Individual cognition
and
collective behaviour in ants

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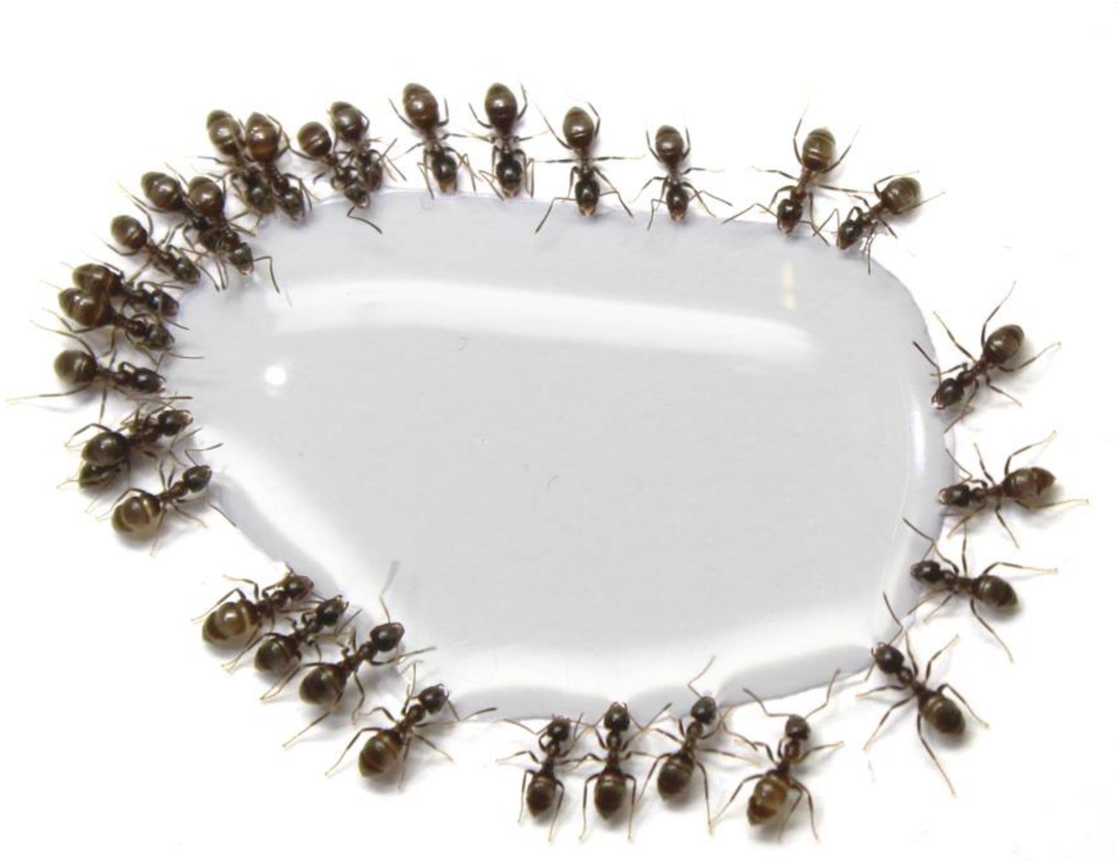

Lasius niger foragers drinking at a sucrose drop, filling their crop before returning to the nest to unload the collected fluid to their nest mates. While feeding, the crop extends and leads to swelling of the gaster, forcing the rigid sclerites apart and revealing the soft membrane underneath. *© Oberhauser FB*

Abstract

Eusocial insects are impressive on individual and collective level. Collectively, they build nests and efficiently exploit and monopolise nearby resources by sharing information amongst their members. Such collective behaviours are shaped by remarkable and multi-facetted individual abilities and decision-making processes. By revealing and investigating factors which influence individual decision making, this thesis provides a deeper insight into the cognitive lives of ants while contributing to a more cohesive understanding of colony behaviour as a whole.

In chapter 2, we demonstrated that foraging ants form expectations about value-neutral qualities of a food source – such as its taste – and dislike food which deviates from those expectations. As such dislike is translated into reduced recruitment, it will have a direct impact on colony-level behaviour. Expectations are thus a potential driver of foraging decisions.

Ants excel in navigation, and we showed in chapter 3 that ants can rapidly learn olfactory or spatial cues to localise food sources. Moreover, when those two types of private information where put into conflict, ants exclusively relied on olfaction. This demonstrates that one type of information can entirely dominate decisions in certain situations.

While we demonstrated that ants learn simple associations rapidly, nothing was known about their ability to learn abstract relations between stimuli, although concept learning has been reported in honeybees. Thus, in chapter 4, we trained ants to learn a relational rule of same/different, but found that the ants, instead of solving such a complex task via learning, resorted to heuristics such as ‘go left’ or ‘go to the more salient cue’. Intriguingly, the heuristics used varied between individual ants.

Learning is dependent on reward and motivation and in chapter 5 we investigated whether those factors alone could explain individual and collective foraging behaviour. While we only found small effects of reward magnitude and motivation on learning, persistence rates varied dramatically in lower motivated colonies. An agent-based model using the empirical data further demonstrated that individual decisions alone can cause ecologically sensible colony-level foraging behaviour.

Finally, in chapter 6 we explored whether colonies could make sensible collective decisions in the context of trail-clearing by Australian meat ants. We found that ant colonies preferentially cleared trails toward a food source if the alternative detours were long. The underlying mechanisms seem to be dependent on individual propensities to remove obstacles which leads to the emergence of paths.

Taken together, the results of this thesis broaden our understanding of individual cognition in ants and demonstrate that properties inherent to individuals, such as experience or task propensities, systematically influence decisions and thus impact the collective.

Work arising from this thesis

This thesis is composed of the following five manuscripts, three of which are published, one is in revision and one in preparation for publication:

A. **Oberhauser FB**, Czaczkes TJ (2018) Tasting the unexpected: Disconfirmation of expectations leads to lower perceived food value in an invertebrate. Biol Lett 14. doi: 10.1098/rsbl.2018.0440
B. **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 72:1–10. doi: 10.1007/s00265-018-2583-6
C. **Oberhauser FB**, Schlemm A, Wendt S, Czaczkes TJ (2019) Private information conflict: *Lasius niger* ants prefer olfactory cues to route memory. Anim Cogn. doi: 10.1007/s10071-019-01248-3
D. **Oberhauser FB**, Middleton EJT, Latty T, Czaczkes TJ (under review, J Exp Biol) Meat ants cut more trail shortcuts when facing long detours.
E. **Oberhauser FB**, Koch A, de Agrò M, Czaczkes TJ (to be submitted) Ants resort to heuristics when facing complex tasks.

During the course of this thesis I also contributed to manuscripts and projects which are not part of this work:

F. Jones S, Czaczkes TJ, Gallager AJ, **Oberhauser FB**, Gourlay E, Bacon JP (accepted) Copy when uncertain: Lower light levels increase trail pheromone deposition and reliance on pheromone trails in ants. Anim Behav
G. de Agrò M., **Oberhauser FB**, Loconsole M, Galli G, Dal Cin F, Moretto E, Regolin L (In preparation) Episodic-like memory and information integration in the black garden ant.
H. **Oberhauser FB**, Wendt S, Czaczkes TJ (In preparation) Social information in the form of pheromone trails does not distort perceived value in ants.
I. **Oberhauser FB**, Bogenberger K, Czaczkes TJ (In preparation) Primacy effect in ants.

Author contributions

Manuscript A

Tomer J. Czaczkes (TJC) and Felix B. Oberhauser (FBO) conceived the experiment, FBO collected, analysed and visualised the data, FBO wrote the manuscript, FBO and TJC revised the manuscript.

Manuscript B

TJC conceived the experiment, FBO and Alexandra Koch (AK) collected the data, TJC wrote the agent-based model, FBO analysed the data, FBO and TJC visualised the data, TJC and FBO wrote ESM5-1 & ESM5-2, FBO wrote the manuscript, FBO and TJC revised the manuscript.

Manuscript C

TJC and FBO conceived the experiments, Annika Schlemm (AS) and Stephanie Wendt (SW) collected the data, FBO analysed and visualised the data, FBO wrote the manuscript, FBO, TJC, AS and SW revised the manuscript.

Manuscript D

TJC, Tanya Latty (TL) and FBO conceived the experiment, FBO and Eliza J. T. Middleton (EJTM) collected the data, FBO analysed and visualised the data and wrote the ant tracker, FBO wrote the manuscript, FBO, TJC, TL and EJTM revised the manuscript.

Manuscript E

FBO and TJC conceived the experiment, FBO and AK collected the data, FBO and Massimo de Agrò (MdA) analysed the data, FBO visualised the data, FBO wrote the manuscript, FBO, TJC and MdA revised the manuscript.

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How this thesis developed

Conducting research on living beings is always a challenge. Laboratory experiments on animal behaviour create a strange situation, lying between unpredictable nature and controlled conditions. By taking animals out of their natural context, we aim to control and reduce the number of possible confounding variables. However, this means confronting the animals with a situation which differs from what they were selected for: to handle the diversity of a natural environment. During the course of this thesis, I dared to venture into ant cognition, an undertaking which soon turned out to be much more complicated than expected. In order to unveil cognitive processes by observation of behaviour, one must design conceptually robust experiments, which will allow alternative explanations to be ruled out. This turned out to be the greatest challenge, as there is seldom a case in which all alternative explanations can be clearly excluded, especially associative learning.

My PhD began in summer, which meant I had to jump right into work to collect data before our ants were put into hibernation. Luckily, I could already collect ants and prepare setups before my official start and was able to collect my first data on the first day of my PhD. The model organism used for my studies was *Lasius niger*, the common black garden ant, and they were a joy to work with. They are fast, resilient to disruptions such as marking, and learn rapidly. Moreover, due to social food sharing via trophallaxis, one ant readily makes multiple visits to a food source (over 40, see chapter 4), often interrupted by only a few (< 3) minutes.

With the first data, I also faced new challenges. I wanted to transition to R for statistical analyses, as it allows the creation of protocols which can be shared amongst scientist and greatly improve transparency. I enjoyed learning it, and ever since published ‘data handling protocols’ alongside the raw data of my manuscripts (see ESMs within this thesis).

The next season turned out to be the greatest challenge of my PhD. Almost all the experiments I had planned and conducted did not work out to some extend due to various reasons. In one case, all the data had a systematic bias and could not be used. Another time, ants did not lay pheromone, for no apparent reason, or began accepting food they usually reject. Things became even more complicated when we tried to test conceptual learning abilities in ants. Thus far, concept learning in insects has been successfully demonstrated in bees by using abstract relational concepts. In such experiments, the animal is trained to set pairs of stimuli, where only the relationship between them leads to reward. Such relations can be 'above-below' or 'same-different'. The later one was chosen, as spatial concepts might be less relevant to a substrate-bound ant. However, we failed to train ants to coloured shapes, restricting us to olfactory cues. Accordingly, we tried to modify the canonical approach of including transfer tests and instead presented the ants with a different odour pair every visit, so that they were constantly confronted with new stimuli – a continuous transfer test, in a way. Still, this restriction to the olfactory modality may have prevented us from showing true concept learning, as this is defined to be transferable across modalities. It turned out that the ants performed surprisingly well in the non-matching treatment, while they failed in the 'go to same odour' treatment. Possible reasons for that are discussed in chapter 4.

Right after collecting the concept learning data, I went to Toulouse to investigate the role of the dopamine receptor AmDop2 in appetitive learning of *Apis mellifera*. It involved proboscis extension response (PER) assays on a lot of bees, which I collected, fully protected by a bee suit, each morning. However, again, I faced serious challenges during the course of the experiment, as the bees generalised heavily to novel odours in differential conditioning. After a lot of effort, I managed to adjust the protocol and could collect sensible data to contribute to a very interesting topic and I left Toulouse with a feeling of accomplishment. This season was very much a transition point for me as a researcher, as I learned to not blame myself for mishaps, but instead to analyse critically and systematically what the cause of unexpected outcomes might be.
On a side note, it was amazing to see that some beehives in Toulouse had adapted strategies to deal with invasive *Vespa velutina* hornets, which hover in front of the entrance and intercept and kill returning bees. Some hives formed clumps of bees at the entrance and the returning foragers would fly directly into them without slowing down. Other hives had swarms of bees flying in front of the hive, which seemed to distract the hornet from focussing on individuals. Unfortunately, there was not enough time to follow up on this.

Winter was writing time, and I could finally finish projects to get them off my desk. I will never forget the relief when my first two projects (chapter 2 & 5) got accepted for publication. It meant more capacity for the rest, of which there was a lot. The next experiment, which I was very excited about, was the investigation of meta-memory-like abilities in ants. Metamemory is the ability of an animal to reflect on its' own memory state (not necessarily consciously) to make appropriate decisions and we came up with a clever experiment to test it. However, after conducting a pilot and sending a description of our experiment to renown researchers in the field, we soon faced serious alternative explanations to overcome. How would we disentangle a ‘vacillation’ response, where ants switch states from “knowing” to “not knowing”, induced by low-memory traces, from a true meta-memory-like decision? At the time, we could not come up with a good solution in a reasonable amount of time (which is always the limiting factor), although it surely exists! Thus, with a very heavy heart, we abandoned this project.

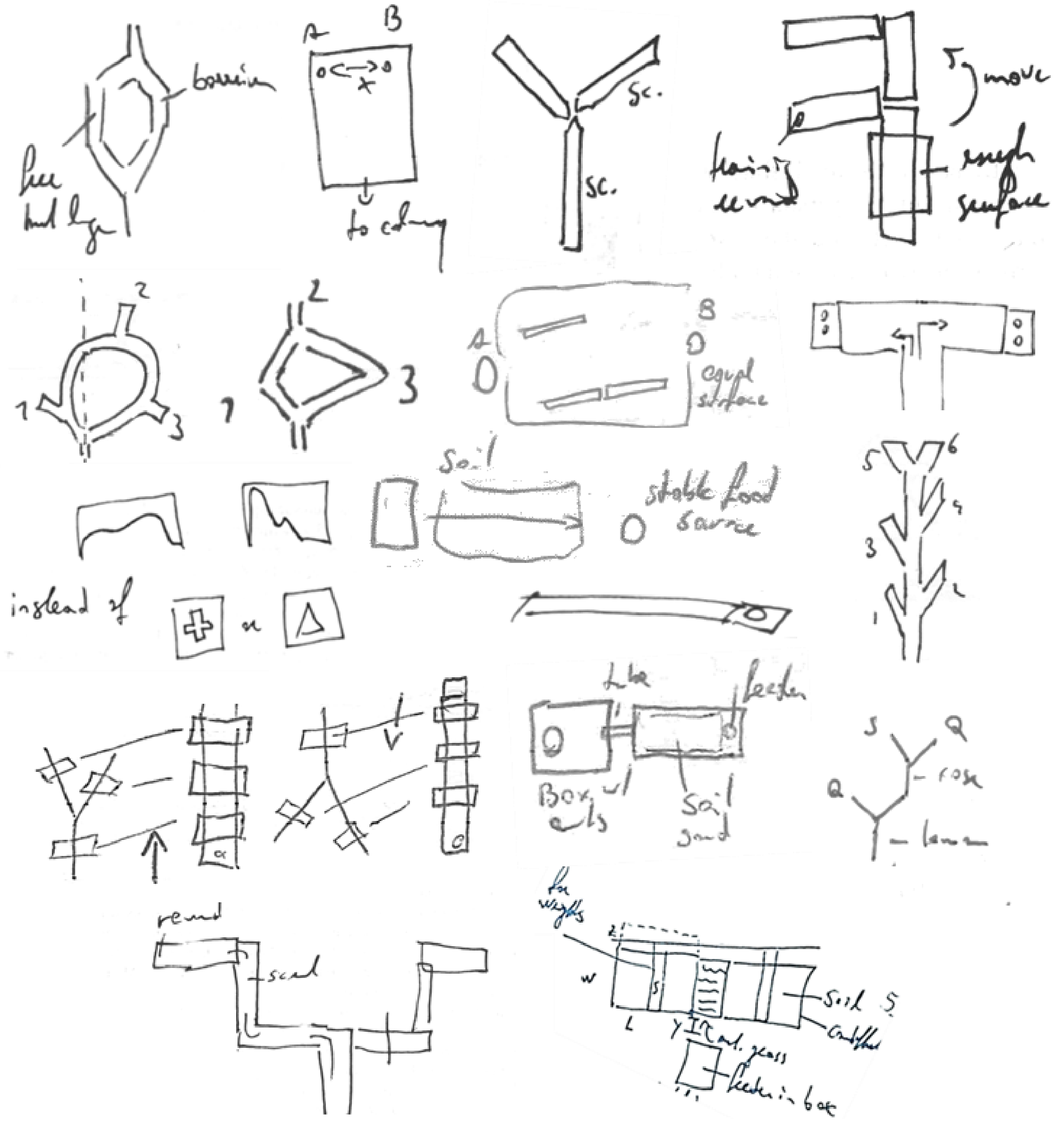
At the same time another pressing question came up. We had already made progress on how ants’ expectations affect the perceived value of a food (see chapter 2) and that different private information types can totally dominate others (see chapter 3). Still, one obvious unanswered question was how social information can affect value perception. Social information can, depending on the individual experience, be preferred to private information (Grüter et al. 2011), but little is known about how the presence of pheromone shapes the expectations of a food source. A heavily ‘advertised’ food might be overrated by ants, similar to a food containing a deterrent is accepted by ants if it tastes like food received from others via trophallaxis (Josens et al. 2016). To this end, we performed a whole test battery trying to unveil the effect of pheromone on decisions and also on the perceived value of the food and could show that pheromone elicits no systematic effect. (Oberhauser et al., in preparation). However, as the last data were collected recently in this season, this study was not included in the thesis due to time constraints.

All of the experiments described until here were carried out in the laboratory, which was a necessity due to the nature of those experiments. However, I love field work, and accordingly was more than happy when I got the opportunity to venture out in the field for a study on meat ants in Australia. This study was challenging mainly for one reason: very limited time. We therefore prepared three possible experiments to do in the field, just to be sure that one would work with a species I had only read about thus far, but never actually observed in the wild. After two field days, it became obvious that two of the experiments would not work without serious adjustments and piloting, but that collective path cutting would be feasible and promising. Thanks to Tanya Latty and Eliza JT Middleton, the carpool service of the University of Sydney (left side driving!) and, last but not least, the absence of rain, I was able to push through the experiment with long working days. It absolutely paid off in the end.

Three years is a very short time to develop and conduct research projects. Many other setups were created and data collected, but the projects were put on hold due to time constraints. One of them was an investigation whether ants can learn a sequence of odour landmarks, resembling an experiment on visual sequence learning by Chameron et al. (1998). In another experiment, I used microcapillary tubes to track ingested volume of drinking ants in real-time – a delightful thing to watch!

So, in retrospect, this is how this thesis evolved over time and came together in its current form. It was an adventure with innumerable lessons. I am excited about all the future questions awaiting me, desperately in need of answers.

to CURIOSITY



A selection of various drafts of potential experiments which arose during the course of the thesis.

Chapter 1 General introduction

1.1 Overview

Social insects are immensely successful. Ants, bees, wasps and termites are estimated to constitute around 75% of the total insect biomass in tropical rainforests (Fittkau and Klinge 1973; Hölldobler and Wilson 1990), and ants and termites alone make up more than half of the insect biomass, while composing only around 2% of the globally known insect species (Wilson and Hölldobler 2005). A major contribution to the global success is their eusociality – the differentiation between reproductive and (mostly) sterile workers caring for the young (Hölldobler and Wilson 1990; Wilson and Hölldobler 2005). Successful reproduction in eusocial organisms thus not only relies on individual capabilities but also on their collective performance. Accordingly, they provide invaluable insights to the intersection between individual and collective behaviour and cognition (Bose et al. 2017; Czaczkes et al. 2015a; Feinerman and Korman 2017; Sasaki and Pratt 2018). While it is often the collective which catches our attention through elaborate nest structures or massive trails, it is the individuals which collect, process and provide information and whole colonies can act on information provided by a few individuals (Feinerman and Korman 2017; Stroeymeyt et al. 2011).

Cognition, be it individual or collective, is missing a consensus definition. It is often used in reference to learning processes which involve more than basic associative learning (Giurfa 2013; Perry et al. 2013). In this thesis, I adhere to a more general definition following Shettleworth (2010), in which cognition comprises all mechanisms which allow animals to acquire, process, store and act on information obtained from the environment. Over the course of the thesis, different stages of information processing were investigated.

How and which information is acquired is heavily dependent on the ecology of a species and the evolutionary demands on its sensory organs (Briscoe and Chittka 2001; Hansson and Stensmyr 2011; Niven and Laughlin 2008). Honeybees, for instance, perform well in colour learning tasks, as memorising floral cues is paramount for their survival (Chittka et al. 2012; Chittka and Menzel 1992). In the soil-dwelling lifestyle of ants, olfaction plays a very important role (Gronenberg and Hölldobler 1999; Hölldobler and Wilson 1990), while reliance on vision is highly variable among ants (Bulova et al. 2016; Gronenberg 1999; Gronenberg and Hölldobler 1999), including eyeless species (e.g. driver ants, Gotwald 1978). The information available to guide decisions is thus restricted by the lifestyle of the animal, and some modalities might be preferred over others due to a better signal-to-noise ratio (Niven and Laughlin 2008) in general or context-dependently – e.g. a switch to olfactory instead visual cues under low light levels (Cammaerts et al. 2012; Jones et al. in press). Such information-use asymmetry, where one type of information or modality is preferred over the other, will be discussed in chapter 3.

The way animals process, store and act on information is not only shaped by their sensory system, but also by processes which evaluate the input and integrate it with past experiences. It is the very essence of learning to predict and re-evaluate outcomes to ensure adaptive behaviour (Byrom and Murphy 2016). The effect of expectations on behaviour will be discussed in chapter 2, while effects of motivation and the quality of encountered stimuli on learning of information are discussed in chapter 5. Other strategies employed by insects to deal with complex tasks will be discussed in chapter 4.

Importantly, cognitive processes are not restricted to individuals, but can also be extended to the collective (Feinerman and Korman 2017; Sasaki and Pratt 2018) which can pool information collected by individuals. Social insect colonies can collectively decide on options such as a new nesting space without the need for individuals to actively compare all options (Mallon et al. 2001; Robinson et al. 2009; Robinson et al. 2014; Seeley and Buhrman 2001) or regulate foraging activities through feedback mechanisms (Beckers et al. 1990; 1993; Czaczkes et al. 2015b; Deneubourg et al. 1990; Goss et al. 1989). An example for collective decision making in the context of trail clearing is presented in chapter 6.

1.2 Foraging and signalling

Since all individuals of a colony benefit from efficient foraging, dishonesty about food locations or eliciting of false alarms is thought to be minimal in eusocial societies, as it incurs fitness costs to all individuals (Heinze and d'Ettorre 2009). This is illustrated by honeybee scouts, a subset of foragers which can act as information producers by informing other foragers about a lucrative food source. Afterwards, they often venture out again to find new locations, thereby maximising collective, but not individual foraging success (Grüter and Leadbeater 2014; Liang et al. 2012). The absence of deception tactics and beneficial impact of information sharing in foraging contexts favoured sophisticated communication systems to increase information flow between individuals. Waggle dances in honeybees (Dyer 2002; Riley et al. 2005; von Frisch and Jander

1957) and pheromone trail recruitment in ants (Czaczkes et al. 2015b) are prominent examples of such systems for information sharing and allow those organisms to decide collectively on the best food sources available (Beckers et al. 1990; de Biseau et al. 1991; Price et al. 2016; Seeley et al. 1991).

Importantly, even very busy foraging trails originate from an initial assessment by single individuals. If a newly encountered food source is deemed to be of sufficient quality and quantity, the ant deposits trail pheromone on the way back to the nest to recruit nestmates (Beckers et al. 1993; Mailleux et al. 2000; 2003). Pheromone deposition is a stereotyped behaviour in which the ant quickly pauses and touches the tip of its gaster on the substrate (Beckers et al. 1992a; Czaczkes et al. 2013a; Fonio et al. 2016) and the number of depositions is proportional to the subjective evaluation of the encountered food source (Beckers et al. 1993; de Biseau et al. 1991; Hangartner 1969; Jackson and Châline 2007; Price et al. 2016; Verhaeghe 1982; Wendt et al. 2019). Quantifying pheromone laying events can thus yield important insights in how insects perceive the quality of a food, i.e. insect value perception (see below and chapter 2). At this stage of recruitment, the whole process is thus dependent on the capabilities of one individual and can be affected by learning and experience (see below, chapter 2 and chapter 5).

Freshly recruited naïve ants then follow the pheromone trail to the new food source and further strengthen the new trail on return to the nest only if they independently found it profitable (Beckers et al. 1993; Feinerman and Korman 2017; Mailleux et al. 2003). Mass recruitment in ants thus constitutes a conditional amplification process, in which an initial value assessment is double-checked by nestmates to reduce response to ants which are misled in their judgment (Feinerman and Korman 2017). Accordingly, collectives are often robust to biases of individuals, such as decoys (Edwards and Pratt 2009; Sasaki and Pratt 2011; 2018).

1.3 Value perception

Colony fitness is thus dependent on the subjective evaluation of food sources by individuals, which requires the integration of many different aspects. One important step towards an understanding of value perception, and perception in general (Weber's law, Weber 1834), is that options are evaluated relatively instead of absolutely. This understanding was emphasised by the Prospect Theory, a pivotal theoretical contribution to economics proposed by Kahneman and Tversky (1979). They, and others, provided convincing evidence that human decision making is heavily influenced not by absolute outcomes, but based on relative perceptions of losses and gains towards a reference point formed by experience or the *status quo* (Kahneman and Tversky 1979; Tversky and Kahneman 1992; Vlaev et al. 2011).

In other animals, such references can be formed by information that the individual has gathered about a food source, be it individually through previous visits (Couvillon and Bitterman 1984; Flaherty 1982) or socially, for example through reciprocal food sharing via trophallaxis (Hölldobler and Wilson 1990; Wheeler 1918) in social insects (Wendt et al. 2019). Multiple studies have confirmed that animals are very sensitive towards quality shifts of an exploited food source. If the food is of lower quality than on previous visits, this often leads to dislike or even its rejection –so-called negative successive contrast effects (e.g. Bitterman 1976; Flaherty 1982; Wendt et al. 2019). When Bitterman (1976) provided honeybees a sucrose solution and lowered the sucrose concentration between visits, he observed that returning bees showed strongly decreased food acceptance and frequent interruptions while drinking, indicating that they experienced a mismatch between expected and experienced value. Bees whose initial experience was with the lower sucrose concentration accepted it readily. Such relative value perception can be seen as an adaptive response to uncertainty from changing qualities of available food. It allows the internal reference of animals to be updated according to the current environmental state (McNamara et al. 2013). If, for instance, an ant is exposed to high-quality food all around or the colony is satiated, it might reject an otherwise acceptable food source (see also chapter 5). Similarly, an ant can shift to a new food source if it is of better quality than the currently exploited food (Czaczkes et al. 2018).

Figure 1-1. A marked Lasius niger forager is drinking on a sucrose drop presented on the end of a runway.
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Yet, not only the energetic value of a food source is important, but also its other properties. Recruiting scouts can inform their nest mates not only about the energetic quality (Wendt et al. 2019), but also about the taste and odour of the found food via trophallaxis (Farina et al. 2007; Gil and de Marco 2005; Josens et al. 2016; Provecho and Josens 2009). This provides an efficient way to share information about the currently exploited food sources within the colony (de Marco and Farina 2003). Ants can memorise the odour of the received food (Gil and de Marco 2005) and later actively approach food sources which resemble the sample received via trophallaxis during recruitment (Provecho and Josens 2009). Similarly, ants and bees can recall previously visited locations in which food-related odours were experienced (Czaczkes et al. 2014; Reinhard et al. 2004).The taste of a food source received socially can even cause ants to accept deterrent food they would otherwise reject (Josens et al. 2016).

If social information can have such an impact on food perception, then private information should be expected to have equally strong effects, as ants often prioritise their own information over information acquired socially (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). However, a direct effect of value-neutral aspects of a food source on its perceived value had not been directly demonstrated so far. In chapter 2, we thus investigated and demonstrated that a value-neutral change of a food source – its taste – can lead to dislike of food, highlighting that aspects of a food source apart from its energetic or nutritional profitability influences its perceived value

1.4 Learning in social insects

Once a new food source has been found, a successful scout has to relocate the nest. Indeed, one of the proposed factors promoting the outstanding learning abilities of Hymenoptera is their territoriality and the fact that many species are central-place foragers, with a reliance on ephemeral resources (Collett et al. 2013; Hölldobler and Wilson 1990; Jones and Agrawal 2017). Studies on insect foraging, especially navigation and route learning, have revealed a variety of mechanisms which allow accurate pinpointing of the nest or food patches (Collett et al. 2003; 2013; Knaden and Graham 2016). It is important to note that selective pressures such as relocating the nest and learning the profitability of food sites are also faced by solitary insects. Indeed, the rise of many impressive cognitive abilities preceded the advent of eusociality and might account for the high reliance on individuals in many of the collective decision making systems of social insects (Farris and Schulmeister 2011; Feinerman and Korman 2017).

Individual learning abilities are often highly variable between individuals and colonies (Chittka et al. 2012). This indicates that it is not cognitive ability *per se* which is selected for, but rather how well cognitive abilities serve in a certain environment (Rowe and Healy 2014). While colonies might profit from good learners in some conditions (Raine and Chittka 2008 but see Evans et al. 2017), it might be fast but inaccurate foragers when variation between food qualities is low (Burns 2005). Therefore, colonies with mixed foraging types might perform best in heterogenous habitats (Burns and Dyer 2008). Variability between individuals also incorporates other potential benefits. A certain noise in evaluation processes can aid the tracking of environmental changes (Deneubourg et al. 1983; Dussutour et al. 2009) and inaccurate foragers might encounter other, higher-yielding food sources (Evans and Raine 2014). Furthermore, parameters affecting learning could influence collective decisions. If poor food qualities were more likely to be forgotten by individual foragers, colonies might focus on higher-quality food sources (see chapter 5).

Learning allows events in an environment to be predicted based on past experiences and allows animals to anticipate and prepare actions before the onset of an event, thus constituting a more rapid adaptation mechanism than evolution. Most research on insect learning has been focussed on forms of associate learning, namely classical (Pavlov 1927) and operant conditioning (Skinner 1938). In classical conditioning, animals learn that a previously neutral, conditioned stimulus (CS) is associated with a stimulus which is of intrinsic importance to the animal due to its appetitive (such as good food) or aversive (such as an electric shock) nature (unconditioned stimulus, US) (Pavlov 1927). Thus, animals learn about relationships in the world (Brembs and Heisenberg 2000). Importantly, the resulting behaviour has no effect on either CS or US presence. This is in contrast to operant (or instrumental) conditioning, where a behavioural action (CR) is associated with an outcome (US), resulting in learning the consequences of one’s own actions (Brembs and Heisenberg 2000). A famous example is the lever pressing of rats for reward in Skinner boxes (Skinner 1938). While in classical conditioning it is the (controllable) environment which predicts the outcome, it is the animal’s own behaviour in operant conditioning (Brembs and Heisenberg 2000). In many cases, the associations made by an insect can be classical, operant or both, such as walking towards the end of a scented Y-maze arm to receive a reward (Dupuy et al. 2006; see chapter 3).

In 1972, Rescorla and Wagner established a model to directly calculate the associative strength of the CS for the US and emphasised that surprise, i.e. prediction error, is a driving factor in learning (Byrom and Murphy 2016). While the model has some limitations, which gave rise to alternative models (see Pearce and Bouton 2001 for an overview), it is able to predict learning performances and to explain learning phenomena such as blocking (Kamin 1969) or overshadowing (Pavlov 1927). The model is encapsulated in a relatively simple formula:

$$_{\Delta}V_A = \alpha\beta\ (\lambda - V_T)$$

which states that the variation in associative strength of the CS A ($_{\Delta}V_A$; i.e. the change in the capacity of stimulus A to elicit a conditioned response) depends on the discrepancy between the US strength (asymptote, λ; i.e. the capacity of the US to elicit an unconditioned response) and the cumulated associative strength gained by A throughout previous trials (V_T). This discrepancy is modified by the learning rate ($\alpha\beta$, slope), which is determined by the salience of the CS (α) and US (β), and which ranges from 0 to 1. In other words, throughout successive learning trials, the CS responses become closer to those originally elicited by the US, which corresponds to the principle of stimulus substitution (Pavlov 1927). An example of predicted learning curves for different parameter settings can be seen in figure 1-2 (the code is provided in ESM1-1).

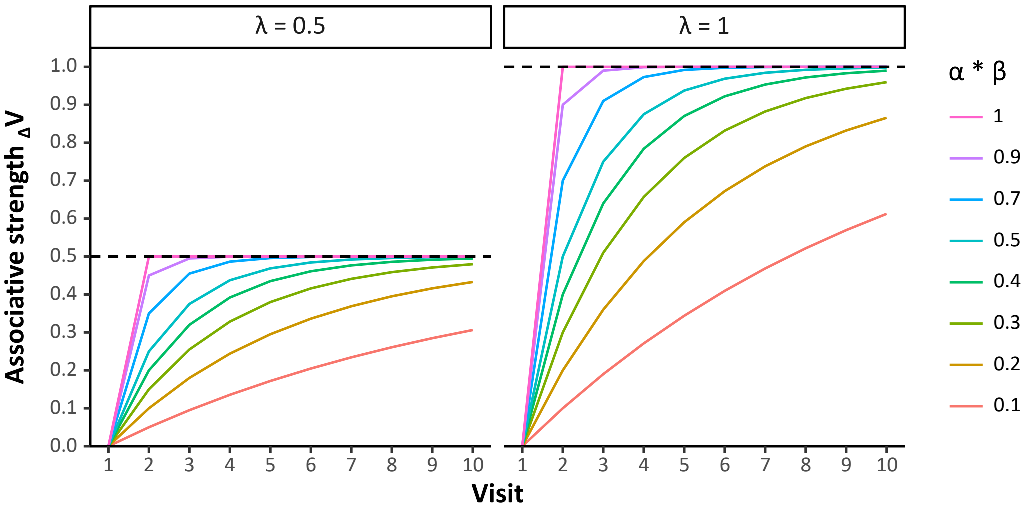

Figure 1-2. Increase of associative strength over repeated visits to a CS – US pair. The higher the learning rate (αβ), the steeper the slope, with a value of 1 equalling immediate learning. λ defines the asymptote of the learning curve.

One important implication of the formula is that the associative strength of a CS at a given trial is dependent on the summed associative strength of all present stimuli. By the use of one equation for each stimulus, the total association strength can be calculated as the sum of all CS:

$$_{\Delta}V_A = \alpha_A \beta \, (\lambda - V_{AB}) \text{ AND } _{\Delta}V_B = \alpha_B \beta \, (\lambda - V_{AB}) \text{ AND } V_{AB} = V_A + V_B$$

As learning speed depends on the salience of the US and CS, a weak US or CS can increase the amount of iterations needed. In contrast, a cue of a preferred modality can be very salient and might contribute a disproportionate amount to the associative strength and, accordingly, will be chosen in a conflict situation because it is more predictive. This is relevant for chapter 3 & 4.

Furthermore, the associative strength is limited by the asymptote and reflects the interaction of the US and CS. The quality of a reward (US) defines the asymptote, thus leading to overall stronger associative memory. This has, for instance, been shown in bees for different sucrose concentrations (Scheiner et al. 1999). Those findings are important for and are discussed in chapter 5.

Moreover, if the CS can serve as reliable predictor for the US, classical conditioning can be described as the transfer of US properties to the CS (Brembs and Heisenberg 2000). This is discussed in chapter 2.

Associative learning is thus more than learning the contiguity of events; it is about learning the predictive power of events or actions. Due to the high adaptive value of associative learning, differentiating it from other cognitive abilities constitutes a serious challenge (Perry and Barron 2013). One suggested practice is to follow Morgan’s canon, which is a version of Occam’s razor applying specifically to animal cognition and urges to only accept an explanation based on complex cognition when simpler learning mechanisms, such as associative learning, cannot account for the observed phenomenon (Morgan 1894; Zentall and Bshary 2018). After all, it is not the goal of learning to reveal the underlying truth, but to solve the problem the animal is facing by any means necessary (Haselton et al. 2015) and some seemingly complex behaviours can be achieved via mechanisms of sensory adaption and habituation (see chapter 4). However, Morgan’s canon introduces its own set of problems, such as theoretical conservatism and the need for objective assessments of cognitive complexity (Fitzpatrick 2008). In either case, considering simple explanations for complex behaviour can help to design elegant experiments which allow to discount such explanations in favour of complex cognition (Zentall and Bshary 2018).

1.5 Collective behaviour

As illustrated above and in discussed in Chapter 5, collective behaviour must be based on individual capabilities and assessments, and knowledgeable individuals can greatly influence decisions or participate disproportionally in colony tasks (Bochynek et al. 2019; Feinerman and Korman 2017; Stroeymeyt et al. 2011). Yet other collective behaviours can arise from pooling many individual solutions. A famous example is the gradual optimisation of foraging trails by ants which leave the trail and enter it shortly after. In cases where this leads to a shortcut, these paths are amplified, thus shortening the trails (Deneubourg et al. 1983; Goss et al. 1989; Reid et al. 2011). The important point here is that ants which contribute to this system do not need to assess the quality of their new solution, but rather collective processes strengthen or ignore this solution due to positive feedback (Feinerman and Korman 2017).

Figure 1-3. Picture of a collective foraging experiment in Lasius niger providing the colony with high-quality (1M, green sucrose drop at the bottom) and poor quality (0.25M, red drop on top) food. At this stage (~10 minutes after first encounter), we can see the emergence of a second, more efficient, route connecting the bridge diagonally to the high-quality food instead of following the wall.
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The clearing of foraging trails from vegetation, as performed to a remarkable extend in some ant species (see chapter 6), is another good example for such collective behaviour based on many individual decisions. No single ant decides on the direction or necessity of a trail, but it is the orchestrated activity of many ants which leads to the emergence of impressive trail systems. Chapter 6 discusses trail clearing in Australian meat ants (*Iridomyrmex purpureus*) and demonstrates that trail construction can be collectively optimised by colonies via an encounter-based removal of obstacles by individuals, as has been proposed for leaf-cutter ants (Bochynek et al. 2019).

1.6 *Lasius niger* as a model organism

While most research on insect cognition has been conducted on model organisms such as the honeybee or *Drosophila*, ants also offer great opportunities for the study of insect cognition both on individual and collective level (Dornhaus and Franks 2008). Many species can be easily maintained in the laboratory and mazes constitute an efficient and reliable way to test individual cognition abilities (Czaczkes 2018, chapters 3,4 & 5). All studies but the last presented in this thesis were conducted on the common black garden ant *Lasius niger* (Hymenoptera: Formicidae) (Linnaeus, 1758), which is a well-researched, very widespread and strong pioneer species in Europe (Seifert 2018). The monogynous colonies can contain 10,000 workers (Seifert 2018; Sommer and Hölldobler 1995) with a mean life expectancy in the laboratory of around 1 year while the queen reportedly can reach an age of over 20 years (Keller 1998; Kramer et al. 2016). *Lasius niger* tends various groups of trophobionts such as aphids, and often visits floral and extrafloral nectaries (Seifert 2018). They are adept learners (see chapters 2,3 & 5) and combine mass recruitment via pheromone trails with context-dependent information use by individuals. The importance of both private information (memory) and social information (trail pheromone) in *L. niger* ecology thus make them a well-suited model species for the intersection of individual and collective behaviour and information processing.

1.7 Aims of this thesis

Ant societies constitute complex systems which can give rise to huge nests (Moreira et al. 2010) or well-organised traffic systems, including traffic lanes (Couzin and Franks 2003). Yet, unlike cells in a body, individuals maintain their autonomy and single abilities and decisions can have a disproportionate effect on the colony. Studying how individuals perceive and act on information thus constitutes an important part for the understanding of the inner workings of ant colonies.

The aim of this thesis was to investigate how individual experience and perception can affect decisions (chapters 2,3 & 5) and how this could translate to colony level effects (chapter 5). Ants are demonstrated to be fast learners and display great flexibility in solving problems by applying strategies specific to individuals (chapter 4). The last sections highlight a potential effect of individual learning speed on collective foraging efficiency (chapter 5) and that colonies use trail clearing to optimise their collective foraging efficiency (chapter 6).

Chapter 2

Tasting the unexpected: disconfirmation of expectations leads to lower perceived food value in an invertebrate

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2.1 Abstract

To make sensible decisions, both humans and other animals must compare the available options against a reference point – either other options or previous experience. Options of higher quality than the reference are considered good value. However, many perceptible attributes of options are value-neutral, such as flower scent. Nonetheless, such value-neutral differences may be part of an expectation. Can a mismatch between the expectation and experience of value-neutral attributes affect perceived value? Consumer psychology theory and results suggest it can. To test this in a non-human animal, we manipulated a value-neutral aspect of a food source – its taste – while keeping its absolute value – its sweetness – the same. Individual ants (*Lasius niger)* were allowed to drink either lemon or rosemary flavoured 1M sucrose. After 3 successive visits to the food, we switched the taste in the last, 4th, visit to induce a disconfirmation of expectations. In control trials ants received the same taste on all 4 visits. Disconfirmed ants showed lower food acceptance and laid less pheromone on the way back to the nest, even though the molarity of the food was unchanged. As ants recruit nestmates via pheromone depositions, fewer depositions indicate that the ants valued the food less. Thus, an expectation of value-neutral attributes can influence the perceived value of a resource. Such influences of value-neutral variables on value perception may affect how animals interact with, and exploit, their environment, and may contribute to phenomena such as flower constancy.

Key words

Expectation disconfirmation; associative learning; irrationality; flower constancy; incentive contrasts; value perception

2.2 Introduction

Making decisions can be a complex and difficult task. In the process, humans and other animals often compare the available options against each other (Kahneman and Tversky 1979; Tversky and Kahneman 1992) or against some previous experience (Flaherty 1982). Discrepancies between a reference point and the chosen option can influence and alter behaviour, especially if the decision turns into a loss (Baumeister et al. 2001; Kahneman and Tversky 1979; Tversky and Kahneman 1992). The influence of previous experience on responses to a newly presented stimulus can be reliably demonstrated by negative successive contrast effects: after receiving a poorer reward than previously, many animals, including insects, decrease their performance or food acceptance (Flaherty 1982; Wendt et al. 2019).

In theory, contrast effects are a means to up- or down-regulate responses to a changing environment or novel situations to increase foraging efficiency (McNamara et al. 2013). However, unlike negative contrasts, evidence for positive successive contrasts (increase in performance) is inconsistent at best (Annicchiarico et al. 2016). This asymmetry in losses and gains is in accordance with Prospect Theory (Kahneman and Tversky 1979; Tversky and Kahneman 1992), which describes decision under risk in humans. It proposes a reference point, relative to which losses or gains are assessed. Importantly, perceived value is more negatively affected by losses than it is positively affected by gains (Baumeister et al. 2001; Kahneman and Tversky 1979; Tversky and Kahneman 1992). Crucially, both prospect theory and successive contrasts do not address situations in which a value-neutral quality of the incentive is altered, for instance when the taste - but not the molarity - of a sucrose solution changes.

Such an effect has been reported in consumer psychology studies investigating effects of expectations on food perception (Piqueras-Fiszman and Spence 2015). When expectations and actual quality of a certain food quality (e.g. its taste) diverge, the caused disconfirmation of expectation can affect perception and hedonic ratings of the food. Such an expectancy effect was shown in a classic study (Carlsmith and Aronson 1963), where people who expected a sweet drink but got a bitter one rated the drink as being more bitter and thus less pleasant (or less sweet when they expected a bitter drink) than subjects with confirmed expectations, so expectations alone affected perception.

Intriguingly, indications that food scent alone can influence value perception in invertebrates were reported by Lindauer over 70 years ago (1948). By using the number of waggle dances of honey bees as proxy for perceived food attractiveness, he found that adding a scent to a previously unscented food source led to an initial decrease in waggle dances compared to a control group, but then rose to be higher than the control group after extended foraging.

In our study, we specifically test whether invertebrate value perception is distorted by disconfirmed expectations. Instead of a downshift in sucrose solutions, which directly affects energetic gain, we manipulate taste - a value-neutral attribute in respect to energy content.

2.3 Methods overview

For a detailed description of experimental and statistical methods see ESM2-1, a shortened version of ESM2-1 is presented at the end of this chapter.

In brief, an ant was allowed to make three return visits to a drop of 1M sucrose solution at the end of a runway (figure 2-1). The drop was flavoured with either lemon or rosemary, odours which were equally attractive to the ants in a pilot study (see table S2-1). On the fourth visit we presented either the same flavour as before (confirmation) or the opposite flavour (disconfirmation). On all visits we scored food acceptance (1 = full acceptance, 0 = partial acceptance or rejection) and pheromone deposition on the 20cm runway from the food to the nest. Ants deposit more pheromone for higher quality (= sweeter) food sources (Beckers et al. 1993). Thus, the intensity of pheromone deposition reflects perceived value. Using innate behaviours such as acceptance or pheromone deposition provides feedback without the need for pretraining (Lydall et al. 2010). Data were analysed using generalized linear mixed-effect models (GLMMs) (Bates et al. 2015) In total, 327 ants from 8 colonies were analysed (table S2-2). All scoring was performed blind to treatment.

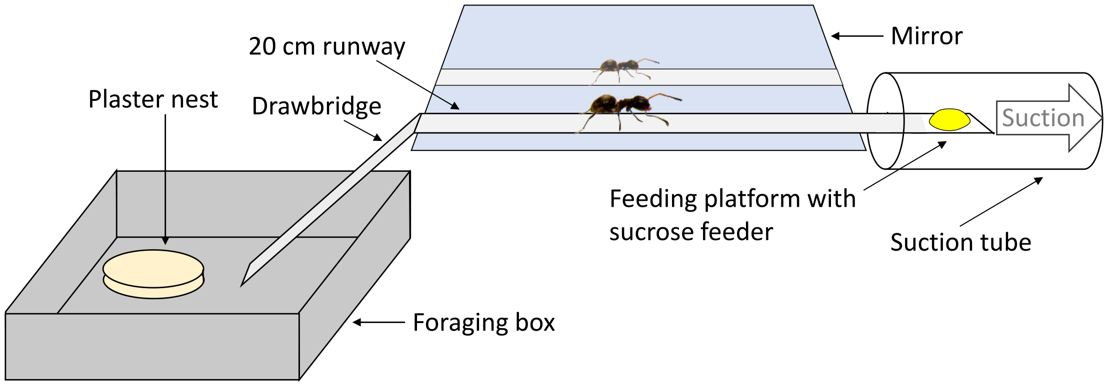

Figure 2-1. Experimental setup. The ants entered the plastic runway via a moveable drawbridge. A flavoured sugar droplet was presented inside a tube with air suction at the end of the runway. Pheromone depositions were counted on the 20cm runway. Each time the ant passed the runway, the paper overlay was replaced.

2.4 Results

We found that disconfirmed ants showed significantly lower food acceptance than ants which found the expected taste (binomial GLMM, z = -4.124, p < 0.0001, figure 2-2a). Likewise, disconfirmed ants deposited significantly less pheromone when returning to the nest than ants which encountered the expected food taste (Quasi-Poisson GLMM, t = -3.102, p< 0.0026, figure 2-2b).

Comparing the final and initial visits, we found a significantly lower food acceptance on the final visit in both confirmation and disconfirmation treatments (z = -5.118, p <0.001; z = -5.171, p < 0.0001, respectively). This was caused by an overall drop in food acceptance after the initial visit (figure S2-5). Nonetheless, disconfirmed ants showed another significant drop in food acceptance on the 4th visit (see above, figure S2-4a). However, disconfirmed ants showed no significant difference in pheromone depositions between the 1st and 4th visit (t = -1.684, p = 0.0942), while confirmed ants deposited significantly more pheromone on the 4th visit (t = 4.648, p > 0.0001, figure S2-4b).

No significant differences were found between tastes, nor was the interaction between treatment and food taste significant (ESM2-1, figure S2-5 & figure S2-6).

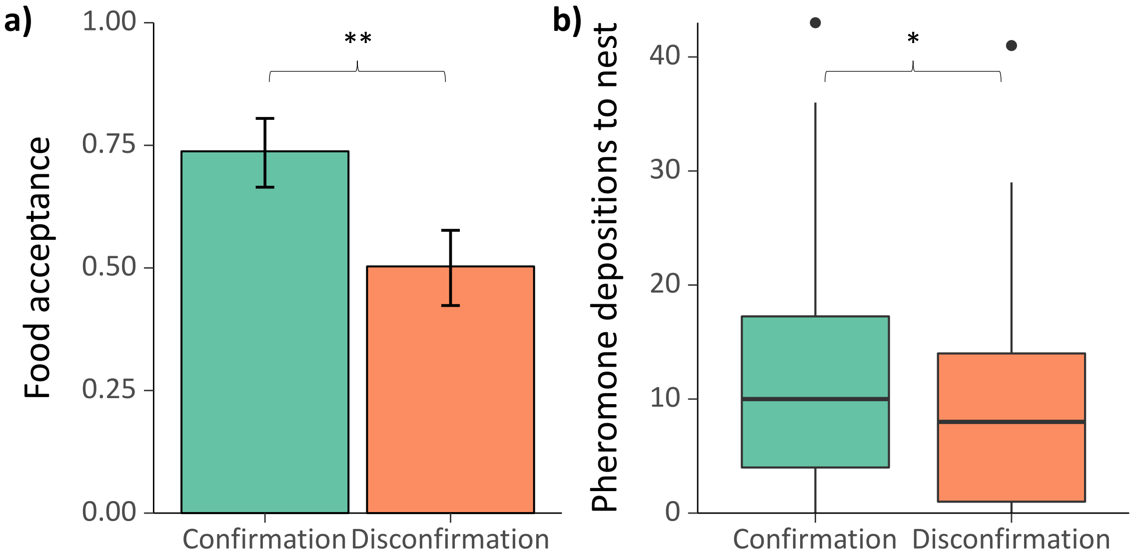

Figure 2-2. ***a)*** *Food acceptance scores (1 = full acceptance, 0 = interrupted drinking) on the 4th visit.* ***b)*** *Pheromone depositions to the nest on the 4th visit. Bars depict means, error bars 95% confidence intervals. Horizontal lines are medians, boxes correspond to first and third quartile and whiskers extend to the largest value within 1.5 * inter-quartile range (IQR). Dots represent values outside of the IQR. Confirmation: n = 164, Disconfirmation: n = 163; * = p < 0.01, ** = p < 0.001.*

2.5 Discussion

Our results demonstrate a negative effect of disconfirmed expectations on value perception in invertebrates, which has only been fully demonstrated in humans so far (Carlsmith and Aronson 1963; Piqueras-Fiszman and Spence 2015). Although it has been reported that the addition of scent to sucrose can affect the number of waggle dances in honey bees (a proxy for food value), that study only compared dances between unscented and scented food (Lindauer 1948) and the reported results might be mediated by scent preferences, or simply the presence versus absence of a scent. In our study, ants perceived the food as less valuable, even restricting recruitment in spite of food deprivation. Our results resemble results obtained in negative contrast experiments (Couvillon and Bitterman 1984; Flaherty 1982), even though we did not manipulate the energetic content of the food. Consequently, in energetic terms, the ants reacted irrationally.

Contrast effects are thought to be beneficial for adaptive behaviour in changing environments. Theory suggests that it is beneficial to react to varying food value by up- or down-regulating effort in the face of changing environmental quality (McNamara et al. 2013). Optimal foraging theories do not offer a viable explanation for the effects of value-neutral attributes on value perception. However, the effect of value-neutral stimuli can be explained in the context of basic learning theory (Rescorla and Wagner 1972), as a cue associated with a reward can gain its own value. In our case, the ants learned the taste to be part of the reward, so taste gained predictive power. When the taste cue was missing, the ants may have experienced this as a loss. Thus, they may have attributed value to a formerly value-neutral quality.

Our findings may offer insights to flower constancy, the tendency to forage on just one type of flower at a given time, often displayed by bees and other pollinators (Chittka et al. 1999). While this might lead to optimisation processes, as each animal learns to handle flowers faster (Ishii and Kadoya 2016), the proximate driver might be that flowers with a different appearance (colour, shape) could be neglected because they are perceived as less valuable. Likewise, ants are known to associate odours with food or other stimuli and to form olfactory memories (Guerrieri and d'Ettorre 2010; Josens et al. 2009; Roces 1990). Associations of food qualities and other cues could thus facilitate site fidelity, a behaviour often described in ants exploiting semi-permanent food sources like honeydew (Quinet and Pasteels 1996).

Attributes of food, like taste, can represent important information about the food location. If route memory and food attributes mismatch, an ant might have made a navigational error. Social insects gather information about the food source via trophallaxis (Farina et al. 2007; Josens et al. 2016; Provecho and Josens 2009; Wendt et al. 2019). The decrease in recruitment in disconfirmed ants may be due to the ant's uncertainty about its location. The reduction in recruitment may thus be ecologically rational.

An alternative explanation of our finding would be that the ants simply "reset" upon discovery of the unexpected food source and behave as if they encountered an unknown food. Despite a decline, disconfirmed ants show similar deposition rates after manipulation to those in the initial visit (figure S2-4b). However, a "reset" cannot explain the lower food acceptance on the final, manipulated, visit compared to the first visit (figure S2-4a). Another possibility is that ants exhibit neophobia to new tastes. However, the significantly higher food acceptance in the initial visit suggests that it is not the "newness", but rather the "unexpectedness" which is driving the observed behaviour. Nonetheless, neophobia effects could be counteracted by high motivation stemming from starvation, which is indicated by the drop in acceptance after the first visit (figure

S2-1), and thus neophobia as a proximate mechanism cannot be ruled out. Interestingly, neophobia could also well explain similar results in human experiments (Carlsmith and Aronson 1963).

Our results show that the foraging behaviour of insects is not only mediated by rational energetic decisions, but also encompasses value-neutral expectations about the food source. Ants, like humans, devalue things with unexpected attributes. The use of pheromone depositions and drinking acceptance as proxies for value perception provide valuable insights how invertebrates perceive value.

Acknowledgments
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Data accessibility
Raw data and a data handling protocol containing all data analysis can be accessed as electronic supplementary material (ESM).

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Conflict of interest
The authors declare no competing interests.

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

2.6 Supplementary manuscript (shortened version of ESM2-1)

Supplement to: Tasting the unexpected: disconfirmation of expectations leads to lower perceived food value in an invertebrate

Felix B. Oberhauser & Tomer J. Czaczkes

2.6.1 Material & Methods

2.6.1.1 (a) Collection and rearing of colonies

Eight colonies of the black garden ant, *Lasius niger*, were collected on the campus of the University of Regensburg, and kept in plastic foraging boxes. Colonies were kept at 500 workers each and were queenless, as is frequently done in foraging experiments (Devigne and Detrain 2002; Dussutour et al. 2004). Colonies were kept in a 12:12 day/night rhythm and were fed three times per week with Bhatkar diet (Bhatkar and Whitcomb 1970) and water *ad libitum*. Colonies were deprived of food for 4 days prior to each trial.

2.6.1.2 (b) Experimental procedure

Several ants were allowed onto a plastic runway (20x1cm) covered with paper overlays via a drawbridge (see figure 2-1). A droplet of 1M sucrose solution (Merck KGaA, Darmstadt, Germany) scented with 0.005% by volume of either rosemary or lemon 100% essential oil (Seeger, Springe, Germany) was presented on a plastic feeder at the end of the runway. Essential oil was used to assure that the ants could perceive and discriminate the odours due to the various components included. Rosemary and lemon odours were found to elicit similar food acceptance and pheromone depositions in a pilot study in which ants made 1 visit to a drop of either rosemary, lemon, lavender or peppermint (see table S2-1 & electronic supplementary material (ESM) 2-2). The setup was the same as described below.

The end of the runway was located inside a wide tube providing light air suction to prevent the ant from smelling the scent before touching the droplet. Although we are referring to taste in the manuscript for sake of simplicity, we cannot be certain that suction alone is preventing the ant from smelling the drop before tasting it. Thus, the ant might experience the drop via smelling and tasting. The setup was filmed from above with a Panasonic DMC-FZ1000 camera.

The first ant at the feeder was marked with a dot of acrylic paint on the abdomen and all other ants were returned to the colony. Only the marked ant was allowed to perform 4 visits to the feeder. The first three visits were considered “training visits” and only either rosemary (R) or lemon (L), was offered so as to build up an expectation for that scent. On the fourth visit, the ants experienced a *confirmation* (same scent as in training, L-L or R-R) or a *disconfirmation* (different scent than in training, L-R or R-L) treatment. The experimenter was blind to the current treatment. Each time an ant walked over the runway, the paper overlay was replaced. After the test, the ant was freeze-killed.

During each outward and return visit, the number of pheromone depositions (ant stops walking and touches the substrate with the tip of its abdomen, Beckers et al. 1993) performed by the ant was counted by eye. In unclear cases, pheromone depositions were extracted from the video.
Ants were also scored each visit for their apparent acceptance of the food: drinking from the sucrose drop immediately and uninterruptedly upon contact was scored 1. Stop drinking within 3 seconds of contact, resuming later was scored 0.5. Ants which did not drink fully within 10 minutes were scored as 0. However, as only 8 ants in total received a score of 0 (all in the disconfirmation treatment), we reclassified all 0.5 scores as 0, to obtain binomial data. Treatment order was pseudo-randomised.

2.6.1.3 (c) Statistical analysis

For a detailed step by step record of the entire statistical analysis procedure, see supplement ESM2-2. Please note that ESM2-2 is provided to ensure transparency but has not been optimised for legibility.

From all 353 ants tested, we removed 26 ants which did not deposit any pheromone from the food to the nest on visits 2 & 3 to prevent non-layers from affecting the analysis. This resulted in 327 ants used for all the analyses. Air pressure and air temperature were excluded from the models, as Mann-Whitney-U tests revealed no significant differences between treatments.
Data were analysed using generalized linear mixed-effect models (GLMMs) (Bolker et al. 2009) in R version 3.4.4 (R Core Team 2018). GLMMs were fitted using the lmer function (Bates et al. 2015). As multiple ants were tested per colony, the colony was added as random effect in all models. The model variables and interactions were defined *a priori*. All models were validated using the DHARMa package (Hartig 2018). Significant effects of the main model were explored by contrasts using the EMMEANS package (Lenth 2018). All contrast *P*-values presented are

corrected for multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg 1995).

As only 8 ants (all in the disconfirmation treatment) did not drink at all, we recoded our food acceptance score to 1 (= full acceptance of food) or 0 (interrupted drinking within first 3 seconds or rejected food) to obtain binomial data. These data were then modelled using a binomial distribution and logit link function and following model formula:

*Food acceptance ~ Treatment * Trained Taste + (1|Colony)*

For the counted number of pheromone depositions, a Quasi-Poisson distribution was used to control for overdispersion (Zuur et al. 2009). To account for variance in pheromone depositions between ants, mean depositions on visit 2 & 3 (*MeanPriorDeposition*) of each ant were added as random factor. The model formula was:

*Pheromone deposition ~ Treatment * Trained Taste + (1|Colony) +(1|MeanPriorDeposition)*

In models comparing 1st and 4th visit we added ant ID nested in Colony as random factor to the corresponding models to account for repeated measures.

2.6.2 Figure Supplements

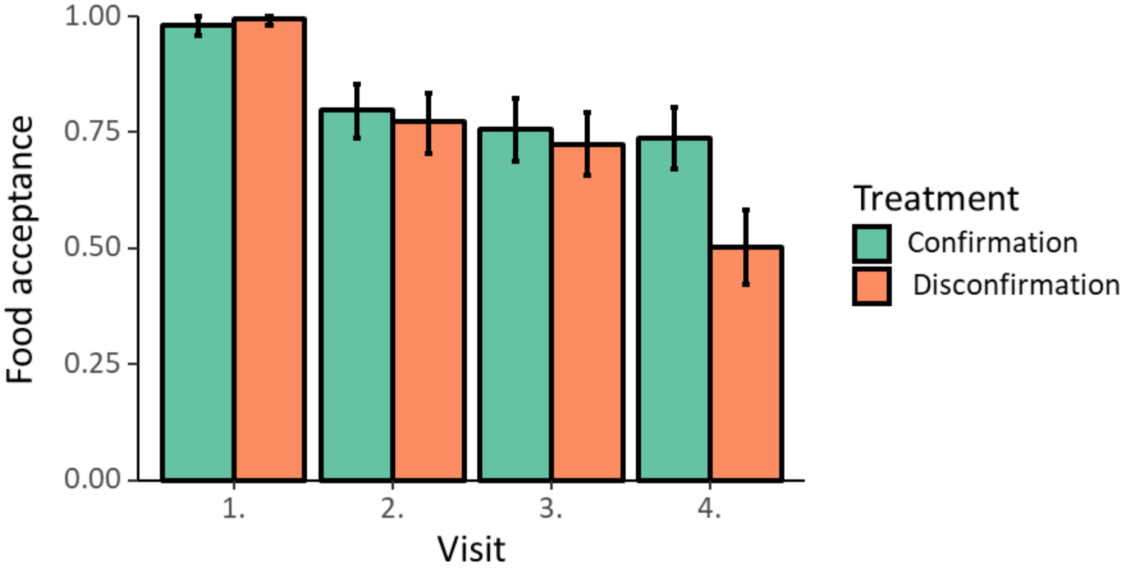

Figure S2-1. Mean food acceptance on each visit for both treatments. 1 = uninterrupted drinking, 0 = interrupted drinking within first 3 seconds. Bars depict mean, error bars depict 95% confidence interval.

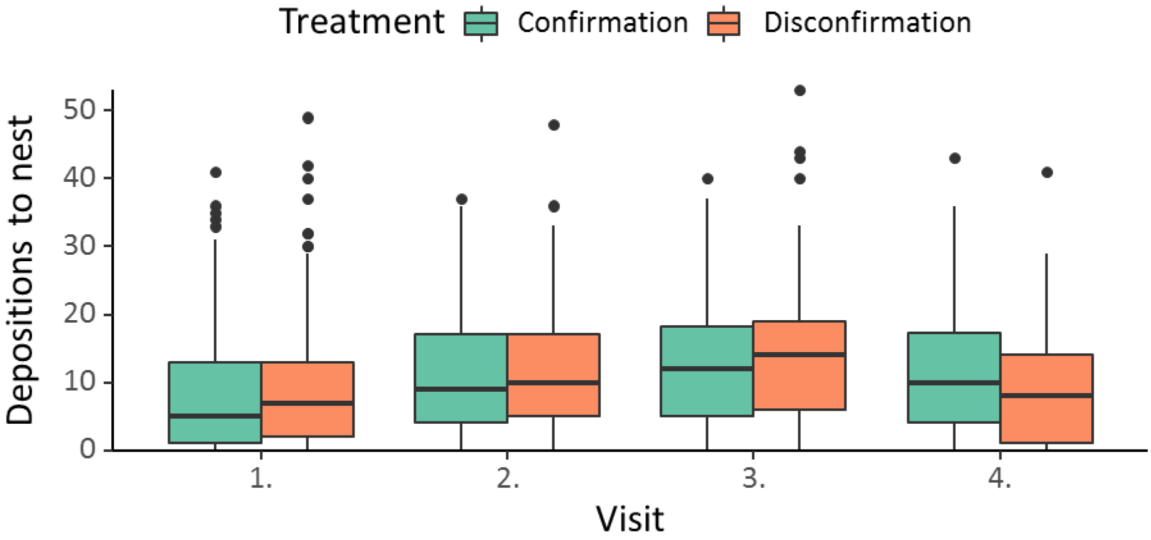

*Figure S2-2. Pheromone depositions on return to the nest on each visit for both treatments. Horizontal lines are medians, boxes correspond to first and third quartile and whiskers extend to the largest value within 1.5 * inter-quartile range (IQR). Dots represent values outside of the IQR.*

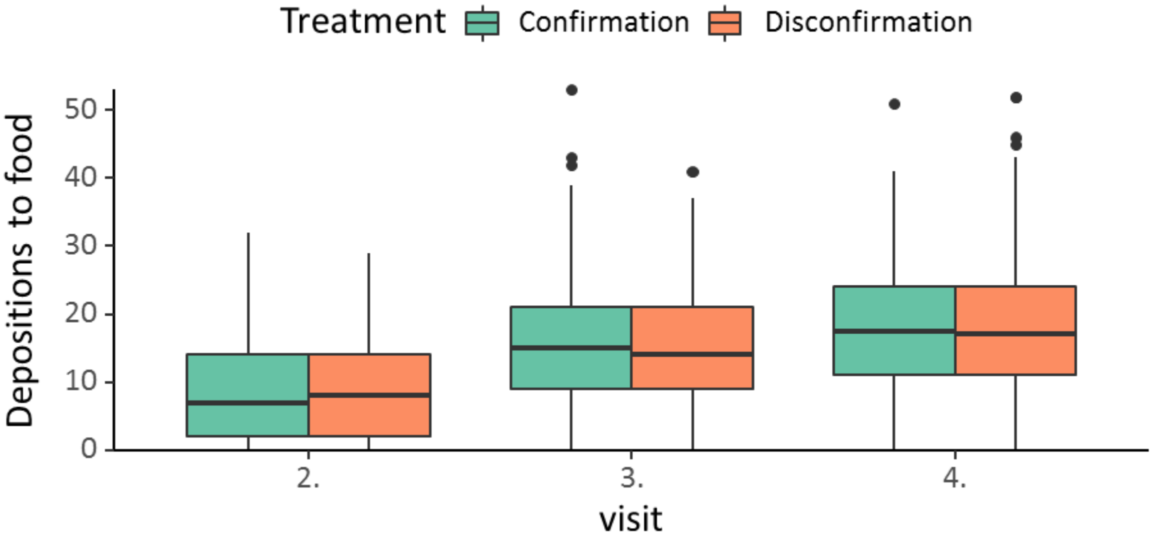

*Figure S2-3. Pheromone depositions to the food on each visit for both treatments. Horizontal lines are medians, boxes correspond to first and third quartile and whiskers extend to the largest value within 1.5 * inter-quartile range (IQR). Dots represent values outside of the IQR.*

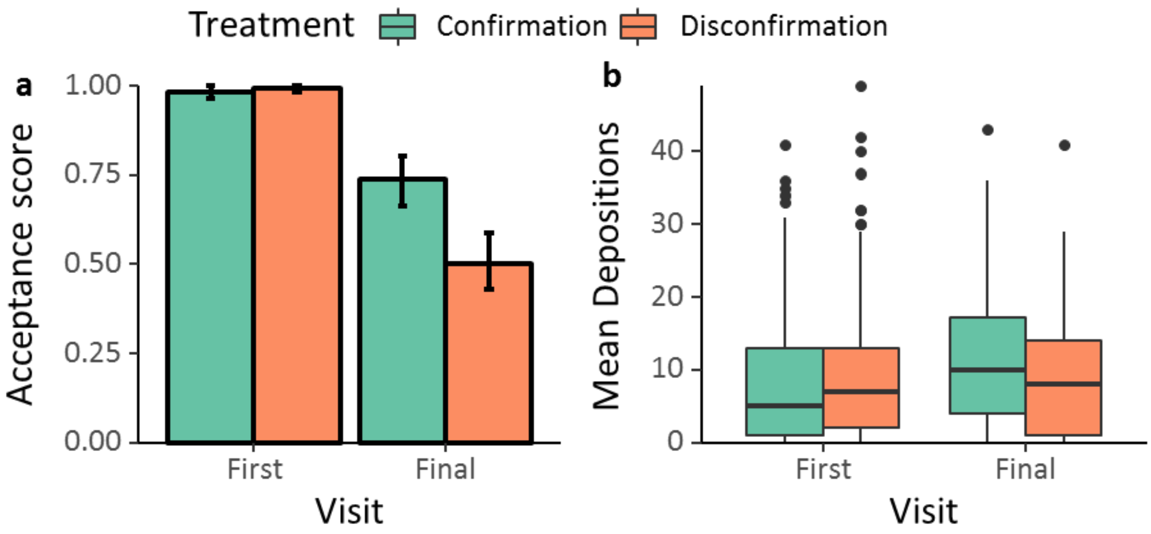

*Figure S2-4. Food acceptance (a) and pheromone depositions on return to the nest (b) on the first visit (discovery of food source) and final visit (after manipulation) for both treatments. Bars depict mean, error bars depict 95% confidence interval in (a); Horizontal lines are medians, boxes correspond to first and third quartile and whiskers extend to the largest value within 1.5 * inter-quartile range (IQR). Dots represent values outside of the IQR.*

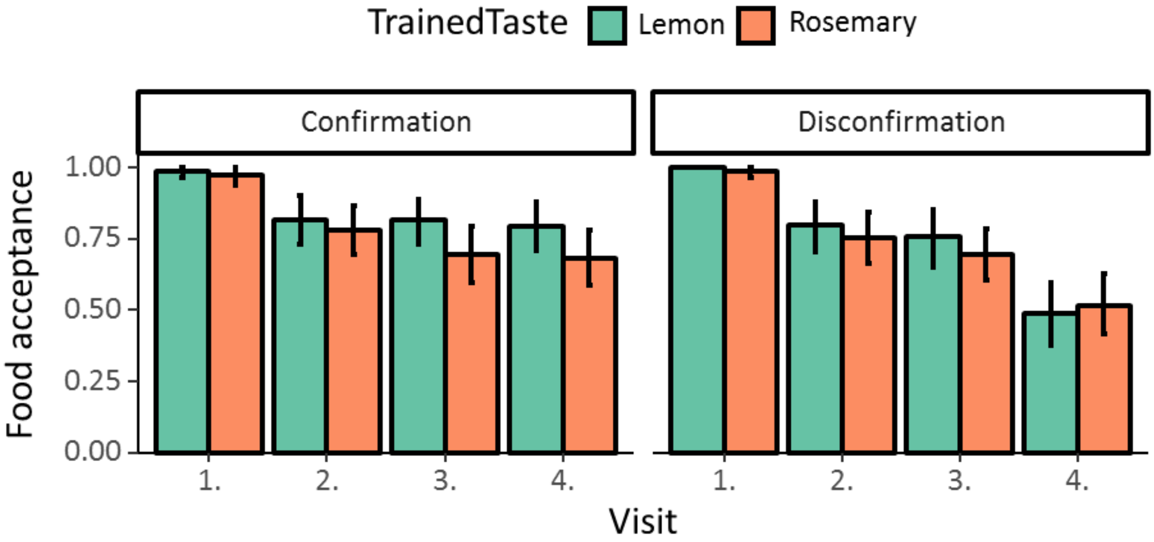

Figure S2-5. Mean food acceptance over all visits for both food tastes. Each panel represents one treatment. Bars depict mean, error bars depict 95% confidence interval.

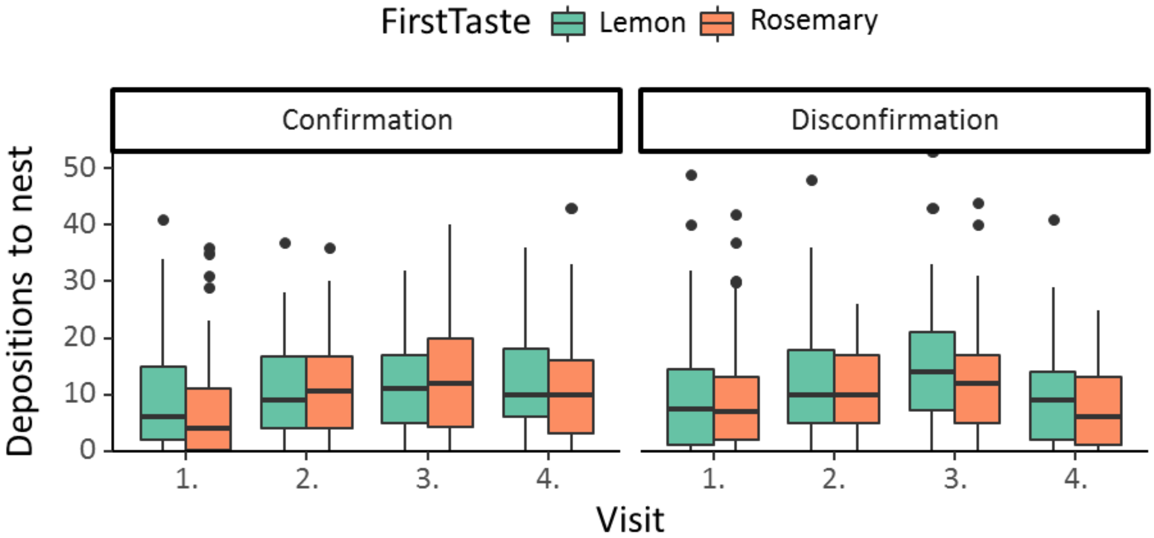

*Figure S2-6. Mean pheromone depositions on the way to the nest over all visits for both food tastes. Horizontal lines are medians, boxes correspond to first and third quartile and whiskers extend to the largest value within 1.5 * inter-quartile range (IQR). Dots represent values outside of the IQR.*

2.6.3 Results

An overview of samples sizes and model results is provided here. For details see ESM2-2.

2.6.3.1 Odour preference pilot

Table S2-1. Mean food acceptance and pheromone depositions ± standard error of means and sample size for each odour.

Odour	**Mean food acceptance**	**Mean pheromone depositions**	**Sample size**
Lavender	0.97 ± 0.02	7.22 ± 0.88	74
Lemon	0.92 ± 0.03	7.58 ± 0.9	73
Peppermint	0.95 ± 0.02	6.81 ± 0.87	74
Rosemary	0.95 ± 0.02	7.62 ± 0.9	73

2.6.3.2 Sample size

From all 353 ants tested, we removed 26 ants which did not deposit any pheromone from the food to the nest on visits 2 & 3 to prevent non-layers from affecting the analysis. This resulted in 327 ants used for all the analyses (see table S2-2).

Table S2-2. Overview of sample size before and after exclusion of ants which did not lay any pheromone on the second and third visit

Treatment	**Trained taste**	**Total ants tested**	**Ants analysed**
Confirmation	Lemon	88	82
Confirmation	Rosemary	91	82
Disconfirmation	Lemon	82	74
Disconfirmation	Rosemary	92	89
Total		353	327

2.6.3.3 Food acceptance

Food acceptance (figure 2-2a) on the 4th visit was significantly lower in disconfirmed ants compared to confirmed ants experiencing the same taste ($z = -4.124$, $p < 0.0001$). Neither was the difference between tastes significant ($z = -1.724$, $p = 0.0846$), nor the interaction of treatment and taste ($z = 1.509$, $p = 0.1314$). Pairwise post-hoc comparisons revealed a significant difference between treatments in ants trained with lemon as well as ants trained with rosemary (odds ratio = 4.918, $p < 0.0001$; odds ratio = 2.275, $p = 0.017$, respectively).

2.6.3.4 Pheromone deposition

Pheromone depositions (figure 2-2b) on the 4th visit were also significantly lower in disconfirmed ants compared to confirmed ants experiencing the same taste ($t = -3.102$, $p < 0.01$). Neither was the difference between tastes significant ($z = - -0.866$, $p = 0.388$), nor the interaction of treatment and taste ($z = 0.334$, $p = 0.739$). Pairwise post-hoc comparisons revealed a significant difference between treatments in ants trained with lemon as well as ants trained with rosemary (ratio = 1.450, $p < 0.01$; ratio = 1.371, $p < 0.01$, respectively).

2.6.3.5 Comparison of 1st and 4th visit

To investigate differences between the initial discovery visit and the final manipulated visit, we compared food acceptance and pheromone depositions of 1st and 4th visit. Each treatment was analysed separately (figure S2-4).

2.6.3.5.1 Food acceptance

Food acceptance was significantly lower on the 4th compared to the 1st visit, irrespective of treatment (confirmation: $z = -5.118$, $p < 0.0001$; disconfirmation: -5.171, $p < 0.0001$). No effect of taste was found (confirmation: $z = -1.890$, $p = 0.0588$; disconfirmation: $z = 0.260$, $p = 0.795$).

2.6.3.5.2 Pheromone depositions

Pheromone depositions were significantly higher on the 4th than on the 1st visit in confirmed ants ($t = 4.648$, $p = 0.0001$), while no such difference was found in disconfirmed ants ($t = -1.683$, $p = 0.094$). No effect of taste was found (confirmation: $t = -0.919$, $p = 0.3659$; disconfirmation: $t = 0.292$, $p = 0.773$).

Chapter 3

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

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3.1 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, in order 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 alternatingly 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 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.

Key words

Route learning; information conflict; private information; odour learning;

3.2 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. In order 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 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. 2015b; 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 so-called olfactory landmarks (Steck 2012). 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; 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 hours (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. 2013a; Czaczkes et al. 2015b; Devigne and Detrain 2002; Evison et al. 2008a; 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 *Lasius 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. in press), 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.

3.3 Material and methods

3.3.1 (a) Collection and rearing of colonies

Colonies of the black garden ant *Lasius 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; Dussutour 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 0.5M sucrose solution. The colonies were deprived of food for 4 days prior to each trial. In total, 22 colonies were tested, with at least 6 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.

3.3.2 (b) Sucrose reward and usage of essential oils

Either 1.5M or 0.25M sucrose solutions (Merck KGaA, Darmstadt, Germany) were used as reward during training. In experiments using odours (experiments 1, 4, 6 & 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 100µl of the corresponding essential oil on filter paper for >2 hours. Essential oil was used to ensure that the ants could perceive and discriminate the smell due to the various components included.

3.3.3 (c) Setup and experimental procedure

During training, we provided either 1.5M sucrose or 0.25M sucrose droplets (~20µl) which were replaced each visit and greatly surpassed *Lasius niger*'s crop capacity (<2µl, Mailleux et al. 2000). The droplets were placed on acetate sheets located at the end of either a straight runway (experiments 1 & 4, see figure 3-1) or at the end of a Y-maze (experiments 2,3,5-7, see figure 3-1) covered with disposable paper overlays. The straight runway was 1cm wide and 20cm long while the Y-maze had 1cm wide and 10cm long stem and arms and was tapered to 2mm 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, figure 3-1) before a test was conducted (see Test phase below, figure 3-1). Y-maze setup and training methods follow Czaczkes (2018).

3.3.3.1 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.

3.3.3.1.1 Experiment 1 – Odour training (figure 3-1a)

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 0.25M or a 1.5M sucrose solution droplet on alternating visits at the end of a 20cm 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).

3.3.3.1.2 Experiment 2 – Side training (figure 3-1b)

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 (1.5M) 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 0.25M 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).

3.3.3.2 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.

3.3.3.2.1 Experiment 3 – moderate side training (figure 3-1b)

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.

3.3.3.2.2 Experiments 4 & 5 – weak odour/ weak side training (figure 3-1a, 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.

3.3.3.3 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.

3.3.3.3.1 Experiment 6 – odour vs. side conflict (figure 3-1c)

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 (0.25M) or a high-quality (1.5M) 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.

3.3.3.3.2 Experiment 7 – unrestricted side vs. odour conflict (figure 3-1d)

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 0.25M or 1.5M (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.

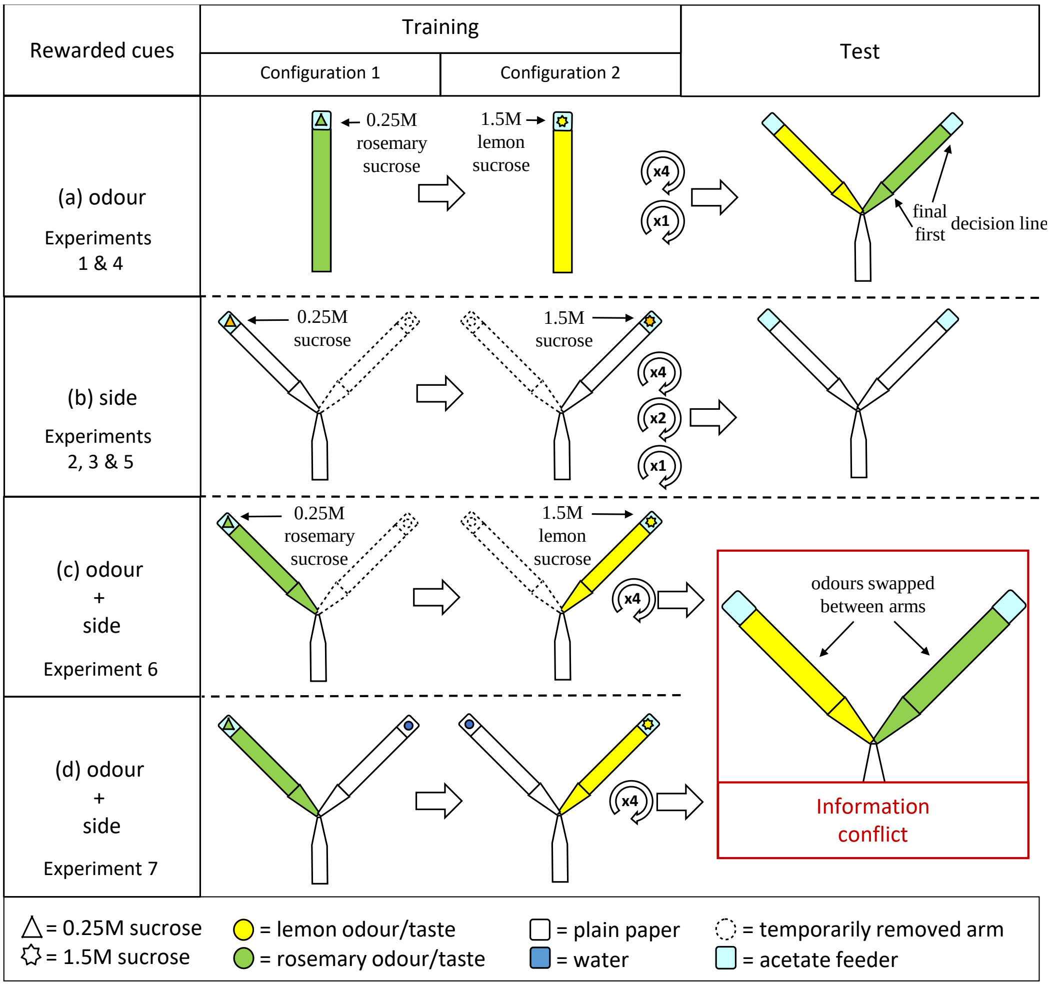

Figure 3-1. Setups used for experiment 1-7. Ants were trained to associate an odour, a side, or both with 1.5M and 0.25M sucrose rewards. After training, ants were tested in unrewarded test Y-mazes for odour learning (first row, a), side learning (second row, b) or preference between odour and side information (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 & 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 & 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).*

3.3.3.4 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 & 5, which were conducted without odours, ants had to decide between two unscented arms. For experiments 1 & 4, which were using odours, ants had to decide between two arms of different odour (lemon and rosemary). Lastly and importantly, in experiments 6 & 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 (figure 3-1 bottom right), while the odour associated with high-quality reward was presented on the opposite arm.

As soon as the ant crossed a line 2cm 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 figure 3-1 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.

3.3.4 c) 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 2016). 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 ESM3-1 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:

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

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 is presented in electronic supplementary material (ESM3-1). The complete raw data is presented in ESM3-2.

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 measure of performance in the analysis.

3.3.5 d) Data availability

All data generated or analysed during this study are provided as electronic supplementary files (ESM).

3.4 Results

For a detailed step-by-step protocol of the statistical analysis, please refer to ESM3-1.

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 figure 3-2A). 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 ESM3-1).

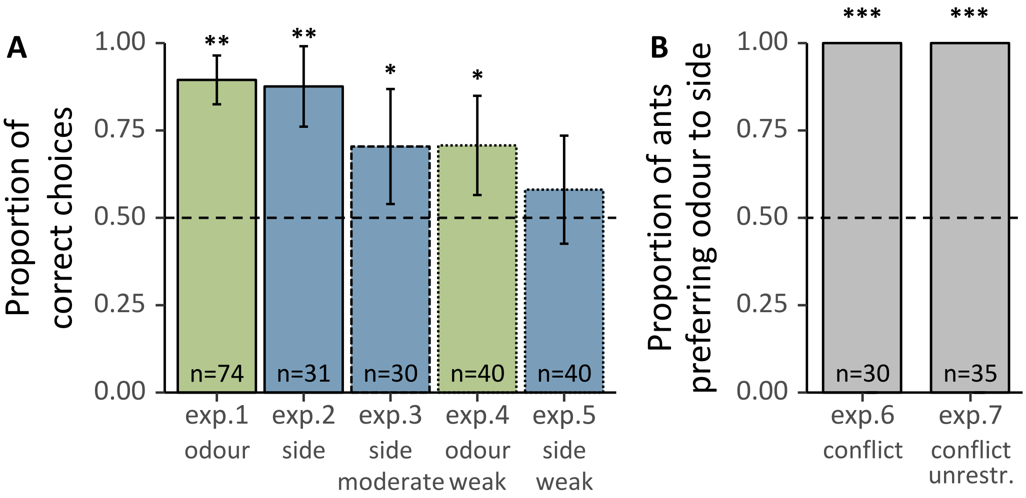

Figure 3-2. ***A)*** *Proportion of ants deciding initially for side or odour associated with high-quality food. When odour alone had predictive power (experiment 1 odour & experiment 4 odour weak), the majority of ants chose the arm with the odour associated with high-quality sucrose (1.5M). When trained for side/quality associations for four or two visits to each combination (experiment 2 side & 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, 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*

3.5 Discussion

Our results show an overwhelming preference for one private information type (olfactory association) over another (route memory) in *Lasius 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 (see figure 3-2B). 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 figure 3-2A), 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%) 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 reduces 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 (figure 3-2A). 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. in press). 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 (Wehner et al. 2016) 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; 2013a).

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. in press), although they learn well under identical setups in the light (Grüter et al. 2011; Oberhauser et al. 2018). Thus, it seems 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. in press; 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; Buehlmann et al. 2015; Steck et al. 2011; Steck 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.

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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.

Chapter 4

Ants resort to heuristics when facing complex tasks

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In preparation

4.1 Abstract

As humans, we often apply relational rules such as ‘same’ or ‘different’ to collections of objects in order to better understand the world around us. Such abstract concepts are independent of the physical nature of the linked objects, and can be, once acquired, transferred to novel objects. Fascinatingly, honeybees were also found to learn abstract concepts, yet little evidence for this ability exists in other insects with similar brain architecture. However, rule learning is not the only alternative to calculating a correct answer for every posed question. Animals can resort to heuristics, which provide fast, robust and energetically cheap decision rules at the cost of accuracy. We investigated whether ants (*Lasius niger*) can learn a relational rule of ‘same’ or ‘different’ by training ants in an odour match-to-sample test over 48 visits. Ants in the ‘different’ treatment improved significantly over time, reaching around 65% correct decisions, but ants in the ‘same’ treatment did not improve. Detailed inspection of individual behaviour revealed that half (51%) of the ants adopted alternative, occasionally unhelpful, heuristic-like rules. Instead of trying to solve a difficult task via complex cognition, ants might have resorted to heuristics such as ‘go left’ or ‘go to more salient cue’ instead, which systematically biased their decisions.

Key words

Heuristics; rule learning; concept learning; ants; cognition;

4.2 Introduction

We frequently apply concepts in our daily lives in order to group objects which belong together. Once we have established a concept, we can apply it to novel situations and objects, thus bypassing costly trial and error learning and allowing adaptive responses based on prior experience. In perceptual concepts, objects are categorised by their shared physical features such as their similar appearance (e.g. trees or rocks). Relational concepts, by contrast, are more abstract and use non-physical features such as the relationship between objects (e.g. ‘same’ or ‘different’) (Lazareva and Wasserman 2008; Zentall et al. 2002; 2008; 2014). Once an animal has learned an abstract concept of ‘same’, it can be transferred and used on other stimuli irrespective of the employed sensory modality. The formation of abstract concepts has traditionally been considered a higher-order ability (Katz et al. 2002) and accordingly most concept learning research focussed on vertebrates such as primates (Basile et al. 2015; Wasserman et al. 2001; Wright and Katz 2007), birds (Gibson et al. 2006; Martinho and Kacelnik 2016; Pepperberg 1987; Wright et al. 2017) or rats (Peña et al. 2006; Wasserman et al. 2012).

Yet, honeybees have also repeatedly been shown to successfully learn and apply abstract concepts, such as same/different (Giurfa et al. 2001), above/below (Avarguès-Weber et al. 2011; 2012) or numerosity (Howard et al. 2018). In the first demonstration of concept learning (Giurfa et al. 2001), honeybees were trained in a ‘same’ or ‘different’ learning task using delayed match-to-sample and non-match-to-sample tests (DMTS, DNMTS, respectively). In the experiment, bees were first trained to find a reward by choosing the Y-maze arm bearing the same (or different in DNMTS) sample as presented shortly before on the maze entrance. After training, bees underwent an unrewarded transfer test on novel stimuli. Crucially, bees could not only transfer the learned concept from colour to pattern stimuli (or vice versa), but also from one modality (colour) to the other (odour).

However, there has been extensive debate regarding how honeybees solve those complex tasks and whether they require top-down control by the animal (Cope et al. 2018; Guiraud et al. 2018; Peng and Chittka 2017; Roper et al. 2017; Vasas and Chittka 2019). It has been argued that honeybees’ success in same/different tasks could be mediated by appetitive or aversive modulation of their innate tendency to revisit similar flowers (Collett 2005, but see Brown and

Sayde 2013) or by sensory accommodation, i.e. reduced response to repeated stimuli (Cope et al. 2018), without the involvement of complex cognition.

A recent experiment closely investigating the strategies deployed by bees while learning an above/below concept found that they can use sequential inspection of the presented items to succeed in the task, without the need for a spatial concept (Guiraud et al. 2018). Similarly, tasks that require animals to discriminate numerosities could be achieved by a small subset of neurons (Skorupski et al. 2017; Vasas and Chittka 2019) and the number of objects in a set can be considered a sensory characteristic of the target, perceivable by the animals through their number sense (Arrighi et al. 2014).

Irrespective of the underlying mechanisms, it is evident that the usage of concepts can vary between individuals: Successful training requires many visits (60 in Giurfa et al. 2001) and not all bees afterwards apply the concept to novel stimuli (60-80% successful transfer in Avarguès-Weber et al. 2011; 2012; Giurfa et al. 2001; 50-70% in Guiraud et al. 2018, 60-70% in Brown and Sayde 2013 in bumblebees), suggesting that some bees were relying on other strategies to solve the tasks. This is in accordance with the finding that animal species deemed to be unable to perform conceptual learning succeeded after the number of training pairs was increased to a point were associative learning became inefficient (Wright and Katz 2006), which indicates that animals employ concept learning only after other strategies fail.

Cognitive mechanisms have not evolved to reveal the truth, but to provide decisions which maximise fitness gains (Haselton et al. 2015). Sometimes, quick heuristics (rules of thumb) can surpass more sophisticated strategies by cheaply and rapidly finding acceptable solutions to a problem at the cost of accuracy or robustness (Gigerenzer and Gaissmaier 2015; Haselton et al. 2015; Mhatre and Robert 2018). In other words, if false-positives (actions which lead to an error) only induce minor costs or if the foraging context is highly variable, animals might resort to heuristics instead of learning (Arkes 1991; Haselton et al. 2015). Heuristics can pre-equip animals to solve complex problems such as nest size estimation by scouting ants using the frequency of own trails crossings (Buffon’s needle; Mallon and Franks 2000), best-of-N rule in nest-searching honeybee swarms (Seeley and Buhrman 2001) or prey interception in dragonflies (Lin and Leonardo 2017).

While research on concept learning (Avarguès-Weber et al. 2011; 2012; Brown and Sayde 2013; Giurfa et al. 2001; Guiraud et al. 2018; Howard et al. 2018) and other complex cognitive abilities of honey bees such as metacognition (Perry and Barron 2013) are now being supplemented by studies on potential underlying neural mechanisms (MaBouDi et al. 2017; Peng and Chittka 2017; Seilheimer et al. 2014; Vasas and Chittka 2019), studies on complex cognition in insects other than honeybees remain very scarce (Brown and Sayde 2013; Tibbetts et al. 2019).

Yet, prerequisites assumed to be crucial for concept learning are met by other Hymenoptera, such as ants, as well (Avarguès-Weber and Giurfa 2013). *Lasius niger* ants are adept learners and can quickly form associative memories for odours (Oberhauser et al. 2019) which they use to remember food locations (Czaczkes et al. 2014) and can retain information about a value in their memory to compare it to sensory input (Wendt et al. 2019), which is a prerequisite for DMTS.

We therefore investigated *Lasius niger* ants' ability to learn a relational rule of 'same' or 'different'. Ants were trained on a Y-maze and continuously confronted with new odour pairs, and only matching (or non-matching in the 'different' treatment) the stem odour to the arm led to a reward. Thus, ants could only succeed when using the relationship between the stimuli as guidance. We then analysed the overall performance of the ants as well as the potential presence of heuristics during this learning phase and found that approximately half of the ants used heuristics to guide their decision.

4.3 Material and methods

4.3.1 Collection and rearing of colonies

Eight stock colonies of the black garden ant *Lasius 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 500-1000 workers. Queenless colonies forage and lay pheromone trails, and are frequently used in foraging and learning experiments (Devigne and Detrain 2002; Dussutour et al. 2005; Grüter et al. 2015). All colonies were kept on a 12:12 day/night cycle and were provided *ad libitum* with water and 1M sucrose solution and supplemental drosophila feeding. The colonies were deprived of food for 4 days prior to each trial. Tested ants were permanently removed from the colony to prevent pseudo-replication.

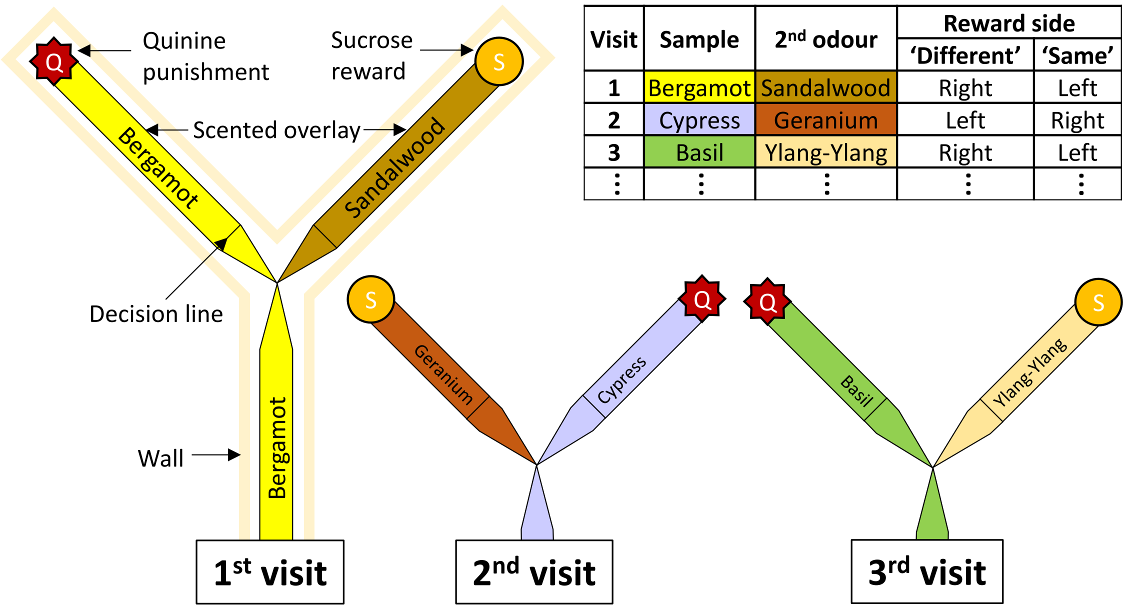
4.3.2 Solutions and odours

1M sucrose and 60mM quinine (both Merck KGaA, Darmstadt, Germany) solutions were used as reward and aversive stimulus during the experiment, respectively. Quinine punishment was found to improve visual discrimination learning in honeybees (Avarguès-Weber et al. 2010). Paper runways were impregnated with one of 12 different essential oils (mitallen5sinnen, Grünwald, Germany; see table S4-1 & electronic supplementary material (ESM) 4-3) by keeping runways in an enclosed box containing 100 µl of the corresponding essential oil on filter paper for > 2 h (see also Oberhauser et al. 2019). To control for potential influences of shared compounds between oils (see below), we obtained all identified compounds of each corresponding oil from literature. Linalool was found to be present in 6 of the tested odours (see ESM4-1) at a percentage of at least 5%, and thus added as potential control heuristic (see below). A list of all oils and their compounds are provided in ESM4-3.

4.3.3 Experimental procedure

Ants were allowed onto a Y-maze (following Czaczkes 2018) via a drawbridge. The Y-maze was surrounded by cardboard to prevent landmark orientation (see figure 4-1). Each visit, we presented the ant with a new combination of odours (new odour pair) by placing scented overlays over the Y-maze stem and arms which were replaced after each visit. One odour was present on one arm, while the other was present on both stem and arm (see figure 4-1). In order to find a reward (1M sucrose), ants had to non-match ('different' treatment) or match ('same' treatment) the odour present on the stem with that on the arm, while the incorrect arm led to quinine punishment. This way, the only predictor for the rewarded Y-maze side available to the ants was the relationship between odours, not the odour identity. As soon as an ant crossed a decision line 2cm inwards of either arm, this was scored as its first decision. Touching either the sucrose or quinine droplet was scored as final decision.

To begin an experiment, 3-5 ants were allowed onto the maze. The first ant to reach the reward was marked with acrylic paint and all other ants were returned to the nest. From now on, only the marked ant was allowed onto the setup via the drawbridge to make 48 visits to as many different odour pairs. While each pair was unique, each odour was presented multiple times over the course of the experiment. To prevent differences in reward association strengths between odours, each was presented as rewarded or unrewarded odour in alteration, so each odour was rewarded approximately once in 12 visits, resulting in 4 rewarded visits per odour (see ESM4-1). As we did not have preference data on all odour pairs, we used a fixed experimental procedure, in which all ants experienced the same odour sequence (see table S4-1). This allowed us to investigate possible odour pair induced effects (e.g. ants always prefer odour A to B). Moreover, as the procedure was fixed, we could compare the two concepts (same/different) at each ant visit by taking the inverse performance of one of them (correct choice in the difference treatment = went to different odour = incorrect choice in the 'same' treatment). The sequence of left and right was fixed, with each side being rewarded in half of the visits and half of the ants starting with either side being rewarded.



Visit	Sample	2nd odour	Reward side	
			'Different'	'Same'
1	Bergamot	Sandalwood	Right	Left
2	Cypress	Geranium	Left	Right
3	Basil	Ylang-Ylang	Right	Left
⋮	⋮	⋮	⋮	⋮

Figure 4-1. Setup used for the 'different' and 'same' treatment. The Y-maze shown on the left depicts the first visit of the 'different' treatment (see table on the top right for details). Each visit, ants encountered a new odour pair by walking over scented paper overlays. The sample odour was present on the stem and on one arm. In the 'different' treatment, the ant had to go to the arm with the odour different from the sample to find reward (1M sucrose, right on the first visit). In the 'same' treatment, the rewarded and punished (quinine) side were swapped (see table inset). The procedure was then continued for the remaining 45 visits with other unique odour pairs, the first three of which are shown here. All scented paper overlays were white, colours are only used for illustration purposes.

4.3.4 Statistical analysis

All statistical models were generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) produced with the glmmTMB function (Brooks et al. 2017) in R version 3.5.1 (R Core Team 2018). Since ants from 8 different colonies were tested, each of which made repeated visits, we included each ant ID nested in colony as random intercept factors in all models. Each model was tested for fit and dispersion using the DHARMa package (Hartig 2018). Post-hoc tests were conducted using estimated marginal means (Lenth 2018). Recipient Operant Characteristic (ROC) curves (Fawcett 2006) were calculated using the package pROC (Robin et al. 2011). An annotated script and output for all data handling and statistical analysis is presented in ESM4-1. The complete raw data is provided in ESM4-2.

4.3.4.1 Rule learning performance

The performance (correct/incorrect decisions) of ants in the two treatments was analysed separately for the ‘same’ and the ‘different’ treatment. The binomial GLMM predictors were defined *a priori*, following Forstmeier and Schielzeth (2011), as:

$$\textit{Decision}_{\textit{(correct/incorrect)}} \sim \textit{Visit}_{\textit{(1:48)}} * \textit{Side}_{\textit{(left/right)}} + \textit{random intercept}_{\textit{(Colony/Ant\_ID)}}$$

Furthermore, as the succession of odours was identical in both treatments, an incorrect choice in the ‘same’ treatment corresponds to a correct choice in the ‘different’ treatment. Therefore, we could directly compare performance between the treatments by calculating an inverse performance for the ‘same’ treatment (correct decision scored as incorrect and vice versa). For this comparison, we ran a binomial GLMM with performance as dependent variable and treatment (‘different’, ‘same inversed’) as predictor (see ESM4-1).

4.3.4.2 Streak lengths

To obtain an estimate of individual performance consistency, we calculated the longest streaks (visits in a row) of correct, incorrect, left, and right decisions, and the visit the corresponding streak started at (referred to as streak onset). For both strike length and strike onset, we ran separate GLMMs for correct/incorrect streaks or side streaks as predictors:

$$\textit{Streak length OR Streak onset} \sim \textit{Streak type}_{\textit{(correct/incorrect OR left/right)}} * \textit{Treatment}_{\textit{(same/different)}} + \\ \textit{random intercept}_{\textit{(Colony/Ant\_ID)}}$$

In case a Poisson error distribution was inadequate, a negative binomial distribution was used (see ESM4-1).

4.3.4.3 Heuristics

To analyse whether ants might have used rules to guide their decisions, we identified three potential heuristics, ‘go different’, ‘go left’ and ‘go last rewarded’ (see table 4-1). A score was calculated for each ant and heuristic, by scoring 1 for each visit the decision was following the corresponding rule, and 0 if it did not. In ‘go different’, each visit was scored 1 when the ant went to the odour different to the stem. This corresponds to correct decisions in the ‘different’ treatment, and the inverse performance in the ‘same’ treatment. In ‘go left’, we scored 1 for each visit the ant went left. In ‘go last’ we scored 1 when an ant went to the Y-maze arm which was rewarded on the previous visit.

Additionally, we introduced a fourth heuristic, ‘go linalool’ to account for it being a shared compound of 6 of the used odours (see Solutions and odours section) and scored 1 if ants chose an odour containing linalool. However, in 22 visits linalool was either present or missing on both arms thus making it impossible to conclude whether ants were using it as a heuristic or choosing randomly in those visits. Accordingly, we also performed an analysis including only visits in which linalool was present on one arm per visit (see ESM4-1 and figure S4-1).

To identify usage of the potential heuristics on individual level, we counted for each heuristic all ants which chose according to the heuristic’s rule at least in 66% of visits (32/48).

To estimate the predictive power of the heuristics on group level, we produced a model for each heuristic using the formula:

$$Performance_{\text{(correct/incorrect)}} \sim Heuristic_{\text{(correct/incorrect)}} + random\ intercept_{\text{(Colony/Ant)}}.$$

For each visit of each ant, the model thus compared the ant’s decision (performance) with the predicted decision of the heuristic. To compare the predictive power of each model, we established Recipient Operant Characteristic (ROC) curves (Fawcett 2006) for each heuristic and a null model containing only the random effect (ant ID and colony). The predictive power of all models was then compared using Area under the Curve (AUC) values of each ROC.

4.4 Results

In total, 55 ants were tested. However, 7 ants performed fewer than 48 visits and one ant was accidentally trained on a different sequence of odour pairs. These ants were excluded from the analysis, resulting in 19 and 18 tested ants in the ‘different’ and ‘same’ concept treatments, respectively.

4.4.1 Rule learning performance

If ants learned the relational rule to go to same or different, we should observe a significant increase in correct choices over time. In the ‘different’ treatment, ants indeed improved significantly over 48 visits (binomial GLMM, χ^2 = 5.724, p = 0.0167, see figure 4-2A). Furthermore, ants’ performance was significantly higher when the reward was presented on the left ($\chi 2$ = 5.8525, p = 0.0155). Note that we included ‘reward side’ in the model and present it here due to *L. niger*’s tendency for left biases (Oberhauser et al. 2018; 2019), and we therefore chose to include it in the a priori model. A separate test for the ‘go left’ heuristic is presented below.

By contrast, no improvement over visits was found in the ‘same’ treatment (binomial GLMM, χ^2 = 0.2546, p = 0.6138, see figure 4-2B), but a significantly higher proportion of correct visits was made when the reward was on the left (χ^2 = 34.4, p < 0.001). In both treatments, the majority of ants (~92%) did not switch sides between entering one arm (first decision) and touching the droplet (final decision), the rest switched from the correct to the incorrect side (~5%), or vice versa (~3%, see figure 4-2). For simplicity, due to these small differences, we only used the first decision of each ant as measure of performance in subsequent analyses. An examination of the odour pairs revealed that in two pairs, one odour was always rejected. Excluding those two pairs did not alter the results presented above (see ESM4-1).

When we directly compared the two treatments, we found a significantly better performance in the ‘different’ treatment (binomial GLMM, χ^2 = 20.6961, p < 0.001, see figure 4-2C). However, there was no difference between performance in the ‘different’ task and the inverse of performance on the ‘same’ task (χ^2 = 0.4255, p = 0.5142, see figure 4-2C). This implies that ants responded in a similar way towards the encountered stimuli irrespective of the treatment.

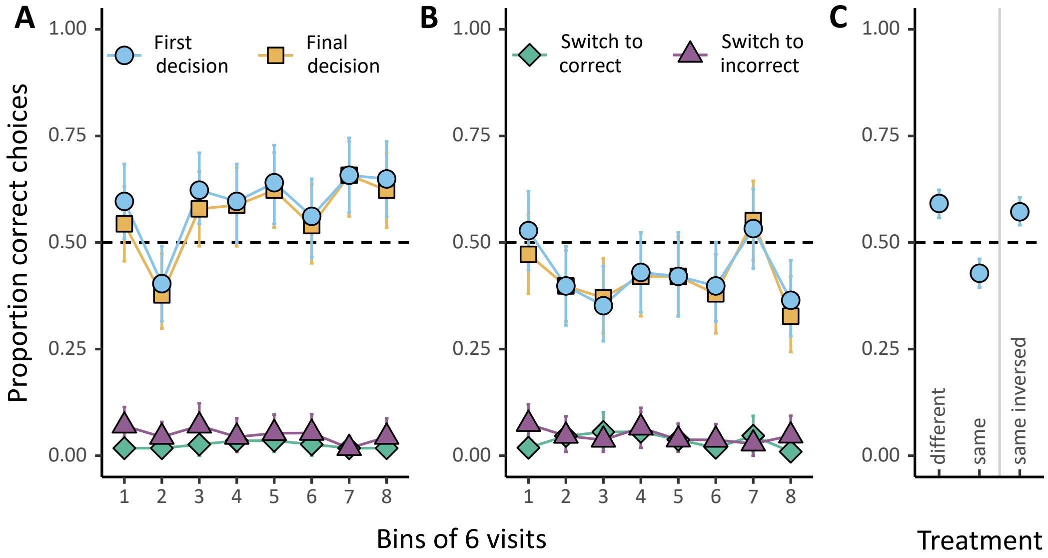


Figure 4-2. Performance of ants over subsequent visits in ***(A)*** *the 'different' treatment (n = 19) and* ***(B)*** *the 'same' treatment (n = 18).* ***(C)*** *Performance averaged over all 48 visits. The inverse performance (correct = incorrect and vice versa) of ants in the 'same' experiment ('same inversed') resembles performance of the 'different' treatment. Dashed line represents chance level of 50%. Symbols are means, error bars represent 95% bootstrapped confidence intervals.*

4.4.2 Streaks

Group level analyses do not adequately capture individual behaviour (Pamir et al. 2011) and a poor group performance can mask individuals which managed to learn the task. On individual level, consistency is a good measure. If ants learned the task, we would expect them to display longer streaks of correct decisions, as they repeatedly choose the correct Y-maze arm. Our analysis revealed a significant interaction between treatment (same/different) and streak type (correct/incorrect) (χ^2 = 8.9718, p = 0.0027) which is reflected by significantly longer correct than incorrect streaks in the 'different' treatment, but the opposite pattern in the 'same' treatment (Estimated marginal mean contrasts, ratio = 1.3718, p = 0.0374; ratio = 0.7228, p = 0.0382, see figure 4-3A). The longest correct streaks were 9 visits long in both treatments, whereas longest incorrect streaks spanned 10 and 11 ('different' and 'same', respectively).

Furthermore, we found that some ants displayed strong side biases. The length of left side streaks was significantly longer than right streaks (χ^2 = 5.0779, p = 0.0242), and estimated marginal mean contrasts revealed that this was within ants in the 'same' treatment (ratio = 0.6354, p = 0.0219), but not within the 'different' treatment (ratio = 0.851, p = 0.3788). The longest left streak in the different treatment was 17 visits, while one ant in the 'same' treatment went left 27 times in a row (56% of visits, an event that is expected to happen by chance at a rate of less than 1 per twelve thousand billion). Longest right streaks were 19 and 9 visits long (‘different’ and ‘same’, respectively).

The onset of correct streaks during training started consistently later than incorrect streaks (χ^2 = 8.4317, p = 0.0037, see figure 4-3B), but the effect was significant in the ‘different’ treatment only (contrasts: ‘different’: ratio = 2.107, p = 0.0034; ‘same’: ratio = 1.093, p = 0.2782). The onsets of left and right streaks did not differ significantly (χ^2 = 0.0001, p = 0.9941).

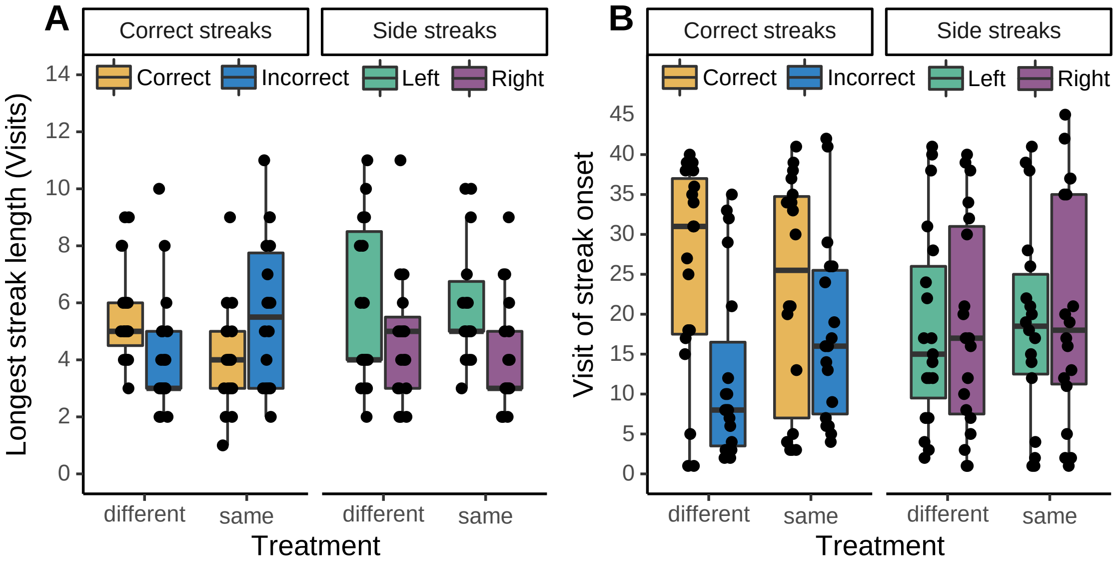


*Figure 4-3. **(A)** The longest streaks of correct and incorrect decisions (left) and left and right decisions (right for each ant and treatment. Three ants with very long streaks (2 left streaks, 1 right streak) are not shown **(B)** Visits until streak onset for correct and incorrect streaks (left) and left and right streaks (right). Points represent individual ants, horizontal lines in boxes are medians, boxes correspond to first and third quartiles and whiskers extend to the largest value within 1.5 x IQR.*

4.4.3 Heuristics

To analyse which potential heuristics were used by individual ants (see table 4-1), we assigned each ant a heuristic if it chose the arm the heuristic would suggest in at least 66% of its visits. Using this method, we found that 58% (10/19) of ants in the ‘different’ treatment deployed a heuristic according to our criterion, compared to 50% (9/18) of ants in the ‘same’ treatment (two ants could have been using two heuristics, see table 4-1 and figure 4-4). The most prominent heuristic in both treatments was to go to the odour different from the stem (‘go different’). Further analyses including a potential ‘go linalool’ heuristic on the 26 visits in which linalool was exclusive to one arm revealed similar results (see figure S4-1). No indications of a ‘go linalool’ heuristic were found.

Table 4-1. Definitions of potential heuristics and how many ants chose corresponding to them for ≥ 66% of 48 visits. Ants which could be assigned to two heuristics, are indicated by + and shown in both heuristics. * Linalool heuristic was calculated from subset of 22 visits.

Heuristic	**Description**	**Number of ants with chose according to heuristic at least 66% of visits**	
		‘different’	**‘same’**
‘Go different’	Choose maze arm with odour different to stem	6 (32%)	4 + 2 (22% - 33%)
‘Go left’	Choose the left arm of the maze	4 (21%)	3 + 1 (17% - 22%)
‘Go last’	Choose maze arm rewarded on last visit	0	0 + 1 (0% - 5%)
‘Go linalool’*	Choose maze arm with odour containing linalool	0	0 + 1 (0% - 5%)
	Total	**19**	**18**

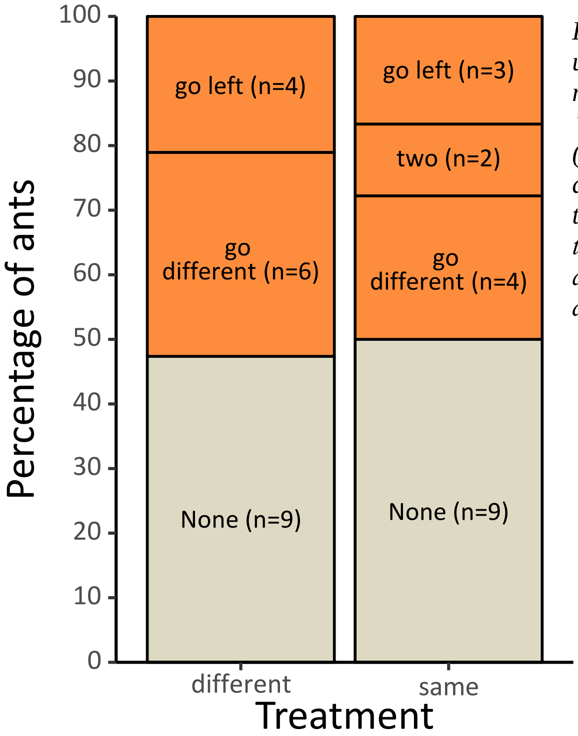


Figure 4-4. Percentage of ants which used each of the defined strategies for a minimum of 66% of their visits in the ‘different’ (n = 19) and ‘same’ treatment (n = 18). Over half of the ants acted according to a certain heuristic. Note that the choices of two ants could be assigned to two different heuristics (once ‘go left’ and ‘go different’, once ‘go last’ and ‘go different’, labelled ‘two’).

To estimate how well performance of ants can be classified using heuristics on group level, we compared Area under the Curve values (AUC) of each ROC model based on heuristic to a null model (only including colony and ant). The AUC values differed only slightly (±0.02) from the null model with an AUC of 0.651 (see ESM4-1). The null model was thus highly explanatory and demonstrated that performance is best described by the individual ant, with no dominant heuristic on group level.

4.5 Discussion

Our experiment revealed that ants were able to significantly improve their performance in a non-matching-to-sample (NMTS) task, where they had to choose a Y-maze arm odour which was different from a sample odour presented on the stem to find a reward. However, ants failed to improve in a match-to-sample (MTS) task. Our analyses suggest that ants did not use a relational rule of same/different to guide their decisions, but rather based their decisions on heuristics such as ‘go left’ or ‘go to most salient cue’.

Although significant, the increase in performance in the ‘different’ treatment was very modest, with 65% correct decisions in the last 6 visits (see figure 4-2A). While we do expect a higher proportion of ants failing to learn complex tasks due to individual variation in learning abilities (Chittka et al. 2012), this final performance is undermined by a high initial performance of 60% correct decisions in the first 6 visits, and the fact that the ants’ performance did not resemble an asymptotic learning curve. Such behaviour is indicative of heuristic use. Moreover, the fact that the inverse overall performance of ants in the ‘same’ treatment resembled that of the ‘different’ treatment (figure 4-2C) also suggests that ants were not using a relational rule of ‘different’ but rather other cues common to both treatments.

However, considering averaged performance might mask individuals which did manage to learn to go to ‘same’ or to ‘different’. To estimate individual performance consistency, we analysed the length of observed streaks, i.e. visits in a row which were correct or incorrect. If learning occurred, we would expect longer correct streaks with an onset in the latter part of the visits. Conversely, incorrect streaks should be short and their onset randomly distributed. Indeed, ants in the ‘different’ treatment had significantly longer correct than incorrect streaks, which started significantly later than incorrect streaks (see figure 4-3). This suggests that ants did modify their behaviour over the course of the treatment. Conversely, ants had significantly longer incorrect streaks in the ‘same’ treatment, again indicating that ants acted similarly in both treatments.

We believe that these response similarities are due to ants' attempts to follow heuristics unrelated to the treatments. The frequency at which ants decided to 'go different' is particularly noteworthy, as its successful application seems to suggest that ants did use a relational rule of 'different'. However, our setup lacked a 'neutral' area devoid of the sample odour (no *delayed* MTS), as the scented stem paper overlay extended until the decision area. Therefore, while walking over the stem overlay, the ant was continuously exposed to the same odour right until the decision point. This could have caused sensory adaptation – the gradual adaptation of receptors to continuous stimulation – which leads to reduced sensation. At the decision point, a new odour would then be perceived as more salient, which, in turn, could be the target of associative learning. Similar to the explanation of Collett (2005) that honeybees could modify their tendency to approach flowers experienced previously, the tested ants might have associated the more salient cue as rewarding. This also well explains the similarity of the inverse performance of the 'same' treatment with that of 'different' (see figure 4-2C) and that ants improved their performance over visits in the 'different' treatment. In other words, the ants might have not used the heuristic 'go different' but rather 'go to the more salient cue'.

At a first glance, the sensory adaptation hypothesis does not seem to explain why the ants also used this strategy in the 'same' treatment. However, in our experiment, once the ant had made a wrong decision, it was allowed to correct itself by walking to the other arm. It thus again experienced a change in odours. In other words, using 'go to the more salient odour' leads to reward in both treatments, but in the 'same' treatment requires two choices to follow the different odour. Such repetition of erroneous behaviour was also reported, but not quantified, in other studies on ants and bees (Macquart et al. 2008; Zhang 2000)

Heuristics can provide a strategy which may be better than stepwise optimisation through learning in cases of highly complex or uncertain information, and where the costs of errors are low (Gigerenzer and Gaissmaier 2015). Our analyses of ants' decisions revealed that that half of the ants in both treatments chose in a manner consistent with 'go to different (the more salient odour)' or 'go left' in at least 66% (32/48) of their visits (figure 4-4). This was not the case for other potential heuristics such as 'go to the last rewarded side' or 'go to linalool'. It is important to note that our assignment of heuristics is not mutually exclusive. In some visits, ant could have chosen in a manner consistent with more than one heuristic. A ROC analysis further showed that no single heuristic could predict the pooled performance of the ants. This is interesting, as it highlights that heuristics are ant specific, i.e. each ant chooses differently.

Many ants also chose to 'go left' during our treatment. Side biases are commonly observed in many animals (Andrade et al. 2001; Bell and Niven 2014; Hunt et al. 2014), and *Lasius niger* are no exception. They were found to display right (Vallortigara and Rogers 2005) and also left biases (Oberhauser et al. 2018). In our study, ants displayed very long streaks to both sides (see figure 4-3A), but the majority were to the left. The left bias was especially strong in the 'same' treatment, with one ant choosing left 27 visits in a row. Such a consistent side bias is intriguing, as the reward side was balanced and led to only 50% success. The lack of improvement and the high prevalence of a left bias in the 'same' treatment indicates that a fraction of ants tend to 'default' to a side bias when failing to extract a rule from a constantly changing environment.

Use of heuristics is promoted when error costs are low (Arkes 1991; Haselton et al. 2015). The costs of making a wrong decision might have been too small to promote careful decisions in our setup. If wrong, ants encountered quinine instead of sucrose at the arm's end. However, after the first encounter with quinine, ants usually approached the droplet very carefully and identified the quinine with their antennae, thereby diminishing its effect as negative reinforcer (Avarguès-Weber et al. 2010). Furthermore, the cost of moving from one arm to the other is likely negligible for the ant in terms of both time and energy.

In conclusion, no convincing evidence for relational rule learning was found. Rather, we found that ants have a high propensity to resort to heuristics in the face of a complex challenge, sacrificing accuracy for speed and ease of applicability. Cognitive processes have not evolved to ascertain objective reality, but to provide decisions which maximise fitness gains (Haselton et al. 2015). Heuristics often provide decision rules which can solve a given task quickly and with reasonable error and can range from simple rules such as 'go left' to sophisticated sets of rules orchestrating behaviours with highly complex outcomes, such as honeycomb construction by bees (Nazzi 2016). Facing a complex challenge, animals might change heuristics or even modify them by learning (Mhatre and Robert 2018). And indeed, ants in our study showed striking individual differences, with different ants settling on different heuristics such as "go left", "go to the more salient cue", or "choose randomly".

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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.

4.6 Supplementary material

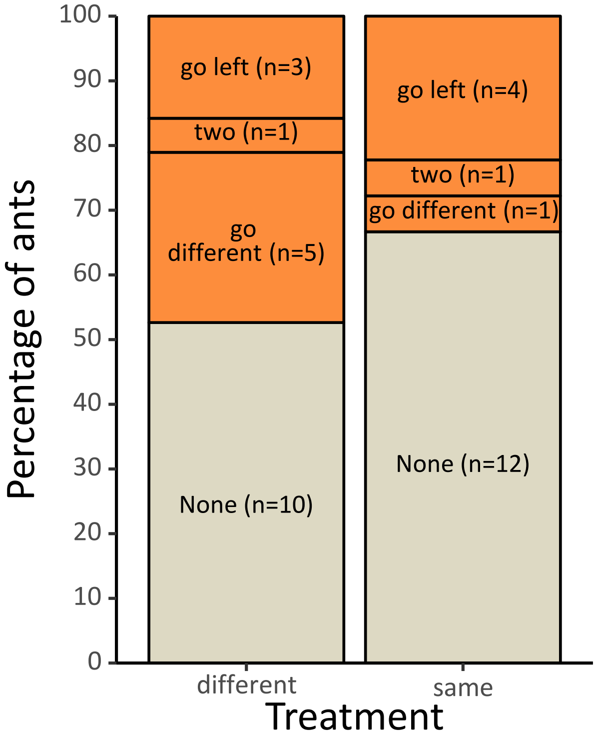

Figure S4-1. Subset of 22 visits in which linalool was present on one arm only. Percentage of ants which used each of the defined strategies for a minimum of 66% of their visits in the 'different' (n = 19) and 'same' treatment (n = 18). Note that the choices of two ants could be assigned to two different heuristics (once 'go left' and 'go different', once 'go different' and 'go linalool', labelled 'two').

Table S4-1. Sequence of odours used for both ‘same’ and ‘difference’ treatments. On each visit, ants encountered a different unique odour pair. The sample odour on the stem had to either be matched to the target odour on one arm (‘same’) or non-matched (‘difference’). This table shows the 'difference' treatment, in which reward was placed on the end of the arm with an odour other than the stem (non-match rewarded). In the ‘same’ treatment, the succession of pairs was identical, but reward was on the arm with the same odour as the stem. Half of the ants started with L (left), half with R (right, shown here).

Visit	**Sample**	**Target**	**Reward side**	**Visit**	**Sample**	**Target**	**Reward side**
1	bergamot	sandalwood	R	**25**	bergamot	peppermint	L
2	cypress	geranium	L	**26**	cypress	melissa	L
3	basil	ylang-ylang	R	**27**	basil	sandalwood	R
4	clove	lemon	L	**28**	clove	geranium	L
5	rosemary	peppermint	R	**29**	rosemary	ylang-ylang	R
6	lavender	melissa	L	**30**	lavender	lemon	L
7	geranium	bergamot	R	**31**	melissa	bergamot	R
8	ylang-ylang	cypress	L	**32**	sandalwood	cypress	R
9	lemon	basil	L	**33**	geranium	basil	L
10	peppermint	clove	R	**34**	ylang-ylang	clove	L
11	melissa	rosemary	R	**35**	lemon	rosemary	R
12	sandalwood	lavender	L	**36**	peppermint	lavender	L
13	bergamot	ylang-ylang	R	**37**	bergamot	clove	L
14	cypress	lemon	L	**38**	cypress	rosemary	R
15	basil	peppermint	R	**39**	basil	lavender	R
16	clove	melissa	R	**40**	sandalwood	lemon	L
17	rosemary	sandalwood	L	**41**	geranium	peppermint	R
18	lavender	geranium	L	**42**	ylang-ylang	melissa	L
19	lemon	bergamot	R	**43**	rosemary	bergamot	R
20	peppermint	cypress	L	**44**	lavender	cypress	L
21	melissa	basil	R	**45**	clove	basil	L
22	sandalwood	clove	L	**46**	peppermint	sandalwood	R
23	geranium	rosemary	R	**47**	melissa	geranium	R
24	ylang-ylang	lavender	R	**48**	lemon	ylang-ylang	L

Chapter 5

Small differences in learning speed for different food qualities can drive efficient collective foraging in ant colonies

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5.1 Abstract

Social insects frequently make important collective decisions, such as selecting the best food sources. Many collective decisions are achieved via communication, for example by differential recruitment depending on resource quality. However, even species which only rarely recruit can respond to a changing environment on a collective level by tracking food source quality. We hypothesised that an apparent collective decision to focus on the highest quality food source can be explained by differential learning of food qualities. Overall, ants may learn the location of higher quality food faster, with most ants finally congregating at the best food source. To test the effect of reward quality and motivation on learning in *Lasius niger*, we trained individual ants to find sucrose molarities of varying concentrations on one arm of a T-maze in spring and in autumn after 1 or 4 days of food deprivation. As predicted, ants learned fastest in spring and lowest in autumn, with reduced food deprivation leading to slower learning. Surprisingly, the effect of food quality and motivation on the learning speed was small. However, persistence rates varied dramatically: All ants in spring made all (6) return visits to all food qualities, in contrast to only 33% of ants in autumn after 1 day of food deprivation.

Fitting the empirical findings into an agent-based model revealed that even a weak tendency of ants to memorise routes to high-quality food sources faster can result in ecologically sensible colony-level behaviour. This collective-seeming decision is non-interactive, and thus resembles an annealing process.

Significance statement

Collective decisions of insects are often achieved via communication and/or other interactions between individuals. However, animals can also make collective decisions in the absence of communication. We show that foraging motivation and food quality can affect both route memory formation speed and the likelihood to return to the food source and thus mediate selective food exploitation. An agent-based model, implemented with our empirical findings, demonstrates that at the collective level even small differences in learning lead to ecologically sensible behaviour: mildly food deprived colonies are selective for high quality food while highly food deprived colonies exploit all food sources equally. We therefore suggest that non-interactive factors such as individual learning and the foraging motivation of a colony can mediate or even drive group-level behaviour in a process resembling annealing. Instead of accounting collective behaviour exclusively to social interactions, a possible contribution of individual processes should also be considered.

Key words

Lasius niger, differential learning, route memory, agent-based modelling, collective decision making, annealing

5.2 Introduction

The ability to choose which resource to exploit is of key importance to both individual animals and animal groups. Groups need to decide where to go while maintaining cohesion or when to fission or fuse in response to resource availability (Couzin and Krause 2003; Sueur et al. 2011). Eusocial insects are of particular interest for collective decision-making, as they form colonies consisting of many individuals acting as one reproductive unit, which favours the development of elaborate communication systems and food sharing while restricting intra-group conflicts (Ratnieks and Reeve 1992).

Social insects frequently use recruitment to collectively decide on and select a nest site (Mallon et al. 2001; Seeley and Buhrman 2001), to make efficient path choices (Beckers et al. 1992b; Reid et al. 2011), and to rapidly exploit food sources (Beckers et al. 1993; Czaczkes and Ratnieks 2012; Deneubourg et al. 1983; Seeley et al. 1991).

Trail pheromones constitute one way of transmitting location information, and are widely used in ants (Czaczkes et al. 2015b; Morgan 2009). By depositing pheromone on the substrate on the way back to the nest, successful foragers can both attract their conspecifics and provide an orientation cue to direct them to the food (Beckers et al. 1993; Czaczkes and Ratnieks 2012; Wilson 1962). Naïve ants relying on social information can thus locate new food sources quickly while avoiding trial and error learning and costly mistakes (Galef, Jr and Giraldeau 2001) and building up a route memory (Collett et al. 2003).

Yet even species with no recruitment or communication manage to make sensible decisions at group level and to adjust their behaviour to a changing environment: Cockroaches (*Blattella germanica*) were found to aggregate at shelters or food sources based on a retention effect – individuals stayed longer at food sources with high neighbour density (Amé et al. 2006; Lihoreau et al. 2010). A similar mechanism was found in greenhead ants (*Rhytidoponera metallica*), a species which does not recruit to food sources. The ants successfully tracked the highest food quality in the environment without comparison between the available food sources. This ability is thought to be achieved by both the tendency of individuals to feed for longer at higher quality food sources and an important interactive retention effect of conspecifics to newcomers (Dussutour and Nicolis 2013). As a result, a gradual improvement is observed: While animals are spread to all food sources initially, they concentrate on certain food sources over time until most animals feed on the best food source.

Another possible mechanism for driving apparent collective decisions without active recruitment could be based on learning. Many social insects learn the location of food sources very quickly, often after only a single visit (Aron et al. 1988; Grüter et al. 2011) and can have retention times up to months (Salo and Rosengren 2001). They can form associations of odour and food locations and use them to recall and navigate to at least two different feeder locations (Czaczkes et al. 2014; Reinhard et al. 2006). Thus, factors affecting memory formation could have significant impact on the foraging efficiency of a colony. Two particularly ecologically relevant factors are (i) food quality (i.e. reward magnitude) and (ii) motivation (for example hunger level or season), both of which are known to affect collective foraging (Seeley 1986).

An effect of reward magnitude on memory formation is a key part of theories of learning (Rescorla and Wagner 1972) and has been reliably demonstrated in many animals including honey bees. Higher sucrose reward concentrations, for example, increase the probability and retention time of proboscis extension response (PER) conditioning (e.g. Scheiner et al. 1999; 2004; 2005). Honey bees make more return visits to feeders offering sweeter rewards (Seeley 1986) and display higher persistence to return to depleted once-profitable foraging locations which used to offer high concentrations of sucrose (Al Toufailia et al. 2013). Higher persistence leads to more visits to the same food location which facilitates learning and positively influences memory retention (Menzel 1999).

Food deprivation is known to heavily affect foraging motivation in social insects (e.g. Cosens and Toussaint 1986; Mailleux et al. 2006). Honey bees differ in the amount of sucrose concentration needed to recruit others and have higher thresholds when plenty of food is available (Lindauer 1948; Seeley 1986). Moreover, high satiation levels can disrupt memory formation in honey bees (Friedrich et al. 2004).

Foraging motivation and sucrose thresholds also vary between seasons (Beekman and Ratnieks 2000; Quinet et al. 1997; Ray and Ferneyhough 1997; Scheiner et al. 2003). In temperate regions many animals, including ants (Cook et al. 2011; Quinet et al. 1997), show reduced foraging towards autumn as their reproductive period is over, resources decline (Mailleux et al. 2006) and they prepare to overwinter.

Taken together, we hypothesised that both the motivation of foraging ants, influenced by season and food deprivation levels, and the quality of the food source they find, will affect the ant's route memory formation. High reward and/or high hunger levels could facilitate rapid learning, while ants at low motivation levels or ants finding low quality food may form memories less rapidly or be more likely to deviate from their memories. If learning is more likely for higher quality food sources, we hypothesise that ants will tend to memorise and return to higher quality food sources. This should result in an annealing process taking place (Černý 1985; Kirkpatrick et al. 1983): at the beginning, ants forage at all food sources, but as ants finding low quality food are more likely to deviate from their memories, they might end up at the best food source as time progresses. Eventually, most ants should be foraging at the highest quality food source.

Here, we investigated the effect of reward quality and motivation level on memory formation in *Lasius niger* foragers. Reward quality (molarity) was varied by offering different concentrations of sucrose solutions. Motivation levels were varied by testing in spring after 4 days of food deprivation (high motivation), in autumn after 4 days of food deprivation (moderate motivation) and in autumn after 1 day of food deprivation (low motivation). We then used this data to build an agent-based model to understand how reward magnitude and motivation level affect the ability of ant colonies to selectively choose the best of multiple foraging locations.

5.3 Material and methods

5.3.1 (a) Collection and rearing of colonies

Stock colonies of the black garden ant, *Lasius 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 500+ workers. Queenless colonies still forage and lay pheromone trails, and are frequently used in foraging experiments (Devigne and Detrain 2002; Dussutour et al. 2004). All colonies were kept in a 12:12 day/night cycle and were provided *ad libitum* with water and Bhatkar diet, a mixture of egg, agar, honey and vitamins (Bhatkar and Whitcomb 1970). Data was collected in spring (April - May 2016) and in autumn (September - November 2016) to test for seasonal effects (referred to as spring or autumn, respectively). The spring colonies were deprived of food for 4 days prior to each trial (referred to as spring high food deprivation) and the autumn colonies for either 4 or 1 days (referred to as autumn high food deprivation and autumn low food deprivation, respectively). Seven colonies tested in spring were retested in the autumn high food deprivation treatment. For the autumn low food deprivation treatment, we used four colonies tested in the other treatments. The remaining colonies were previously untested.

5.3.2 (b) Experimental procedure

Several ants were allowed onto a plastic T-maze covered with paper overlays via a drawbridge. The runway towards the maze head was tapered to prevent guidance by pheromone before the ant had to choose a side (figure 5-1) (Popp et al. 2017). A sucrose solution droplet (Merck KGaA, Darmstadt, Germany) was presented on a plastic feeder at one side of the T-maze head while a droplet of water was presented on a feeder on the opposite side. The side containing the sugar reward remained the same throughout all seven visits. The T-maze head was recorded from above with a Panasonic DMC-FZ1000 camera, facilitating the observation of ants' decisions.

Each colony was tested with three different concentrations of sucrose solution (0.125, 0.5 or 1.5M). Furthermore, to control for possible side preference, as is often reported in ants (Hunt et al. 2014), each colony was tested on both sides of the T-maze.

The first six ants (five in the autumn low food deprivation treatment) to reach the feeder were individually marked with a dot of acrylic paint on the abdomen and all other ants were returned to the colony. The drawbridge was used to selectively allow only the marked ants onto the runway thereafter for 6 successive visits, resulting in a total of 7 visits (familiarisation + 6 visits) per ant. Each time an ant walked over the maze head the paper overlay was replaced to remove any trail pheromone that might be deposited. On each outward visit for each ant, the first decision line (situated 3cm inwards on each arm, figure 5-1) crossed by the ant with its antennae was scored as its' initial decision. The first droplet contact on each visit was scored as its' final decision. Ants were allowed to drink fully. The experiments were not conducted blind to treatment, but ants rarely changed arms after their initial decision (see table S5-1), and decisions were unambiguous, thus restricting observer bias. Neither the position of the sucrose solution nor its concentration was changed during each trial. Colonies were tested once per week except for the autumn low food deprivation treatment, where they were tested once per 2 weeks. All marked ants were freeze-killed after testing to prevent pseudo-replication.

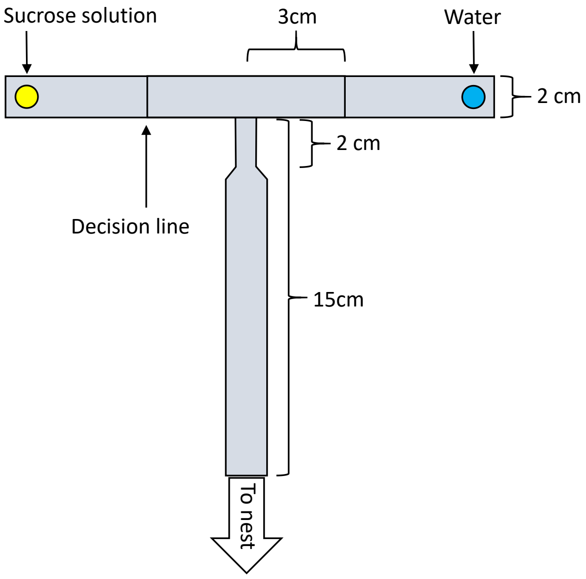

Figure 5-1. Experimental setup. The ants entered the plastic runway via a moveable drawbridge. The end of the 20cm-long runway leading to the maze head was tapered. On one maze arm, a sucrose droplet of either 0.125, 0.5 or 1.5M was presented while a water droplet was presented on the other arm

5.3.3 (c) Statistical analysis

Data were analysed using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) in R version 3.4.1 (R Core Team 2018). GLMMs were fitted using the lmer function (Bates et al. 2015). As the data were binomial (correct / incorrect), a binomial distribution and logit link were used. Since multiple ants were tested per colony and each ant made repeated visits, we included ant ID nested in colony as random factors. Each model was tested for fit, dispersion and zero inflation using the DHARMa package (Hartig 2018).

The model predictors and interactions were defined *a priori*, as suggested by Forstmeier and Schielzeth (2011), as:

*Decision (correct/incorrect) ~ Motivation * Molarity + Side + (1|Colony/Ant_ID)*

Molarity of the food was included as continuous variable. All p-values presented were corrected for multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg 1995). In total, 688 ants were tested, 104 of which performed fewer than 6 return visits (referred to as dropped out ants) and were excluded from the analysis to decrease variance in motivation effects, resulting in 584 ants used for analysis. A complete annotated script and output for all data handling and statistical analysis is presented in electronic supplementary material (ESM) 5-3. The complete raw data is presented in ESM5-4.

In only a small fraction (2.09%) of visits, the initial and final decision differed. In most such cases, ants chose the correct side in their initial decision and then U-turned and chose the wrong side on their final decision (table S5-1). For simplicity, due to these small differences, we used only the final decision of each ant as measure of performance in the final analysis.

5.3.4 (d) Agent-based model

We developed an agent-based model in order to study the effect of individual learning rates on colony-level foraging behaviour. The model is an adaptation of an earlier model of *L. niger* foraging (Czaczkes et al. 2015a) and was coded in Netlogo 6 (Wilensky 1999). Here we provide an overview of the model, a detailed description following the ODD (Overview, Design concepts, Details) protocol (Grimm et al. 2006; Grimm et al. 2010) is provided in ESM5-1, and the annotated model itself is provided in ESM5-2.

In the model, an ant colony in the centre of the model environment was surrounded by three food sources, either in random positions or in fixed positions equidistant from the nest. The resources were of quality 0, 1 and 2, which respectively represent molarities of 0.125, 0.5 and 1.5. Ants explored the environment using a correlated random walk. If they found a food source, the ants learned the location of the food source and returned to the nest. On returning to the nest, the probability of the ant following its memory or beginning scouting again depended on the motivation level of the colony (a global variable) and the quality of the resource. The probability of memory following was taken directly from the empirical data gathered in our experiments. Memory was modelled in one of two ways. First, we modelled memory in the standard manner, with each ant showing the same behaviour, based on the average behaviour of ants taken from the empirical data ("average ants model"). However, the average behaviour of individuals is a poor description of each individual's behaviour (Pamir et al. 2011). Thus, we also modelled the ant's memory use to be variable between individuals, with the average centred on the empirical data, but individuals varying around that ("variable ants model"). This implementation is based on the extended learning curves model of Pamir et al. (2011). The model was run for 3000 time steps, and the proportion of food returned from each food source was recorded at the end of the model run.

5.3.5 (e) Data availability

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

5.4 Results

Our statistical model revealed that ants significantly improved their performance (correct decisions) with increasing visits ($z = 15.33$, $p < 0.0001$), demonstrating that the ants learned the route to the food source.

Interestingly, reward magnitude, i.e. the molarity of the food, did not significantly affect performance positively ($z = 1.88$, $p = 0.35$; figure 5-2). Altogether, molarity effects were surprisingly modest, especially at high motivation levels, with 75% accuracy on the first visit even for the lowest molarity at the highest motivation level (see figure 5-2a and figure S5-1).

It is important to note that we only included ants which finished all 6 visits in our analysis. However, the number of ants which dropped out (i.e. did not finish all 6 visits) was not randomly distributed: When presented with 0.125M sucrose, most (>65%) of the ants in autumn after 1 day of food deprivation did not finish all 6 visits (table 5-1). The number of drop-outs decreased with increasing molarity (~17% for 1.5M) but remained high in comparison to highly food deprived autumn or spring ants (<10% and 0%, respectively). By contrast, all ants completed all 6 visits in spring, irrespective of the molarity, highlighting the importance of motivation on persistence.

Seasonal effects were more prominent: Ants tested in spring showed faster learning than ants tested in autumn after 4 days ($z = 4.29$, $p = 0.0001$) or only 1 day of food deprivation ($z = 5.29$, $p < 0.0001$). No significant difference was found between ants tested in autumn with 4 days or 1 day of food deprivation ($z = 1.88$, $p = 0.35$), although the performance of the 1-day-deprived ants tended to be lower (figure 5-2b).

Table 5-1. Total number of ants tested and number of ants which finished all 6 return visits. Ants with less visits were excluded from the analysis (dropped). All ants finished in spring, while many in autumn after 1 day of food deprivation (autumn low) did not finish.

Motivation	**Molarity**	**# ants tested**	**# ants with 6 visits**	**% dropped**
Autumn low	0.125	76	25	67.1
	0.5	81	57	29.6
	1.5	80	66	17.5
Autumn high	0.125	104	98	5.8
	0.5	100	91	9
	1.5	103	103	0
Spring	0.125	48	48	0
	0.5	48	48	0
	1.5	48	48	0
	Total	**688**	**584**	**15.1**

No significant interactions between motivation (season and hunger level) and reward magnitude (molarity) were found (spring: z = 0.36, p = 0.99; autumn high: z = 0.36, p = 0.99, autumn low: z = 1.67, p = 0.44).

We also noted a side bias, as ants had higher proportions of correct decisions when they had to learn to go to the left (z = 5.59, p < 0.0001). Detailed statistical outputs for all tests are provided in ESM5-3.

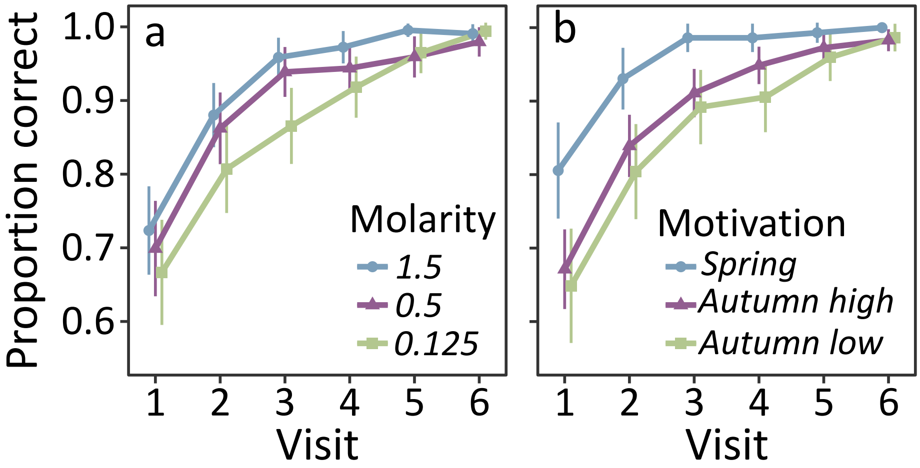

*Figure 5-2. The proportion of correct decisions made by ants as a function of **(a)** food quality or **(b)** motivation level. The colonies of the "autumn low" experiment were food deprived for only 1 day, as opposed to 4 days in the other two treatments. Visit 0 (not shown) is the discovery visit, where ants find the food for the first time. Points show means and error bars show 95% confidence intervals.*

An examination of the individual-level learning rates revealed that most (~70%) ants learned rapidly, already choosing the correct side on the first return visit to the food source (chi-squared test against chance level p = 0.5, two sided, $\chi^2 = 91.372$, df = 1, $p < 0.0001$; see figure 5-3a, b). An interesting pattern can be seen when considering the number of successive correct visits: The majority (80%) of ants in spring made no errors over all 6 visits, while this was only achieved by ~65% of ants of the autumn colonies (chi-squared test, two sided, $\chi^2 = 17.885$, df = 1, $p < 0.001$; figure 5-3d).

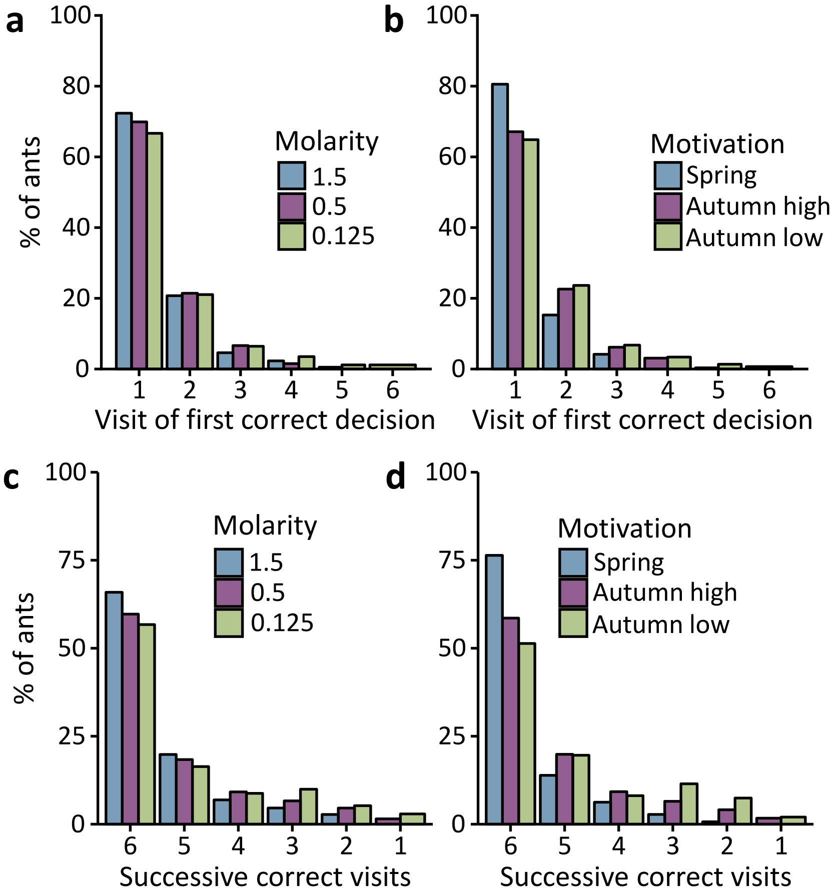

Figure 5-3. ***a, b****: Percentage of ants making their first correct decision on the first down to the last visit. Most ants made their first correct decision on the first return visit to the food. While very small differences are seen between different food qualities (a), they become more prominent between different motivational levels (b).* ***c, d****: Percentage of ants making 6 correct visits in a row (100% correct) down to only 1 (no two correct visits in a row) as a measure of consistency of ant behaviour. While there is a trend for longer streaks with increasing food quality (c), motivational effects are stronger, with the majority of ants tested in spring making no error at all (d). Spring and Autumn high: 4 days of food deprivation; Autumn low: 1 day of food deprivation.*

5.4.1 Agent-based model results

The poor quality (0.125M) feeder was usually collectively avoided by the end of the model run (figure 5-4). When motivation levels were low or medium, the good quality (1.5M) feeder had the highest proportion of ants exploiting it. When motivation levels were high, however, colonies generally showed less “choosiness”, having a more equal distribution of foragers, with the majority of foragers exploiting the medium quality (0.5M) feeder. It should be noted that effect sizes are not large: at most 40% of ants exploited the most strongly chosen feeder, as compared to the null situation of one third.

Increasing motivation level tended to increase the total food returned (due to more ants exploiting their memory rather than scouting), but the average quality of food returned to the nest was lower at higher motivation levels (see figure S5-2-1). Differences between the “average ants” and “variable ants” models were subtle, with “variable ants” returning less food and being more choosey (see supplementary discussion in ESM5-2).

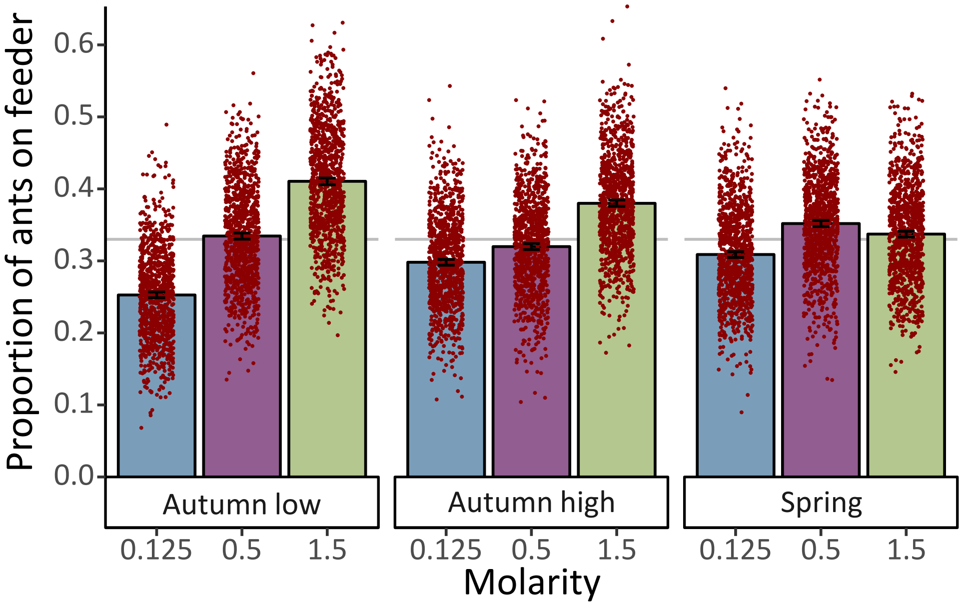

Figure 5-4. Proportion of agents exploiting each of the three feeders. These simulations used the “average ants” model of memory formation. Bars show means, error bars show 95% confidence intervals, dots show individual model run results. The dashed grey line shows the null situation of one third. For further model results see ESM5-2.

5.5 Discussion

In our study, we investigated whether the effect of reward magnitude (sucrose concentration) and motivation (season and hunger level) on differential learning is strong enough to explain apparent collective decisions of ants in the absence of communication.

Highest learning rates were found in spring and lowest in autumn in slightly food-deprived colonies. However, surprisingly, the molarity of the reward did not significantly affect learning speed, although on average ants learned fastest when presented with the highest reward (figure 5-2a). Nonetheless, when these weak effects were built into an agent-based model of ant foraging, colonies were found to send the largest proportion of foragers to the best food sources. This was especially the case at lower motivation levels: as foraging motivation drops, colonies become more collectively ‘choosey’. These findings suggest that at least part of an apparent collective decision to congregate at a high-quality food source could be accounted for by an as yet neglected annealing mechanism (Černý 1985; Kirkpatrick et al. 1983) based on learning: By preferentially memorising the best food source, ants will gradually improve until most ants forage at the best food source, in the absence of communication. The collective choice mechanism at play in our model differs from other non-recruiting systems such as cockroach aggregation (Amé et al. 2006; Lihoreau et al. 2010) in that it is completely non-interactive.

It is noteworthy that even a weak and non-significant effect of food quality on learning could, in the agent-based model, drive apparently ecologically sensible collective behaviour. We do not dispute that in laboratory experiments with mass-recruiting ants, collective feeder selection is driven mainly by pheromone deposition (Beckers et al. 1990; 1993; Wilson 1962), although memory undisputable can play a role in triggering collective behaviour (Czaczkes et al. 2016). However, such near-unanimous collective decisions do not well represent collective foraging on carbohydrate sources by ants in the wild (Devigne and Detrain 2005). In natural situations with limited, depleting but replenishing food sources, collective foraging is better described by models in which memory plays the biggest role in individual decision making (Czaczkes et al. 2015a). Understanding the effect of memory on foraging decisions is thus key to understanding real-world colony foraging decisions. One could argue that in natural situations such weak learning effects found could be masked by pheromone-mediated decisions, thus preventing any effect on colony level. However, only around one third of *L. niger* workers deposit pheromone on their first return trip from a food source (Beckers et al. 1992a) and workers follow their own memory over a pheromone trail even after only one visit (Czaczkes et al. 2018; Grüter et al. 2011). As a result, even in the presence of pheromone, memory-based decision making likely plays an important role in colony-level foraging decisions.

Overall, the learning rates of *L. niger* ants were very fast, with most ants (~70%) choosing the correct path already on the first visit after familiarization (figure 5-2a, b), resembling results of other studies (Czaczkes et al. 2013a; Grüter et al. 2011). This highlights the ecological importance of route memory in *L. niger,* allowing the ants to find distributed, semi-permanent food sources repeatedly. In accordance with studies conducted on honey bees, decreasing rewards (1.5, 0.5, 0.125M) led to decreasing learning rates (e.g. Scheiner et al. 1999; 2004; 2005), although the differences were subtle and not significant in our study. Especially in highly food deprived ants, differences in learning rates were small, and these only became more prominent under low foraging motivation (figure S5-1). This finding is surprising in itself, as basic learning theory would predict a strong and consistent link between reward strength and learning (Rescorla and Wagner 1972). In our setup, however, it is impossible to distinguish between actual learning and persistence effects: A well-satiated ant might just alternate T-arms to explore. Moreover, more than 65% of the 1-day-deprived ants did not perform all 6 runs when presented with the lowest, 0.125M, reward, but rather remained in the nest after some visits, as opposed to only ~6% in the 4-days-deprived autumn group and 0% in spring colonies (table 5-1). Under low hunger pressure, ants thus ignore poor quality food, which was also found in our agent-based model and is ecologically sensible (Seeley 1986). By this mechanism, at intermediate and low foraging motivation conditions, ant colonies will concentrate their foraging effort only on high-quality food sources, with workers simply not returning to low quality feeders (table 5-1). This mechanism again relies on memory and persistence rather than differential recruitment. However, it is important to note that our model is offered as a proof of concept that individual behaviour, driven by differential learning, sums to a collective-seeming response. We do not suggest that this is truly the mechanism used by *L. niger* to make collective food choices.

Learning curves (figure 5-2a, b) are often used to present learning rates of groups, under the assumption that the group probability is a good representation of individual performance. This approach, however, neglects individual differences in learning rates (Pamir et al. 2011; 2014).

Motivational differences between individuals are masked by overall performance and individuals seldom display a gradual improvement, but rather a binary response (figure 5-3a-d, and Pamir et al. 2011; 2014). Furthermore, group level representations cannot show inconsistent behaviour of individuals, such as making errors after being correct before. We found such inconsistent behaviour in our study (figure 5-3c, d), with some ants in autumn, especially in the low motivation condition, not making two correct visits in a row. Nonetheless, the majority (75% in spring, >50% in autumn) of ants not only decided correctly on the first revisit, but also continued to be correct for the remaining 5 visits. These observations might be explained in terms of an exploration/exploitation trade-off (Cohen et al. 2007; Mehlhorn et al. 2015; Patrick et al. 2017). Animals have to decide between either exploiting available food or exploring the surroundings in order to find new food sources. In our experiment, hungry ants readily exploited the available food and thus maximised their energy intake, while well-satiated ants were more likely to explore (move to the other arm), especially when the encountered food was of low quality. Unfortunately, our setup does not allow us to differentiate ants which did not learn the route from ants which know the way but are exploring instead. Even though we suspect hunger levels influence learning of the animals *per se*, hunger is also expected to affect the probability of exploration. Both mechanisms likely play a role in driving our results.

As the experimental setup only allowed individual ants to be tested, and completely preventing pheromone-mediated recruitment in a whole colony is not technically feasible, we used an agent-based model (see ESM5-1) to explore the impact of the observed learning rates on colony level. In the model, low motivation colonies mostly retrieved high-quality food. This is sensible, as the processing and storage capacity of a colony is limited in such situations. By contrast, highly motivated colonies maximised food intake by collecting all food sources equally, leading to a decrease in energetic gain per load while maximising total energetic input. Such decreased choosiness when deprived of food can also be observed in other animals such as spiders (Pruitt et al. 2011). As the model was designed to explore the effects of differential learning on foraging, we purposefully did not include differential drop-out rates. Such a model would have to account for the costs of foraging, for which we lack good parameterisation. Including such an effect would most likely strengthen the patterns we see in our model: We would expect much greater ‘choosiness’, higher efficiency, and lower overall food intake at lower motivation levels.

The season of the year is known to affect foraging in social insects (Al Toufailia et al. 2013; Beekman and Ratnieks 2000; Cook et al. 2011; Mailleux et al. 2006; Quinet et al. 1997; Ray and Ferneyhough 1997; Scheiner et al. 2003), but effects on laboratory-reared colonies are less clear (Ray and Ferneyhough 1997). Our tested laboratory ant colonies clearly displayed decreased learning rates in autumn (figure 5-2b). In spring, *L. niger* is usually in dire need of food to begin worker production while being exposed to fluctuations in food supply, as the aphid colonies first need to establish (Mailleux et al. 2006). In such a variable environment, it is ecologically sensible to fully exploit all available sources. In autumn, ants usually stop egg production (Kipyatkov 1993) and activity and energy needs decrease with falling temperature (MacKay 1985). In our study, this variable response to food over the seasons seems to prevail in the laboratory colonies as well. Importantly, our queenless colonies are reinforced multiple times per year with workers from outside stem colonies, constituting a possible source of seasonal behaviour. Furthermore, we observe very large fluctuations in the amount of food consumed by colonies over the course of the year even in unreinforced colonies, with consumption rates falling dramatically towards autumn (FBO and TJC, pers. obs.). However, as we have only tested once per season, the observed effects might not be only attributable to seasonal effects.

To conclude, both reward quality and motivation can in principle lead to an adaptive increase in foraging efficiency via differential learning, without the need for any communication. This is in line with Dussutour and Nicolis (2013), who demonstrated that even ants which do not recruit to food sources can nonetheless focus on the best food source in a changing environment. They demonstrated, as we have, that such collective behaviour can be achieved by the increased retention of individuals to the best option, although their proposed retention mechanism relies on conspecifics and the quality of the food source while ours is likely based on memory and motivational effects. Both retention due to occupancy time at a food source and due to persistent visiting likely play a role in real-world situations. Such a collective decision without communication or comparison is conceptually identical to an annealing process and represents one of the simplest types of collective behaviour. Route memories alone have been demonstrated to be sufficient to trigger collective behavioural patterns in ants, such as becoming stuck in local foraging optima (Czaczkes et al. 2016). This study shows how memory could also play a role in the selection of the best resource. When studying collective decision-making, researchers tend to focus on interactive effects such as positive feedback and stigmergy. However, simpler non-interactive individual-level behavioural effects should also always be considered as an alternative, or contributing, mechanism driving group-level behaviour.

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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.

5.6 Supplementary material

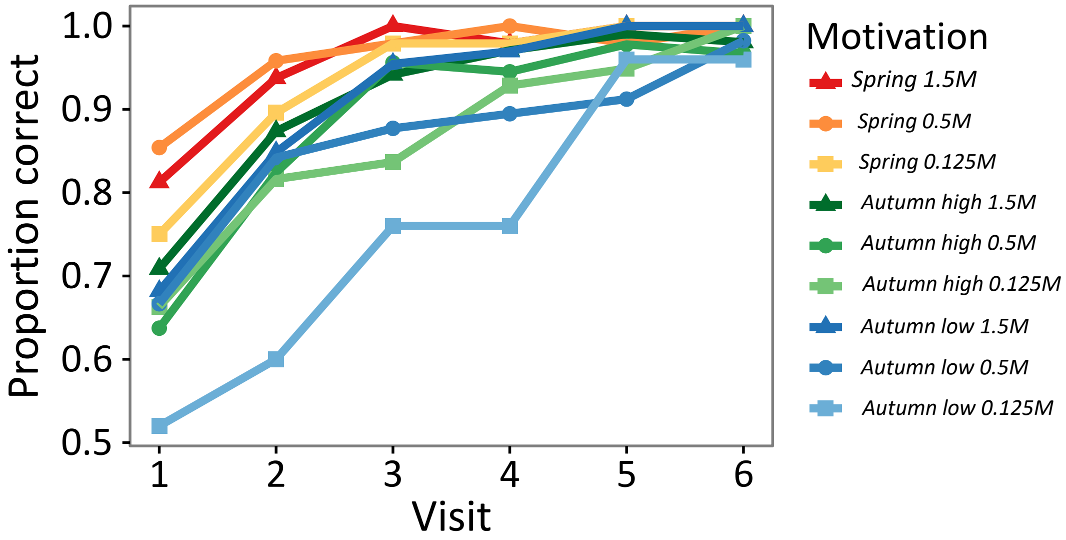


Figure S5-1. Learning curves of all treatments shown in separate coloured lines. Gradients of the same colour correspond to motivation, shapes to molarity. Spring, Autumn were food deprived for four days, Autumn low for only one day. Points show means.

Table S5-1. Percentage of switches (going to other side than initially chosen) of all decisions made.

Motivation	**Molarity**	**correct initial decision**	**correct final decision**	**initial - final decision**	**% switched**
0.125	Autumn low	114	114	0	0.000
0.125	Autumn high	516	509	7	1.190
0.125	Spring	266	269	-3	4.514
0.5	Autumn low	302	295	7	2.047
0.5	Autumn high	488	483	5	0.916
0.5	Spring	275	277	-2	3.472
1.5	Autumn low	365	360	5	1.263
1.5	Autumn high	575	563	12	1.942
1.5	Spring	279	275	4	3.472

5.7 Supplementary protocols (ESM5-1 & ESM5-2)

5.7.1 ESM5-1 ODD protocol for the agent-based model

Supplement to: Small differences in learning speed for different food qualities can drive efficient collective foraging in ant colonies

F. B. Oberhauser, A. Koch, T. J. Czaczkes

5.7.1.1 Table of content

5.7.1.2 Overview

We developed a spatially explicit agent based model using the modelling program Netlogo 5.0.3 (Wilensky 1999). The Netlogo file, including the annotated program code, is provided as electronic supplementary material (ESM) 5-5, and can be run using the Netlogo program which is freely available at http://ccl.northwestern.edu/netlogo/. The model description follows the ODD (Overview, Design concepts, Details) protocol (Grimm et al. 2006; 2010). The current model is adapted from a model published by Czaczkes et al. (2015a).

5.7.1.2.1 Purpose

The purpose of the model was to explore the effect of individual learning on collective behaviour on the efficiency of collective food exploitation by an ant colony. Learning rate was affected by two factors, motivation level and food quality, and is fully parameterized from empirical data. The model is not designed to be an exact model of *Lasius niger* foraging, but instead to capture the salient elements of memory-based foraging.

5.7.1.2.2 Entities, state variables, and scales

The model is spatially explicit, consisting of a grid of 100 by 100 patches (the environment) and populated by agents (the ants). The model is temporally explicit, and runs for 3000 time-steps. This allows for between 20 and 30 return visits for each ant, depending on motivation level and how memory was modelled (see below). Global variables, which can affect the agents or the patches, are also modelled. A list of parameters for agents, patches, and global variables, is provided in table 5-1-1.

Table 5-1-1. Agent, patch, and global variables

Variable	**Values**	**Description**
Agents		
X and Y coordinates	X coordinate: - -50 – 50 Y coordinate: -50 – 50 Non-integer	Every agent has an explicit location on the grid.
State	“scouting”, “following-memory “or “returning”	Ants may be searching for food by random search, may be following their memory to a previously visited food source, or may be returning from a food source to the nest.
$M_{favoured}$	Number, an integer ≥ 0, < number of feeders (3)	The food patch the ant heads towards when following its memory.
$M_{strength}$	Number, an integer ≥ 0, ≤ 5	The strength of the ants’ memory for the current food patch it is favouring ($M_{favoured}$). Every successful visit to this food patch increases $M_{strength}$ by 1, capped at 5 (counting begins at 0, so a total of 6 memory levels). The $M_{strength}$ tells the ant which probability of returning to a food source to pull from an array populated with the empirical data from the behavioural experiments (see table S5-1-2)
Patches		
X and Y coordinates	X coordinate: -50 – 50 Y coordinate: -50 – 50 Integer	Every patch has an explicit location. Patches cannot move.
Patchtype	“nothing”, “food” or “nest”	This defines whether the patch is empty environment (“nothing”), a food patch (“food”) or the nest entrance (“nest”).
Feeder number	Number, 0, 1, or 2	The identity of the feeder, if the patchtype = “food”.

Feeder quality	Number, 0.125, 0.5, or 1.5	The quality of the feeder, if the patchtype = “food”. Feeder number 0 has feeder quality 0.125, feeder 1 has quality 0.5 and feeder 2 has quality 1.5
Global variables		
Motivation level	0, 1, or 2	This describes which empirical dataset the ants should use to form their memory: 0 = Autumn 1 day of food deprivation 1 = Autumn 4 days of food deprivation 2 = Spring 4 days of food deprivation
Total number of feeders (F_{total})	Total number of feeders, integer ≥ 1, modelled as 3	The total number of feeders in the model.
Random-feeder-location	Whether the feeders are randomly placed or not, set as “TRUE” or “FALSE” Modelled as “FALSE”	When randomly placed, feeders appear anywhere in the model, except overlapping the nest entrance. When feeders are non-randomly placed they appear regularly spaced around the nest entrance at a distance of 75 patches.
St-Dev	Number, non-integer, ≥ 0. Modelled as 20.	Affects how sinuous the paths of scouting and returning ants are. A number is randomly chosen from a normal distribution with a mean of 0 and a standard deviation of “St-Dev”.
Ant number	Number, non-integer, ≥ 0. Modelled as 50	The number of active foragers.
Variable-threshhold?	“TRUE” or “FALSE”	Two options for modelling memory formation and use are implemented: an “average ant” method based on standard learning curves, and a “unique ants” method on the extended learning curve model of Pamir et al. (2011). See ‘details’ section below.

5.7.1.2.3 Process overview and scheduling

The environment is formed by defining one patch at coordinates 0, 0 as the nest, and circular areas with a radius of 3 patches as feeders. The feeders are either randomly on non-randomly placed (see Random-feeder-location in table S5-1-1). When not randomly placed the feeders are 75 patches away from the nest. A number of ants defined by AntNumber is spawned either at the nest or randomly placed throughout the environment, facing random directions. Ants begin in the state “scouting”, and travel using a correlated random walk around the environment (see St-Dev in table S5-1-1). In later stages of the simulation, ants can also follow their own memory. On reaching a full food source ants take the state “returning”, turn around and begin walking in the direction of the nest.

If memory is enabled, ants memorise the location of the first feeder they discover, and begin making repeated return trips to this feeder. Every time an ant makes a successful visit to its memorised feeder its memory becomes stronger, up to a limit of 6. If the feeder an ant encounters is not the one it had memorised, it resets its memory and begins the memorisation process again for the new feeder. The memory function is modelled to fit with empirical findings described in the empirical results section of this paper, see table S5-1-2 and details section below. An annotated screenshot of the model is provided in figure S5-1-1.

During the simulation, the behavioural states and variables of each agent and patch are updated asynchronously, in the order: ants check location (nest, feeder, elsewhere) and change state accordingly → ants behave according to their state (random walk/follow pheromone, return to nest, or follow memory) → global measurements taken. See the fully commented code for details.

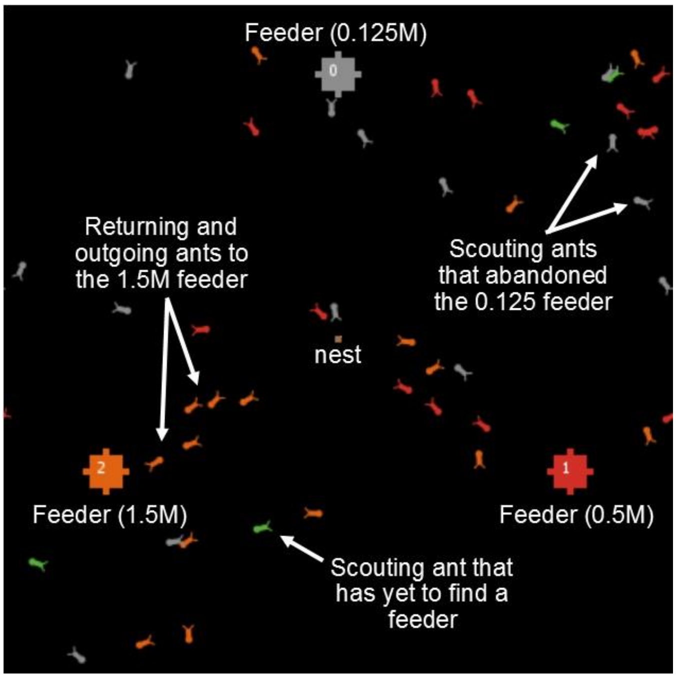


Figure S5-1-1. Annotated screenshot from the model after 900 time-steps. The colour of an ant denotes its current favoured feeder (Mfavoured).

5.7.1.3 Design concepts

The model purposefully ignores interactions between ants, such as trail pheromone recruitment, so as to focus on the potential for individual level decisions to sum up to apparently collective coherent colony-level behaviour. Even ants which do not show any type of recruitment can, for example, track food quality over time, preferentially concentrating on the most profitable feeder (Dussutour and Nicolis 2013). The proportion of agents exploiting each food source is an emergent property of the system.

Agents (ants) thus have a limited ability to sense their environment. Agents can sense when they have reached a feeder, whether this feeder is their favoured feeder ($M_{favoured}$) or not, and the quality of the feeder. Agents returning to the nest are assumed to know the location of the nest (Collett and Collett 2000). Agents do not interact with each other. Agents have a memory of the location of their favoured food source ($M_{favoured}$), and the number of times they have successfully visited $M_{favoured}$. Stochasticity is used to introduce variation into the paths of the ants (St-Dev, see table S5-1-1), both during scouting and when returning to the nest. It is also used to influence the likelihood of following a memory or returning to a scouting state (see tables S5-1-2 and S5-1-3). Lastly, when testing the effect of a changing environment, stochasticity is present in the quality location. Observations are made at the end of every model run, and include the mean number of visits made by each ant to a food source, and the mean proportion of ants which are exploiting each of the three feeders. This is a measure of how efficiently the ant colony is exploiting the resources available: the higher proportion of ants exploiting the best feeder, and the lower the proportion of ants exploiting the worst feeder, the better. This is reflected in the total food returned: ants add 0.125 if returning from feeder quality 0, 0.5 if returning from feeder quality 1, and 1.5 if returning from feeder quality 2. Agents have no explicit objectives, do not learn apart from the memory abilities outlined above, and do not attempt to predict the future state of the model. There are no intermediate levels of organisation, such as groups or collectives, in the model.

5.7.1.4 Details

At the beginning of the model, the environment was set up as above. Memory arrays are populated in a fixed manner from the empirical data.

Memory is modelled as follows: When the ant first visits a feeder, it sets this feeder as its favoured feeder ($M_{favoured}$), and increases its memory score for the preferred feeder ($M_{strength}$). Every further visit to this feeder increased $M_{strength}$ by one, up to a maximum of 6. If the ant visits a feeder other than $M_{favoured}$ (see below), the ant sets its favoured feeder to the current feeder, and resets its $M_{strength}$. When an ant returns to the nest from a feeder, it either returns directly towards $M_{favoured}$ or begins scouting. Two methods for making this decision are implemented: an "average ant" method, and a "variable ants" method. These are detailed below.

In the "average ant" method (in the model the "variable-threshhold = FALSE") all ants behave identically. The chance of returning to $M_{favoured}$ ($prob_{switch}$) is drawn from a look-up table (memory array), populated with data taken directly from the empirical observations described in this study. The return chance is affected by the motivation level, the feeder quality, and the number of consecutive visits the ant has made to $M_{favoured}$. Two tables are provided – an unmodified one, and one in which the final $prob_{switch}$ at $M_{strength}$ 6 is constrained to 0.98. We used only the modified table in our analysis, as according to the empirical data collected for visits past visit 6, a return probability of 1 is not a good description of reality. The unmodified table is provided in table 5-1-2 below.

In the "variable ants" method ("variable-threshhold = TRUE") each ant is spawned with a unique learning rate. This is drawn from a normal distribution with a mean of 0 and a standard deviation set by the variable 'learning-variability'. Note that the learning rate can thus be either positive of negative. When an ant returns to the nest it looks up $prob_{switch}$ for the look-up table and adds the 'learning-variability' score (either increasing or decreasing the resultant number, depending on the sign of the learning variability score). The ant then checks whether the resultant number is above a threshold set by the 'threshhold' variable. If it is, there is a 0.98 probability that the ant returns to $M_{favoured}$. Otherwise, the ant begins scouting again, but does not reset its memory. This implementation is based on the extended learning curves model of Pamir et al. (2011).

Table 5-1-2. $\text{Prob}_{\text{switch}}$ lookup table. The table provided is the unmodified version. For the modified version used in the model, simply replace $\text{prob}_{\text{switch}}$ to 0.98 in all instances of M_{strength} = 6.

Molarity	**Motivation**	**M_{strength}**	**$\text{Prob}_{\text{switch}}$**
0	0	1	0.5526
0	0	2	0.6935
0	0	3	0.7826
0	0	4	0.8537
0	0	5	0.9677
0	0	6	0.96
1	0	1	0.679
1	0	2	0.8052
1	0	3	0.863
1	0	4	0.8788
1	0	5	0.8852
1	0	6	0.9825
2	0	1	0.6625
2	0	2	0.8228
2	0	3	0.9333
2	0	4	0.9714
2	0	5	0.9853
2	0	6	1
0	1	1	0.6538
0	1	2	0.8039
0	1	3	0.82
0	1	4	0.9286
0	1	5	0.949
0	1	6	1
1	1	1	0.65
1	1	2	0.8
1	1	3	0.9474
1	1	4	0.9468
1	1	5	0.9783
1	1	6	0.967
2	1	1	0.7087
2	1	2	0.8738
2	1	3	0.9417
2	1	4	0.9709
2	1	5	0.99029
2	1	6	0.9806
0	2	1	0.75
0	2	2	0.8958
0	2	3	0.9792
0	2	4	0.9792
0	2	5	1
0	2	6	1
1	2	1	0.8542
1	2	2	0.9583
1	2	3	0.9792
1	2	4	1
1	2	5	0.9792
1	2	6	1
2	2	1	0.8125
2	2	2	0.9375
2	2	3	1
2	2	4	0.9792
2	2	5	1
2	2	6	1

The model was run with the variable states shown in table S5-1-3. Each combination of all the variables shown is repeated for 1000 model runs. Each model run lasts for 3000 time-steps. Further details of all sub-models can be found in the fully annotated code (see ESM 5-5). Netlogo code is both free and very intuitive and will be comprehensible even for readers with little or no programming experience.

Table S5-1-3. Variable levels used when running the main model. Note that the combinations of “threshold” and “learning-variability” were only tested when “variable-threshold” was set to “TRUE”, as they are not used when “variable-threshold” is set to “FALSE”.

Variable	**Levels modelled**
Motivation level	1,2,3
Variable threshold	“true” “false”
Threshold	0.75, 0.85, 0.95
Learning-variability	0.1, 0.2, 0.3
Memory enabled?	“TRUE”
Pheromone enabled?	“FALSE”
Random feeder locations?	“FALSE”
Random ant location start location?	“TRUE”
Number of feeders	3
St-Dev	20
Peak-at-98%-accuracy	“TRUE”

5.7.1.5 Sensitivity analysis

There are several variables and parameters in the model which, while not being directly relevant to testing our hypotheses, may have influenced the results of model. Such variables include the world size, the number of ants, the variability of the ant’s correlated random walk, whether the feeders and the ants begin in a random or fixed location, and the order in which procedures were carried out in the model. Some of these will have trivial and fully predictable effects. These include world size (bigger worlds will reduce the number of return trips and reduce the probability of finding a feeder – the equivalent of running the model for fewer time steps) and the number of ants (as ants do not interact, increase ant number is the equivalent to running more model runs). The non-trivial variables were systematically varied, and their effect on the collective behaviour of the ant colonies examined.

5.7.1.5.1 Sinuosity of the random walk

Changing the path sinuosity of the ants had no major effect on the model (see figure S5-1-2). At higher path sinuosity the variability between different model runs increased somewhat, with more extreme individual model run outcomes.

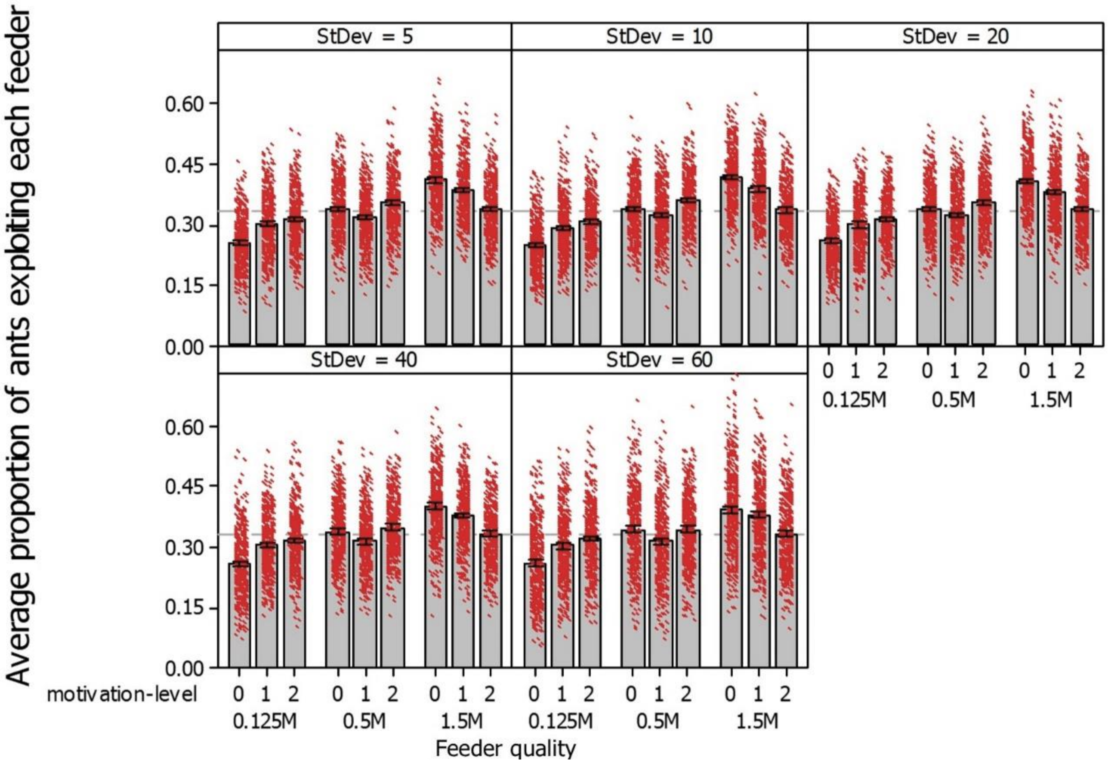


Figure S5-1-2. The effect of varying path sinuosity (St-Dev) on the model. Increased sinuosity causes increased variability between model runs but does not affect overall response patterns.

5.7.1.5.2 Feeder location and ant starting location

Ant starting location (in the nest or randomly in the environment) does not affect model outcomes. Random feeder locations result in greater inter-model run variability but does not affect overall response patterns in the model (see figure S5-1-3).

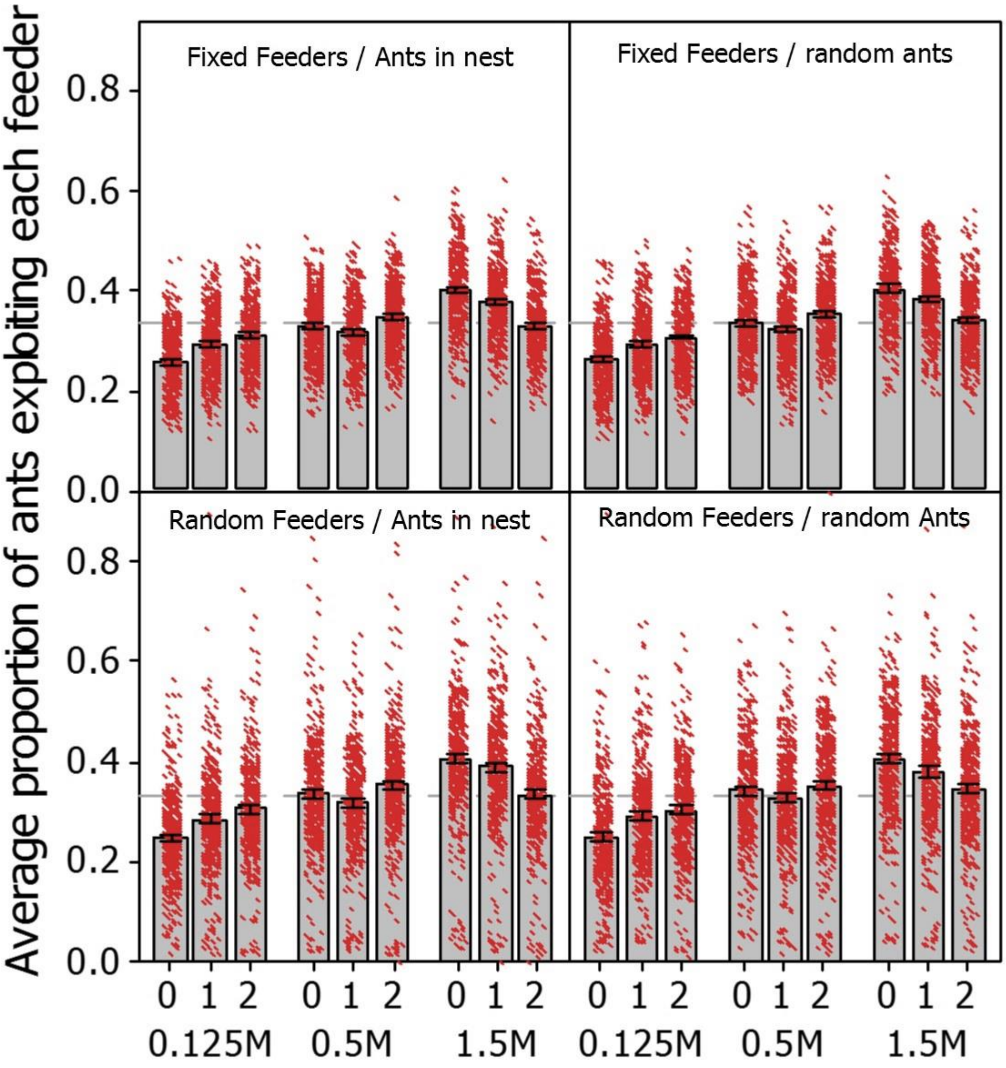


Figure S5-1-3. The effect of randomising ant starting location and feeder location. Ant starting location (in the nest or randomly in the environment) does not affect model outcomes. Random feeder locations result in greater inter-model run variability, but does not affect overall response patterns in the model.

5.7.1.5.3 Procedure order

Reversing the procedure order (the main ‘go’ procedure in the model) had no influence on the results of the model (figure S5-1-4). The results of the reversed-order model are qualitatively identical to the main model.

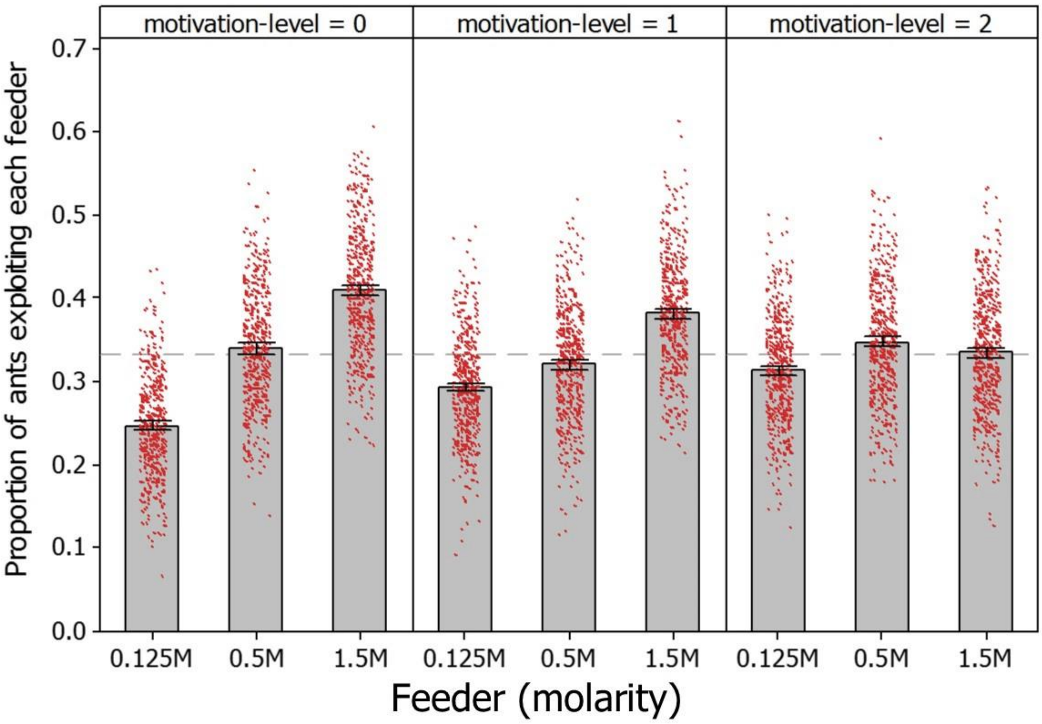


Figure S5-1-4. The results of models in which the main procedure order was reversed are not different from the original model

5.7.2 ESM5-2 Agent based model results and discussion

Supplement to: Small differences in learning speed for different food qualities can drive efficient collective foraging in ant colonies

F. B. Oberhauser, A. Koch, T. J. Czaczkes

5.7.2.1 Table of contents

5.7.2.2 Overview

The aim of this agent-based model was to examine how memory formation affects collective foraging in ants. Specifically, we explored the role of food quality and motivation on learning. Our models are populated with empirical data drawn from behavioural assays (see main document for details). In addition, we used the model to explore how different models of learning influence collective behaviour. In particular, we compared a standard learning model (the “average ant” model”), in which all agents act identically according to the average behaviour of the ants recorded in the empirical study, with an arguably more realistic model. In this more realistic model, the “variable ants” model, the agents vary in their learning ability, and must therefore make more or fewer repeated visits to a food source for their memory strength to overcome a threshold and trigger a behavioural response. The “variable ants” model is based loosely on the extended learning curve model proposed by (Pamir et al. 2011).

The model consists of ants foraging from a central nest on three different feeders of different qualities: 0.125M, 0.5M and 1.5M. Ants may have different motivation levels; 0, 1, or 2, which reflect the data collected after 1 day of food deprivation in autumn, after 4 days of food deprivation in autumn, or after 4 days of food deprivation in spring, respectively. Details of the model are available in a standard ODD format in ESM5-1 (see 5.7.1 above).

As sample sizes for model runs are arbitrary, statistical analysis of model-derived data is not appropriate. Results are the presented graphically.

5.7.2.3 Results

5.7.2.3.1 Results from the "average ants" model

5.7.2.3.1.1 Proportion of ants foraging on each feeder

The poor (0.125M) feeder was collectively avoided (see figure S5-2-1). When motivation levels were low or medium, the good (1.5) feeder had the highest proportion of foraging ants. When motivation levels were very high, however, colonies generally showed less "choosiness", having a more equal distribution of foragers, with the majority of foragers exploiting the medium quality (0.5) feeder. It should be noted that effect sizes are not large: at most 40% of ants exploited the most strongly chosen feeder, as compared to the null situation of one third.

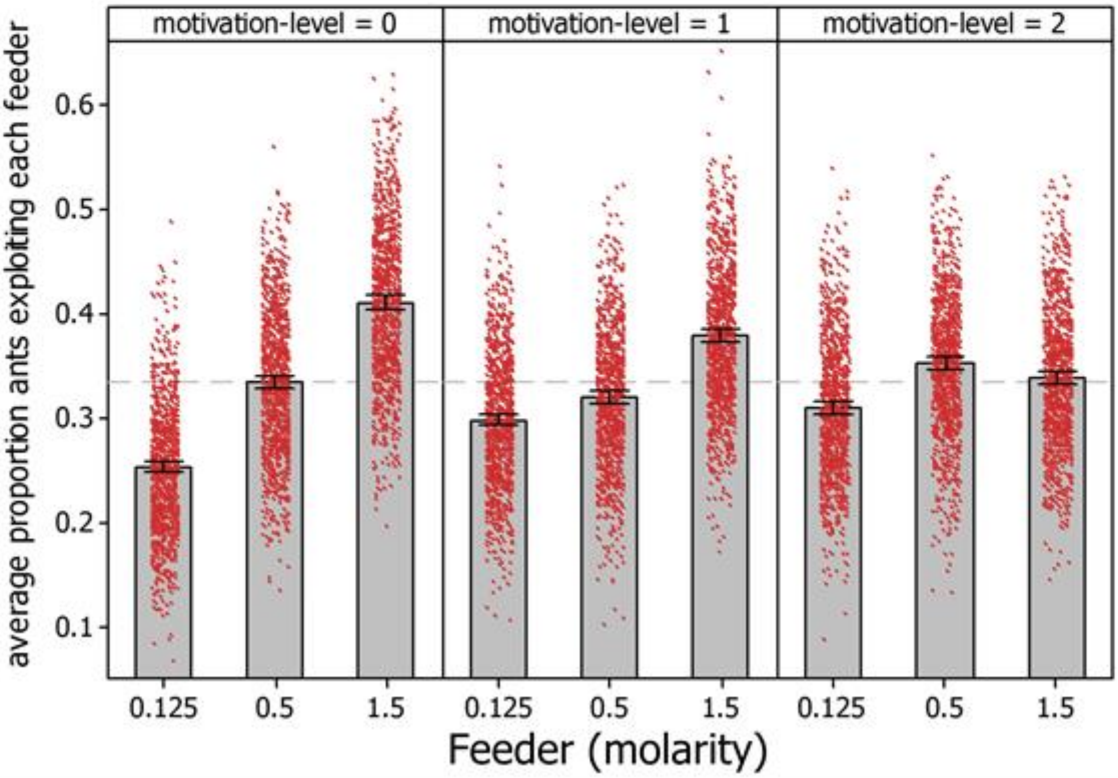


Figure S5-2-1 – Proportion of agents with $M_{favoured}$ as each of the three feeders. These simulations used the “average ants” model of memory formation. Bars show means, error bars show 95% confidence intervals, dots show individual model run results. The dashed grey line shows the null situation of one third.

5.7.2.3.1.2 Food returned, average number of return visits, and food returned per visit

In this model, food return was equal to the feeder quality: ants returning from the 0.125M feeder returned 0.125 food units, and so on. Collectively, as motivation level increased more food was returned (figure S5-2-2A). This was driven by the number of return visits the ants made: the higher the motivation level, the less time ants spent scouting, and so the higher the number of return visits they made (figure S5-2-2B). However, the average quality of food returned per visit declined as motivation level increased (figure S5-2-2C), as a larger proportion of ants were returning from the low or medium quality food source (figure S5-2-1). Again, the differences are not large, but are within a biologically relevant range.

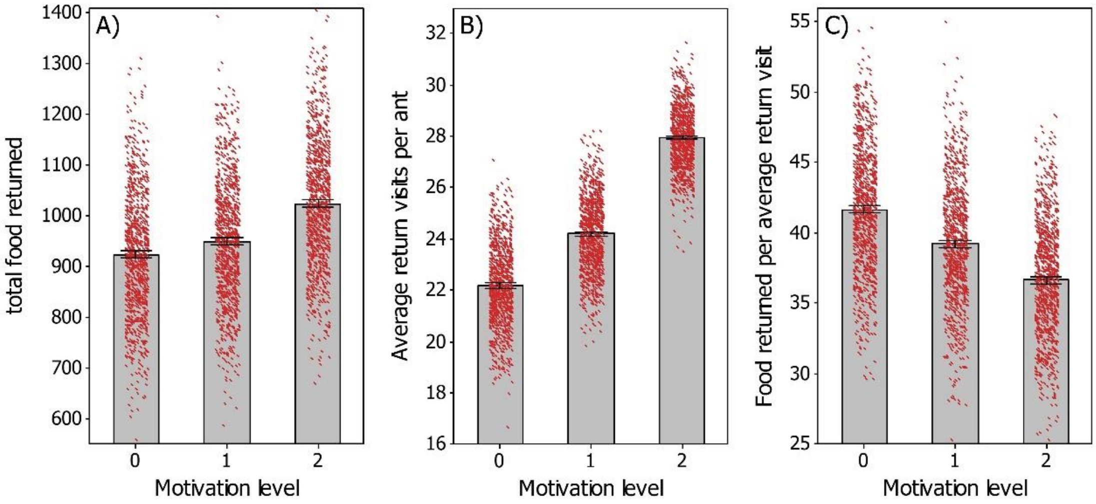


Figure S5-2-2 – the effect of motivation level on A) total food returned, B) the average number of return visits per ant, and C) average quality of the food returned, as measured by the total food returned divided by the average return visits per ant.

5.7.2.3.2 Results from the "variable ants" model

The models run with the "variable ants" model of memory use showed broadly the same pattern as those run with the "average ants" model.

5.7.2.3.2.1 Proportion of ants foraging on each feeder by threshold level and learning variability

Overall, the poor feeder was again avoided (see figure S5-2-3), while the proportion of ants exploiting the medium and good feeder were more or less equal. Noteworthy is that low variability (0.2 or 0.1) coupled with a low threshold (0.85 or 0.75) resulted in a relatively strong avoidance of the poor feeder (as low as 20% exploitation).

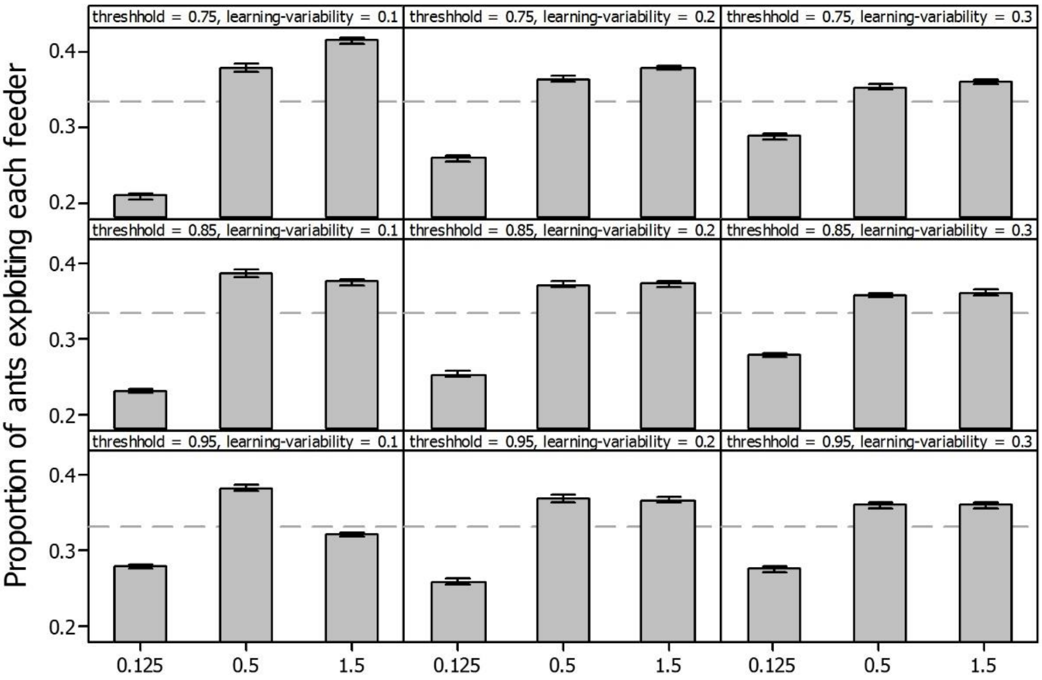


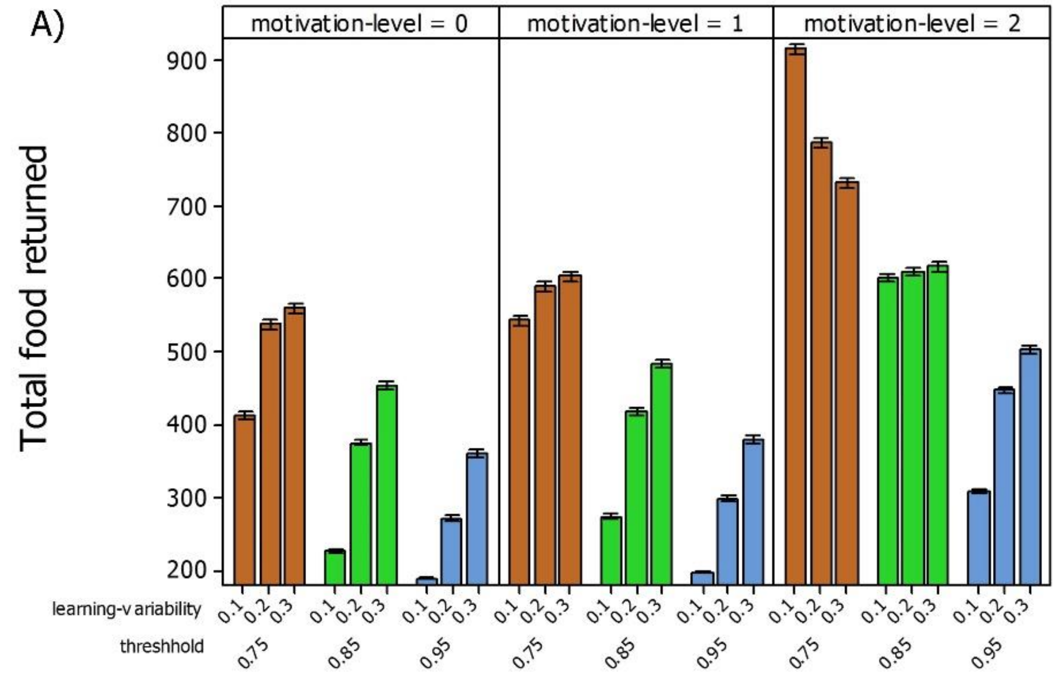
Figure S5-2-3. Proportion of agents with Mfavoured as each of the three feeders. These simulations used the "variable ant" model of memory formation. Bars show means, error bars show 95% confidence intervals. We omit individual model run data for clarity, but variability is comparable with that seen in figure S5-2-1. The dashed grey line shows the null situation of one third.

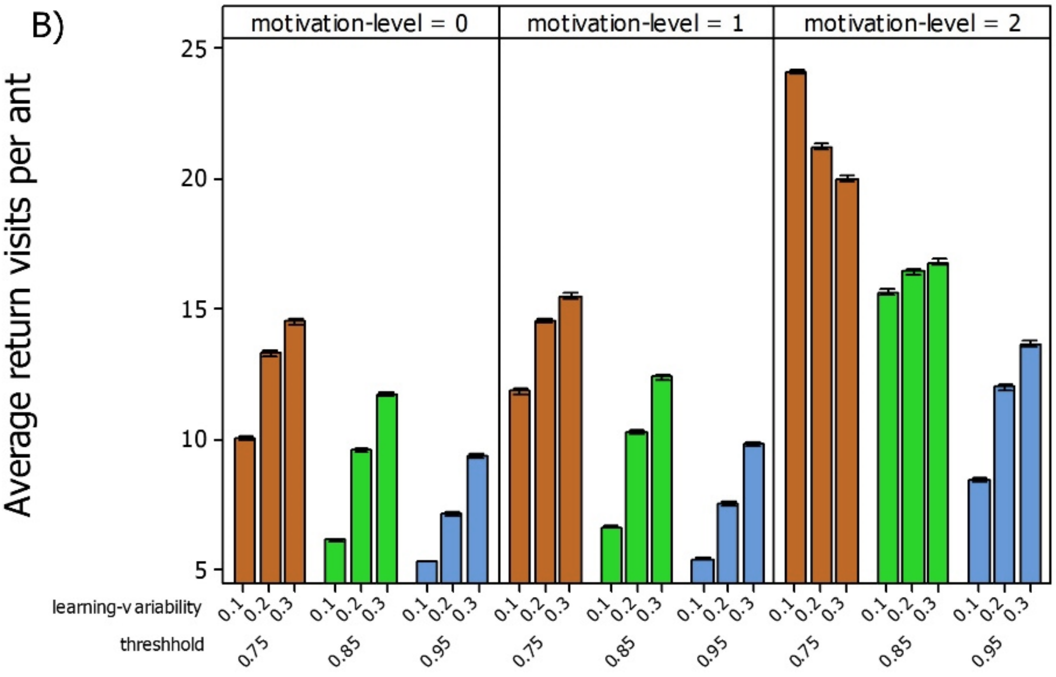
5.7.2.3.2.2 Food returned, average number of return visits, and food returned per visit by threshold level and learning variability

Results were broadly similar to those from the “average ants” models – more food was returned at higher motivation levels (see figure S5-2-4A), driven mostly by more return visits (figure S5-2-4B), but driving down “choosiness” resulting in a lower average quality of returned food (figure S5-2-4C).

Some higher-level interactions can be noted. Increased learning variability increased food return at low and medium motivation levels, or at high motivation levels with a high learning threshold (figure S5-2-4A). However, if motivation was high but the learning threshold was not, variability had either no or a negative effect on food return. This can be understood in terms of the effect of variability on “choosiness”. When thresholds are low (as in high motivation or high threshold situations), most ants will quickly fall over the threshold, but by increasing variability, more agents will have extremely low learning rates, and thus fall below threshold. Conversely, when thresholds are high, the opposite applies, and variability will act to put more agents over the threshold.

Learning variability tended to decrease the average quality of food returned per ant (see figure S5-2-4C), with the exception of situations in which thresholds were high (either due to the set threshold or to the motivation level).


A)
Total food returned
motivation-level = 0
motivation-level = 1
motivation-level = 2
200
300
400
500
600
700
800
900
learning-variability
0.1
0.2
0.3
threshhold
0.75
0.85
0.95


B)
Average return visits per ant
motivation-level = 0
motivation-level = 1
motivation-level = 2
5
10
15
20
25
learning-variability
0.1
0.2
0.3
threshhold
0.75
0.85
0.95

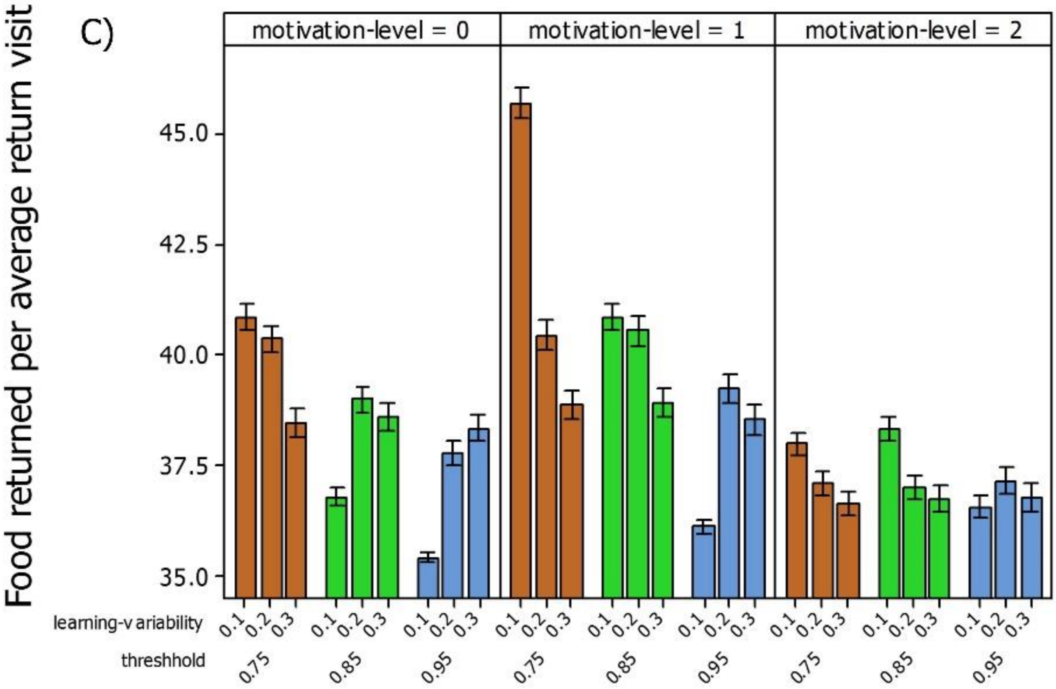


Figure S5-2-4 – the effect of motivation level on ***A)*** *total food returned,* ***B)*** *the average number of return visits per ant, and* ***C)*** *average quality of the food returned, as measured by the total food returned divided by the average return visits per ant. Data is shown for the "variable ant" model of memory formation. The effect of increasing the variability in the learning propensity of the ants, and of increasing the threshold for memory strength to trigger behaviour, is also shown.*

5.7.2.4 Supplemental discussion

The route memory of ants that exploit honeydew or nectarines is well developed (Czaczkes et al. 2016; Czaczkes and Heinze 2015; Grüter et al. 2011; Nicholson et al. 1999; Salo and Rosengren 2001) and plays a large role in their foraging, even being preferentially followed when it conflicts with other information sources, such as trail pheromones (Grüter et al. 2011; Salo and Rosengren 2001). Individual memories can also have large effects on the collective behaviours of ants, such as reactivating foraging routes after winter dormancy (Salo and Rosengren 2001) or triggering trapping of collective foraging decisions in local optima (Czaczkes et al. 2016).

In the empirical portion of this work, we found that route memory formation is rapid even when motivation levels are low, either due to internal reasons (hunger, season) or external reasons (the quality of the food source). While motivation level does cause some variation in learning rates,

this variation is low. As such, the effect of this variation on the collective behaviour of the ants, as shown in the agent-based models, is also generally low, with high stochasticity.
Nonetheless, although effect sizes were not large, the collective decisions which resulted from this variable learning were ecologically sensible: Overall, better food sources were preferentially exploited. When colonies had low motivation, i.e. were close to satiation, they were choosey, and mostly retrieved high quality food. This is sensible, as the processing and storage capacity of a colony is limited in such situations. When colonies had high motivation (i.e. were hungry) they showed little or no choosiness, but maximised food intake. In such situations processing and storage capacity is not limited, and food intake should be maximised.

Increasing biological realism by implementing a “variable ants” model of memory formation (Pamir et al. 2011) did not increase colony efficiency. Indeed, “variable ant” models always returned less food overall than their “average ant” counterparts, and even returned lower food qualities per foraging trip, so ants both spent less time foraging and yet were less choosey overall in their food selection. Much theoretical research has been devoted to the role of interindividual variability on colony performance (Jeanson and Weidenmüller 2014), but empirical results are mixed. Some studies have reported a benefit of increased genetic diversity (which presumably translates to inter-individual behavioural variability) for colony level foraging success, but other studies have failed to find such effects (reviewed in Jeanson and Weidenmüller 2014). The costs of inter-individual variation have rarely been studied (Jeanson and Weidenmüller 2014), and our model suggests that such costs could be large. However, our model necessarily neglected many subtleties, such as a fluctuating environment, depleting resources, or recruitment, the inclusion of which may have demonstrated beneficial aspects of behavioural variation (Czaczkes et al. 2015a).

Unlike most simulations of social insect collective foraging, we explicitly excluded communication from our model. We did this in order to focus on the role of memory, and to demonstrate that individual behaviour, with no interaction between agents, could result in biologically sensible collective behaviour. Dussutour and Nicolis (2013) demonstrated that ants which do not recruit to food sources could nonetheless focus on the best food source in a changing environment. Using mathematical models conceptually very similar to the agent-based models implemented here, they demonstrated, as we have, that such collective behaviour can be achieved just by the increased retention of individuals by the best option. Such a collective decision without communication or comparison is conceptually identical to an annealing process (Černý 1985; Kirkpatrick et al. 1983), and represents one of the simplest types of collective behaviour.

Chapter 6

Meat ants cut more trail shortcuts when facing long detours

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6.1 Abstract

Engineered paths increase efficiency and safety but also incur construction and maintenance costs, leading to a trade-off between investment and gain. Such a trade-off is faced by Australian meat ants, which create and maintain vegetation-free trails between nests and food sources, and thus their trails are expected to be constructed selectively. To test this, we placed an artificial obstacle consisting of 300 paper grass blades between a sucrose feeder and the colony, flanked by walls of either 10 or 80cm length. To exploit the feeder, ants could detour around the walls or take a direct route by traversing through the obstacle. We found that, when confronted with a long alternative detour, 76% of colonies removed more paper blades and ants were also 60% more likely to traverse the obstacle instead of detouring. An analysis of cut patterns revealed that ants did not cut randomly, but instead concentrated on creating a trail to the food source. Meat ants were thus able to collectively deploy their trail clearing efforts in a directed manner when detour costs were high, and rapidly established cleared trails to the food source by focussing on completing a vertically aligned trail which is then followed by the ants.

Key words

Meat ants; route selection; trail clearing; trade-off; optimisation; ant foraging;

6.2 Introduction

Trails are often an integral part of collective movements. They are broadly deployed, from game trails of herd animals such as elephants (Blake and Inkamba-Nkulu 2004) or deer (Etzenhouser et al. 1998) up to complex road structures built by human (Buhl et al. 2006; Lämmer et al. 2006) and social insect societies (Latty et al. 2011). The nature of trails depends on their function, be it to reduce energy expenditure (Bochynek et al. 2017; Halsey 2016; Howard 2001) or travel time (Ydenberg et al. 1994) or to increase the speed or safety of travel (Bochynek et al. 2017; Loreto et al. 2013). Trails are often used by central place foragers - animals which make multiple trips between fixed destinations - such as ants (McIver 1991; Perna and Latty 2014).

In ants, trails last variable amounts of time, ranging from mere minutes in the form of volatile pheromone trails used in some ant species (Czaczkes et al. 2013b; Jeanson et al. 2003) or the constantly adapting living bridges built by *Eciton* army ants (Reid et al. 2015), up to months or years in large insect societies (Howard 2001; Lanan 2014). Longer usage of trails allows for more sophisticated trail construction, as their costs can be ameliorated by continued energy savings over time (Bochynek et al. 2017). Accordingly, cleared trails are often constructed towards stable food sources or resource-rich regions, where ants can disperse to various end points (Farji-Brener and Sierra 2016; Gordon 1991; Greaves and Hughes 1974; Hölldobler and Wilson 1990).

Such large trunk trails, which are actively cleared of vegetation to create and maintain highways, are created by many ant species. Removal of vegetation allows for fast travel to stable food sources (Bochynek et al. 2017; Bruce and Burd 2012; Fowler 1978; Greaves and Hughes 1974; Hölldobler and Lumsden 1980; Howard 2001; Lanan 2014; Plowes et al. 2013; Shepherd 1982) and thus differs from most animal trails, which are created passively by trampling of vegetation (Bates 1950; Blake and Inkamba-Nkulu 2004). Trunk trails leave the nest and bifurcate repeatedly, ramifying into the foraging areas (Hölldobler and Lumsden 1980; Hölldobler and Wilson 1990; Salo and Rosengren 2001). They allow expansion to new resources in their vicinity (Vasconcelos 1990) and are defended as colony territory (Hölldobler and Lumsden 1980). Trunk trails allow most foragers to navigate easily and efficiently between nest and foraging sites while also being provided with guidance by trail pheromones (Czaczkes et al. 2015b) and the polarity inherent to the trail (Jackson et al. 2004); trails can thus be considered as a form of 'external memory' for the colony (Shepherd 1982).

Long-term trail networks also connect nests in polydomous colonies, allowing for food and brood transfer between the spatially separated nests (Debout et al. 2007; Lanan 2014; McIver 1991; van Wilgenburg and Elgar 2007). However, trails devoid of vegetation are costly both to create and to maintain. Thus, colonies need to attain a balance between efficient travelling and time and effort expended on trail maintenance (Bochynek et al. 2017; Bouchebti et al. 2018; Bruce and Burd 2012; Farji-Brener et al. 2015; Howard 2001; Shepherd 1982). The importance of efficiency is shown by many ants forming trails along fallen tree trunks which are not aligned with their goal, but allow for easier travel and faster travel speed (Denny et al. 2001; Farji-Brener et al. 2007; Frank et al. 2018; Loreto et al. 2013), while decreasing risks of predation and substrate contacts (Loreto et al. 2013) as well as construction costs. Nonetheless, trails might be optimised to reduce travel time in areas exposed to sun, weather events or high predation risks (Farji-Brener et al. 2015) or when built underground (Mintzer 1979).

Large trails devoid of vegetation are constructed by the Australian meat ant (*Iridomyrmex purpureus*), which forms trail networks both between nests and to food trees, where workers harvest honeydew from hemiptera (Greaves and Hughes 1974; van Wilgenburg and Elgar 2007). The connections between the nests are usually a trade-off between stability (resilience to disruptions) and efficiency (least amount of trails) (Cabanes et al. 2015; Cook et al. 2014). However, vulnerability to obstructions like falling branches is high, and trails without maintenance can quickly become unusable (Evison et al. 2008b). The benefits of more efficient travel must surpass costs of clearing, and thus trails are expected to be constructed and maintained selectively (Bochynek et al. 2017).

Many studies of trail clearing focus on movable obstacles on trails (Bochynek et al. 2019; Howard 2001), which are easily quantifiable and can be dragged off trails quickly. However, grass and other low vegetation can also constitute a significant clearing effort (Farji-Brener et al. 2015), especially in ant species living in open habitats, as is the case in meat ants (Greaves and Hughes 1974). A recent study found that meat ants prefer shorter routes covered with turf grass to smooth but longer routes, but no such preference was found for routes of equal length, indicating that meat ants ignored the surface structure despite slower walking speeds (Luo et al. 2018). Yet, the use of turf grass as an obstacle did not allow clearing outcomes and cutting patterns to be assessed. Another study employed artificial grass obstacles made of 300 hard cardboard or thin paper blades as obstacles to a food source. Yet, quantification of clearing effort showed that meat ants allocated the same number of workers to both obstacle types, irrespective of the longer clearing times for cardboard (Middleton et al. 2019). These studies thus suggest that trail clearing is not an optimized process.

These findings are surprising, as optimisation is to be expected for such costly behaviours, and can be seen in trail clearing by leaf-cutter ants (Bochynek et al. 2017; Farji-Brener et al. 2015; Howard 2001; Shepherd 1982 but see Cevallos Dupuis and Harrison 2017). In the present study, we used an array of artificial grass blades to directly quantify clearing activity, which is hard to do in natural settings (Bouchebti et al. 2018). We also analysed clearing patterns and their emergence over time. This allowed us to demonstrate, for the first time, cost-dependent deployment of goal-directed trail-clearing behaviour.

6.3 Material and methods

6.3.1 (a) Studied species and field site

Meat ants (*Iridomyrmex purpureus*) are a widespread species of Dolichoderinae and endemic to Australia. They are polydomous, with their large mounds housing tens of thousands of workers (Greaves and Hughes 1974). Trail networks cleared from vegetation allow the efficient exchange of food and brood between nests and trees infested by honeydew-secreting insects (Greaves and Hughes 1974; van Wilgenburg and Elgar 2007).

All experiments were conducted in a forest area at the Hawkesbury campus of Western Sydney University in Richmond, New South Wales, Australia (33°38′S, 150°46′E) between March and April 2018. The area has a high density of meat ant colonies which are located along a road through a *Eucalyptus* forest. Only colonies which were on even ground and surrounded by clear ground or little vegetation were used for the experiments, resulting in a total of 17 tested colonies.

6.3.2 (b) Experimental procedure

A 1M sucrose feeder was placed 5cm behind an artificial paper grass obstacle (10cm x 23cm) (see figure 6-1A). To reach it, ants had to either traverse the obstacle or detour around it. The obstacles were identical to those used in Middleton et al. (2019) (see figure 6-1B) and ants crossing the obstacle were found to be ~3.5 times slower than ants traversing cleared trails (Middleton et al. 2019). Obstacles contained 15 rows and 20 columns of laser-cut green paper strips (henceforth “paper blades”). Each row comprised 20 paper blades 2mm apart from each other, each 2mm wide and 1.5cm high, resulting in a total of 300 artificial paper blades. Rows were 1cm apart from each other. An acetate sheet was placed over the obstacle to protect it from sporadic rain and litter. The obstacle was flanked by 10cm high Corflute™ walls. Meat ants were found to rarely climb the walls, thus making it a reliable barrier without any chemical treatment. The walls flanking the obstacle where either 10cm or 80cm wide (causing a short or long detour, respectively). At the end of these detour walls, another sham obstacle plate was placed devoid of paper blades to mimic the surface structure of the shortcut (see figure 6-1A). The walls were partially sunk into the soil to prevent the ants from passing underneath. Each colony was tested on both the long and the short detour in a randomised order, which ensured that half of the colonies started with either treatment. Ambient temperature in the shade was recorded directly after the apparatus was installed.

For each detour length, we revisited the setup 24 and 48 hours after instalment, resulting in four data points. Each visit, we noted the number of cut paper blades, and recorded the ambient temperature. Incompletely cut paper blades were also counted as ‘cut’ if they laid flat on the ground. A camera mounted on a tripod above the setup recorded all ant activity for 1 minute. From those videos we obtained an ant flow rate, i.e. the number of ants passing through the obstacle or sides per minute. The person analysing the video was unaware of the study’s hypotheses and instructed to count separately all ants moving through the obstacle and ants moving around it. An ant was counted as soon as it moved onto the obstacle. If it traceably re-entered the obstacle, this did not count as additional visit. Ants which were walking through the 5cm gap between the walls flanking the obstacle and the feeder box (see figure 6-1A) were counted as detouring ants. Each video was analysed twice to ensure reliability of the data.

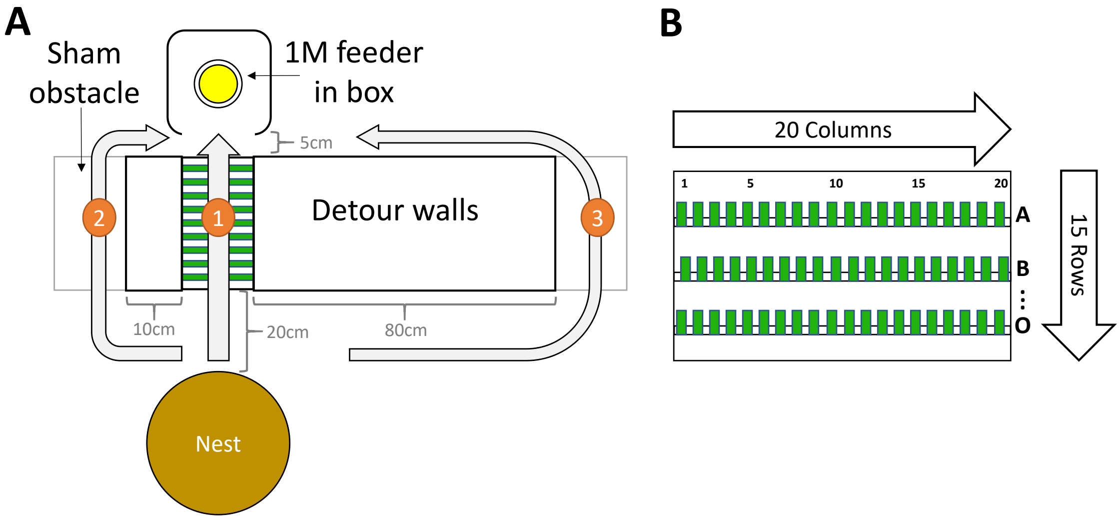

Figure 6-1. ***A)*** *Schematic illustration of the setup. The direct path (1) to a newly placed feeder was obstructed by an obstacle containing paper blades and flanked by 10cm high walls. The walls were either 10cm wide causing a small detour (2) or 80cm wide causing a long detour (3). Note that both detour routes are shown here, but in the experiment, both sides always had the same length (either 80cm or 10cm). To retrieve the food, the ants could either traverse the obstacle (1) or detour around the wall (2,3).* ***B)*** *The obstacle consisted of 15 rows (A-O) each holding 20 paper blades (1-20). Row A was placed next to the nest.*

6.3.3 (c) Statistical analysis

All analyses were conducted in R version 3.5.1 (R Core Team 2018). Multiple packages were used for data preparation (Dowle and Srinivasan 2018; Dragulescu and Arendt 2018; Xie 2018), analysis (Brooks et al. 2017; Fox and Weisberg 2018; Hartig 2018; Pohlert 2018) and plotting (Wickham 2016; Wilke 2018). See ESM6-1 for a protocol leading through all analysis steps. All models were tested for model fit and overdispersion using the DHARMa and sjstats packages (Hartig 2018; Lüdecke 2019).

6.3.3.1 Overall pruning activity

To assess the overall difference in pruning activity between the detour treatments, we compared how many of the 300 potential paper blades were cut after 24 and 48 hours on each obstacle. Due to the balanced design of the study with two treatments and two time points per colony, we used a non-parametric Friedman test for dependent samples followed by post-hoc Conover tests with p-value adjustments following Holm (Holm 1979).

To test temperature effects on pruning activity, we calculated Spearman’s rank correlations between the number of cut blades and the average temperature of 24 and 48 hours.

6.3.3.2 Ant flow rate

For each treatment, we counted the number of ants walking through the shortcut or detouring around it for one minute. In order to test for possible effects of detour length and duration on flow rate, we used a proportional binomial GLMM with logit link and colony as random intercept. The model formula was

$\textit{Ants shortcut / Ants detour} \sim \textit{Detour length}_{\textit{(short or long)}} * \textit{Duration}_{\textit{(after 24 or 48 hours)}} + \textit{random effect: Colony}$.

In addition, we investigated a possible correlation between flow rate and number of cut paper blades. We ran two separate Spearman's rank correlations, one for the number of ants walking through the obstacle, and one for the number of detouring ants for each treatment.

6.3.3.3 Trail cutting

As each paper blade was numbered, we could obtain its exact location and were able to reconstruct the cutting pattern accordingly. If ants cut a trail from the nest to the food, we would expect higher spread of blades along the vertical axis (along rows), as ants cut from the start to the end. By contrast, the width of the trail, i.e. the horizontal spread (along columns) of cut blades, should be narrow.

We used the interquartile range (IQR) as a measure of dispersion. The IQR is non-parametric and provides the range in which 50% of the data is found by subtracting the 25% quartile from the 75% quartile. The IQR was calculated for rows and columns separately. In our case, an IQR_{Rows} of 4 would tell us that 50% of all cut paper blades were found within 4 rows. This method makes reasonable assumptions: trails are straight, not diagonal, and only one main trail exists per obstacle.

As the obstacles had 15 rows but 20 columns, we then normalised the IQRs by dividing row IQRs by 15 and column IQRs by 20, to make rows and columns comparable. This resulted in values ranging between 0 – 1. A value of 0.5 means that the IQR was half of the total rows or columns. To compare the normalised IQR between rows and columns, we modelled a GLMM with beta distribution ($0 < y < 1$). The model formula was

$$\textit{IQR}_{\textit{normalised}} \sim \textit{Detour length}_{\textit{(short or long)}} * \textit{Duration}_{\textit{(after 24 or 48 hours)}} + \textit{IQR}_{\textit{(rows or columns)}} + \textit{random effect: Colony}.$$

Furthermore, to show that meat ants clear trails rather than cut blades randomly, we also created random cut patterns by using a random binomial distribution (see ESM6-1). For each treatment of each colony we created random cut patterns with the exact same number of cut blades. Those patterns then underwent the same procedure of calculating IQRs. Then, we modelled two GLMMs with beta distribution, one for IQR_{rows}, one for $IQR_{columns}$. The model formula was

$$IQR_{normalised} \sim Detour\ length_{\ (short\ or\ long)} * Duration_{\ (after\ 24\ or\ 48\ hours)} + Data_{\ (empiric\ or\ randomly\ generated)} + random\ effect{:}\ Colony.$$

6.3.3.4 Ant movement

In order to visualise ant movement trails and compare them to cut patterns, we wrote a motion tracking program using the OpenCV 3.4.1 library (Bradski 2000) in Python 3.7 (https://www.python.org/) to extract ant movement through the obstacle from the ant flow rate videos. Each ant was detected via background subtraction and its position was tracked frame to frame. As in most videos multiple ants were present simultaneously and ants were not individually marked, each ants’ frame-to-frame position was assumed to be that with minimum Euclidian distance to the last position. This method is prone to identity switches when ant paths cross but probable switches do not affect visualisation. We analysed 20 frames per second for the whole duration of the video. The tracking data was then freed from false-positive detections and visualised using R. The tracking program and R code are provided in ESM6-5.

6.3.4 (d) Data availability

All data analysed during this study are available in ESM6-2, data handling protocol and ant tracker code in ESM6-1 & ESM6-5, respectively.

6.4 Results

Altogether, 17 colonies were tested for both long and short detours. One colony was missing the long detour treatment after 48 hours, and thus only 24 hours data points were used for flow rate and trail analysis, and this colony was excluded from the Friedman test to maintain a balanced design.

6.4.1 Overall pruning activity

In 3 colonies, ants managed to rip out parts of the rows without pruning individual paper blades. The corresponding parts were removed in all treatments of those colonies (see ESM6-1).

A Friedman test revealed a highly significant difference in cut blade numbers between treatments and time points (χ^2 = 18.79, df = 3, $p < 0.001$). Post-hoc tests found that ants cut significantly more blades in the long detour treatment than in the short detour after 48 hours ($p = 0.0105$, see figure 6-2A), but not after 24 hours ($p = 0.2289$). No significant difference in cut blades was found after 24 and 48 hours within treatments (short detour: $p = 0.6301$; long detour: $p = 0.1176$). A significant difference was also found between short detour after 24 hours compared to long detour after 48 hours ($p = 0.0011$).

Except for one colony, which never cut, all zero-cutters were exclusively found in the short detour treatment (6 out of 17) (χ^2 = 0.94, df = 1, $p = 0.332$). Furthermore, 76% (13/17) of the colonies cut more blades in the long detour treatment (χ^2 = 3.76, df = 1, $p = 0.0523$), resulting in overall 760 more blades cut in the long detour. After 24 hours, ants had removed 1881 blades, which rose to 3032 after 48 hours, excluding one colony which had no data for 48 hours. Temperature did not correlate significantly with the number of blades cut (Spearman rho: -0.06, $p = 0.6192$).

6.4.2 Ant flow rate

Overall, significantly more ants walked through the shortcut in the long compared to the short detour treatment (proportional binomial GLMM; Est. = 1.0242, $z = 6.809$, $p < 0.0001$, see figure 6-2B). No significant difference was found between 24 and 48 hours (Est. = 0.1498, $z = 0.924$, $p = 0.3555$) nor was the interaction between treatment and time point significant (Est. = 0.4200, $z = 1.877$, $p = 0.0606$).

We found a significant correlation between the number of blades cut and number of ants walking through the shortcut in both treatments (short detour: Spearman rho = 0.67, $p < 0.0001$, long detour: Spearman rho = 0.42, $p = 0.0128$). Number of ants detouring did not correlate significantly with number of cut blades (short detour: Spearman rho = -0.14, $p = 0.4467$, long detour: Spearman rho = 0.14, $p = 0.4394$).

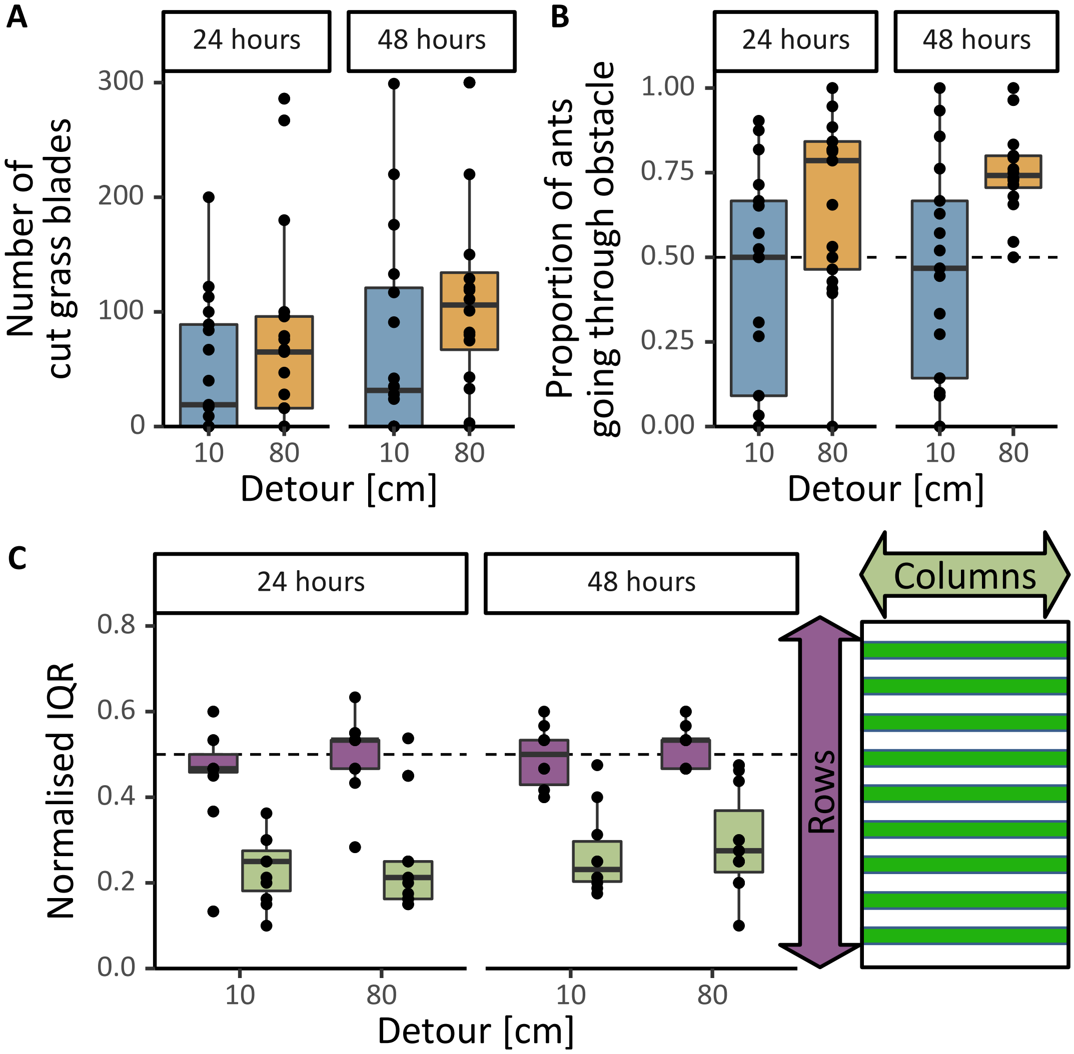

Figure 6-2. ***A)*** *Number of paper blades cut by each colony after 24 and 48 hours in the short and long detour treatment. After 48 hours, a significantly higher cutting activity was found colonies faced with the long detour (post-hoc Conover test, p = 0.0105), which was not the case after 24 hours (p = 0.2289). Sample sizes from left to right: n=16, n=16, n=17, n=17.* ***B)*** *Proportion of ants going through the obstacle or detouring around after 24 and 48 hours in the short and long detour treatment. 1 corresponds to all ants going through obstacle. A higher fraction of ants was found to walk through the obstacle in the long detour treatment (binomial GLMM, p < 0.0001), while no significant effect of time was found (p = 0.3555). Sample sizes from left to right: n=17, n=17, n=17, n=16.* ***C)*** *Normalised interquartile range (IQR) of rows (vertical spread of cut blades, see obstacle scheme on right) and columns (horizontal spread) after 24 and 48 hours in the short and long detour treatment. A normalised IQR of 0.5 (dotted line) means that 50% of blades were cut in 50% of rows/columns, i.e. were cut randomly. The lower the IQR, the less spread was found. Column IQRs were significantly smaller than row IQRs (beta regression GLMM, p < 0.0001), while detour treatments and time points did not differ significantly (p = 0.0629, p = 0.0772, respectively). Sample sizes from left to right (note that only colonies that cut blades were used): n=11, n=11, n=13, n=13, n=10, n=10, n=15, n=15. Each colony is represented by a dot. Horizontal lines in boxes are medians, boxes correspond to first and third quartiles and whiskers extend to the largest value within 1.5 x IQR.*

6.4.3 Trail cutting

Comparing the normalised interquartile ranges (IQR) of rows (spread of vertical cuts from nest to food) and columns (spread of horizontal cuts) showed that column IQRs were significantly smaller than row IQRs (beta regression GLMM, Est. = -0.9938, z = -13.293, $p < 0.0001$, see figure 6-2C). No difference was found between detour lengths and between 24 and 48 hours (est. = 0.2069, z = 1.860, p = 0.0629; est. = 0.203, z = 1.767, p = 0.0772, respectively), nor did they interact significantly with each other (est. = -0.0658, z = -0.436, p = 0.6629).

When we compared empiric IQRs to randomly generated IQRs of columns, we found that the empiric IQR of columns was significantly lower than the IQR of randomly distributed cut patterns (est. = 0.8956, z = 11.509, $p < 0.0001$, see figure S6-1), whereas detour length and duration had no effect (est. = 0.1612, z = 1.391, p = 0.164; est. = 0.1089, z = 0.913, p = 0.361, respectively) and their interaction was not significant (est. = -0.0618, z = -0.393, p = 0.694).

In contrast, no significant differences were found between the row IQRs of empiric and randomly distributed cut patterns (est. = -0.0249, z = -0.409, p = 0.6828, see figure S6-1). Again, no effect of duration was found (est. = 0.1829, z = 1.95, p = 0.0512, respectively), but detour length had a significant effect (est. = 0.1977, z = 2.188, p = 0.0287). The IQR data demonstrate that the cutting pattern of ants formed vertically-oriented, narrow trails from the entrance to the exit of the apparatus.

6.4.4 Ant movement

As can be seen in figure 6-3 and ESM6-4, ant movement was often very well aligned with the cutting pattern of the colonies. Due to the noisy nature of the field videos and varying ant flow rates, we could not obtain quantitatively comparable data for each colony. Nonetheless, visual inspection of ant trails and cut patterns clearly demonstrates that ants preferentially travel along cleared trails (see figure 6-3).

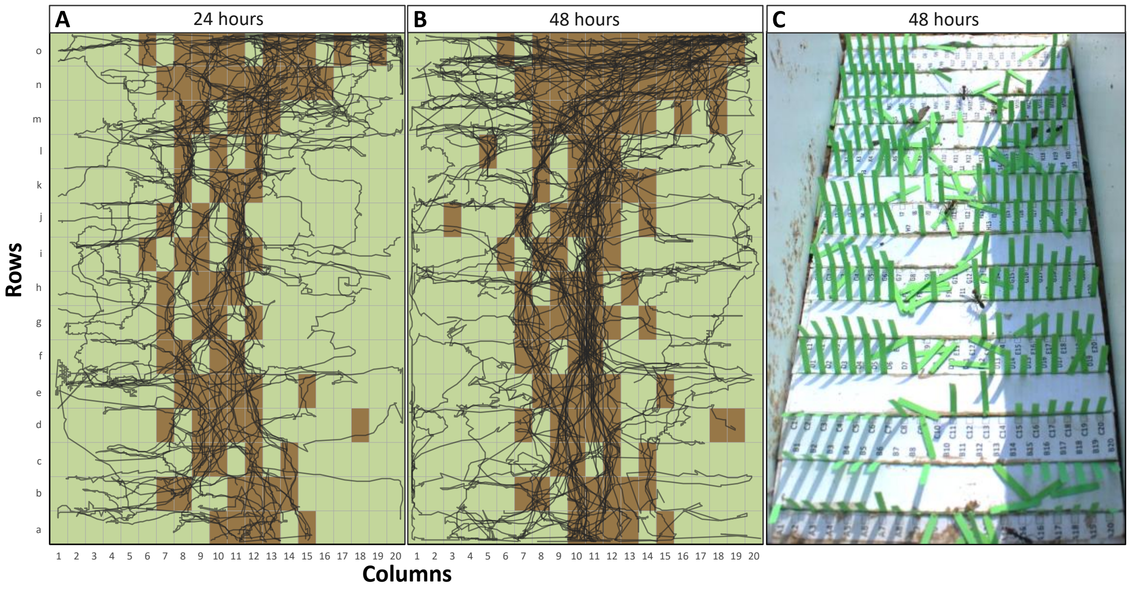

Figure 6-3. Cut patterns and tracked ant movement through the obstacle from the nest (bottom) to the food 24 and 48 hours after placing the long detour setup at colony 33. ***A, B)*** *Representations of obstacle, green rectangles correspond to uncleared fractions of the obstacle, brown rectangles depict cut blades. Each black line shows the trajectory of an individual ant over time. Note the frequent horizontal movement caused by the blade obstacle rows, and the broadening of the cut trail over time.* ***C)*** *Picture of the same obstacle after 48 hours, with a clearly visible cleared trail through the blades.*

6.5 Discussion

Our study demonstrated that meat ants (*Iridomyrmex purpureus*) readily cleared artificial obstacles between a food source and their nest, and that 76% of the colonies removed more paper grass blades when they faced longer detours, resulting in 60% more cut blades compared to the short detour treatment after 48 hours. This suggests that meat ants can adjust their trail clearing effort context dependently.

The number of cut blades did not differ significantly within treatments after 24 and 48 hours, suggesting a rapid onset of pruning activity. Indeed, 62% of all blades were cut within the first 24 hours. Such high activity is impressive and may be further facilitated by the close proximity of the setup to the nest. The colonies varied dramatically in their clearing onset speed: some colonies had removed all 300 paper blades after 48 hours while others, mainly in the short detour treatment, removed none. As multiple colonies were tested on the same day, it is unlikely that weather or other environmental factors were the drivers of behavioural variation. Instead, meat ant colonies might display different trail clearing propensities, with some colonies consistently investing in trails early on. Such behavioural syndromes are usually consistent across situations and stable over time (Jandt et al. 2014). This is indicated by our finding that some colonies consistently had high or low clearing activity in both treatments (see ESM6-3, c29, c31 and c40). However, the data collected in our study is not sufficient to conclude whether meat ant colonies display stable trail clearing behaviour over time.

Meat ants could reduce both the amount of time an uncleared trail slows down foragers and occupies clearers by first establishing a passage through the obstacle to increase forager speed, and then successively widening the trail. Such creation of trails instead of random cutting was demonstrated by the comparisons of the vertical (nest to food) and horizontal (wall to wall) spread of cut paper blades (see ESM6-3). While blades were cut evenly on the food-nest axis, ants focussed their horizontal blade removal on fewer columns, resulting in significantly less spread and often clear trails from the food to the nest (see figure 6-2 & figure 6-3, ESM6-3 & ESM6-4). This was the case in both detour lengths, indicating that ants generally aim to clear trails. The horizontal spread increased over time, indicating that meat ants focus first on establishing a trail to the food source, which is then successively broadened, although this result was not significant.

Our study found no indications that ants stopped their clearing activity at a certain stage. Some colonies cleared all or almost all artificial blades, which might be wider than needed for the observed number of ant foragers. This is in accordance with studies in leaf-cutter ants, where trails in the field are often reported as wider than needed by peak traffic (Farji-Brener et al. 2012). It was suggested that ants are slowed by encroaching vegetation at the trail’s edges, and widely cleared trails thus aid efficiency (Farji-Brener et al. 2012). However, a simple behavioural rule such as “cut encountered paper blade with a certain probability” could also well explain the clearing patterns found in this study, especially the emergence of trails through the obstacles. Ants often moved horizontally along blade rows until they found a missing blade to go through (see figure 6-3, ESM6-4). This favours emergence of trails, as blades directly after a removed blade have higher encounter rates. Such encounter-based mechanism could also explain the different cut rates between long and short detour treatments. The long walls could act as funnel, causing more ants to walk through the middle. Indeed, consistent with the differences in clearing activity, ant workers predominantly walked through the obstacle when facing a long detour, while they did not favour the shortcut in the short detour treatment (median ~50%). Also in line with this mechanism is the significant correlation between number of blades cut and the flow rate of ants through the shortcut. However, it is important to note that causation here may be reversed, as a higher ant flow rate could also be caused by the reduction of hindering paper blades.

As with many collective organisation systems based on positive feedback, the initial conditions may strongly influence cutting behaviour. If a ‘funnelling’ effect is taking place, when and if an initial decision to cut is made will strongly influence where the trail forms, and whether it forms at all. This sensitivity to one initial, stochastic choice may explain the high variation in cutting behaviour observed among colonies.
A strictly encounter-based cutting strategy would suggest that each ant has a certain threshold to initiate cutting upon contact with an paper blade and continues to do so until it is removed or a certain amount of time has elapsed (Bochynek et al. 2019). This is in accordance with findings by Middleton et al. (2019), which report that ants only cleared for 5.5 seconds on average before continuing their travel. However, some clearing ants we observed in the field were very persistent, consistently biting the stem of single blades for minutes at a time, and were also found to switch blades (Oberhauser, pers. obs.). Individuals which are more likely to remove obstacles were also reported in leaf-cutter ants (Bochynek et al. 2019; Howard 2001). This suggests that, while ultimately encounter-based, paper blade removal might be driven by a few persistent ant workers in meat ants. Such ‘elite worker’ behaviour is widespread in social insects, especially ants (Hölldobler and Wilson 1990; Mersch et al. 2018). It is worth noting that cut-initiation behaviour seems to be stereotyped, with most ants first walking up to the tip of the paper blades, then turning around and walk down again until they touch the substrate with their head, after which they initiate cutting at the base of the blade (Middleton et al. 2019; Oberhauser, pers. obs.). A similar behaviour was reported in grass-cutting ants, were it is thought to enable ants to estimate the length of the grass fragment to be cut (Roces and Bollazzi 2009).

While the highest clearing activity clearly took place in colonies facing long detours, around 65% of colonies nevertheless initiated cutting in the short detour treatment, and no significant difference between treatments was found after 24 hours. In other words, colonies also began to remove paper blades in situations where they could have easily circumvented them with low energetic or time costs. Such removal of blades irrespective of alternative low-cost detours in meat ants was also reported in Luo et al. (2018). This low threshold to initiate cutting is surprising, but the cost of a clearing workforce consisting of a few persistent workers at a time might be comparably low in colonies comprising thousands of workers (Greaves and Hughes 1974). Moreover, the provided sucrose might be perceived as stable resource similar to honeydew, which is a crucial source of energy and water required for meat ant colony survival and connected to nests via trails (Greaves and Hughes 1974). Such stable and high-quality resources would favour early trail clearing onset to maximise gain (Bochynek et al. 2017; Shepherd 1982) but also to monopolise the resource in the territory (Ettershank and Ettershank 1982).

Our study demonstrated that meat ant colonies clear trails economically, preferentially cutting when alternative routes are long. Trail clearing is goal-directed and not random, with meat ants tending to first create paths to the food. The low threshold to initiate cutting and the rapid emergence of trails indicates a fast and adaptive system, whose cost might be balanced by employing only a few workers at a time. Taken together, our results demonstrate that trail clearing in meat ants results from a collective decision-making system which allows adaptive and robust collective behaviour.

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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.

6.6 Supplementary material

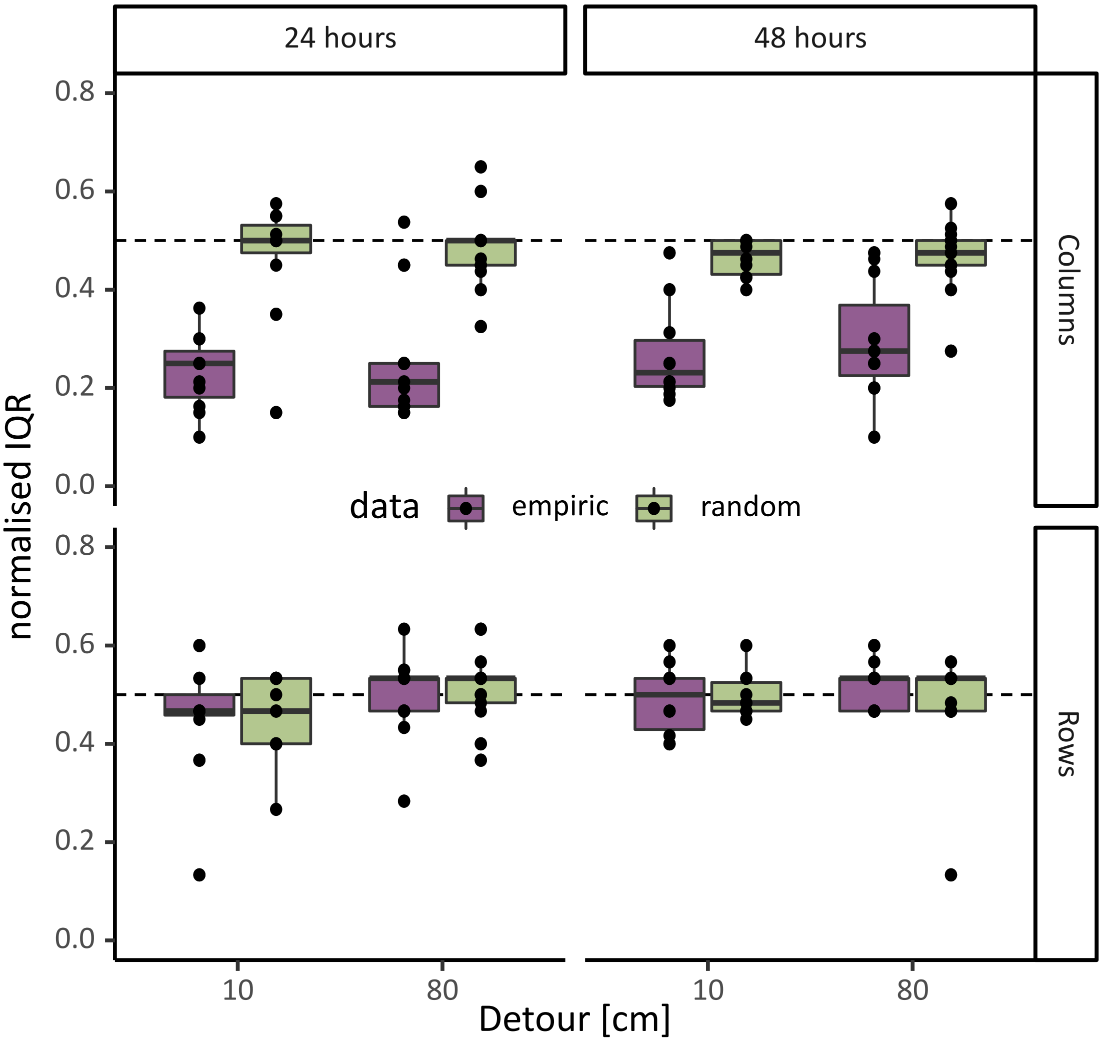

Figure S6-1. Normalised interquartile range (IQR) obtained from the experiment (empiric) or from randomly generated cut patterns (random) for columns (horizontal spread of cut blades) and rows (vertical spread) after 24 and 48 hours in the short and long detour treatment. A normalised IQR of 0.5 (dotted line) means that 50% of blades were cut in 50% of rows/columns, i.e. were cut randomly. The lower the IQR, the less spread was found. Empiric column IQRs were significantly lower than random IQRs (p < 0.0001), while detour length and duration had no effect (p = 0.164, p = 0.361, respectively). Empiric row IQRs were not significantly different from random IQRs (p = 0.6828), with a small effect of detour length (p = 0.0287) and no significant effect of duration (p = 0.0512). The IQR data thus demonstrate that the cutting pattern of the ants formed narrow, vertically oriented trails from the entrance to the exit of the apparatus.

Chapter 7 Comprehensive summary, discussion and outlook

Eusocial insect colonies display an impressive degree of organisation. Yet, unlike cells in a body, individuals maintain a high degree of autonomy, and individual members' abilities and decisions can have a disproportionate effect on the collective. Identifying factors which impact individual behaviour is thus of major importance for a cohesive understanding of the workings of a colony as a whole. Over the course of this thesis, different aspects of information processing were investigated, and the findings provide a more detailed insight into factors which influence ants' decisions and reveal promising directions for future studies, as will be outlined below.

Colonies can collectively choose the best food source or nesting site without the need for direct comparisons of the options by individuals (Beckers et al. 1990; Mallon et al. 2001; Robinson et al. 2009; 2014; Seeley et al. 1991; Seeley and Buhrman 2001). This is possible because individuals can independently estimate the quality of options and adjust their recruitment according to the perceived value (Abbott and Dukas 2009; Beckers et al. 1993; Robinson et al. 2009; Seeley et al. 2000; Seeley and Buhrman 2001; Wendt et al. 2019), which elevates value perception to an integral part of collective decision making. Many studies have shown that foraging insects are very sensitive to changes in reward quality (e.g. Bitterman 1976; Couvillon and Bitterman 1984; Flaherty 1982; Wendt et al. 2019), but less is known about the effect of changes in perceptible attributes of food sources such as taste. Nonetheless, a mismatch between the expected and encountered attributes of a food source might affect its perceived value, as was reported in humans (Carlsmith and Aronson 1963; Piqueras-Fiszman and Spence 2015).

In chapter 2 we therefore investigated the effect of expectations on value perception, and demonstrated that ants which encountered a food of different taste than in training displayed lower food acceptance and pheromone depositions – both proxies of perceived food value (Wendt et al. 2019) – than ants which had their expectations confirmed. As sucrose concentration of the food was unchanged, the ants reacted, in energetic terms, irrationally. This finding suggests that learned value-neutral attributes of food can drive foraging decisions and might contribute to phenomena such as site fidelity in ants (Quinet and Pasteels 1996) or flower constancy in bees (Chittka et al. 1999) by discounting options which deviate from expectations.

An important point to discuss concerning our findings in chapter 2 is whether the observed behaviour can also be explained by neophobia, as the unexpected taste introduced in the disconfirmation treatment was never presented during training. While the high acceptance for both tastes on the first visit can be raised as an argument against neophobia, we cannot rule out that the starvation of the ant drove the high initial acceptance. Such an effect of neophobia is hard to disentangle from expectations, however, because neophobia requires the ant to recognise a novel taste, which is only possible if the ant has learnt the currently exploited food attributes in the first place, be it via associative (expectations) or habituation (neophobia) learning. In fact, neophobia can also well explain the results of human studies on this topic (Carlsmith and Aronson 1963). In either case, it can be concluded that ants dislike food with unexpected attributes.

Odour information about a food was also found to bias individual perception after being acquired socially via trophallaxis (Josens et al. 2016). However, as in the current study, ants had directly experienced the food prior to the test – there was always a first-hand experience of the food by the focal ant. A source of purely social information about a food quality is provided by pheromone depositions, whose strength is proportional to the perceived quality of the food in *Lasius niger* (Wendt et al. 2019). This raises the question of whether ants form expectations about a food while following a highly-advertised, i.e. strongly-marked, pheromone trail towards a food source. Such bias could potentially affect the conditional amplification process, during which newly recruited individuals independently evaluate the food and only deposit pheromone themselves if the food is deemed profitable (Beckers et al. 1993; Feinerman and Korman 2017; Mailleux et al. 2003). In a recent study, which could not be finished in due time to be included in this thesis, we conducted an extensive battery of different tests in which we applied extracted pheromone from *Lasius niger* workers (following von Thienen et al. 2014) on a runway to a food. The results revealed no effect of pheromone on food acceptance, drinking time and other parameters. Ants also showed no preference for odours associated with foods found in the presence of pheromone (Oberhauser et al., in prep.). It thus seems as if expectation effects are restricted to directly acquired, i.e. private, information.

How insects choose between private and social information has been the subject of much attention (Almeida et al. 2018; Grüter et al. 2008; 2011; Middleton et al. 2018; Smolla et al. 2016). Yet insects often have access to multiple private information sources which can concur or conflict. How insects deal with conflicting private information has been mostly studied by inducing conflicts between visual navigation cues and path integration during route learning in desert ants (Collett 2012; Legge et al. 2014; Wehner et al. 2016; Wystrach et al. 2015), although olfactory cues can also be of central importance in ant navigation (Buehlmann et al. 2013; 2015; Steck et al. 2011; 2012; Wolf and Wehner 2005). In chapter 3, we investigated multimodal navigation abilities of *Lasius niger* based on olfactory and spatial cues and found very fast acquisition rates for both modalities. However, when those two memories were put in conflict after training, all ants exclusively oriented using the olfactory cues. This highlights that one type of information can completely drive an ant's decision.

This clear main finding has other interesting implications. While all ants followed the odour cue in a conflict situation, such consistent performance was not observed in the other experiments providing either spatial or olfactory cues. This allows speculation about the involvement of attentional mechanisms (de Bivort and van Swinderen 2016; Spaethe et al. 2006). The presence of conflicting information might induce more careful choices, as wrong decisions bear unknown costs for the ant (Chittka et al. 2009). This is also consistent with Grüter et al. (2011), who found higher reliance on memory when it conflicted with pheromone trails than when no pheromone was present. Future studies could thus more systematically investigate whether conflicting information increases decision accuracy, with complementary analyses of decision times of ants on the first, intermediate and conflict visits to identify potential speed/accuracy trade-offs (Chittka et al. 2009).

Furthermore, to reveal whether higher accuracy is mediated via the 'novelty' of a situation, or due to evaluation processes between known options, it would be of interest whether learning performance could be improved by the addition of a novel stimulus on the incorrect arm acting as distractor and whether such potential effects are modality-specific (Nityananda and Chittka 2015).

The clear odour preference found in our conflict experiment further seems to suggest that ants decided in a hierarchical way. This is in contrast to models which propose that navigating ants weigh each available information by its certainty (Hoinville and Wehner 2018; Wehner et al. 2016). However, a Y-maze does not offer intermediate choices and we thus cannot deduce whether the spatial information was weakly weighed, ignored or not recalled by the ant. Adding an additional test without odour cues at the end of the conflict experiment would have provided an answer to the last option. Yet, in another experiment (de Agrò et al, in prep.) we trained ants on a Y-maze in which one odour always led to reward on alternating arms. Wall colours around the maze were consistent with the rewarded arm side. When the odour cue was made uninformative during the test by presenting it on both arms, the ants were still able to localise the correct arm by relying on the wall colour. In other words, the ants could not only learn contextual information (colour of maze wall) along with a highly predictive odour cue, but also integrated spatial information (Y-maze arm side) with wall colour to localise food in the presence of an uninformative odour cue (de Agrò et al, in prep.). Thus, it is very likely that the ants memorised both olfactory and spatial cues in our experiment, but heavily preferred one over the other.

Given the ability of ants to process and learn various cues simultaneously, it is an open question whether they are also able to extract and act on relevant information provided by the relationships between stimuli. Accordingly, chapter 4 investigated the ability of *Lasius niger* ants to learn a relational rule of go to ‘different’ or ‘same’. We found no evidence for such an ability, but instead observed that a high proportion of ants (>50%) seemingly resorted to heuristics such as ‘go left’. Interestingly, no single heuristic could reliably predict performance on group level, because individual ants chose different heuristics. This finding reflects the discussion of chapters 4 & 5 that measures of group performance often do not well represent individual behaviour and highlights the potential of studies investigating the individual behavioural strategies used to solve tasks in social insects (Guiraud et al. 2018).

One restriction of our study was that ants were assigned to a heuristic if they chose according to the heuristics’ prediction in at least 66% of visits. We cannot differentiate a heuristic ‘choice’ from an innate bias for this behaviour, e.g. ‘go left’, which could be independent from the complexity of the task. In fact, we observed a left bias in another study of this thesis as well (chapter 5). One interesting question for future studies would thus be whether individual ants tend to display stable biases over time, resembling a personality trait (Jandt et al. 2014; Pinter-Wollman 2012) and how they are distributed within and between colonies (Carere et al. 2018; Jandt and Gordon 2016). Biases might even systematically affect foraging success of colonies (Raine and Chittka 2007) and side biases could explain why *Lasius niger* ants were found to perform better on repeating routes (e.g. left-left) than alternating routes in double Y-mazes (Czaczkes et al. 2013a).

Following from this, it is interesting to ask when heuristics or biases are deployed. They might be favoured initially, providing the ant with consistency until it has learnt the environment, or after unsuccessful learning attempts. Our data about longest streak onsets (figure 4-3B) suggest

a tendency of side streaks and incorrect streaks to start in the first half of the 48 visits, while correct streaks start in the later half. Therefore, ants seem to resort to heuristics or biases initially, which are then modified by learning, although more data is needed to form a firm conclusion.
To advance our knowledge about rule learning abilities in ants, the introduced methodology needs to be improved to overcome the limitations of the presented setup. A new setup solving the issues reported in the discussion of chapter 4 was trialled, but introduced other restrictions which are not of interest here (Oberhauser & de Agrò, unpubl. results).

If and how fast an ant learns is not only dependent on the complexity of a task, but also on other factors such as reward quality and motivation. In chapter 5, we investigated how those factors affect learning speed and discussed whether efficient foraging in ant colonies could be achieved in the absence of recruitment through an annealing process based on faster learning of better food. And indeed, a model based on the individual decisions of ants resulted in sensible collective foraging behaviour also found in bees (Seeley 1986).
Surprisingly, reward quality only had a weak effect on ant performance, while the strongest effects arose from motivation. Ants displayed a poor performance and high-dropout rate when food was of lowest quality and colonies were weakly food deprived (see figure S5-1 & table 5-1). In this case, it is not clear how much of the observed performance can be attributed to learning, as it is possible, even likely, that ants recalled the food location but chose to explore.
This constitutes an intriguing exploration/exploitation trade-off (Cohen et al. 2007; Mehlhorn et al. 2015), which is linked to colony satiation. Ants can share and store food via a social stomach (crop) and an individual’s crop fill is proportional to colony satiation (Greenwald et al. 2018). With increasing colony satiation, returning ants take longer to release their load to conspecifics which provides foragers information about the current storage of the colony (Greenwald et al. 2018; Seeley 1986). In case of poor available food and little remaining storage capacity, it might be optimal to reduce foraging activity (Dornhaus and Chittka 2004), which is in accordance with the high observed drop-out rates. Those few ants which persisted seemed to focus more on exploration, which is reflected by the low performance and raises questions about the reasons for their persistence.
In contrast to satiation and motivation effects, the effects of different food qualities on learning speed were relatively small. However, higher reward consistently led to better performance, and it is reasonable to assume that a small effect in a navigation task as simple as a T-maze might be

more pronounced in a natural setting, where ants have to navigate through heterogenous terrain or when ants face a more complicated maze (Czaczkes et al. 2013a; Pasquier and Grüter 2016).

Heterogenous terrain is also faced by Australian meat ants, through which they frequently clear vegetation-free trails to stable food sources. In chapter 6, we showed that colonies allocated their trail-clearing effort in a situation-dependent manner, by removing more obstacles when alternative detours to a food source were long and by creating defined paths through the obstacle. The use of artificial paper obstacles is proving to be a simple but powerful method to quantify removal activity.

An important aspect of that experiment to discuss is how the observed collective decision emerged. The crucial parameter of the setup is, evidently, the length of the obstacle arms. The arms can act like a funnel, with longer obstacles leading a higher proportion of ants through the obstacle. If we assume a certain probability of removing obstacles upon encounter for each ant (Bochynek et al. 2019), we would expect higher clearing activity as the 'funnel effect' increases. This is reminiscent of studies on mazes, where fluids put under pressure can also use the fastest way through a maze (Fuerstman et al. 2003). To test such a 'funnel effect', one could use triangles instead of rectangles to flank the obstacle. While inducing a detour, this would refract ants away from the obstacle. If we saw a higher cutting activity in this scenario, we could attribute it to a collective decision originating from the ants, not the environment. Yet, if a funnel effect mediated all trail clearing, we would expect highest trail clearing along the edges to the arms, which was not the case (see ESM6-3 & figure 7-1).

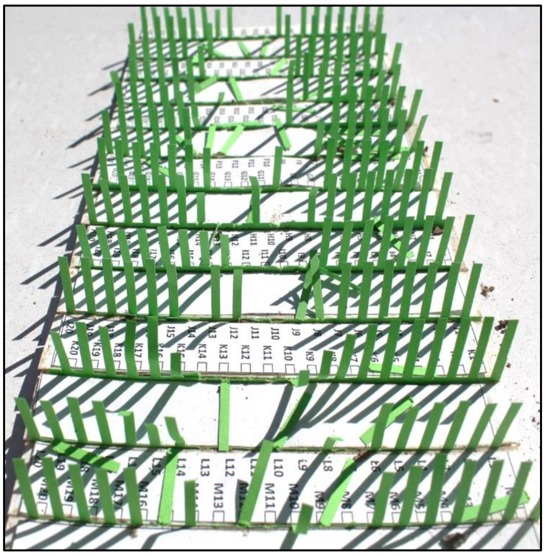

Figure 7-1. Artificial paper grass obstacle after 48 hours of meat ants exposure. The path created by the ants to traverse the obstacle is visible by eye. *© Oberhauser FB*

The emergence of paths through the obstacle can be accounted for by another interesting finding about the individual behaviour of ants – their propensity to walk horizontally until they find a gap to pass through the paper blades (figure 6-3). After short unsuccessful searches, they climb over the paper blades (Oberhauser, pers. obs.). While not quantified in this study, this seems to be a crucial element in the emergence of trails, as blades immediately after a removed one will experience more frequent contacts with ants and are thus more likely to be removed, in a process similar to a river eroding a path through soil.

Due to field conditions, only short sequences of the clearing process were recorded on video. Although trail clearing appears to be a distributed task with many involved individuals, it nonetheless became apparent that only a few workers at a time removed paper blades and did so very persistently (Oberhauser pers. obs., see also Bochynek et al. 2019; Howard 2001). Future studies with longer video recordings of clearing processes and individual tracking could thus potentially unveil the most important mechanisms at play to shortest path identification and clearing in ants.

7.1 Conclusion

An understanding of the factors which influence, or bias, individual information processing and decision making are central to gaining a more coherent understanding of animal behaviour. All of the studies presented here investigated effects on individual decisions, whose outcome has also ramifications for the collective, be it through expectations (chapter 2), asymmetric use of information (chapter 3), individual use of heuristics (chapter 4), motivation-dependent foraging (chapter 5) or obstacle removal (chapter 6). The findings presented here highlight promising new avenues for future research, some of which have been outlined above.

As discussed in chapter 4, behavioural observations of whether animals can succeed in tasks are not sufficient, but must be supplemented with an understanding of how they do so. While much research now focusses bottom-up approaches to improve our understanding of cognitive mechanisms (Cope et al. 2018; Devaud et al. 2015; Li et al. 2017; Peng and Chittka 2017; Roper et al. 2017; Vasas and Chittka 2019), the availability of accurate and more widely applicable tracking software provides a powerful tool for the advancement of the study of the intersection between individual and collective cognition (Graving et al. 2019). More detailed long-term observations can help to elucidate complex interaction mechanisms, such as trophallactic (Greenwald et al. 2015; 2018) or disease transmission networks (Stroeymeyt et al. 2018), to understand how information passes from one individual to the next. To gain resolution on individuals, such studies could be complemented by tracking foraging individuals outside the nest (Robinson et al. 2014; Woodgate et al. 2016) and by assessing individual learning abilities (Evans et al. 2017; Raine and Chittka 2008). This way, the contributions of individual experiences and abilities to collective choices could be exposed in more detail to identify the role of individual cognition within a colony.

Feinerman and Korman (2017) argue that the high cognitive abilities of social insects make contributions of individuals very valuable and allow social insect collectives to outperform more distributed algorithms which are constrained by their loose network. This might have restricted social insects to a local fitness optimum, where each individual still maintains autonomy within the colony. As a result, even highly sophisticated collective behaviours pale in comparison to the neuronal networks acting in each of the individuals' brains. Yet, this autonomy within a collective is what renders social insects special. One can hardly deduce from group behaviour how a particular individual will decide. We can, however, do so by observing that individual.

Supplementary data

All electronic supplementary material (ESM) referred to in this thesis, including all raw data used in the studies as well as annotated data handling scripts, can be found on the accompanying compact disc.

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