizontal lines are medians, boxes are along with interquartile ranges, whiskers are 5%/95% ranges and dots are outliers. * $p < 0.05$, n.s.: $p > 0.05$.

they fed alone (GLMM: estimate = -0.13 , $z = -2.4$, $p = 0.03$, figure 3). However, when beads were used as a social cue, there was no significant effect of social cue presence on the number of pheromone depositions during the ants' return to the nest (GLMM: estimate = -0.01 , $z = -0.18$, $p = 0.85$, figure 3). The order of social cue presentation (first presentation of a social cue on the first or second training visit) did not have a significant effect on number of pheromone depositions in both treatments (GLMM for live nest-mates treatment: estimate = 0.03 , $z = 0.52$, $p = 0.8$; GLMM for dummy ant treatment: estimate = 0.08 , $z = 1.29$, $p = 0.26$).

4. Discussion

Ants showed a significant preference for food sources at which they fed alone over food sources at which other ants were feeding, and also deposited more pheromone when returning from solitary feeding (figure 2). However, surface lipid-coated beads failed to elicit this effect.

These results demonstrate that ants actively avoided feeding at already occupied food sources and recruited more heavily to unoccupied food sources. The results further suggest that the attractiveness of a food source is not solely based on direct traits such as sugar concentration, flow rate or distance to the nest [4,22,75,76], but can also be affected by the status of occupancy and most likely also by other indirect traits. The reduction of pheromone depositions on crowded trails has already been described in *Lasius niger* ants [30]. In addition to a similar effect from occupancy at the food source, here we report an apparent aversion to occupied food sources. Both behaviours may combine and lead to the exploitation of numerous valuable food sources in the environment rather than overexploiting only one good food source. This may counteract the tendency of the positive-feedback component of the ant recruitment system to result in only choosing one option, a phenomenon termed symmetry breaking [11,25,52,77,78]. This may be beneficial for two reasons: Firstly, overexploitation can lead to queuing at

the food source and slower travel speed due to crowded trails [15,16,18]. A reduction of pheromone strength on already occupied trails and preference for unoccupied food sources may lead to a more evenly distributed food exploitation and thus a higher colony-level food intake. Secondly, an aversion to occupied food sources may act as a negative feedback system, preventing colonies from becoming trapped in local foraging optima. Nonlinear positive feedback systems in general, and pheromone-mediated recruitment, particularly in ants, can result in such a strong recruitment that the system cannot react to changing environments. Thus, if an ant colony is allowed to forage extensively at a good food source, and then the quality of the food is reduced, colonies often fail to refocus their foraging effort to newly available, better food sources [11,12,25,78]. In this and other species, trails cannot realistically become strong enough to cause aversion [53]. The negative feedback system we describe may be an effective method of mitigating these effects, especially in combination with other negative feedback mechanisms, such as a simple downregulation of pheromone depositions [52,77]. Note that there is no explicit switch from positive- to negative-feedback causing behaviours—rather, both take place simultaneously. In the current study, the negative feedback is a downregulation of positive feedback behaviour (pheromone deposition), which can proceed until positive feedback is completely shut down, allowing negative feedback processes, such as pheromone decay, to take over.

However, the presence of only five nest-mates at a relatively large (≈ 5 mm) food source might not be reasonably considered as a crowded food source. Furthermore, the presence of nest-mates at a food patch may serve as an indicator for a safe and productive food source which is worth exploiting and should be concentrated on while it is not yet completely crowded [50]. Why then do the ants reduce exploitation of such food sources? In Czaczkes *et al.*'s study [50], even though ants downregulated pheromone depositions on crowded trails, colonies showed a clear preference for paths on which dummy ants were present compared to control paths. The authors argue that the presence of nest-mates and a simultaneous absence of alarm pheromones on a path inform foragers that the path is safe and productive and is thus preferred over one at which nest-mates are absent [50]. Furthermore, colonies may benefit from increased information transfer and recruitment potential on paths where nest-mates are present [17,50,79–81]. Why then do ants reduce their pheromone deposition and preference for a food source which is occupied by just a few nest-mates? Nest-mate density at a food source may be an indicator for how many foragers are already exploiting a food source and may also inform ants about whether additional nest-mates should be recruited [82]. Given the positive-feedback nature of recruitment in these ants, even the presence of a few nest-mates suggests that this food source will soon be well occupied. Foragers with experience of other, unoccupied, food sources could thus concentrate on recruiting to other food source, or on scouting for new ones. These foragers may thus accept the risk of feeding alone at a newly discovered food source until more nest-mates have been recruited. Such scouting ants have been described in various ant species [59,83–85]. A similar pattern was reported in foraging bumblebees: Bees which were experienced with a food source avoided occupied food

sources, but naive bees preferred them [47]. The behavioural pattern reported here and in Czaczkes *et al.* [50] can also be seen in this light: In the current study, we trained individual ants to food sources over the course of eight visits, allowing them to become familiar with the food, odour and nest-mate presence or absence. By contrast, Czaczkes *et al.* [50] investigated path preference of complete colonies in which path choice was driven by the initial decisions of the first few, naive foragers. These naive ants would be more likely to visit occupied food sources, while informed ants would rather avoid them. Importantly, individual *Lasius niger* workers are very flexible in their use of pheromone trails. While naive and recruited workers can follow pheromone trails with high fidelity [86], ants are not dependent on pheromone decay to maintain flexibility: knowledgeable individuals can completely ignore pheromone trails in preference for conflicting route memories [87]. However, knowledgeable individuals can again switch back to high fidelity pheromone following, even in the face of conflicting memories, when other third-source information sources become available [88]. The current results build on this picture, demonstrating how flexible individuals, changing their preferences due to perceived food crowding, may provide information used to maintain colony flexibility.

The fact that dummy ants (black surface lipid-coated glass beads) did not elicit a decrease in recruitment strength and food attractiveness suggests that the mere presence of a nest-mate odour may not be sufficient for nest-mate recognition in this context. Although surface lipid-coated glass beads have successfully been used in previous studies on recruitment behaviour [44], including in *Lasius niger* [30,66], the lack of other stimuli such as movement, home-range markings [89–91], or feeding signals (e.g. local pheromone recruitment or stridulation) [64,81,92,93] may have caused the ants to underestimate the local density of ants, or have prevented them from being perceived as nest-mates.

Ants showed a stronger preference for the odour associated with the absence of social cues when it was first experienced on the first training visit, with only 34.1% of choices for the odour at which a social cue was present. By contrast, if the social cue was first presented on the first training visit, 51.8% of choices were for the Y-maze side covered in

social cue odour. This strongly suggests a primacy effect, in which memory of the first-exposed cue is stronger than memory of a cue experienced later [94–96]. Ants also chose the social cue odour more often when it was associated with lemon odour and placed on the left side of the Y-maze, suggesting an innate odour preference for lemon over rosemary odour and a side bias. Side biases especially have been widely reported in ants and other animals [97–104]. However, as our treatments were fully balanced by treatment presentation order, neither the primacy effect nor the innate biases should interfere with our interpretation.

We demonstrate that negative feedback is not only elicited by nest-mate presence on paths in *Lasius niger*, but also through nest-mate presence during food consumption. Moreover, ants also prefer food sources without fellow foragers. The avoidance of already occupied food sources allows ants to distribute their foraging effort and exploit multiple food sources simultaneously. This can result in a more efficient exploitation of the environment [105]. In addition, such behaviour may increase the amount of information about available food sources and may thus increase the colony-level food intake rate. Such negative-feedback systems may play a critical role in maintaining collective flexibility and preventing trapping in local optima. Taken together, the combination of passive negative feedback from reduced pheromone deposition, and active negative feedback via occupied food avoidance, may be a powerful mechanism for increasing collective foraging efficacy.

Ethics. All animal treatment guidelines applicable to ants under German law have been followed.

Data accessibility. Raw data collected in the presented experiments can be found on Dryad via <https://dx.doi.org/10.5061/dryad.qz612jm8x>.

Authors' contributions. N.K. performed the experiments. S.W. supervised the experiments and analysed the data. S.W. and T.J.C. designed the study, interpreted the data and wrote the manuscript. All authors gave final approval for publication and agree to be held accountable for the content therein.

Competing interests. The authors declare that they have no conflict of interest.

Funding. We thank the DFG (Deutsche Forschungsgemeinschaft) which funded S.W. and T.J.C. with an Emmy Noether grant to T.J.C., grant no. CZ 237/1-1.

References

1. von Frisch K. 1946 Die Tänze der Bienen. *Österr Zool.*, 1–48.
2. Seeley T, Camazine S, Sneyd J. 1991 Collective decision-making in honey bees: how colonies choose among nectar sources. *Behav. Ecol. Sociobiol.* **28**, 277–290. (doi:10.1007/BF00175101)
3. Seeley T, Mikheyev A, Pagano G. 2000 Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *J. Comp. Physiol. A.* **186**, 813–819. (doi:10.1007/s003590000134)
4. Beckers R, Deneubourg J-L, 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)
5. Hangartner W. 1970 Control of pheromone quantity in odor trails of the ant *Acanthomyops interjectus*. *Experientia* **26**, 664–665. (doi:10.1007/BF01898753)
6. Beckers R, Deneubourg J-L, Goss S. 1992 Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). *Insectes Soc.* **39**, 59–72. (doi:10.1007/BF01240531)
7. Detrain C, Deneubourg J-L. 2008 Collective Decision-Making and Foraging Patterns in Ants and Honeybees. In *Advances in insect physiology* (ed. S Simpson), pp. 123–173. New York, NY: Academic Press.
8. Wendt S, Strunk K, Heinze J, Roider A, Czaczkes T. 2019 Positive and negative incentive contrasts lead to relative value perception in ants. *eLife* **8**, e45450. (doi:10.7554/eLife.45450)
9. von Thienen W, Metzler D, Witte V. 2015 Modeling shortest path selection of the ant *Linepithema humile* using psychophysical theory and realistic parameter values. *J. Theor. Biol.* **372**, 168–178. (doi:10.1016/j.jtbi.2015.02.030)
10. Wilson E. 1962 Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith) 1. The organization of mass-foraging. *Anim. Behav.* **10**, 134–147. (doi:10.1016/0003-3472(62)90141-0)
11. Beckers R, Deneubourg JL, Goss S, Pasteels JM. 1990 Collective decision making through food recruitment. *Insect. Soc.* **37**, 258–267. (doi:10.1007/BF02224053)
12. Czaczkes T, Salmane A, Klampfleuthner F, Heinze J. 2016 Private information alone can trigger trapping

- of ant colonies in local feeding optima. *J. Exp. Biol.* **219**, 744–751. (doi:10.1242/jeb.131847)
13. Frizzi F, Talone F, Santini G. 2018 Modulation of trail laying in the ant *Lasius neglectus* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *Ethology* **124**, 870–880. (doi:10.1111/eth.12821)
14. Latty T, Beekman M. 2013 Keeping track of changes: the performance of ant colonies in dynamic environments. *Anim. Behav.* **85**, 637–643. (doi:10.1016/j.anbehav.2012.12.027)
15. Burd M. 1996 Server system and queuing models of leaf harvesting by leaf-cutting ants. *Am. Nat.* **148**, 613–629. (doi:10.1086/285943)
16. Burd M. 2000 Foraging behaviour of *Atta cephalotes* (leaf-cutting ants): an examination of two predictions for load selection. *Anim. Behav.* **60**, 781–788. (doi:10.1006/anbe.2000.1537)
17. Dussutour A, Beshers S, Deneubourg J-L, Fourcassié V. 2007 Crowding increases foraging efficiency in the leaf-cutting ant *Atta colombica*. *Insect. Soc.* **54**, 158–165. (doi:10.1007/s00040-007-0926-9)
18. Burd M, Aranwela N. 2003 Head-on encounter rates and walking speed of foragers in leaf-cutting ant traffic. *Insect. Soc.* **50**, 3–8. (doi:10.1007/s000400300001)
19. Fischer M, Völkl W, Hoffmann K. 2005 Honeydew production and honeydew sugar composition of polyphagous black bean aphid, *Aphis fabae* (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. *Eur. J. Entomol.* **102**, 155–160. (doi:10.14411/eje.2005.025)
20. Dreisig H. 2000 Defense by exploitation in the Florida carpenter ant, *Camponotus floridanus*, at an extrafloral nectar resource. *Behav. Ecol. Sociobiol.* **47**, 274–279. (doi:10.1007/s002650050666)
21. Völkl W, Woodring J, Fischer M, Lorenz MW, Hoffmann KH. 1999 Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**, 483–491. (doi:10.1007/s004420050751)
22. Schilman P, Roces F. 2003 Assessment of nectar flow rate and memory for patch quality in the ant *Camponotus rufipes*. *Anim. Behav.* **66**, 687–693. (doi:10.1006/anbe.2003.2242)
23. Dreisig H. 1988 Foraging rate of ants collecting honeydew or extrafloral nectar, and some possible constraints. *Ecol. Entomol.* **13**, 143–154. (doi:10.1111/j.1365-2311.1988.tb00342.x)
24. Shaffer Z, Sasaki T, Pratt SC. 2013 Linear recruitment leads to allocation and flexibility in collective foraging by ants. *Anim. Behav.* **86**, 967–975. (doi:10.1016/j.anbehav.2013.08.014)
25. Sumpter D, Beekman M. 2003 From nonlinearity to optimality: pheromone trail foraging by ants. *Anim. Behav.* **66**, 273–280. (doi:10.1006/anbe.2003.2224)
26. Beckers R, Deneubourg J-L, Goss S. 1992 Trails and U-turns in the selection of a path by the ant *Lasius niger*. *J. Theor. Biol.* **159**, 397–415. (doi:10.1016/S0022-5193(05)80686-1)
27. Goss S, Aron S, Deneubourg J-L, Pasteels J. 1989 Self-organized shortcuts in the Argentine ant. *Naturwissenschaften* **76**, 579–581. (doi:10.1007/BF00462870)
28. Dussutour A, Nicolis S, Shephard G, Beekman M, Sumpter D. 2009 The role of multiple pheromones in food recruitment by ants. *J. Exp. Biol.* **212**, 2337–2348. (doi:10.1242/jeb.029827)
29. Kietzman P, Visscher P. 2015 The anti-waggle dance: use of the stop signal as negative feedback. *Front. Ecol. Evol.* **3**, 14. (doi:10.3389/fevo.2015.00014)
30. Czaczkes T, Grüter C, Ratnieks F. 2013 Negative feedback in ants: crowding results in less trail pheromone deposition. *J. R. Soc. Interface.* **10**, 20121009. (doi:10.1098/rsif.2012.1009)
31. Kirchner W. 1993 Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **33**, 169–172. (doi:10.1007/BF00216597)
32. Nieh J. 1993 The stop signal of honey bees: reconsidering its message. *Behav. Ecol. Sociobiol.* **33**, 51–56. (doi:10.1007/BF00164346)
33. Nieh J. 2010 A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Curr. Biol.* **20**, 310–315. (doi:10.1016/j.cub.2009.12.060)
34. Pastor K, Seeley T. 2005 The brief piping signal of the honey bee: begging call or stop signal? *Ethology* **111**, 775–784. (doi:10.1111/j.1439-0310.2005.01116.x)
35. Thom C, Gilley D, Tautz J. 2003 Worker piping in honey bees (*Apis mellifera*): the behavior of piping nectar foragers. *Behav. Ecol. Sociobiol.* **53**, 199–205. (doi:10.1007/s00265-002-0567-y)
36. Czaczkes T, Grüter C, Ellis L, Wood E, Ratnieks F. 2013 Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. *J. Exp. Biol.* **216**, 188–197. (doi:10.1242/jeb.076570)
37. Clark CW, Mangel M. 1984 Foraging and flocking strategies: information in an uncertain environment. *Am. Nat.* **123**, 626–641. (doi:10.1086/284228)
38. Krebs J, MacRoberts M, Cullen J. 1972 Flocking and feeding in the great tit parus major—an experimental study. *Ibis* **114**, 507–530. (doi:10.1111/j.1474-919X.1972.tb00852.x)
39. Lahav S, Soroker V, Hefetz A, Vander Meer R. 1999 Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* **86**, 246–249. (doi:10.1007/s001140050609)
40. Wagner D, Tissot M, Cuevas W, Gordon D. 2000 Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *J. Chem. Ecol.* **26**, 2245–2257. (doi:10.1023/A:1005529224856)
41. van Zweden JS, d’Ettorre P. 2010 Nestmate recognition in social insects and the role of hydrocarbons. In *Insect hydrocarbons - biology, biochemistry, and chemical ecology* (eds GJ Blomquist, A-G Bagnères), pp. 222–243. Cambridge, UK: Cambridge University Press.
42. 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. (doi:10.1303/aez.2004.381)
43. Greene M, Gordon D. 2003 Social insects: cuticular hydrocarbons inform task decisions. *Nature* **423**, 32. (doi:10.1038/423032a)
44. Greene M, Gordon D. 2007 Interaction rate informs harvester ant task decisions. *Behav. Ecol.* **18**, 451–455. (doi:10.1093/beheco/arl105)
45. Ozaki M, Wada-Katsumata A, Fujikawa K, Iwasaki M, Yokohari F, Satoji Y, Nisimura T, Yamaoka R. 2005 Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* **309**, 311–314. (doi:10.1126/science.1105244)
46. Avargués-Weber A, Chittka L. 2014 Local enhancement or stimulus enhancement? Bumblebee social learning results in a specific pattern of flower preference. *Anim. Behav.* **97**, 185–191. (doi:10.1016/j.anbehav.2014.09.020)
47. Kawaguchi L, Ohashi K, Toquenaga Y. 2007 Contrasting responses of bumble bees to feeding conspecifics on their familiar and unfamiliar flowers. *Proc. R. Soc. B* **274**, 2661–2667. (doi:10.1098/rspb.2007.0860)
48. Leadbeater E, Chittka L. 2007 The dynamics of social learning in an insect model, the bumblebee (*Bombus terrestris*). *Behav. Ecol. Sociobiol.* **61**, 1789–1796. (doi:10.1007/s00265-007-0412-4)
49. Worden B, Papaj D. 2005 Flower choice copying in bumblebees. *Biol. Lett.* **1**, 504–507. (doi:10.1098/rsbl.2005.0368)
50. Czaczkes T, Franz S, Witte V, Heinze J. 2015 Perception of collective path use affects path selection in ants. *Anim. Behav.* **99**, 15–24. (doi:10.1016/j.anbehav.2014.10.014)
51. Dussutour A, Fourcassié V, Helbing D, Deneubourg J-L. 2004 Optimal traffic organization in ants under crowded conditions. *Nature* **428**, 70–73. (doi:10.1038/nature02345)
52. Grüter C, Schürch R, Czaczkes T, Taylor K, Durance T, Jones S, Ratnieks FL. 2012 Negative feedback enables fast and flexible collective decision-making in ants. *PLoS ONE* **7**, e44501. (doi:10.1371/journal.pone.0044501)
53. von Thienen W, Metzler D, Choe D-H, Witte V. 2014 Pheromone communication in ants: a detailed analysis of concentration-dependent decisions in three species. *Behav. Ecol. Sociobiol.* **68**, 1611–1627. (doi:10.1007/s00265-014-1770-3)
54. Eidmann H. 1927 *Ameisen und blattläuse*. G. Thieme.
55. Novgorodova TA. 2015 Organization of honeydew collection by foragers of different species of ants (Hymenoptera: Formicidae): Effect of colony size and species specificity. *Eur. J. Entomol.* **112**, 688–697. (doi:10.14411/eje.2015.077)
56. Detrain C, Pereira H, Fourcassié V. 2019 Differential responses to chemical cues correlate with task performance in ant foragers. *Behav. Ecol. Sociobiol.* **73**, 107. (doi:10.1007/s00265-019-2717-5)
57. Stroeymeyt N, Grasse AV, Crespi A, Mersch DP, Cremer S, Keller L. 2018 Social network plasticity decreases disease transmission in a eusocial insect. *Science* **362**, 941–945. (doi:10.1126/science.aat4793)
58. Portha S, Deneubourg J-L, Detrain C. 2004 How food type and brood influence foraging decisions of

- 505 *Lasius niger* scouts. *Anim. Behav.* **68**, 115–122.
506 (doi:10.1016/j.anbehav.2003.10.016)
- 507 59. Maillieux A-C, Detrain C, Deneubourg J-L. 2006
508 Starvation drives a threshold triggering
509 communication. *J. Exp. Biol.* **209**, 4224–4229.
510 (doi:10.1242/jeb.02461)
- 511 60. Josens R, Rocas F. 2000 Foraging in the ant
512 *Camponotus mus*: nectar-intake rate and crop filling
513 depend on colony starvation. *J. Insect. Physiol.* **46**,
514 1103–1110. (doi:10.1016/S0022-1910(99)00220-6)
- 515 61. Czaczkes T, Koch A, Fröber K, Dreisbach G. 2018
516 Voluntary switching in an invertebrate: the effect of
517 cue and reward change. *J. Exp. Psychol. Anim.*
518 *Learn. Cognition* **44**, 247–257. (doi:10.1037/
519 xan0000171)
- 520 62. Oberhauser F, Schlemm S, Wendt S, Czaczkes T.
521 2019 Private information conflict: *Lasius niger* ants
522 prefer olfactory cues to route memory. *Anim. Cogn.*
523 **22**, 355–364. (doi:10.1007/s10071-019-01248-3)
- 524 63. Nonacs P. 1990 Death in the distance: mortality risk
525 as information for foraging ants. *Behaviour* **112**,
526 23–35. (doi:10.1163/156853990X00662)
- 527 64. Rocas F, Hölldobler B. 1996 Use of stridulation in
528 foraging leaf-cutting ants: mechanical support
529 during cutting or short-range recruitment signal?
530 *Behav. Ecol. Sociobiol.* **39**, 293–299. (doi:10.1007/
531 s002650050292)
- 532 65. Sturgis S, Gordon D. 2012 Nestmate recognition in
533 ants (Hymenoptera: Formicidae): a review.
534 *Myrmecological News* **16**, 101–110.
- 535 66. Czaczkes T, Grüter C, Ratnieks F. 2014 Rapid up- and
536 down-regulation of pheromone signalling due to
537 trail crowding in the ant *Lasius niger*. *Behaviour*
538 **151**, 669–682. (doi:10.1163/1568539X-00003157)
- 539 67. Guerrieri F, Nehring V, Jørgensen C, Nielsen J,
540 Galizia C, d’Ettorre P. 2009 Ants recognize foes and
541 not friends. *Proc. R. Soc. B* **276**, 2461–2468.
542 (doi:10.1098/rspb.2008.1860)
- 543 68. Guerrieri F, d’Ettorre P. 2008 The mandible opening
544 response: quantifying aggression elicited by
545 chemical cues in ants. *J. Exp. Biol.* **211**, 1109–1113.
546 (doi:10.1242/jeb.008508)
- 547 69. R Core Team. 2016 *R: A language and environment for*
548 *statistical computing*. Vienna, Austria: R Foundation for
549 Statistical Computing. See <https://www.R-project.org>.
- 550 70. Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting
551 Linear Mixed-Effects Models using lme4. *arXiv*.
552 stat:1406.5823.
- 553 71. Hartig F. 2017 Residual Diagnostics for Hierarchical
554 (Multi-Level / Mixed) Regression Models. See [http://](http://florianhartig.github.io/DHARMA/)
555 florianhartig.github.io/DHARMA/.
- 556 72. Forstmeier W, Schielzeth H. 2011 Cryptic multiple
557 hypotheses testing in linear models: overestimated
558 effect sizes and the winner’s curse. *Behav. Ecol.*
559 *Sociobiol.* **65**, 47–55. (doi:10.1007/s00265-010-
560 1038-5)
- 561 73. Benjamini Y, Hochberg Y. 1995 Controlling the false
562 discovery rate: a practical and powerful approach to
563 multiple testing. *J. R. Stat. Soc. B (Methodological)*.
564 **57**, 289–300. (doi:10.1111/j.2517-6161.1995.
565 tb02031.x)
- 566 74. Lipatova O, Wheeler D, Vadillo M, Miller R. 2006
567 Recency-to-primacy shift in cue competition. *J. Exp.*
Psychol. Anim. Behav. Proces. **32**, 396–406. (doi:10.
1037/0097-7403.32.4.396)
75. Fewell J, Harrison J, Stiller T, Breed M. 1992
Distance effects on resource profitability and
recruitment in the giant tropical ant, *Paraponera*
clavata. *Oecologia* **92**, 542–547. (doi:10.1007/
BF00317846)
76. Josens R, Farina W, Rocas F. 1998 Nectar feeding by
the ant *Camponotus mus*: intake rate and crop
filling as a function of sucrose concentration.
J. Insect. Physiol. **44**, 579–585. (doi:10.1016/S0022-
1910(98)00053-5)
77. Czaczkes T. 2014 How to not get stuck—negative
feedback due to crowding maintains flexibility in
ant foraging. *J. Theor. Biol.* **360**, 172–180. (doi:10.
1016/j.jtbi.2014.07.005)
78. de Biseau J, Deneubourg J-L, Pasteels J. 1991
Collective flexibility during mass recruitment in the
ant *Myrmica sabuleti* (Hymenoptera: Formicidae).
Psyche **98**, 323–336. (doi:10.1155/1991/38402)
79. Farji-Brener A, Amador-Vargas S, Chinchilla F,
Escobar S, Cabrera S, Herrera M, Sandoval C. 2010
Information transfer in head-on encounters
between leaf-cutting ant workers: food, trail
condition or orientation cues? *Anim. Behav.* **79**,
343–349. (doi:10.1016/j.anbehav.2009.11.009)
80. Rocas F. 1990 Olfactory conditioning during the
recruitment process in a leaf-cutting ant. *Oecologia*
83, 261–262. (doi:10.1007/BF00317762)
81. Rocas F. 1994 Odour learning and decision-making
during food collection in the leaf-cutting ant
Acromyrmex lundi. *Insect. Soc.* **41**, 235–239. (doi:10.
1007/BF01242294)
82. Jarau S, Hrnrcir M. 2009 *Food exploitation by social*
insects - ecological, behavioral, and theoretical
approaches. CRC Press, Taylor & Francis Group.
83. Breed M, Fewell J, Moore A, Williams K. 1987
Graded recruitment in a ponerine ant. *Behav. Ecol.*
Sociobiol. **20**, 407–411. (doi:10.1007/BF00302983)
84. Chadab R, Rettenmeyer C. 1975 Mass recruitment
by army ants. *Science* **188**, 1124–1125. (doi:10.
1126/science.1215991)
85. Jaffe K, Howse P. 1979 The mass recruitment system
of the leaf cutting ant, *Atta cephalotes* (L.). *Anim.*
Behav. **27**, 930–939. (doi:10.1016/0003-3472(79)
90031-9)
86. Czaczkes T, Castorena M, Schürch R, Heinze J. 2017
Pheromone trail following in the ant *Lasius niger*:
high accuracy and variability but no effect of task
state. *Physiol. Entomol.* **42**, 91–97. (doi:10.1111/
phen.12174)
87. Grüter C, Czaczkes T, Ratnieks F. 2011 Decision
making in ant foragers (*Lasius niger*) facing
conflicting private and social information. *Behav.*
Ecol. Sociobiol. **65**, 141–148. (doi:10.1007/s00265-
010-1020-2)
88. Czaczkes T, Beckwith J, Horsch A-L, Hartig F. 2019
The multi-dimensional nature of information drives
prioritization of private over social information in
ants. *Proc. R. Soc. B* **286**, 20191136. (doi:10.1098/
rspb.2019.1136)
89. Depickère S, Fresneau D, Detrain C, Deneubourg J-L.
2004 Marking as a decision factor in the choice of a
new resting site in *Lasius niger*. *Insect. Soc.* **51**,
243–246.
90. Detrain C, Deneubourg J-L. 2009 Social cues and
adaptive foraging strategies in ants. In *Food*
exploitation by social insects (eds S Jarau, M Hrnrcir),
pp. 29–54. Boca Raton, FL: CRC Press.
91. Devigne C, Detrain C. 2002 Collective exploration
and area marking in the ant *Lasius niger*. *Insect.*
Soc. **49**, 357–362. (doi:10.1007/PL00012659)
92. Bouchebti S, Ferrere S, Vittori K, Latil G, Dussoutour
A, Fourcassié V. 2015 Contact rate modulates
foraging efficiency in leaf cutting ants. *Sci. Rep.* **5**,
Article number: 18650. (doi:10.1038/srep18650)
93. Hölldobler B, Wilson E. 1990 *The ants*. Berlin,
Germany: Springer.
94. Pineño O, Miller R. 2005 Primacy and recency
effects in extinction and latent inhibition: a selective
review with implications for models of learning.
Behav. Processes. **69**, 223–235. (doi:10.1016/j.beproc.
2005.02.006)
95. Wright A, Santiago H, Sands S, Kendrick D, Cook R.
1985 Memory processing of serial lists by pigeons,
monkeys, and people. *Science* **229**, 287–289.
(doi:10.1126/science.9304205)
96. Wright A, Roediger H. 2003 Interference processes
in monkey auditory list memory. *Psychon. Bull. Rev.*
10, 696–702. (doi:10.3758/BF03196534)
97. Buchanan S, Kain J, de Bivort B. 2015 Neuronal
control of locomotor handedness in *Drosophila*.
Proc. Natl Acad. Sci. USA **112**, 6700–6705. (doi:10.
1073/pnas.1500804112)
98. Cooper R, Nudo N, González J, Vinson S, Liang H.
2011 Side-dominance of *Periplaneta americana*
persists through antenna amputation. *J. Insect Behav.*
24, 175–185. (doi:10.1007/s10905-010-9246-4)
99. Frasnelli E. 2013 Brain and behavioral lateralization
in invertebrates. *Front. Psychol.* **4**, 939. (doi:10.
3389/fpsyg.2013.00939)
100. Glick S, Ross D. 1981 Right-sided population bias
and lateralization of activity in normal rats. *Brain*
Res. **205**, 222–225. (doi:10.1016/0006-
8993(81)90737-X)
101. Guo K, Meints K, Hall C, Hall S, Mills D. 2009 Left
gaze bias in humans, rhesus monkeys and domestic
dogs. *Anim. Cogn.* **12**, 409–418. (doi:10.1007/
s10071-008-0199-3)
102. Hunt E, O’Shea-Wheller T, Albery G, Bridger T, Gumn
M, Franks N. 2014 Ants show a leftward turning bias
when exploring unknown nest sites. *Biol. Lett.* **10**,
20140945. (doi:10.1098/rsbl.2014.0945)
103. Kight S, Steelman L, Coffey G, Lucente J, Castillo M.
2008 Evidence of population-level lateralized
behaviour in giant water bugs, *Belostoma*
flumineum Say (Heteroptera: Belostomatidae):
T-maze turning is left biased. *Behav. Processes.*
79, 66–69. (doi:10.1016/j.beproc.2008.04.001)
104. Stancher G, Clara E, Regolin L, Vallortigara G. 2006
Lateralized righting behavior in the tortoise (*Testudo*
hermanni). *Behav. Brain Res.* **173**, 315–319.
(doi:10.1016/j.bbr.2006.06.023)
105. Czaczkes T, Czaczkes B, Iglhaut C, Heinze J. 2015
Composite collective decision-making. *Proc. R. Soc.*
B **282**, 20142723. (doi:10.1098/rspb.2014.2723)