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Ants resort to heuristics when facing relational-learning tasks they cannot solve

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We humans sort the world around us into conceptual groups, such as ‘the same’ or ‘different’, which facilitates many cognitive tasks. Applying such abstract concepts can improve problem-solving success and is therefore worth the cognitive investment. In this study, we investigated whether ants (*Lasius niger*) can learn the relational rule of ‘the same’ or ‘different’ by training them in an odour match-to-sample test over 48 visits. While ants in the ‘different’ treatment improved significantly over time, reaching around 65% correct decisions, ants in the ‘same’ treatment did not. Ants did not seem able to learn such abstract relational concepts, but instead created their own individual strategy to try to solve the problem: some ants decided to ‘always go left’, others preferred a ‘go to the more salient cue’ heuristic which systematically biased their decisions. These heuristics even occasionally lowered the success rate in the experiment below chance, indicating that following *any* rule may be more desirable than making truly random decisions. As the finding that ants resort to heuristics when facing hard-to-solve decisions was discovered *post-hoc*, we strongly encourage other researchers to ask whether employing heuristics in the face of challenging tasks is a widespread phenomenon in insects.

1. Introduction

As humans, we group objects that belong together into categories (classes of items) using learned concepts (mental representations of classes) [1]. While perceptual concepts allow the categorization of objects by their similar appearance or function (e.g. trees or toys), relational concepts, by contrast, are more abstract and use non-physical features such as the relationship between objects (e.g. ‘same’ or ‘different’) [1–4]. Once an animal has learned an abstract concept such as ‘the same’, it can be transferred and used on other stimuli irrespective of their physical nature, i.e. of the employed sensory modality [5]. The formation of abstract concepts has traditionally been considered a higher-order ability [6] and accordingly most concept learning research focussed on vertebrates such as monkeys [7–9], birds [10–13] or rats [14,15].

But abstract association learning is not limited to vertebrates. Honeybees have also repeatedly been shown to successfully learn and apply abstract concepts, such as same/different [16], above/below [17,18] or numerosity [19]. In an important study on concept learning, Giurfa *et al.* [16] demonstrated that honeybees are not only able to learn a ‘same’ or ‘different’ task, but also transfer the learned concept from colour to pattern stimuli (or *vice versa*), and even from one modality (colour) to another (odour).

However, how honeybees solve those complex tasks and whether solving them requires equally complex cognition is still debated. Several honeybee brain models suggest that apparent ‘higher-order’ learning could be based on very simple brain circuits [20–23]. Solving those tasks could be facilitated by behavioural strategies such as sequential scanning of stimuli [23], and

behavioural studies have argued that honeybees' success in same/different tasks could be mediated by appetitive or aversive modulation of their innate tendency to revisit similar flowers [24] (but see [25]) or by sensory accommodation, i.e. reduced response to repeated stimuli [20], without the need to form a concept.

A recent experiment that closely examined 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 [26]. However, this might only be true for close-up inspection of the objects by the bees, which is not possible for decisions made from afar in a Y-maze [16].

Irrespective of the underlying mechanisms, studies have shown that the usage of concepts can vary between individuals: successful training requires many visits (60 in [16]) and not all bees can go on to apply the concept to novel stimuli (60–80% successful transfer in [16–18]; 50–70% in [26], 60–70% in [25] in bumblebees), suggesting that some bees either failed to complete the task or had to rely on other strategies to solve it. This is in accordance with the finding that animal species which were initially unable to learn concepts succeeded after the number of training pairs was increased to a point where associative learning became inefficient [27]. That might indicate that animals employ concept learning only after other strategies fail.

Cognitive mechanisms have not evolved to accurately reflect the real world, but to provide decisions which maximize fitness gains [28]. Sometimes, quick heuristics (rules of thumb) can surpass more sophisticated strategies by rapidly finding acceptable solutions to a problem at the cost of accuracy [28–30]. In other words, if false-positives (actions that lead to an error) only induce minor costs or if the foraging context is highly variable, animals might resort to heuristics instead of learning the precise solution [28,31]. Heuristics could pre-equip animals to solve complex problems, e.g. nest size estimation by scouting ants using the frequency of their own trail crossings (Buffon's needle; [32]), best-of-N rule in nest-searching honeybee swarms [33] or prey interception in dragonflies [34].

While research on concept learning [16–19,25,26] and other abilities of honeybees such as metacognition [35] are now being supplemented by studies modelling potential neural mechanisms [21,23,36,37], studies on complex cognition in insects other than honeybees remain very scarce [25,38]. Yet, the prerequisites assumed to be crucial for concept learning are met by other Hymenoptera, such as ants [5]. *Lasius niger* ants are adept learners and can quickly form associative memories for odours [39] which they use to remember food locations [40] and can memorize information about a value to compare it to sensory input [41].

In this study, we investigated *L. niger* ants' ability to learn the relational rule of 'the 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 odour led to a reward. Thus, ants could only succeed when using the relationship between the stimuli as guidance, i.e. when they applied a relational rule.

2. Material and methods

(a) Collection and rearing of colonies

Eight stock colonies of the black garden ant *Lasius niger* were collected on the University of Regensburg campus 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 [42–44]. All colonies were kept on a 12:12 day/night cycle and were provided *ad libitum* with water and 1 M 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.

(b) Solutions and odours

About 1 M sucrose and 60 mM 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 [45] and has also been used in learning paradigms in ants [46–49]. Paper runways were impregnated with one of 12 different essential oils (Mit allen 5 Sinnen, Grünwald, Germany; see electronic supplementary material (ESM), table S1 and S3) by keeping runways in an enclosed box containing 100 µl of the corresponding essential oil on filter paper for longer than 2 h (see also [39]). To control for potential influences of shared compounds between oils (see below), we compared all the identified compounds of each corresponding oil from the literature. Linalool was found to be present in six of the tested odours at a percentage of at least 5% (see ESM, S1); we therefore considered a potential heuristic under which ants chose whichever scent contained linalool (see below). A list of all oils and their compounds are provided in ESM, S3.

(c) Experimental procedure

Ants were allowed onto a Y-maze (following [50]) via a drawbridge. The Y-maze was surrounded by a barrier to prevent landmark orientation (figure 1 and ESM, figure S1). 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 by new overlays the next visit. One odour was present on one arm, while the other was present on both stem and arm (figure 1). In order to find a reward (1 M 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 2 cm 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 set-up via the drawbridge to make 48 visits to 48 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 odour was presented as rewarded or unrewarded odour in alternation. Thus, each odour was rewarded approximately once in 12 visits, resulting in four rewarded visits per odour (see ESM, S1). 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 ESM table S1). 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 visit by taking the inverse performance

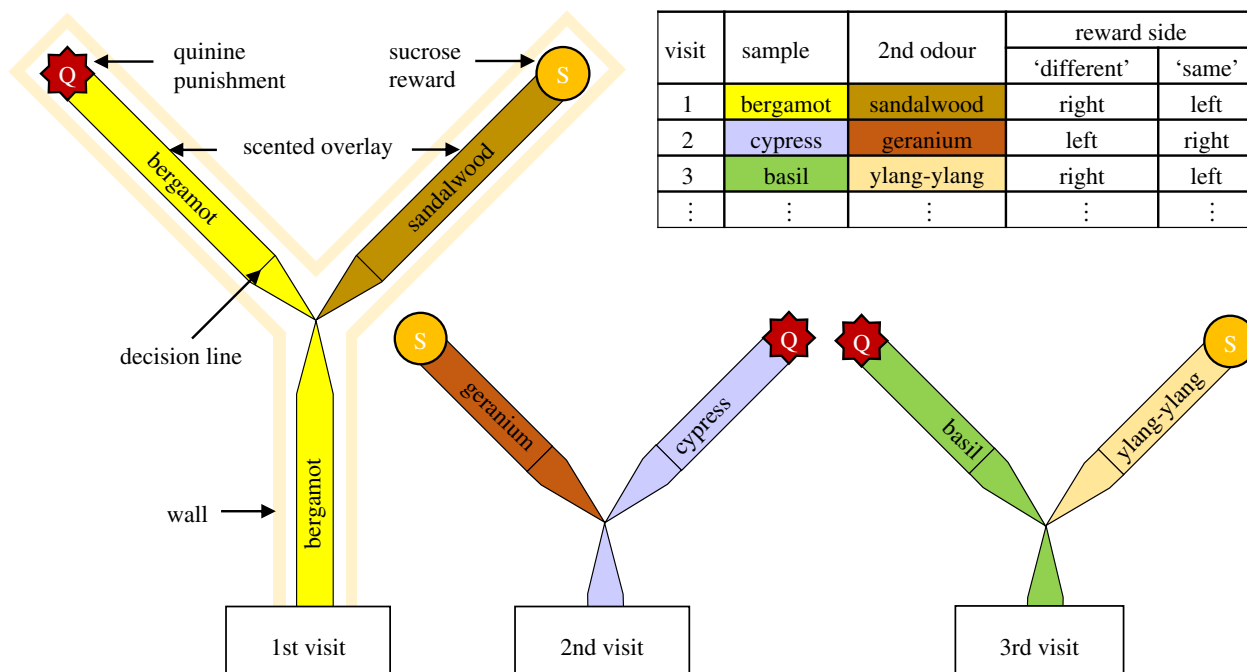


Figure 1. Set-up used for the 'different' and 'same' treatment. The Y-maze shown on the left depicts the first visit of the 'different' treatment (see table on the top right for details). Each visit, ants encountered a new odour pair by walking over scented paper overlays. The sample odour was present 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 (1 M sucrose, right on the first visit). In the 'same' treatment, the rewarded and punished (quinine) side were swapped (see inset table). 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. (Online version in colour.)

of one of them (correct choice in the 'different' 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.

(d) Statistical analysis

All statistical models were generalized linear mixed-effect models (GLMM) [51] produced with the `glmmTMB` package [52] in R v. 3.6.1 [53]. Since ants from eight 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 [54]. Post-hoc tests were conducted using estimated marginal means [55]. Receiver operating characteristic (ROC) curves [56] were calculated using the package `pROC` [57].

(i) Rule learning performance

The performance (correct/incorrect decisions) of ants was analysed separately for the 'same' and the 'different' treatment. The binomial GLMM predictors were defined *a priori*, following Forstmeier and Schielzeth [58] as:

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

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 ESM, S1).

(ii) 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 streak length and streak onset, we ran separate GLMMs for correct/incorrect streaks or side streaks as predictors:

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

In case a Poisson error distribution was inadequate, a negative binomial distribution was used (see ESM, S1).

(iii) Heuristics

To analyse whether ants might have used specific rules to guide their decisions, we considered and then tested six potential heuristics: 'go to different odour than stem', which can also be described as 'go to more salient cue' (see discussion), 'go to odour as stem', 'go left', 'go right', 'go last rewarded' or 'go linalool' (table 1). A score was calculated for each ant and heuristic, by scoring 1 for each visit the ant's decision was following the corresponding rule, and 0 if it did not. In 'go to the more salient cue', each visit was scored 1 when the ant went to the odour different to the stem. In 'go left', we scored 1 for each visit the ant went left and *vice versa* for 'go right'. In 'go last' we scored 1 when an ant went to the Y-maze arm which was rewarded on the previous visit (table 1). As our ants always found a reward at the end of each trial, this corresponds to a win-stay strategy. Please note that both 'go to same/different' and 'go left/right' are mutually exclusive—an ant that always chooses the different odour or left side cannot also chose 'same' and 'right'.

The additional, sixth, heuristic, 'go linalool' was introduced to account for it being a shared compound of six of the odours

Table 1. Definitions of potential heuristics and how many ants chose corresponding to them for at least 2/3 of 48 visits. Please note that two ants in the ‘same’ treatment could be assigned to either ‘go left’ or ‘go last’ in addition to ‘go different’, thus totalling to 20 instead of 18. *Linalool heuristic was calculated from subset of 22 visits.

heuristic	description	number of ants which chose according to heuristic at least 2/3 of visits	
		‘different’	‘same’
‘go different’	choose maze arm with odour different to stem	6	6
‘go same’	choose maze arm with same odour than stem	0	1
‘go left’	choose left arm of maze	4	4
‘go right’	choose right arm of maze	1	0
‘go last’	choose maze arm rewarded on last visit	0	1
‘go linalool’*	choose maze arm with odour containing linalool	0	0
none of the above		8	8

used (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. Similarly, we also investigated whether odour preferences of ants could explain the results (see ESM, S1).

To identify potential heuristic usage at the individual level, we counted, for each, heuristic all ants which chose in accordance to each heuristic in at least 2/3 (66.6%) of visits (32/48). To also assess the false-positive rate of this arbitrarily-set-threshold procedure, we ran a simulation using the same reward side pattern used in the experiments (see ESM, table S1) with random choices. We simulated 40 000 ants (four different random generator seeds, 10 000 iterations each). The result was added in figure 4 to provide information about how often a heuristic would be assigned by chance alone.

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

$$\text{Performance}_{(\text{correct/incorrect})} \sim \text{Heuristic}_{(\text{correct/incorrect})} + \text{random intercept}_{(\text{Colony/Ant})}$$

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 ROC curves [56] 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.

3. Results

In total, 55 ants were tested. However, seven 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. One ant had two visits with a different odour pair combination. It was left in as its removal did not affect the results in a significant way.

(a) 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, $\chi^2 = 5.72$, $p = 0.0167$, figure 2a). Furthermore, ants’ performance was significantly higher when the reward was presented on the left ($\chi^2 = 5.85$, $p = 0.0155$). No significant interaction between visit and reward side was found ($\chi^2 = 0.03$, $p = 0.8537$), so performance was not better, for instance, at early visits and reward on the left. Note that we included ‘reward side’ in the model and present it here due to *L. niger*’s tendency for left biases [39,59], 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, $\chi^2 = 0.25$, $p = 0.6138$, figure 2b), but a significantly higher proportion of correct visits was made when the reward was on the left ($\chi^2 = 34.4$, $p < 0.001$). Again, no significant interaction between visit and reward side was found ($\chi^2 = 0.03$, $p = 0.8549$). In both treatments, the majority of ants (approx. 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 (approx. 5%), or *vice versa* (approx. 3%, figure 2). For simplicity, due to these small differences, we only used the first decision of each ant as measure of performance in subsequent analyses.

When we directly compared the two treatments, we found a significantly better performance in the ‘different’ treatment (binomial GLMM, $\chi^2 = 20.7$, $p < 0.001$, figure 2c). However, there was no significant difference between performance in the ‘different’ task and the inverse of performance on the ‘same’ task ($\chi^2 = 0.42$, $p = 0.5142$, figure 2c). This implies that ants responded in a similar way towards the encountered stimuli irrespective of the treatment.

(b) Streaks

Group level analyses do not adequately capture individual behaviour [60] and a poor group performance can mask individuals which managed to learn the task. On an individual level, consistency is a good measure of learning. 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)

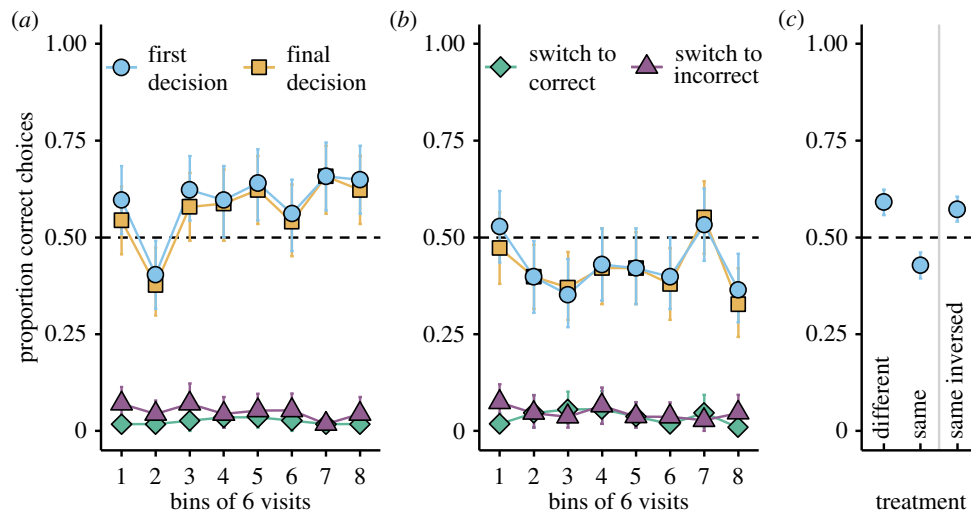


Figure 2. Performance of ants over subsequent visits in (a) the ‘different’ treatment ($n = 19$) and (b) the ‘same’ treatment ($n = 18$). (c) Performance averaged over all 48 visits. The inverse performance (correct = incorrect and vice versa) of ants in the ‘same’ experiment (same inverted) resembles performance of the ‘different’ treatment. Dashed line represents chance level of 50%. Symbols are means, error bars represent 95% bootstrapped confidence intervals. (Online version in colour.)

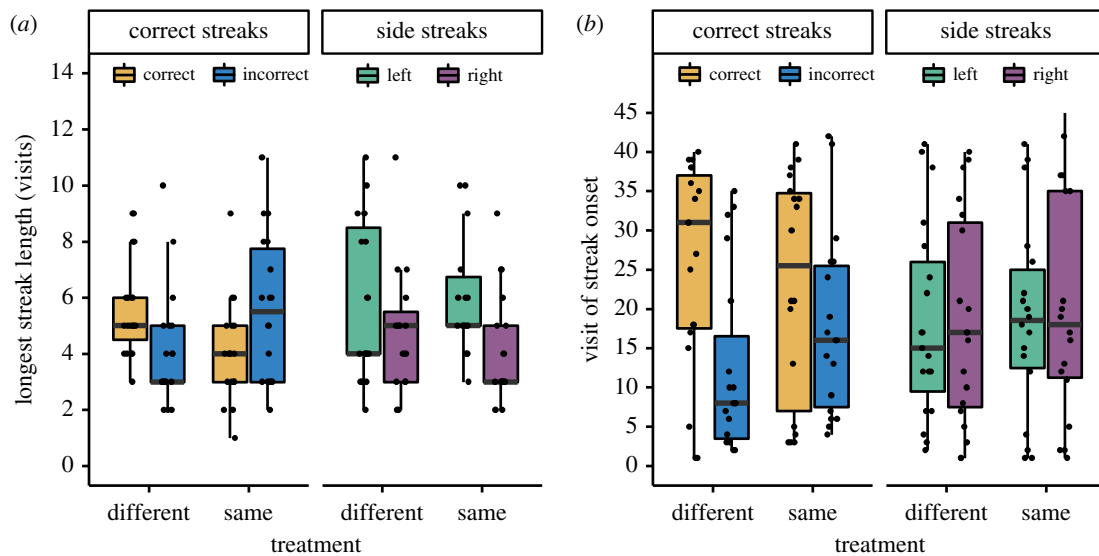


Figure 3. (a) The longest streaks of correct and incorrect decisions (left) and left and right decisions (right) for each ant and treatment. Three ants with very long streaks (two left streaks, 27 and 17 visits long; one right streak, 19 visits) are not shown. Ants made significantly longer correct streaks in the ‘different’ treatment ($p = 0.0374$), while the opposite was found in the ‘same’ treatment ($p = 0.0382$). Left streaks tended to be longer in both treatments, but this difference was significant in the ‘same’ treatment only ($p = 0.0219$). (b) Visits until streak onset for correct and incorrect streaks (left) and left and right streaks (right). Correct streaks started in later visits in both treatments, but this difference was significant in the ‘different’ treatment only ($p = 0.0034$). No difference was found in the onsets of left and right streaks. 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 $1.5 \times$ interquartile range. (Online version in colour.)

($\chi^2 = 8.97$, $p = 0.0027$) 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 = 1.37, $p = 0.0374$; ratio = 0.72, $p = 0.0382$, respectively, figure 3a). The longest correct streaks were nine visits long in both treatments, whereas the longest incorrect streaks spanned 10 and 11 visits (‘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 ($\chi^2 = 5.08$, $p = 0.0242$), and estimated marginal mean contrasts revealed that this was within ants in the ‘same’ treatment (ratio = 0.63, $p = 0.0219$), but not within the ‘different’ treatment (ratio = 0.85, $p = 0.3788$). 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 nine visits long (‘different’ and ‘same’, respectively).

The onset of correct streaks during training started consistently later than incorrect streaks ($\chi^2 = 8.43$, $p = 0.0037$, figure 3b), but the effect was significant in the ‘different’ treatment only (contrasts: ‘different’: ratio = 2.11, $p = 0.0034$; ‘same’: ratio = 1.09, $p = 0.2782$). The onsets of left and right streaks did not differ significantly ($\chi^2 < 0.01$, $p = 0.9941$).

(c) Heuristics

To analyse which potential heuristics were used by individual ants (table 1), we assigned each ant to a heuristic if it chose the arm the heuristic would suggest in at least 2/3

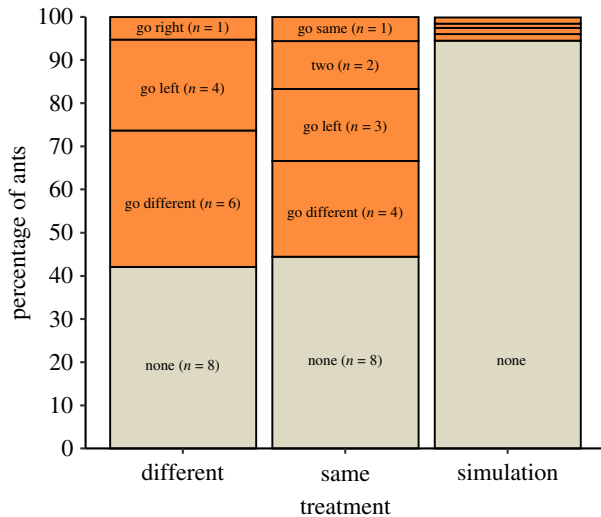


Figure 4. Percentage of ants which used a defined strategy for a minimum of 2/3 of their visits in the ‘different’ ($n = 19$) and ‘same’ treatment ($n = 18$) and in a simulation using random choices. Over half of the ants acted according to a certain heuristic in the two treatments, while random guessing would only lead to such a behaviour in 5.5% of cases according to the simulation results. 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’). (Online version in colour.)

(66.6%) of its visits. This proportion corresponds to $p < 0.05$ in 48 visits when the chance of being correct is 50%. Using this method, we found that 58% (11/19) of ants in the ‘different’ treatment deployed a heuristic according to our criterion, as did 56% (10/18) of ants in the ‘same’ treatment (two ants could have been using two heuristics, table 1 and figure 4). Our simulation result demonstrated that the chance of meeting the criterion by choosing randomly was only 5.5% (2198 of 40 000 simulations).

The most prominent heuristic in both treatments was to go to the odour different from the stem (go different). Further analyses on the 26 visits allowing a potential ‘go linolool’ heuristic did not find any indication of its use. Also, a close look at ants’ odour preferences revealed a preference for sandalwood, which, however, could not explain the observed results (see ESM, S1).

An explicit threshold (here 66.6%) increases clarity but does not provide information on how faithfully an ant follows a given heuristic. Thus, we provide an additional figure with scores for all heuristics per ant in the supplement (see ESM, figure S2A and B).

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 ESM, S1). The null model was thus highly explanatory and demonstrated that performance is best described by the individual ant, with no dominant heuristic at the group level.

4. 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, contrary to our hypothesis. Our analyses suggest that ants did not use a relational rule of same/different to guide their decisions and may not be able to do so. Rather, they seemed to base their decisions on heuristics such as ‘go left’ or ‘go to the most salient cue’ (see below).

Although significant, the increase in performance in the ‘different’ treatment was modest, with 65% correct decisions (74/114) in the last bin compared to 60% (68/114) in the first bin (figure 2a). While we do expect a high proportion of ants failing to learn complex tasks due to individual variation in learning abilities [61], the high initial performance of 60% correct decisions and the fact that the ants’ performance did not resemble an asymptotic learning curve suggests that the majority of ants did not rely on learning. Rather, the high initial performance indicates that some ants used unlearned heuristics to systematically guide their decisions. Moreover, the fact that the inverse overall performance of ants in the ‘same’ treatment resembled that of the ‘different’ treatment (figure 2c) also suggests that ants were not learning and using a relational rule of ‘different’ but rather other cues common to both treatments. It is worth noting that the low performance in the second bin (figure 2a) in the ‘different’ treatment suggests initial learning attempts, as ants predominantly chose the odours presented at the stem which were acting as targets and thus rewarded in the first six visits.

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 (figure 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. These findings do not suggest that ants learned a relational rule, as we originally hypothesized they would.

Instead, 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 set-up 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. Thus, 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’ (figure 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 most salient cue'.

At a first glance, the sensory adaptation hypothesis does not seem to explain why ants would also use this rule 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 persistence of erroneous behaviour was also reported in a study by Macquart *et al.* [62], where ants took longer to learn a new rule once a misleading rule was in place. Zhang [63] also reported that two bees persisted to use a rule to 'always go to one side' which took longer but also allowed them to navigate the maze.

Heuristics can provide a rule that may be better than step-wise optimization through learning in cases of highly complex or uncertain information, and where the costs of errors are low [29]. Our analyses of ants' decisions revealed that half of the ants in both treatments chose in a manner consistent with 'go to different (the more salient odour)' or 'go left/right' in at least 66.6% (32/48) of their visits (figure 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, ants 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 individual specific, i.e. each ant chooses differently. It is important to note that this experiment was not *a priori* designed to demonstrate heuristics in ants, and these interpretations of the results are *post-hoc* in nature. Thus, we strongly encourage other researchers to continue this research direction, validating our findings with dedicated tests of heuristic use.

Many ants also chose to 'go left' during our treatment. Side biases are commonly observed in many animals [64–66], and *L. niger* are no exception. They were found to display right [67] and also left biases [59]. In our study, ants displayed very long streaks to both sides (figure 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. A side bias might lessen the cognitive load of foraging ants, as a sequence does not require the ant to memorize each decision. Indeed, maze studies have been found that

ants and bees best memorize repeating sequences such as left-left [62,63,68].

Use of heuristics is promoted when error costs are low [28,31]. The costs of making a wrong decision might have been too small to promote careful decisions in our set-up. 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. Similarly, Josens *et al.* [49] also found weak effects of quinine on freely moving ants in a Y-maze. 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. They even did so when the chosen heuristic led to poorer results than expected by chance. It thus seems that, in some situations, following even an inappropriate heuristic is easier, or in some way preferable, to random choice. Cognitive processes have not evolved to ascertain objective reality, but to provide decisions that maximize fitness gains [28]. Heuristics often provide decision rules that 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 [69]. Facing a complex challenge, animals might change heuristics or even modify them by learning [30]. And indeed, the 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 'go right'. But many would rather use heuristics than simply leaving matters to chance.

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

Data accessibility. An annotated script and output for all data handling and statistical analyses is presented in ESM, S1. The complete raw data are provided in ESM, S2.

Authors' contributions. F.B.O. and T.J.C. conceived the experiment; F.B.O. and A.K. collected the data; F.B.O. and M.d.A. analysed the data; F.B.O. wrote the manuscript, all authors revised the draft. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. The authors declare that they have no competing interests.

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