



Private information conflict: *Lasius niger* ants prefer olfactory cues to route memory

F. B. Oberhauser¹ · A. Schlemm^{1,2} · S. Wendt¹ · T. J. Czaczkes¹

Received: 21 November 2018 / Revised: 5 February 2019 / Accepted: 8 February 2019 / Published online: 15 February 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Foraging animals use a variety of information sources to navigate, such as memorised views or odours associated with a goal. Animals frequently use different information sources concurrently, to increase navigation accuracy or reliability. While much research has focussed on conflicts between individually learned (private) information and social information, conflicts between private information sources have been less broadly studied. Here, we investigate such a conflict by pitting route memory against associative odour cue learning in the ant *Lasius niger*. Ants were alternately trained to find a high-quality scented food source on one arm of a Y-maze, and a differently scented low-quality food source on the opposite arm. After training, ants were presented with a Y-maze in which the high- and low-quality-associated scents were presented on opposite arms than during training. The ants showed an extremely strong preferential reliance on the odour cues, with 100% of ants following the high-quality odour and thus moving towards the side associated with low-quality food. Further experiments demonstrated that ants also learn odour associations more rapidly, requiring only one visit to each odour-quality combination to form a reliable association. Side associations in the absence of odours, by contrast, required at least two visits to each side for reliable learning. While much attention has been focussed on visual route learning in insect navigation and decision-making, our results highlight the overwhelming importance of odour cues in insect path choice.

Keywords Route learning · Information conflict · Private information · Odour learning

Introduction

When making decisions, animals rely on acquired information to choose the best option. Information can either be private (i.e. only available to the animal) or public (available more broadly) (Danchin et al. 2004). Animals may have access to many concurrent sources of information of multiple types and modalities and use different strategies to most effectively utilise their available information (Grüter and Leadbeater 2014).

Much research into information use strategies focusses on foraging and navigation decisions, particularly in social insects (Almeida et al. 2018; Collett 2012; Grüter et al. 2011, 2015). For social animals, effective information use can be particularly challenging. Animals have to decide whether to follow their own memory, follow available social information, or to explore the available options themselves, with each strategy involving certain amounts of risk and time costs. To optimise information use, many animals display context-dependent strategies for picking the most promising information source (Grüter and Leadbeater 2014). In recruiting animals such as honeybees and many ants, social information is often used as scaffold on which to establish a route memory (Collett et al. 2003). Once in place, route memories are often, but not always, followed preferentially to recruitment signals (Almeida et al. 2018; Aron et al. 1988; Fourcassie and Beugnon 1988; Grüter et al. 2008, 2011; Harrison et al. 1989; Leuthold et al. 1976; Stroeymeyt et al. 2011).

While much attention has been focussed on information use strategies involving mixed information types (usually

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10071-019-01248-3>) contains supplementary material, which is available to authorized users.

✉ F. B. Oberhauser
Felix.Oberhauser@outlook.com

¹ Animal Comparative Economics Laboratory, Department of Zoology and Evolutionary Biology, University of Regensburg, Regensburg, Germany

² Department of Zoology, University of Oxford, Oxford, UK

private memories and social information) (Coolen et al. 2003; Grüter et al. 2008, 2011; Kendal et al. 2005; Middleton et al. 2018; Smolla et al. 2016; Webster and Laland 2008), animals often have access to multiple relevant information sources of the same type, which may concur or conflict. Indeed, private information may also encompass all the features of information use described above, such as the use of context-dependent strategies and synergistic or additive use of composite or separate information sources, as also found in multimodal communication (see Partan and Marler 2005). However, conflicts between different private information types have been mostly studied in the context of route learning in desert ants (Collett 2012; Legge et al. 2014; Wystrach et al. 2015).

During route memory acquisition, ants incorporate various modalities to aid their navigation (for an overview see Knaden and Graham 2016), depending on the ecology and species. These are, amongst others, visual cues (optic flow, landmarks, panoramas; Collett et al. 2003; Esch et al. 2001; Graham and Cheng 2009; Wystrach et al. 2011), internal cues (e.g. step integrator; Wittlinger et al. 2006) or other sensorimotor cues (e.g. Macquart et al. 2008) or chemical cues (trail pheromones, odours; Czaczkes et al. 2015; Steck 2012). Given the amount of different information sources, it is reasonable to assume that animals are occasionally confronted with contradicting cues while foraging. Most of the studies investigating such contradicting cues did so by conflicting visual navigation cues and path integration (the use of monitored distance and direction to calculate a homing vector (Collett and Collett 2000) in desert ants which navigate in the absence of trail pheromones (Collett 2012). It was found that ants confronted with conflicting information from visual cues and path integration integrate both information sources to form a ‘mean’ vector, at the price of systematic navigation errors (Collett 2012). This indicates additive, not exclusive, use of memorised cues.

In terms of chemical cues, ants may rely, apart from pheromones, on the so-called olfactory landmarks (see Steck 2012 for an overview). The importance of odours in foraging by *Cataglyphis* has been repeatedly demonstrated and odour cues seem to constitute an easily overlooked factor driving foraging behaviour (Buehlmann et al. 2013, 2015; Steck et al. 2011; Steck 2012; Wolf and Wehner 2005), also in species in which trail pheromones are not important. The ants’ strong reliance on odours is also reflected in their proportionally large antennal lobes and lip region of the mushroom bodies (Gronenberg 1999; Gronenberg and López-Riquelme 2004), which are brain areas heavily involved in odour identification and learning (Heisenberg 2003; Søvik et al. 2015). This suggests both high discriminative abilities between odours and their ecological importance for ants. Indeed, studies investigating discriminative capabilities in *Camponotus* ants using Y-mazes found that they can

reliably learn to distinguish different odours (Dupuy et al. 2006) and also memorise an odour-food association for at least 72 h (Josens et al. 2009). Furthermore, ants can also learn to associate odours with aversive stimuli such as heat (Desmedt et al. 2017), highlighting their general availability for learning in ants.

Unlike desert ants, which forage on unpredictably located insect carcasses, many ants, such as our study species *Lasius niger*, derive most of their energy from honeydew provided by cultivated aphids (Flatt and Weisser 2000). Such aphid colonies constitute a semi-permanent food source favouring pheromone trails. Foraging *L. niger* ants rely both on pheromone and visual cues to guide themselves, with decreasing importance of pheromone in experienced individuals (Czaczkes et al. 2013, 2015; Devigne and Detrain 2002; Evison et al. 2008; Grüter et al. 2011). While odour as well as visual cues are ecologically important for their route memory acquisition, other information sources may also play an important role in route learning and navigation, such as path integration (Collett and Collett 2000) and sensorimotor sequence learning (Macquart et al. 2008). While we are not aware of any demonstrations of such information use in *L. niger*, it is notable that *L. niger* foragers fail to learn the location of a food source after one visit in total darkness (Jones et al. 2018), while in a lit environment they do so readily (Oberhauser et al. 2018). This implies that sensorimotor learning is not well developed in this species, and that if path integration occurs, it requires vision for orientation.

The aim of this study was to investigate how foraging ants react to a conflict between two memory-based private information sources: route memory and associatively learned olfactory cues. We first characterised route learning and odour association learning abilities in the ants and then conflicted the two sources.

Materials and methods

Collection and rearing of colonies

Colonies of the black garden ant *L. niger* were collected on the campus of the University of Regensburg and kept in plastic foraging boxes with a layer of plaster of Paris on the bottom. Each box contained a circular plaster nest (14 cm diameter, 2 cm high). The collected colonies were queenless and consisted of ~2000 workers. Queenless colonies forage and lay pheromone trails, and are frequently used in foraging experiments (Devigne and Detrain 2002; Dusutour et al. 2005; Grüter et al. 2015). All colonies were kept in a 12:12 day/night cycle and were provided with ad libitum water and 0.5 M sucrose solution. The colonies were deprived of food for 4 days prior to each trial. In total, 22 colonies were tested, with at least six different colonies

tested per experiment except for the ‘side moderate’ experiment (see below), where 4 colonies were tested. All tested ants were permanently removed from the colony to prevent pseudo-replication.

Sucrose reward and usage of essential oils

Either 1.5 M or 0.25 M sucrose solutions (Merck KGaA, Darmstadt, Germany) were used as reward during training. In experiments using odours (experiments 1, 4, 6 and 7), the sucrose solutions were flavoured by adding 0.005% rosemary or lemon essential oil (Seeger, Springe, Germany) by volume. Previous studies have shown that *L. niger* workers show no preference for either rosemary or lemon essential oil flavoured sucrose solutions (Oberhauser and Czaczkes 2018). Paper runways were impregnated with rosemary or lemon scent by keeping runways in an enclosed box containing 100 µl of the corresponding essential oil on filter paper for > 2 h. Essential oil was used to ensure that the ants could perceive and discriminate the smell due to the various components included.

Setup and experimental procedure

During training, we provided either 1.5 M sucrose or 0.25 M sucrose droplets (~ 20 µl) which were replaced each visit and greatly surpassed *Lasius niger*'s crop capacity (< 2 µl, Mailleux et al. 2000). The droplets were placed on acetate sheets located at the end of either a straight runway (experiments 1 and 4, see Fig. 1) or at the end of a Y-maze (experiments 2, 3, 5–7, see Fig. 1), covered with disposable paper overlays. The straight runway was 1 cm wide and 20 cm long while the Y-maze had 1 cm wide and 10 cm long stem and arms and was tapered to 2 mm at the bifurcation point. Up to five ants were allowed onto the setup simultaneously using a bridge. The first ant to reach the sucrose solution was marked with acrylic paint and all other ants were returned to the nest. From this point on, only the marked ant was selectively allowed to move onto the setup and back to the nest on its own using the bridge. Each visit, the paper overlays were replaced with a fresh overlay, to remove any pheromone or cuticular hydrocarbons deposited on the path. Depending on the experiment, the ant then underwent one of the following training conditions (see below, Fig. 1) before a test was conducted (see “Test phase” below, Fig. 1). Y-maze setup and training methods follow Czaczkes (2018).

Odour and side learning capabilities

The first experiments investigated if and how fast *L. niger* can be differentially conditioned to two odours and how fast they can form route memories to a Y-maze side associated with high-quality reward.

Experiment 1: odour training (Fig. 1a)

The aim of this experiment was to teach ants two odour-reward quality associations, i.e. to perform differential conditioning. Ants made eight training visits and encountered either a 0.25 M or a 1.5 M sucrose solution droplet on alternating visits at the end of a 20 cm long straight runway covered by a scented paper overlay. On each visit, the runway paper overlay was scented and the sucrose solution flavoured with either rosemary or lemon essential oil, with the same odour always leading to the high-quality reward. Sucrose droplets and paper overlays were exchanged between visits to prevent guidance by trail pheromone. The odours associated with high- and low-quality rewards were balanced between ants. After training, the odour preference of the ants was tested (see “Test phase” section below).

Experiment 2: side training (Fig. 1b)

The aim of this experiment was to teach ants that one arm of a Y-maze offered high-quality reward, and the other arm low-quality reward. Ants were presented on alternating visits with a Y-maze covered with unscented paper overlays in which only one arm was present at a time. On one side, low-quality (0.25M) sucrose solution was provided at the end of the Y-maze arm, whereas high-quality (1.5 M) solution was provided on the other side. Ants made eight alternating training visits in total—four to the high-quality side, and four to the low-quality side. Ants always encountered the 0.25 M feeder first. The side of each solution was kept constant during the training (for instance high-quality always on the right arm), but was balanced between ants. Paper overlays of the arms were exchanged between visits. After training, the side preference of the ants was tested (see “Test phase” section below).

Reduced training visits

To investigate the speed of memory acquisition, we conducted weakened forms of the training carried out in experiments 1 and 2 by reducing the overall training visits to each odour/reward or side/reward configuration.

Experiment 3: moderate side training (Fig. 1b)

As in experiment 2, ants were trained to associate a Y-maze side with a reward. In this experiment, however, ants conducted four instead of eight training visits—two to each quality side.

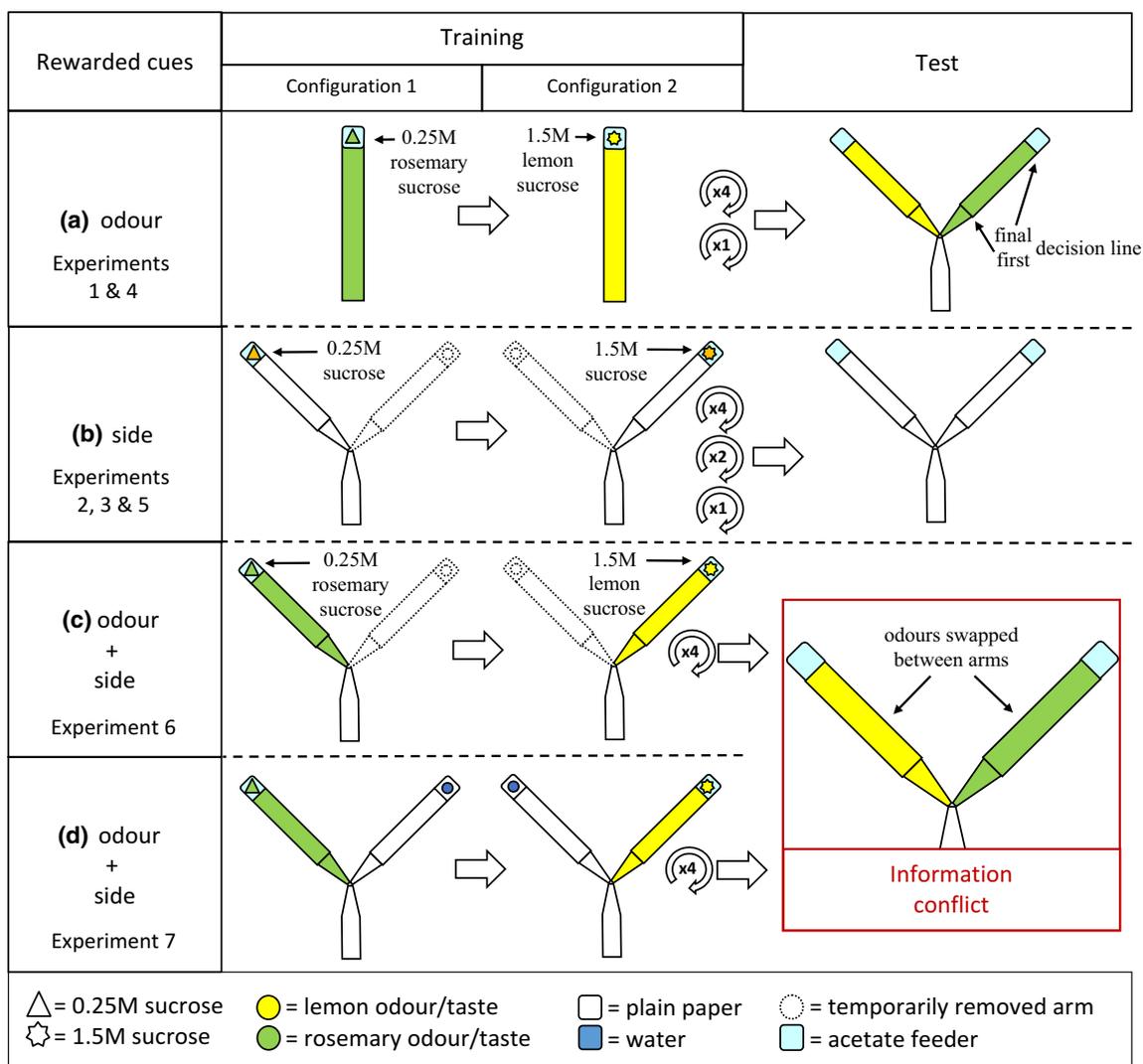


Fig. 1 Setups used for experiment 1–7. Ants were trained to associate an odour, a side, or both with 1.5 M and 0.25 M sucrose rewards. After training, ants were tested in unrewarded test Y-mazes for odour learning (first row, **a**), side learning (second row, **b**) or preference between odour and side information (information conflict, last two rows, **c**, **d**). The numbers within rotating arrows correspond to the number of training visits to each configuration before continuing to the test. **a** During odour training (experiments 1 and 4), ants always encountered low-quality flavoured sucrose in conjunction with one odour and flavour (here rosemary), and high-quality sucrose with the other odour and flavour (here lemon). Ants made either one (experiment 4) or four (experiment 2) visits to each configuration before being tested in a Y-maze without reward with each arm presenting one of the two trained odours. **b** During side training (experiments 2, 3 and 5), ants always encountered low-quality sucrose on one arm

(here left) and high-quality sucrose on the other arm (here right) on the next visit. Ants made either one (experiment 5), two (experiment 3) or four (experiment 2) visits to each configuration before being tested in a Y-maze without reward. **c** A combination of **a** and **b** (experiment 6). On four training visits ants encountered low-quality flavoured sucrose in conjunction with odour (here rosemary) on one arm (here left). On four interspersed visits, ants encountered high-quality sucrose with the other odour and flavour (here lemon) on the other arm (here right). After these eight training visits, ants were tested in a Y-maze without reward in which the odour side was reversed to the training (here rosemary on right). **d** Similar to **c**, but instead of removing one arm, both arms were present at each visit, with one of the arms being unscented and only presenting unflavoured water (experiment 7). After eight training visits, ants were tested as in **c**

Experiments 4 and 5: weak odour/weak side training (Fig. 1a, b)

Experiments were conducted as in experiment 1 (odour) or experiment 2 (side), but ants only underwent two training visits, one to each odour or arm and reward type.

Conflict experiments

After we established a baseline performance for odour and side learning in the previous experiments, we now provided the ants with both olfactory and side cues concurrently in the training.

Experiment 6: odour vs. side conflict (Fig. 1c)

This experiment was a combination of experiments 1 & 2. The aim of this experiment was to train ants to associate different sides with different reward qualities, and also different odours with different reward qualities. Ants again made eight alternating training visits and encountered either a low-quality (0.25 M) or a high-quality (1.5 M) flavoured sucrose solution. As in experiment 2, the side of the high-quality reward was kept constant, but this time, the arm of the Y-maze leading to the reward, and the reward itself, was also scented and quality specific, as in experiment 1.

Experiment 7: unrestricted side vs. odour conflict (Fig. 1d)

In experiment 6, only one arm was presented on each training visit. As a result, the predictive power of directional information might have been compromised, as ants might have learned a rule such as ‘follow path to end’ (Schwarz et al. 2012), while odour cues were always experienced. To prevent such imbalance, in this experiment we presented both arms simultaneously during training in this experiment, to force a decision between arms and thus encourage side learning in trained ants. The training procedure was the same as in experiment 6, except that at each visit, both arms were present. The arm not leading to 0.25 M or 1.5 M (the arm added in this experiment) only offered water and was unscented. The ants were free to go to both arms during training. The ants thus could learn, as in experiment 6, that both scent and side are viable predictors of reward.

Test phase

After undergoing one of the 7 different training routines, ants were confronted with a Y-maze retention test in which no rewards were presented. For experiments 2, 3 and 5, which were conducted without odours, ants had to decide between two unscented arms. For experiments 1 and 4, which were using odours, ants had to decide between two arms of different odour (lemon and rosemary). Lastly and importantly, in experiments 6 and 7, we induced an information conflict, by placing the odour associated with low-quality reward on the side which led to high-quality reward during training (Fig. 1 bottom right), while the odour associated with high-quality reward was presented on the opposite arm.

As soon as the ant crossed a line 2 cm inwards of each arm with the head or antennae, the side was scored as the ant’s initial decision. As soon as the ant crossed a line at the end of the overlay, it was scored as its final decision (see Fig. 1 top right). Furthermore, each ant was allowed onto a sheet of paper at the end of the runway and placed back onto the bridge, permitting her to walk up the maze and to decide again between arms.

This was repeated up to 10 times to get an estimate of consistency in addition to the first choice of each ant.

Statistical analysis

Data were analysed using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) in R version 3.4.4 (R Core Team 2018). GLMMs were fitted using the lme4 package (Bates et al. 2015). As the data were binomial (correct/incorrect), a binomial error distribution and logit link were used. Since multiple ants were tested per colony, we included colony as random factor. In an additional analysis, we also included repeated test visits where each ant was allowed to make up to 10 choices as measure of consistency. In this case, each ant was added as random intercept nested in colony (see ESM 1 for model and results). Each model was validated using the DHARMA package (Hartig 2018). The model predictors and interactions were defined a priori, as suggested by Forstmeier and Schielzeth (2011), as:

$$\text{Decision (correct/incorrect)} \sim \text{Experiment} \\ + \text{RewardSide} + (1|\text{Colony}),$$

where “Decision” is 1 if the ant chose the side (experiments 2, 3, 5) or odour (experiments 1, 4, 6, 7) associated with good food, or 0 if it chose the other arm. Predictors were “Experiment” (experiments 1–7) and “RewardSide” (side of high-quality reward Y-maze arm in test).

We used estimated marginal means contrasts (Lenth 2018) to test each of the experiments against chance level of 50%, the p-values presented were corrected for multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg 1995). Results were plotted using the gglot2 package (Wickham 2016). In total, 250 ants were tested. A complete annotated script and output for all data handling and statistical analysis are presented in electronic supplementary material (ESM 1). The complete raw data are presented in ESM 2.

In only a small fraction (3.6%) of visits, the first and final decision of ants differed. For simplicity, due to these small differences, we used only the first decision of each ant as a measure of performance in the analysis.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Results

For a detailed step-by-step protocol of the statistical analysis, please refer to ESM 1.

89% of ants reliably chose the odour associated with high-quality reward after 4 training visits to each odour (experiment 1, z ratio = 5.661, $p < 0.0001$, see Fig. 2a). Similarly, 87% of ants trained for side/quality associations chose the arm of the maze associated with high-quality reward (experiment 2, z ratio = 3.617, $p = 0.0007$). After two visits to each side/quality combination, 70% of the ants managed to associate the side of the arm with high-quality reward (experiment 3, z -ratio = 2.150, $p = 0.0394$). When training was reduced to only one visit to each quality, 71% of ants trained to odour/quality associations chose the arm with the high-quality-associated odour (experiment 4, z ratio = 2.522, $p = 0.0195$). However, ants failed to associate the side of the arm with high-quality reward after only one visit to each side/quality combination (58%, experiment 5, z ratio = 1, $p = 0.3157$).

When ants were confronted with conflicting information (experiment 6), 100% chose to follow odour cues over side cues. 100% of ants also followed odour cues over side when both arms were present during training (experiment 7). No statistical tests were performed for these clear results. When we included the retention test replicates, resulting in up to 10 choices per ant, the results were similar ($p < 0.0001$ in all experiments except experiment 5 side weak, see ESM 1).

Discussion

Our results show an overwhelming preference for one private information type (olfactory association) over another (route memory) in *L. niger* ants. After we trained ants to associate an odour and a Y-maze side with a high-quality reward, we tested them in a Y-maze where the odour associated with high-quality reward was placed on the opposite arm than in training. Strikingly, 100% of ants confronted with such conflicting odour and side information chose the arm with the high-quality odour, although they never found high-quality reward on that side of the maze. The same result was obtained when we added a second arm during training, offering them a choice rather than forcing them into one direction (Fig. 2b). This was done to encourage side learning and to prevent appliance of a “follow path until end” rule (Schwarz et al. 2012). Thus, despite the ants’ rapid ability to learn the correct side of a Y-maze (see Fig. 2a), ants exclusively oriented themselves using odour cues. However, as the current study was restricted to one pair of odours and one maze design, it would be premature to conclude that our findings represent a general pattern of odour dominance in this species.

Surprisingly, all ants (100%) chose odour when confronted with conflicting information while fewer ants (89%)

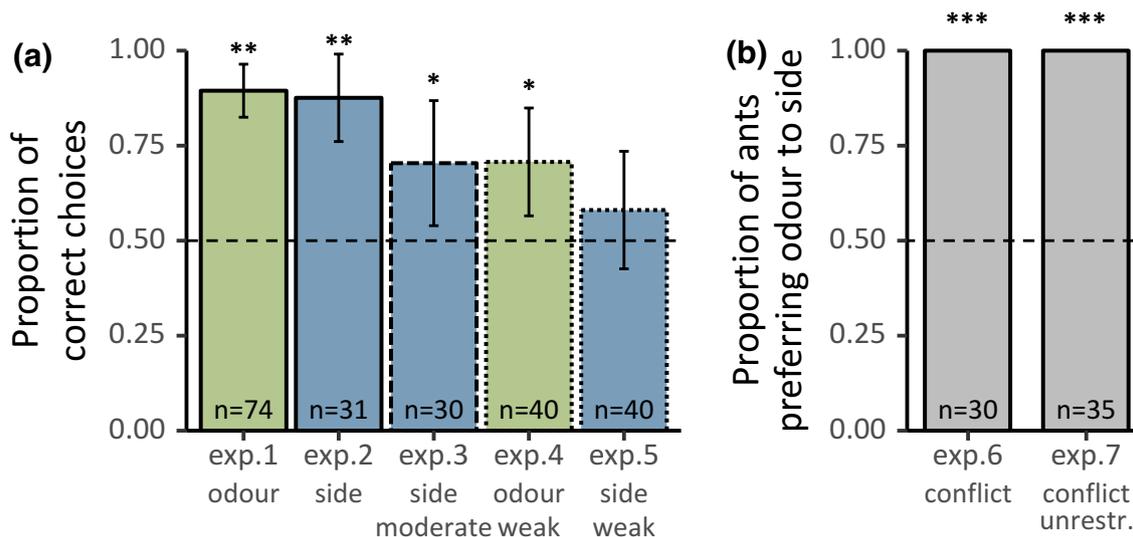


Fig. 2 **a** Proportion of ants deciding initially for side or odour associated with high-quality food. When odour alone had predictive power (experiment 1 odour and experiment 4 odour weak), the majority of ants chose the arm with the odour associated with high-quality sucrose (1.5 M). When trained for side/quality associations for four or two visits to each combination (experiment 2 side and experiment 3 side moderate), the majority of ants chose the side (Y-maze arm) associated with high-quality sucrose in the test. Ants trained with only one visit to each side/odour combination (experiment 5, side weak) showed no preference for the high-quality-associated side. **b** In

conflict experiments, ants overwhelmingly followed odour over side cues, with 100% of ants choosing the arm marked with the high-quality-associated odour. Solid lines reflect experiments with eight training visits, dashed lines four training visits, and dotted lines two training visits. Bars depict means and error bars 95% confidence intervals derived from the fitted GLMM. The dotted horizontal line displays chance level of 50%. * $p < 0.05$; ** $p < 0.001$; ***100% choice for one option, no statistical test was performed. *exp.* experiment, *unrestr.* unrestricted side

followed the odour in a non-conflict situation. While the differences are very small and we cannot exclude possible sampling effects, this finding is consistent with Grüter et al. (2011), who also found higher pheromone following in a conflict group confronted with contradicting social and private information than in ants which experienced no conflict. These findings suggest that contradicting information might increase the salience of a decision and thereby reduce random behaviour in favour of experience-driven decisions. Animals are not aiming to excel in discrimination tasks, but rather are interested in obtaining a reward and avoid punishment (Chittka et al. 2009). In our setup, the costs of “wrong” decisions were low, thus favouring fast but less-accurate decisions. Conflict situations, however, presented a new situation with unknown costs, perhaps causing our ants to invest in higher accuracy. This also suggests that standard discrimination tasks might underestimate the true ability of the tested animal.

Learning of olfactory cues was very fast, with 71% of ants choosing an odour associated with high-quality reward over another associated with low-quality reward after visiting each odour/quality combination only once. Ants failed to learn the side of the maze as a predictor for reward after only one visit to each side (58% correct decisions). If ants are only trained for one side and quality, studies found an accuracy of around 70–80% correct decisions after one visit in T-mazes (Grüter et al. 2011; Oberhauser et al. 2018), thus highlighting a detrimental effect of the lower-quality side association on performance. Ants reliably learned an association after two training visits to each side/quality condition (70% correct decisions) and thus performed at a similar level to ants learning odour cues with only one visit (Fig. 2a). After four visits to each quality, almost 90% of ants chose correctly, irrespective of whether they learned odour or side cues. Such fast acquisition speed is remarkable and, regarding odours, seems to surpass that of *Camponotus mus* ants, which made ~60% correct choices in a Y-maze after 4 training visits in which they were differentially conditioned to two odours (Dupuy et al. 2006). *Camponotus fellah* ants perform at a similar level with ~75–90% correct choices for the rewarded odour after 8 visits (Dupuy et al. 2006; Josens et al. 2009). However, these disparities may also be due to methodological differences.

The clear preference for the olfactory modality in conflict situations may give the impression that ants use their private information in a hierarchical way. Hierarchical information use has been suggested for private and social information (Almeida et al. 2018), although it is clear that when choosing social or private information, information use strategies are more complex, as environmental conditions or other available information sources affect information use choices (Czaczkes et al. 2018; Grüter and Leadbeater 2014; Jones et al. 2018). The preference found for the olfactory modality

is likely strongly linked to the ecology of the investigated species. While our data seem to suggest a strictly hierarchical information use pattern, mounting evidence suggests that different information sources are combined (see Wehner et al. 2016 for an overview) rather than used in a strictly hierarchical way and, if they do not conflict, combining multiple sources of information can improve learning or foraging speed (Czaczkes et al. 2011, 2013).

While our ants were able to learn odours as a predictor for reward after only one training visit to each combination, they failed to do so for side learning. This indicates that, for some reason, side learning is less reliable or less effective for the ants than odour learning in our experiment. The underlying mechanisms are yet unknown. It has been argued that ants have proportionally larger brain regions dedicated to olfaction than vision (Gronenberg 1999; Gronenberg and López-Riquelme 2004), but it is unclear whether this relates to learning abilities. Indeed, which cues are used for side learning is also not clear in this species. The use of landmarks, which were abundantly present in the laboratory room, seems to be well suited for use in side learning, but we do not know whether *L. niger* ants are able to extract single landmarks or use panoramic views for navigation (Wystrach et al. 2011). In pilot studies in which ants were trained to use single distinct visual cues (coloured shapes), ants did not display any learning, thus suggesting that these ants cannot readily extract information from single landmarks (Oberhauser & Czaczkes, unpublished data). Similarly, the addition of simple landmarks on a hard-to-learn trail does not improve navigation in this species (Grüter et al. 2015). However, studies which did not restrict the ants to use single landmarks, but instead provided them a landmark-rich panorama, as the current study, found that *L. niger* ants rely on their visual system to navigate a route (Aron et al. 1993; Carthy 1951; Sakiyama and Gunji 2013), making the visual modality a likely candidate to mediate side learning. Ants may also use path integration (Collett 2000) or sensorimotor learning (Macquart et al. 2008) to navigate to the correct maze arm. However, in near darkness, *L. niger* fail to learn a rewarded side after one visit (Jones et al. 2018), although they learn well under identical setups in the light (Grüter et al. 2011; Oberhauser et al. 2018). Thus, it seems that vision at least is critical for route learning in these ants.

The presence of more than one cue with predictive power can facilitate memory formation (Knaden and Graham 2016; Steck et al. 2011), which could favour the acquisition of multimodal landmarks (cues of different modalities) in insects. Honey bees can also link a certain smell to a colour to find a reward (Srinivasan et al. 1998) and ants recall the location of a food when exposed to its associated odour (Czaczkes et al. 2014). Moreover, redundant information can also serve as backup once the preferred information is not available. For example, *Myrmica ruginodis*, which prefers visual cues

to navigate a complex maze in bright light, readily switches to olfactory cues at low light intensities (Cammaerts et al. 2012). Similarly, *Formica uralensis* and *L. niger* were found to switch to local chemical cues (pheromone) when visual cues are weak (Jones et al. 2018; Salo and Rosengren 2001). Multiple cues can also be integrated: Collett (2012) found that when visual cues and path integration were put in conflict, ants made systematic errors by combining the information to make an ‘average’ heading. Indeed, many studies indicate the presence of weighted cue integration in ants (Wehner et al. 2016). In our conflict test, there was no way for the ants to combine their information, because there was no possibility of making an intermediate decision, thus forcing a hierarchical-like decision. The ants may have made such a ‘compromise’ decision, given the chance.

Finally, the preference of odour cues may not only stem from the ants’ ecology, but also from a particularity of the setup. In training, the scented overlays always extended to the food source, so ants were able to perceive both odour and reward in conjunction, which is required for classical conditioning (Pavlov 1927). In contrast, the decision to move to one particular arm preceded the reward (operant conditioning, Wolf and Heisenberg 1991), although the view seen during the encounter with the reward was available throughout the drinking period. While odour cues were present both at the junction and the reward, we do not know if the cues used for side learning were as readily available at both locations. Thus, preference for odour cues might have arisen from different underlying learning mechanisms. Taking into account the highly context-specific use of different modalities shown in other studies, caution should be used when generalising our findings to a general odour-preference mechanism in *L. niger* ants. Rather, our results provide a proof-of-concept that one modality can entirely dominate over all others.

Despite the clear reliance and preference of odour cues by *L. niger* found in our study, little is known about the usage of odour cues by *L. niger* foragers in the wild. *L. niger* regularly harvest honeydew from phloem-feeding insects and nectaries (Flatt and Weisser 2000), all of which exhibit individual odours which ants can learn (Hayashi et al. 2017; Hojo et al. 2014). It has been shown that *L. niger* ants can use directionless odour cues to retrieve food location information (Czaczkes et al. 2014) and that ants expect the flavour of a food source which was learned either privately (Oberhauser and Czaczkes 2018) or socially (Provecho and Josens 2009). As odour cues were found to play a role in navigation in desert ants (Buehlmann et al. 2013, 2015; Knaden and Graham 2016; Steck et al. 2011, 2012; Wolf and Wehner 2005), it seems probable that *L. niger*, which relies heavily on olfaction for recruitment using pheromone, might extract odour landmarks as well, especially in the cluttered habitats in which it forages. Given ants’ remarkable odour learning abilities

and ability to use odours as navigational landmark, the preferential use of odour cues to visual cues promotes odour to a key player in ant navigation—possibly more important than visual navigation even for very visual species. Studies of navigation in ants should thus fully address the role of olfaction in navigation.

Acknowledgements We thank Frederic Krieger for collecting part of the data. Ken Cheng and two anonymous reviewers are thanked for providing very helpful comments to the manuscript. Also thanks to Anna-Lena Horsch, Emerenz Rosner, Stefan Ried, Julia Held, Lisa Armenia, Johannes Gössele, Philip Pervan, Michael Schachtner, Kristina Pogorelski, Andreas Lösch, Thomas Stadler, Daniel Mederer, Raphael Sedlmaier, Marie van Vugt, Sophie Große-Kathöfer, Christoph Nagler, Sabrina Zölch, Marcus Igl, Robert Zimmermann, Marc Hauber, and Lina Pedraza for gathering pilot data for this project.

Funding FBO, SW and TJC were funded by a DFG Emmy Noether grant to TJC (Grant no. CZ 237/1-1).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

- Almeida NGD, Camargo RDS, Forti LC, Lopes JFS (2018) Hierarchical establishment of information sources during foraging decision-making process involving *Acromyrmex subterraneus* (Forel, 1893) (Hymenoptera, Formicidae). *Rev Bras Entomol* 62:36–39. <https://doi.org/10.1016/j.rbe.2017.11.006>
- Aron S, Deneubourg JL, Pasteels JM (1988) Visual cues and trail-following idiosyncrasy in *Leptothorax unifasciatus*: An orientation process during foraging. *Ins Soc* 35:355–366. <https://doi.org/10.1007/BF02225811>
- Aron S, Beckers R, Deneubourg JL, Pasteels JM (1993) Memory and chemical communication in the orientation of two mass-recruiting ant species. *Ins Soc* 40:369–380. <https://doi.org/10.1007/BF01253900>
- Bates D, Mächler M, Bolker BM, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Soft*. <https://doi.org/10.18637/jss.v067.i01>
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B Met* 57:289–300
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Buehlmann C, Hansson BS, Knaden M (2013) Flexible weighing of olfactory and vector information in the desert ant *Cataglyphis fortis*. *Biol Lett* 9:20130070. <https://doi.org/10.1098/rsbl.2013.0070>
- Buehlmann C, Graham P, Hansson BS, Knaden M (2015) Desert ants use olfactory scenes for navigation. *Anim Behav* 106:99–105. <https://doi.org/10.1016/j.anbehav.2015.04.029>

- Cammaerts M-C, Rachidi Z, Beke S, Essaadi Y (2012) Use of olfactory and visual cues for orientation by the ant *Myrmica ruginodis* (Hymenoptera: Formicidae). *Myrmecol News* 16:45–55
- Carthy JD (1951) The Orientation of two allied species of british ant, I. Visual direction finding in *Acanthomyops (Lasius) niger*. *Behaviour* 3:275–303
- Chittka L, Skorupski P, Raine NE (2009) Speed-accuracy tradeoffs in animal decision making. *Trends Ecol Evol* 24:400–407. <https://doi.org/10.1016/j.tree.2009.02.010>
- Collett TS (2000) Path integration in insects. *Curr Opin Neurobiol* 10:757–762. [https://doi.org/10.1016/S0959-4388\(00\)00150-1](https://doi.org/10.1016/S0959-4388(00)00150-1)
- Collett M (2012) How navigational guidance systems are combined in a desert ant. *Curr Biol* 22:927–932. <https://doi.org/10.1016/j.cub.2012.03.049>
- Collett M, Collett TS (2000) How do insects use path integration for their navigation? *Biol Cybern* 83:245–259. <https://doi.org/10.1007/s004220000168>
- Collett TS, Graham P, Durier V (2003) Route learning by insects. *Curr Opin Neurobiol* 13:718–725. <https://doi.org/10.1016/j.conb.2003.10.004>
- Coolen I, van Bergen Y, Day RL, Laland KN (2003) Species difference in adaptive use of public information in sticklebacks. *Proc Biol Sci* 270:2413–2419. <https://doi.org/10.1098/rspb.2003.2525>
- Czaczkes TJ (2018) Using T- and Y-mazes in myrmecology and elsewhere: a practical guide. *Ins Soc* 65:213–224. <https://doi.org/10.1007/s00040-018-0621-z>
- Czaczkes TJ, Grüter C, Jones SM, Ratnieks FLW (2011) Synergy between social and private information increases foraging efficiency in ants. *Biol Lett* 7:521–524. <https://doi.org/10.1098/rsbl.2011.0067>
- Czaczkes TJ, Grüter C, Ellis L, Wood E, Ratnieks FLW (2013) Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*. *J Exp Biol* 216:188–197. <https://doi.org/10.1242/jeb.076570>
- Czaczkes TJ, Schlosser L, Heinze J, Witte V (2014) Ants use directionless odour cues to recall odour-associated locations. *Behav Ecol Sociobiol* 68:981–988. <https://doi.org/10.1007/s00265-014-1710-2>
- Czaczkes TJ, Grüter C, Ratnieks FLW (2015) Trail pheromones: an integrative view of their role in social insect colony organization. *Annu Rev Entomol* 60:581–599. <https://doi.org/10.1146/annurev-ento-010814-020627>
- Czaczkes TJ, Beckwith JJ, Horsch A-L (2018) Information synergy: Adding unambiguous quality information rescues social information use in ants. *bioRxiv* 219980. <https://doi.org/10.1101/219980>
- Danchin E, Giraldeau L-A, Valone TJ, Wagner RH (2004) Public information: from nosy neighbors to cultural evolution. *Science* 305:487–491. <https://doi.org/10.1126/science.1098254>
- Desmedt L, Baracchi D, Devaud J-M, Giurfa M, d’Ettorre P (2017) Aversive learning of odor-heat associations in ants. *J Exp Biol* 220:4661–4668. <https://doi.org/10.1242/jeb.161737>
- Devigne C, Detrain C (2002) Collective exploration and area marking in the ant *Lasius niger*. *Ins Soc* 49:357–362. <https://doi.org/10.1007/PL00012659>
- Dupuy F, Sandoz J-C, Giurfa M, Josens R (2006) Individual olfactory learning in *Camponotus* ants. *Anim Behav* 72:1081–1091. <https://doi.org/10.1016/j.anbehav.2006.03.011>
- Dussoutour A, Deneubourg J-L, Fourcassie V (2005) Amplification of individual preferences in a social context: the case of wall-following in ants. *Proc Biol Sci* 272:705–714. <https://doi.org/10.1098/rspb.2004.2990>
- Esch HE, Zhang S, Srinivasan MV, Tautz J (2001) Honeybee dances communicate distances measured by optic flow. *Nature* 411:581. <https://doi.org/10.1038/35079072>
- Evison SEF, Petchey OL, Beckerman AP, Ratnieks FLW (2008) Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. *Behav Ecol Sociobiol* 63:261–267. <https://doi.org/10.1007/s00265-008-0657-6>
- Flatt T, Weisser WW (2000) The effects of mutualistic ants on aphid life history traits. *Ecology* 81:3522–3529. [https://doi.org/10.1890/0012-9658\(2000\)081%5B3522:TEOMA0%5D2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081%5B3522:TEOMA0%5D2.0.CO;2)
- Forstmeier W, Schielzeth H (2011) Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner’s curse. *Behav Ecol Sociobiol* 65:47–55. <https://doi.org/10.1007/s00265-010-1038-5>
- Fourcassie V, Beugnon G (1988) How do red wood ants orient when foraging in a three dimensional system? I. Laboratory experiments. *Ins Soc* 35:92–105. <https://doi.org/10.1007/BF02224141>
- Graham P, Cheng K (2009) Which portion of the natural panorama is used for view-based navigation in the Australian desert ant? *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 195:681–689. <https://doi.org/10.1007/s00359-009-0443-6>
- Gronenberg W (1999) Modality-specific segregation of input to ant mushroom bodies. *Brain Behav Evol* 54:85–95. <https://doi.org/10.1159/000006615>
- Gronenberg W, López-Riquelme GO (2004) Multisensory convergence in the mushroom bodies of ants and bees. *Acta Biol Hung* 55:31–37. <https://doi.org/10.1556/ABiol.55.2004.1-4.5>
- Grüter C, Leadbeater E (2014) Insights from insects about adaptive social information use. *Trends Ecol Evol* 29:177–184. <https://doi.org/10.1016/j.tree.2014.01.004>
- Grüter C, Balbuena MS, Farina WM (2008) Informational conflicts created by the waggle dance. *Proc Biol Sci* 275:1321–1327. <https://doi.org/10.1098/rspb.2008.0186>
- Grüter C, Czaczkes TJ, Ratnieks FLW (2011) Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. *Behav Ecol Sociobiol* 65:141–148. <https://doi.org/10.1007/s00265-010-1020-2>
- Grüter C, Maitre D, Blakey A, Cole R, Ratnieks FLW (2015) Collective decision making in a heterogeneous environment: *Lasius niger* colonies preferentially forage at easy to learn locations. *Anim Behav* 104:189–195. <https://doi.org/10.1016/j.anbehav.2015.03.017>
- Harrison JF, Fewell JH, Stiller TM, Breed MD (1989) Effects of experience on use of orientation cues in the giant tropical ant. *Anim Behav* 37:869–871. [https://doi.org/10.1016/0003-3472\(89\)90076-6](https://doi.org/10.1016/0003-3472(89)90076-6)
- Hartig F (2018) DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models. <https://CRAN.R-project.org/package=DHARMA>
- Hayashi M, Hojo MK, Nomura M, Tsuji K (2017) Social transmission of information about a mutualist via trophallaxis in ant colonies. *Proc Biol Sci*. <https://doi.org/10.1098/rspb.2017.1367>
- Heisenberg M (2003) Mushroom body memoir: From maps to models. *Nat Rev Neurosci* 4:266. <https://doi.org/10.1038/nrn1074>
- Hojo MK, Yamamoto A, Akino T, Tsuji K, Yamaoka R (2014) Ants use partner specific odors to learn to recognize a mutualistic partner. *PLoS One* 9:e86054. <https://doi.org/10.1371/journal.pone.0086054>
- Jones S, Czaczkes TJ, Gallager AJ, Oberhauser FB, Bacon JP (2018) Copy when uncertain: Lower light levels result in higher trail pheromone deposition and stronger reliance on pheromone trails in the ant *Lasius niger*. *bioRxiv* 473579. <https://doi.org/10.1101/473579>
- Josens R, Eschbach C, Giurfa M (2009) Differential conditioning and long-term olfactory memory in individual *Camponotus fellah* ants. *J Exp Biol* 212:1904–1911. <https://doi.org/10.1242/jeb.030080>

- Kendal RL, Coolen I, van Bergen Y, Laland KN (2005) Trade-offs in the adaptive use of social and asocial learning, vol 35. Elsevier, Amsterdam, pp 333–379
- Knaden M, Graham P (2016) The sensory ecology of ant navigation: from natural environments to neural mechanisms. *Annu Rev Entomol* 61:63–76. <https://doi.org/10.1146/annurev-ento-010715-023703>
- Legge ELG, Wystrach A, Spetch ML, Cheng K (2014) Combining sky and earth: desert ants (*Melophorus bagoti*) show weighted integration of celestial and terrestrial cues. *J Exp Biol* 217:4159–4166. <https://doi.org/10.1242/jeb.107862>
- Lenth R (2018) Emmeans: Estimated marginal means, aka least-squares means. <https://CRAN.R-project.org/package=emmeans>
- Leuthold RH, Bruinsma O, van Huis A (1976) Optical and pheromonal orientation and memory for homing distance in the harvester termite *Hodotermes mossambicus* (Hagen). *Behav Ecol Sociobiol* 1:127–139. <https://doi.org/10.1007/BF00299194>
- Macquart D, Latil G, Beugnon G (2008) Sensorimotor sequence learning in the ant *Gigantiops destructor*. *Anim Behav* 75:1693–1701. <https://doi.org/10.1016/j.anbehav.2007.10.023>
- Mailleux AC, Deneubourg J-L, Detrain C (2000) How do ants assess food volume? *Anim Behav* 59:1061–1069. <https://doi.org/10.1006/anbe.2000.1396>
- Middleton EJT, Reid CR, Mann RP, Latty T (2018) Social and private information influence the decision making of Australian meat ants (*Iridomyrmex purpureus*). *Ins Soc* 65:649–656. <https://doi.org/10.1007/s00040-018-0656-1>
- Oberhauser FB, Czaczkes TJ (2018) Tasting the unexpected: disconfirmation of expectations leads to lower perceived food value in an invertebrate. *Biol Lett*. <https://doi.org/10.1098/rsbl.2018.0440>
- Oberhauser FB, Koch A, Czaczkes TJ (2018) Small differences in learning speed for different food qualities can drive efficient collective foraging in ant colonies. *Behav Ecol Sociobiol* 72:1096. <https://doi.org/10.1007/s00265-018-2583-6>
- Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. *Am Nat* 166:231–245. <https://doi.org/10.1086/431246>
- Pavlov IP (1927) *Conditioned reflexes*. Oxford University Press, Oxford
- Provecho Y, Josens R (2009) Olfactory memory established during trophallaxis affects food search behaviour in ants. *J Exp Biol* 212:3221–3227. <https://doi.org/10.1242/jeb.033506>
- R Core Team (2018) R: a language and environment for statistical computing. Vienna, Austria. <https://www.R-project.org/>
- Sakiyama T, Gunji YP (2013) Garden ant homing behavior in a maze task based on local visual cues. *Ins Soc* 60:155–162. <https://doi.org/10.1007/s00040-012-0279-x>
- Salo O, Rosengren R (2001) Memory of location and site recognition in the ant *Formica uralensis* (Hymenoptera: Formicidae). *Ethology* 107:737–752. <https://doi.org/10.1046/j.1439-0310.2001.00702.x>
- Schwarz S, Schultheiss P, Cheng K (2012) Visual cue learning and odometry in guiding the search behavior of desert ants, *Melophorus bagoti*, in artificial channels. *Behav Process* 91:298–303. <https://doi.org/10.1016/j.beproc.2012.09.013>
- Smolla M, Alem S, Chittka L, Shultz S (2016) Copy-when-uncertain: bumblebees rely on social information when rewards are highly variable. *Biol Lett*. <https://doi.org/10.1098/rsbl.2016.0188>
- Søvik E, Perry CJ, Barron AB (2015) *Insect reward systems. Genomics, physiology and behaviour of social insects*, vol 48. Elsevier, Amsterdam, pp 189–226
- Srinivasan MV, Zhang SW, Zhu H (1998) Honeybees link sights to smells. *Nature* 396:637–638. <https://doi.org/10.1038/25272>
- Steck K (2012) Just follow your nose: homing by olfactory cues in ants. *Curr Opin Neurobiol* 22:231–235. <https://doi.org/10.1016/j.conb.2011.10.011>
- Steck K, Hansson BS, Knaden M (2011) Desert ants benefit from combining visual and olfactory landmarks. *J Exp Biol* 214:1307–1312. <https://doi.org/10.1242/jeb.053579>
- Stroeymeyt N, Franks NR, Giurfa M (2011) Knowledgeable individuals lead collective decisions in ants. *J Exp Biol* 214:3046–3054. <https://doi.org/10.1242/jeb.059188>
- Webster MM, Laland KN (2008) Social learning strategies and predation risk: minnows copy only when using private information would be costly. *Proc Biol Sci* 275:2869–2876. <https://doi.org/10.1098/rspb.2008.0817>
- Wehner R, Hoinville T, Cruse H, Cheng K (2016) Steering intermediate courses: desert ants combine information from various navigational routines. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 202:459–472. <https://doi.org/10.1007/s00359-016-1094-z>
- Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer, New York
- Wittlinger M, Wehner R, Wolf H (2006) The ant odometer: stepping on stilts and stumps. *Science* 312:1965–1967. <https://doi.org/10.1126/science.1126912>
- Wolf R, Heisenberg M (1991) Basic organization of operant behavior as revealed in *Drosophila* flight orientation. *J Comp Physiol A*. <https://doi.org/10.1007/BF00194898>
- Wolf H, Wehner R (2005) Desert ants compensate for navigation uncertainty. *J Exp Biol* 208:4223–4230. <https://doi.org/10.1242/jeb.01905>
- Wystrach A, Beugnon G, Cheng K (2011) Landmarks or panoramas: what do navigating ants attend to for guidance? *Front Zool* 8:21. <https://doi.org/10.1186/1742-9994-8-21>
- Wystrach A, Mangan M, Webb B (2015) Optimal cue integration in ants. *Proc Biol Sci* 282:1–6. <https://doi.org/10.1098/rspb.2015.1484>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.