ESM for Czaczkes & Heinze (2015):

Ants adjust their pheromone deposition to a changing environment and their probability of making errors

Contents

Detailed results

Average pheromone deposition by number of training visits to first food source

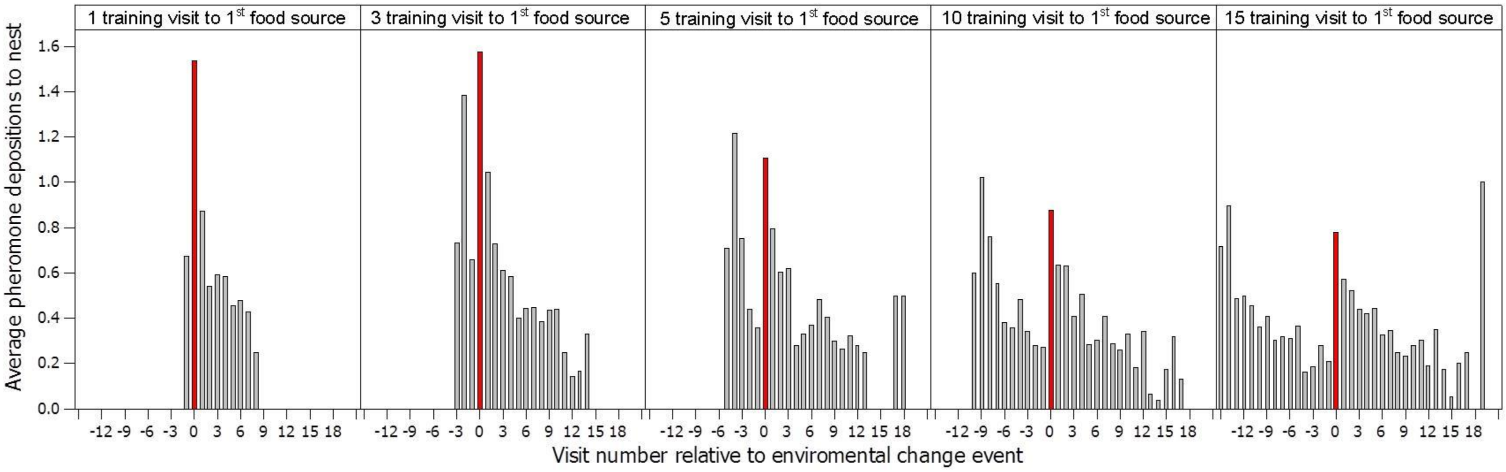

Figure S1 - Average pheromone deposition by number of training visits to first food source

Post-hoc pairwise comparison of ants which had, or had not, just experienced and environmental change, and which had, or had not, just made a navigational error.

Ants returning from a food source to the nest responded differently depending on whether they had just experienced a change in environment (the food source was not where it had been on their previous visit), and whether they chose the branch on which they had found food on a previous visit: ants, which had just experienced an environmental change, deposited significantly more pheromone than ants, which found the feeder in the same location as they had on previous visits. We performed pair-wise post-hoc tests to distinguish the four groups, which the ants could fall into. Table S1 provides the post-hoc comparisons for the number of pheromone depositions performed, including both ants which did and did not deposit trail pheromone, as illustrated in the figure 2 inset in the main manuscript.

Table S1 - *(read all tables as Y compared to X)*	Switch & ant went to new food location	Switch & ant went to previous food location	Not switched & ants made a correct decision	Not switched & ants made an error
Switch & ant went to new food location	NA	No diff Z = 0.263 P = 0.792	More phero Z = 17.333 P < 0.0001	More phero Z = 10.389 P < 0.0001
Switch & ant went to previous food location	No diff Z = 0.263 P = 0.792	NA	More phero Z = 4.938 P < 0.0001	More phero Z = 3.022 P = 0.00251

Not switched & ants made a correct decision	Less phero Z = -4.938 P < 0.0001	Less phero Z = -17.33 P < 0.0001	NA	Less phero Z = -9.061 P < 0.0001
Not switched & ants made an error	Less phero Z = -3.022 P = 0.00502	Less phero Z = -10.39 P < 0.0001	More phero Z = 9.06 P < 0.0001	NA

We also analysed the behaviour of the ants separately for deposition probability (whether an ant deposited pheromone at all, or not – table S2 and figure S2 below), and intensity (of the ants depositing pheromone, the number of depositions performed – table S3 and figure S3 below).

Table S2 – deposition probability	Switch & ant went to new food location	Switch & ant went to previous food location	Not switched & ants made a correct decision	Not switched & ants made an error
Switch & ant went to new food location	NA	No diff Z 1.05 P = 0.295	No diff Z = 1.84 P = 0.066	No diff Z = -1.01 P = 0.311
Switch & ant went to previous food location	No diff Z -1.05 P = 0.295	NA	More phero Z = 10.30 P < 0.0001	More phero Z = 3.022 P = 0.00251
Not switched & ants made a correct decision	No diff Z = -1.84 P = 0.066	Less phero Z = -10.30 P < 0.0001	NA	Less phero Z = -5.34 P < 0.0001
Not switched & ants made an error	No diff Z = 1.01 P = 0.311	Less phero Z = -7.08 P < 0.0001	More phero Z = 5.34 P < 0.0001	NA

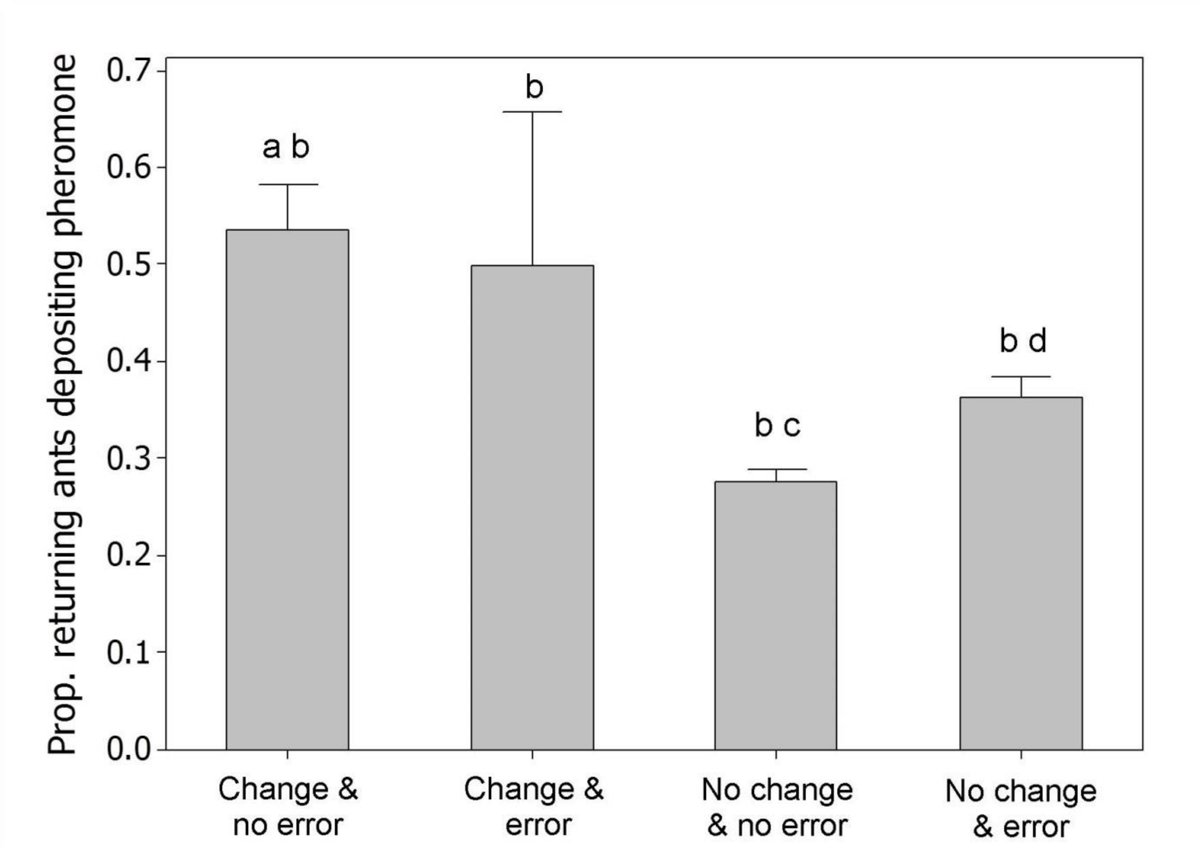

Figure S2 – proportion of ants depositing pheromone on return journey

Table S3 – deposition intensity	Switch & ant went to new food location	Switch & ant went to previous food location	Not at the switch & ants made a correct decision	Not at the switch & ants made an error
Switch & ant went to new food location	NA	No diff Z = 1.14 P = 0.254	More phero Z = 17.333 P < 0.0001	More phero Z = 2.69 P = 0.007
Switch & ant went to previous food location	No diff Z = -1.14 P = 0.254	NA	More phero Z = 7.41 P < 0.0001	More phero Z = -4.45 P < 0.0001
Not at the switch & ants made a correct decision	Less phero Z = 3.6 P = 0.0003	Less phero Z = -7.41 P < 0.0001	NA	Less phero Z = -3.90 P < 0.0001
Not at the switch & ants made an error	Less phero Z = -2.69 P = 0.007	Less phero Z = -4.45 P < 0.0001	More phero Z = 3.90 P < 0.0001	NA

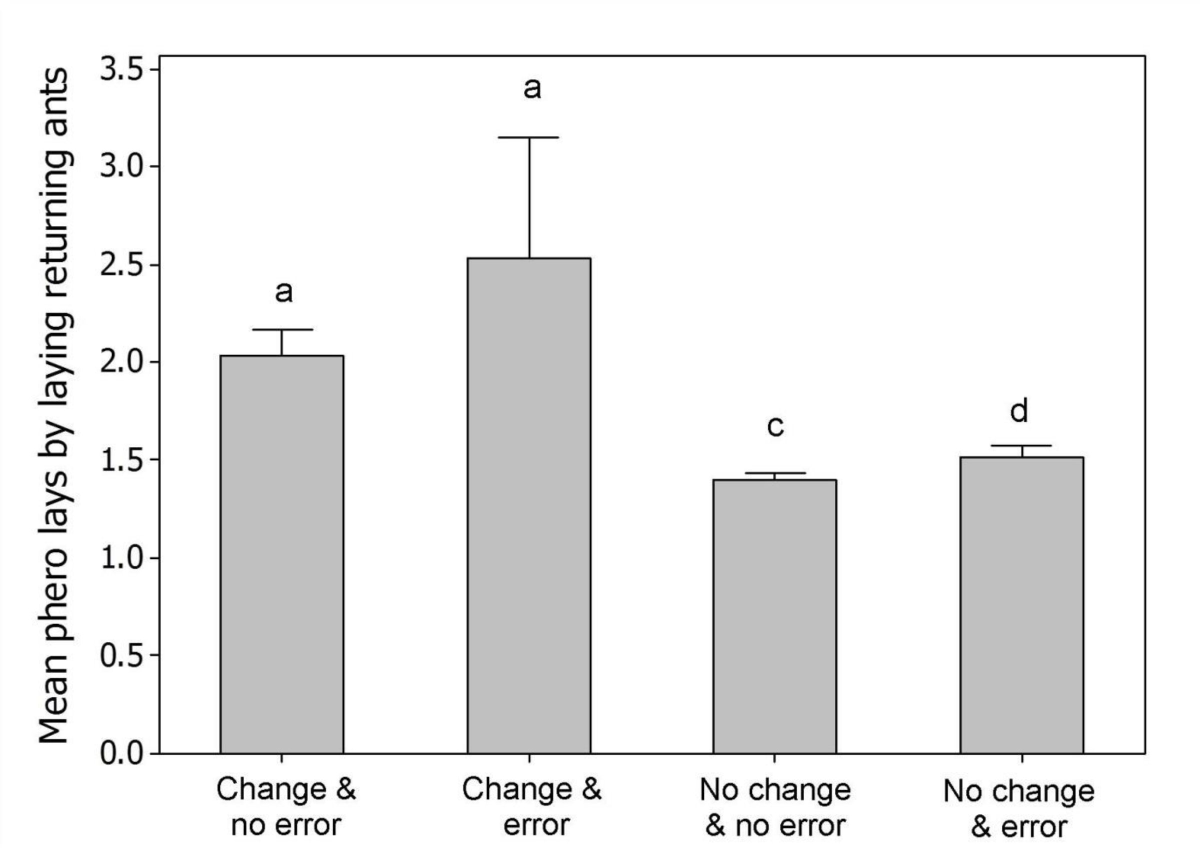

Figure S3 – mean pheromone depositions by ants, which made at least one pheromone deposition on their return journey

Post-hoc pairwise comparison of travelling to (returning) or from (outgoing) the nest, and which had, or had not, just made a navigational error.

Ants, which were about to make an error, deposited less pheromone on their way to a food source than ants in the other possible states (see figure 3 in main text, and table S4 below). Conversely, ants which have just made an error deposit more pheromone when returning to the nest. We performed pairwise post-hoc analyses to explicitly compare the four possible groups ants could fall into. We first performed these tests on the number of pheromone depositions, including ants, which did not deposit pheromone (table S4, figure 3 in main text). We then separated pheromone deposition probability (whether and ant deposits pheromone at all – table S5, figure S4 below) and intensity (if an ant deposits pheromone, how many depositions does she perform – table S6, figure S5 below). As can be seen, the main effects were mostly driven by deposition probability, not intensity.

Table S4 *(read all tables as Y compared to X)*	**To nest & error**	**To nest & correct**	**To feeder & error**	**To feeder & correct**
To nest & error	NA	More phero Z = 7.087 P < 0.0001	More phero Z = 17.579 P < 0.0001	More phero Z = 16.236 P < 0.0001
To nest & correct	Less phero Z = -7.087 P < 0.0001	NA	More phero Z = 14.08 P < 0.0001	More phero Z = 16.236 P < 0.0001
To feeder & error	Less phero Z = -17.579 P < 0.0001	Less phero Z = -14.08 P < 0.0001	NA	Less phero Z = -7.683 P < 0.0001
To feeder & correct	Less phero Z = -16.239 P < 0.0001	Less phero Z = -11.639 P < 0.0001	More phero Z = 7.682 P < 0.0001	NA

Table S4) Pairwise comparisons of the four possible combinations of ants going to the feeder or returning to the nest, and of whether or not an error was/will be made. For ants going to the feeder “correct” or “error” refers to their upcoming decision. For ants returning to the nest, “correct” or “error” refers to the decision the ant has made on the current visit.

Table S5 – deposition	**To nest & error**	**To nest & correct**	**To feeder & error**	**To feeder & correct**

probability				
To nest & error	NA	More phero Z = 9.98 P < 0.0001	More phero Z = 17.88 P < 0.0001	More phero Z = 11.20 P < 0.0001
To nest & correct	Less phero Z = -9.98 P < 0.0001	NA	More phero Z = 16.30 P < 0.0001	More phero Z = 8.93 P < 0.0001
To feeder & error	Less phero Z = -17.88 P < 0.0001	Less phero Z = -16.3 P < 0.0001	NA	Less phero Z = 10.74 P < 0.0001
To feeder & correct	Less phero Z = -11.20 P < 0.0001	Less phero Z = -8.93 P < 0.0001	More phero Z = 10.74 P < 0.0001	NA

Table S5) Pairwise comparisons of the four possible combinations of ants going to the feeder or returning to the nest, and of whether or not an error was/will be made, considering only whether ants deposited pheromone or not. For ants going to the feeder “correct” or “error” refers to their upcoming decision. For ants returning from the nest, “correct” or “error” refers to the decision the ant has on the current visit.

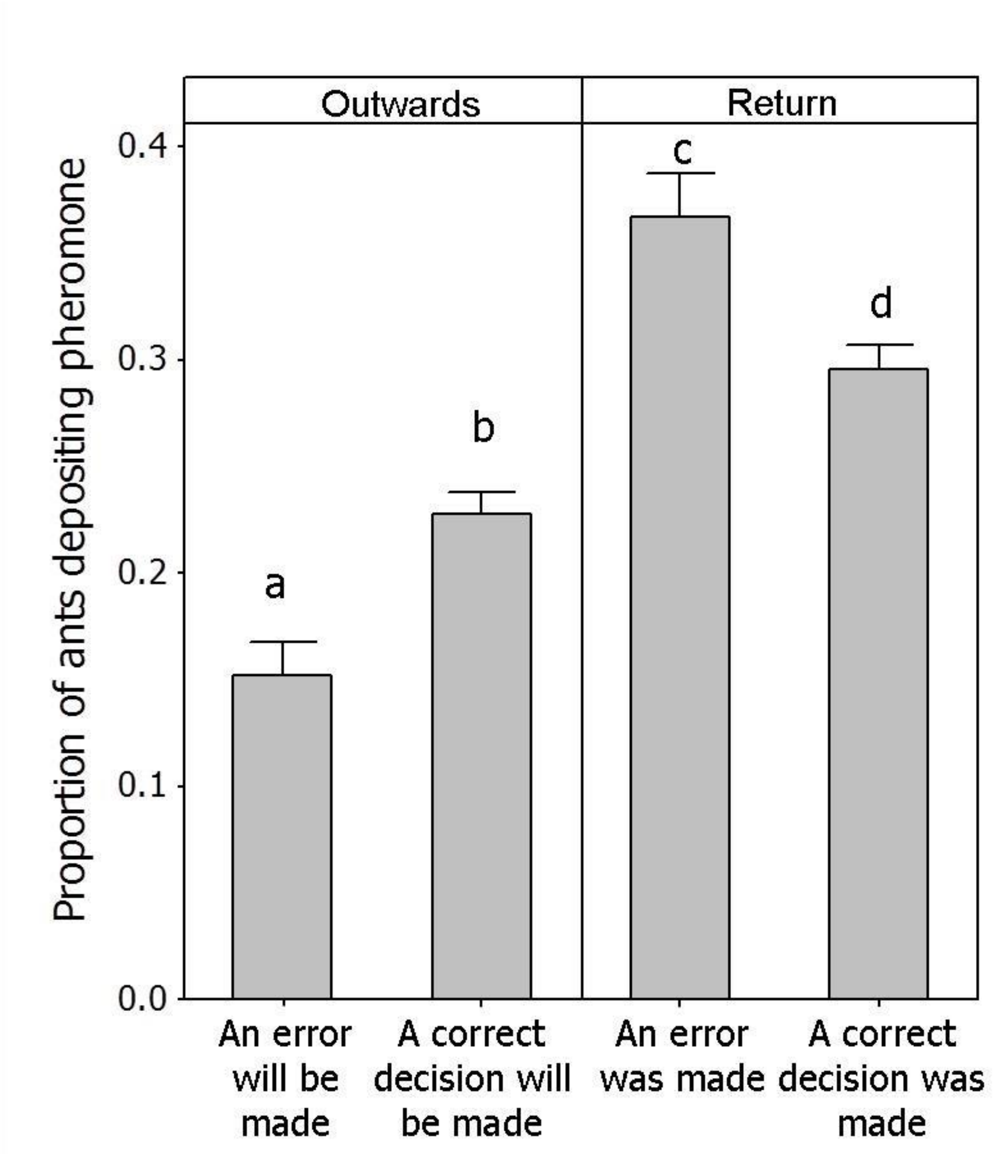

Figure S4 – proportion of ants depositing pheromone by direction and whether they will make/have made an error.

Table S6 – deposition probability	**To nest & error**	**To nest & correct**	**To feeder & error**	**To feeder & correct**
To nest & error	NA	More phero Z = 2.72 P = 0.0066	More phero Z = 17.88 P < 0.0001	More phero Z = 11.20 P < 0.0001
To nest & correct	Less phero Z = -2.72 P = 0.0066	NA	More phero Z = 16.30 P < 0.0001	More phero Z = 8.93 P < 0.0001
To feeder & error	Less phero Z = -4.10	Less phero Z = -2.71	NA	No diff Z = -1.26

	P < 0.0001	P = 0.0069		P = 0.21
To feeder & correct	Less phero Z = -4.75 P < 0.0001	Less phero Z = -2.63 P = 0.0087	No diff Z = 1.26 P = 0.21	NA

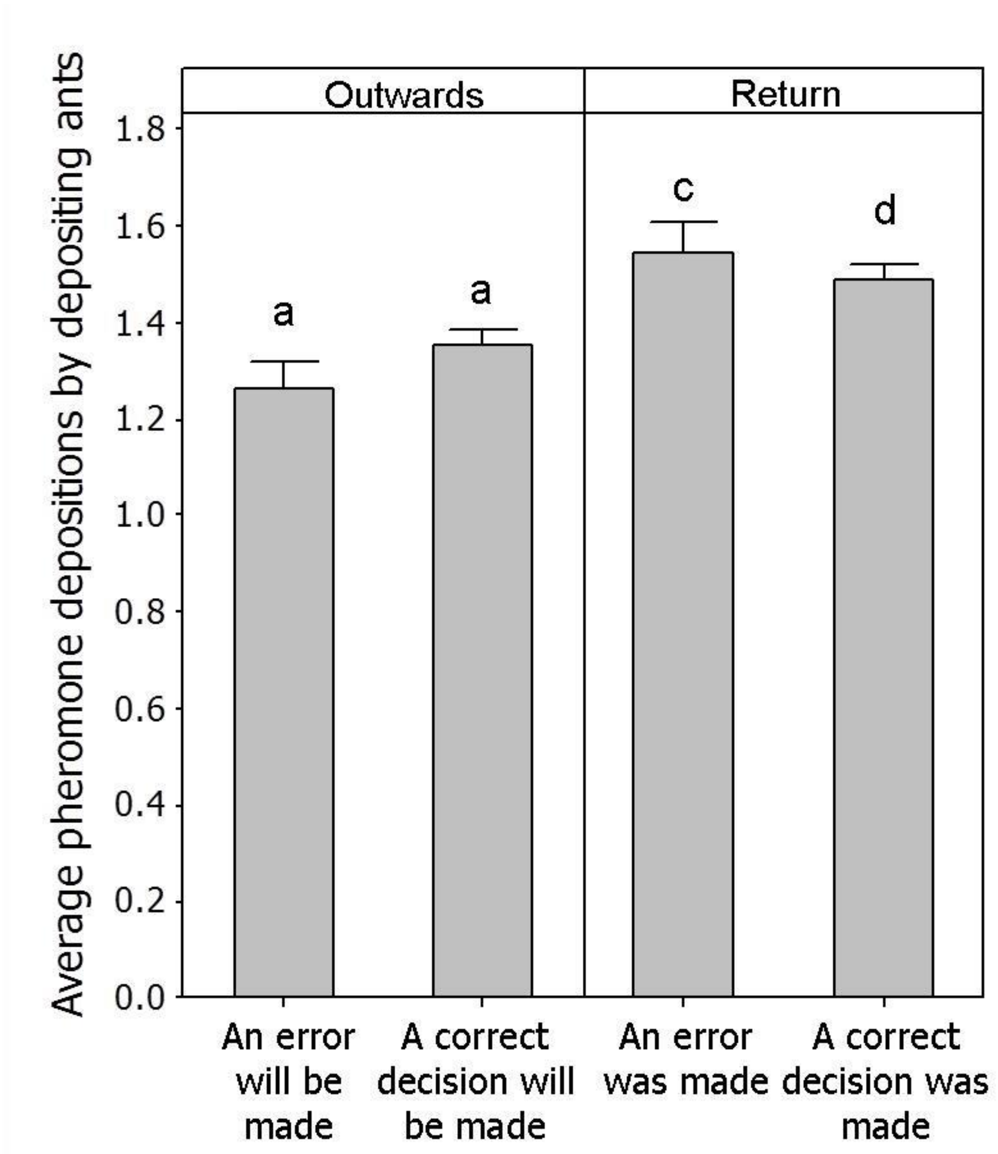

Figure S5 – mean pheromone depositions by ants, which made at least one pheromone deposition, by travel direction and whether or not they will make / have made an error

Supporting the claim of metamemory judgements

Extraordinary claims have a high burden of proof. While Smith et al [1] convincingly warn that over-restrictive thresholds in the interpretation of metacognition risk 'throwing the baby out with the bathwater', we feel that any claim of metacognition in an invertebrate requires the explicit exclusion of alternative, low-level interpretations of their behaviour. In this supplement we discuss the major pitfalls in attempts to experimental ly demonstrate metacognition in animals, and describe how our data avoid these and other pitfalls. However, our experiment was not specifically designed to demonstrate metacognition. Our data could, with sufficient creativity, also be explained by lower level processes. For example, it is conceivable (but perhaps unlikely) that a factor which we did not control for (e.g. age, corpulence, etc) may result in one subset of ants making more learning errors, depositing more pheromone on return journeys, and depositing less pheromone on outwards journeys. We thus are not making a claim for an incontrovertible proof of metacognition in an invertebrate.

The endeavour of assigning metacognitive abilities to non-human animals has been plagued by concerns that the evidence provided does not support high-level cognitive claims [1–3]. Smith et al [1] provide a list of four basic pitfalls which must be avoided by comparative psychologists when ascribing metacognitive abilities.

Concerns traditionally ascribed to classical studies of animal metacognition

Classically, the study of animal metacognition involves providing animals with a series of trials in which the task performed becomes increasingly difficult. Animals may make a choice and are rewarded for correct choices (and usually punished for incorrect choices). Animals are also provided with an 'uncertain' response, which allows the animal to forgo answering the challenge, while either receiving a small reward or avoiding punishment. A correlation of the use of the 'uncertain' response with the difficulty of the task is taken as a signal of metacognitive abilities.

Reinforcement of uncertainty responses

In studies where choosing 'uncertain' is rewarded (e.g.[4,5]), choosing 'uncertain' may become attractive in and of itself, and independent of its' metacognitive role. This concern is ruled out in the current experiment as the ants studied were rewarded regardless of the accuracy of their decision, and regardless of their choice to deposit pheromone or not.

Reaction to primary stimulus quality

In classical metamemory experiments, animals are asked whether one stimulus is similar to or different from another. For example, a dolphin (*Tursiops truncatus)* had to decide whether an audio tone heard was high (2100 Hz) or low (below 2100 Hz)[6]. Thus, values at one end of the stimulus range can become aversive or associated with the 'uncertain' response. In the dolphin example, higher pitched tones, not necessarily uncertainty, could be responded to with an 'uncertain' response. This concern is ruled out in the current experiment as the stimuli to which the ants were exposed were disassociated from task difficulty. Indeed, the stimuli to which the ants were exposed were not changed.

Entrainment to reinforcement contingencies

If reward or punishment occurs after every trial, animals may learn to associate certain stimulus-response pairs with reward or punishment. Thus, for example, monkeys shown two similar stimuli may learn to associate seeing such a stimulus and choosing 'uncertain' with a positive outcome. This would not require making a metacognitive judgement. This possibility is ruled out in the current experiment as the ants do not have to be trained to declare their uncertainty. Moreover, the ants are rewarded regardless of their behavioural choices.

Representational specificity

In this criticism of metacognitive abilities, it is claimed that the response may only be possible in a narrow range of situations. This may or may not be the case in the current experiment, as the ants were only interrogated as to whether they remember the location of a feeder. However, while this criticism may weaken claims of ant metacognition being analogous to human metacognition, it does not weaken the claim that metacognition is occurring.

Other possible objections to the current claim of metacognition in ants

Pheromone deposition rates may simply correlate with the progression of the learning process

As the ants in this study learned over time (data to be published separately in [7]), it is conceivable that the correlation between future decision accuracy and pheromone deposition may be due to ants in later visits depositing more pheromone, and ants in later visits also being more accurate. However, if the number of previous visits each ant has made is added to the model, the upcoming choice of the ants still significantly predicts pheromone deposition behaviour (GLMM, Z = 2.54, P =

0.022). Indeed, the number of pheromone depositions made by ants drops in later visits (Z = -2.40, P = 0.022).

Pheromone deposition rates may be controlled by previous success rates

To test the possibility that the reduction in pheromone deposition could be explained by ants tracking their previous errors rather than predicting future performance, we attempted to model pheromone deposition by the number of cumulative errors each ant had made as well as whether their upcoming decision would be correct or not. While the upcoming decision of the ant significantly predicted pheromone deposition (Z = 7.236, P < 0.0001), the number of previous errors the ant had made did not significantly predict pheromone deposition (Z = -1.51, P = 0.131, see figure S6 below).

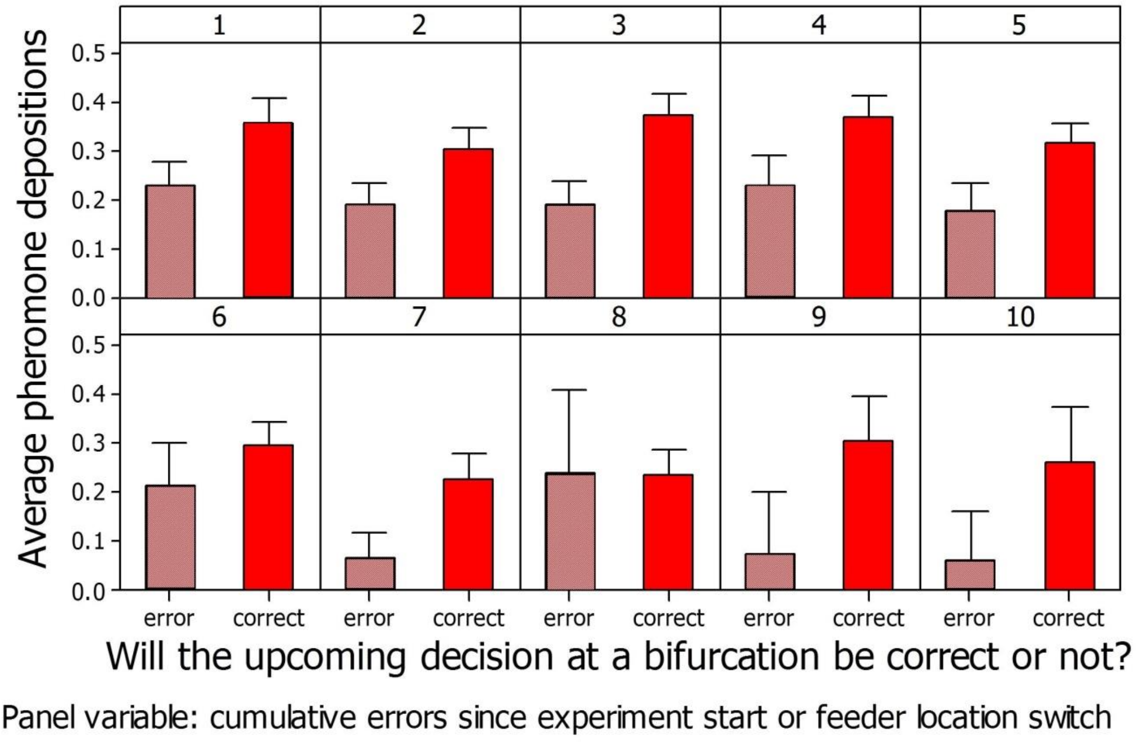

Figure S6) average pheromone depositions for ants which will either make an error or a correct decision at an upcoming bifurcation. Each panel shows ants with a different number of cumulative errors since the start of the experiment or the point at which the feeder locations were switched. Ants deposited less pheromone when they were about to make an error regardless of the number of previous errors they have already made.

Individual variation drives the correlation between making errors and lower pheromone deposition

An alternative hypothesis that could explain our findings would be that some ants are less capable of learning, and these same ants also deposit less pheromone on the outwards journey. However, the identity of individual ants was added as a random effect during the statistical analysis. If individual variation were the only driver of this pattern, no significant effect of error making would be found after taking individual identity into account.

Related to this alternative hypothesis is the possibility that ants vary in their motivation or enthusiasm for the food source (see below).

Pheromone deposition rates and making "errors" may be both due to a lack of enthusiasm for the known food source

An alternative hypothesis that could explain our findings would be that some ants do not rate the quality of the feeder they know the location of as high. These ants may deposit less pheromone on the way to the feeder, and may also search in other locations for a food source, instead of returning to the known food source. However, this hypothesis is not well supported by the data. All colonies were identical in their level of starvation, and the food quality remained the same. This, and the fact that individual variation cannot explain the results (see above), requires the individual ants to change their enthusiasm over the course of the experiment for the alternative 'enthusiasm' hypothesis explanation to be correct. However, even if one takes the visit number into account, whether the ant will make a correct decision or not still significantly accounts for its' pheromone deposition rate (see detailed results). Moreover, there is no significant interaction between visit number and whether the ant will make a correct decision or not. One would expect the enthusiasm of ants to change in a regular manner over consecutive visits. The fact that this change in pheromone deposition number over multiple visits does not account for the effect of the upcoming decision makes suggests that a different mechanism, namely metacognition, is responsible for the observed behaviours. Moreover, overall the ants deposit less pheromone in later visits, but make fewer errors in later visits. If the ants were making 'errors' due to lack of enthusiasm, we would expect the ants either to make more 'errors' over time (as they became less enthusiastic) or deposit more pheromone over time (as they became more enthusiastic).

Lastly, a lack of enthusiasm for a food source is clear when it is observed, with the ants not drinking fully at a food source, walking away many times from the foods source, or leaving the food source with their crop not completely full (pers. obs.). This did not happen during these experiments, and the ants were always apparently very 'enthusiastic' about their food. Occasionally, in other experiments, we noted a lack of enthusiasm in ants, but such a lack of enthusiasm is always apparent from the beginning of the experiment. A change in enthusiasm during a foraging bout is only ever noted when the ant fails return to the food source from the nest.

Alternative explanations for the up-regulation of pheromone deposition by returning ants

Ants deposit more pheromone on their return journey after making a navigational error

We believe that this finding reflects the ants responding to an apparent need for more information by providing it in the form of more trail pheromone.

An alternative explanation could be that making an error results in a more time or energy consuming trip. Similarly, ants may be responding to a more 'frustrating' trip. However, it does not seem reasonable that ants would increase their pheromone deposition rates in response to such negative aspects of their trip.

A more plausible possibility is that the ants are 'summing' their total pheromone depositions on the outwards and return journey in such a way as to produce a steady level of trail pheromone. Thus, when they must reduce pheromone deposition due to uncertainty on their outwards journey, they must increase it on the return journey to compensate. Indeed, the difference in average pheromone deposition by ants which had or had not made a navigational error is almost exactly the opposite of average pheromone deposition by ants on their outwards journey which will or will not make an error (see table S7 below). This alternative explanation is not easily discounted, and indeed both this explanation and our preferred reasoning may be correct.

Table of mean pheromone depositions	*error*	*no error*	*difference*
Travel direction: to nest	0.592	0.474	0.118
Travel direction: to food	0.201	0.325	-0.124
sum	0.793	0.799	-0.006

Table S7– average pheromone depositions by ants on their outward or return journey, when they either have made (or will make) and error or not.

Ants deposit more pheromone on their return journey after an environmental change

We believe that this finding reflects the ants responding to an apparent need for more information, due to the old information becoming outdated, by providing it in the form of more trail pheromone.

A potential alternative explanation is that the ants are simply responding to the discovery of a new food source, not the disappearance of the old food source as well. Indeed, there was no significant difference in the pheromone deposition of returning ants which had or had not made a navigational error (and thus had or had not experienced the lack of the original food source). However, only 26 ants had made an error just as the environment was changed, and thus went directly to the new food location. The statistical power of this comparison is thus rather weak. Moreover, it is very likely that most of the ants had explored the unrewarding arm of the T-maze before it became rewarding, and so the discovery of a food source there may still be considered an environmental change, not a simply the discovery of a new food location. The current data does not allow us to rule our either of these two explanations.

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